Revista Iberoamericana de Psicología y Salud
ISSN: 2171-2069
rips@usc.es
Sociedad Universitaria de Investigación en Psicología y Salud
España

López, Laudino; Méndez, Marta; Méndez-López, Magdalena; Aller, María Ángeles; Arias, Jaime; Díaz, Fernando; Arias, Jorge L.

THE PORTACAVAL SHAM OPERATION IN RATS AFFECTS ACQUISITION BUT NOT MEMORY OF AN ACTIVE AVOIDANCE TASK

Revista Iberoamericana de Psicología y Salud, vol. 1, núm. 1, enero, 2010, pp. 81-99
Sociedad Universitaria de Investigación en Psicología y Salud
A Coruña, España

Revista Iberoamericana de Psicología y Salud, 2010, 1(1): 81-99
www.usc.es/suips

THE PORTACAVAL SHAM OPERATION IN RATS AFFECTS ACQUISITION BUT NOT MEMORY OF AN ACTIVE AVOIDANCE TASK

Laudino López*, Marta Méndez*, Magdalena Méndez-López*, María Ángeles Aller**, Jaime Arias**, Fernando Díaz***, and Jorge L. Arias*

* Laboratorio de Neurociencias. Departamento de Psicología. (Universidad de Oviedo)

** Departamento de Cirugía I, Facultad de Medicina, (Universidad Complutense de Madrid)

*** Departamento de Biología Funcional (Fisiología). Facultad de Medicina (Universidad de Oviedo).

(Received 8 June 2009; revised 2 September 2009; accepted 4 September 2009)

Abstract

Hepatic encephalopathy is one of the most important diseases and is the focus of investigation by many research groups. One of the most frequently used models is the portocaval shunt. This requires a surgically intervened group and also a sham operated group as control. Our objective was to examine whether the sham operation had physiological or behavioural consequences for the animals. Two groups of rats were studied: rats that had undergone a sham operation consisting in a laparotomy followed by clamping of the portal vein and inferior vena cava for 15 min; and an unoperated control group. The animals were then submitted to behavioural tests and plasma testosterone and corticosterone levels were determined. The sham-operated rats behaved slightly differently to the control rats in the open field. They tended to walk more in the central area. In the Morris pool, they learnt the task one day later than the control group. In the associative learning test, the sham operation prevented rats from being able to learn the task. However, the sham operation did not interfere with the rats remembering a previously learnt task. The sham group also presented higher levels of plasma corticosterone than controls. It seems necessary to reconsider what would constitute the most appropriate control group for portacaval shunt.

Keywords: sham-operation; open field; Morris water maze; active avoidance; Wistar rat.

Resumen

La encefalopatía hepática es una de las enfermedades más importantes y es objeto de estudio por muchos grupos de investigación. Uno de los modelos más frecuentemente utilizados para su estudio es la derivación portocava. El estudio de este modelo requiere de la realización de un grupo intervenido quirúrgicamente y un grupo pseudo-operado que funciona como control. Nuestro objetivo es examinar si la pseudo-operación produce alteraciones fisiológicas o comportamentales en los animales. Estudiamos dos grupos de ratas: ratas que fueron sometidas a una pseudo-operación, consistente en una laparotomía seguida de clampaje de la vena porta y la vena cava inferior durante 15 minutos, y un grupo control no operado. Los animales fueron sometidos a pruebas de comportamiento y se determinaron los niveles de testosterona y corticosterona en plasma. Las ratas pseudo-operadas se comportaron de forma ligeramente diferente a las ratas control en el campo abierto, tendiendo a caminar más en la zona central. En la piscina de Morris, aprendieron la tarea un día después que el grupo control. En la prueba de aprendizaje asociativo, la pseudo-operación impidió que las ratas fueran capaces de aprender la tarea. Sin embargo, la pseudo-operación no interfirió en la capacidad de las ratas para recordar una tarea que anteriormente habían aprendido. El grupo pseudo-operado presentó niveles más altos de corticosterona en plasma que los controles. Sería necesario reconsiderar cuál es el grupo control más apropiado para la derivación portocava.

Palabras clave: pseudo-operación; campo abierto; Laberínto acuático de Morris; Evitación activa; rata Wistar.

Correspondence: Laudino López, Ph. D. Laboratorio de Neurociencias. Departamento de Psicología, Plaza Feijoo s/n. 33003 Oviedo, Spain,. E-mail: llopez@uniovi.es

Introduction

Hepatic encephalopathy (HE), mainly associated with viral or alcoholic cirrhosis, is one of the most important diseases of the Western World. The most accepted and used classification for HE is the one proposed by Ferenci, Lockwood, Mullen, Tarter, Weissenborn, & Blei (2002), according to which it is classified into three main types, depending on the origin or cause. The first is type A, which refers to encephalopathy associated with acute liver damage. The next is type B, which concerns HE related to portosystemic shunt, which does not require any hepatocytic alteration. The third, referred to as type C, is associated with cirrhosis and portal hypertension with portosystemic shunt. However, in spite of advances in recent years there are still many unanswered questions about HE, in relation to its ethiopathogeny (Butterworth, 2003; Hazell & Butterworth, 1999; Ong & Mullen, 2001), treatment (Blei & Córdoba, 2001) or diagnosis (Montagnese, Amodio, & Morgan, 2004; Quero & Herrerías, 2006). It is, therefore, necessary to develop different experimental models of hepatic insufficiency and to recur to further animal experimentation (Bhatnagar & Majumdar, 2003; Blei, Omary, & Butterworth, 1992; Chamuleau, 1996). Moreover, since HE can be derived from three types of hepatic insufficiency, it is necessary to develop experimental models that can imitate, as closely as possible, the characteristics of type A, B or C HE.

The models proposed for type A HE (associated with acute liver failure) include treatment with hepatotoxic drugs, total or partial devascularization and total or partial hepatectomy (Chamuleau, 1996; Filipponi & Mosca, 2001; Terblanche & Hickman, 1991). Combined techniques can also be used such as portocaval anastomosis and posterior ischaemia (Benoist et al., 2000) or portacaval anastomosis and partial hepatectomy (Filipponi et al., 1991). However, none of these models reproduce all the characteristics of acute human HE, although the individual features of each model can help, independently, to study specific aspects, such as how to prolong survival or control intracranial pressure (Filipponi & Mosca, 2001). Nor is there a good model either for chronic HE. Perhaps the most effective and also the most used is that of portocaval anastomosis, but this is only a good model of type B HE (Jover, Madaria, Felipo, Rodrigo, Candela, & Compan, 2005) although it does not reproduce many characteristics of portosystemic encephalopathy (Blei et al.,

1992). Other models of type B HE use partial ligature of the vena porta to simulate the portosystemic shunt (Abraldes, Pasarín, & García-Pagán, 2006; Diéguez et al., 2002). Finally, the models proposed for chronic type C HE (associated with cirrhosis and portal hypertension) require the use of hepatotoxins, such as intoxication with carbon tetrachloride or with thioacetamide (Chamuleau, 1996; Laleman et al., 2006; Li, Benjamin, & Alexander, 2002) or obstruction of the bile duct (Jover et al., 2005; Kountouras, Billing, & Scheuer, 1984).

It is clear from the above, that many models have to use surgery to obtain the hepatic insufficiency. This is the case of hepatectomies, devascularization, portocaval anastomosis or portal hypertension. One methodological consequence of practising surgery in experimental models is the need to use animals in a sham operation. To date, the possible physiological or behavioural consequences of sham operation have not been sufficiently investigated, although our team has already revealed differences between these animals and unoperated controls (López et al., 1997).

In our laboratory, and during behavioural studies, we have repeatedly found ourselves dealing with sham operated animals with a slightly different behaviour to unmanipulated controls, although it is also true that the former did present positive differences in most tasks compared to animals with portocaval anastomosis or portal hypertension. To date, we have not yet observed learning differences in passive avoidance tasks or alterations in circadian rhythm or motor alterations in sham operated animals compared to unmanipulated controls. However, differences between both groups of animals were observed in other types of learning, such as active avoidance, spatial learning or open field, with postoperative periods, in the case of sham-operated rats of between 30 and 40 days.

We, therefore, designed four experiments in order to determine whether animals commonly used as controls of portocaval anastomosis intervention, in other words, sham operated animals, present any differences compared to unmanipulated controls in three behavioural tests: open field, spatial memory and associative learning, and differences in the plasma levels of corticosterone or testosterone.

Method

Performing the sham operation

The operations were done under anaesthesia with ketamine (90 mg/Kg, ip) and xylazine (8 mg/Kg). A bilateral subcostal laparotomy with prolongation to the xyphoid apophysis, followed by dissection of the portal vein and inferior vena cava in its infrahepatic path with later clamping for 15 min was performed. The operative field was irrigated with saline solution during the intervention. Finally, the laparotomies were closed by continuous suture on the two planes. All the experiments were performed according to the European Communities Council Directive 86/609/EEC, and the study was approved by the local committee for animal studies (Oviedo University). Wistar rats from the vivarium of Oviedo University (Oviedo, Spain) were used. All the animals had *ad libitum* access to food and tap water and were maintained at constant room temperature (21 ± 2 ºC), with a relative humidity of 65 ± 10% and an artificial light-dark cycle of 12 h (08:00-20:00 h/ 20:00-08:00 h).

Experiment 1. Open Field

Open field is the method most used to study an animal's general motor activity (Kelly, 1993). The objective of this experiment was to study the animals' exploratory behaviour before and after the sham operation. The results are compared with those of another group of unmanipulated animals that also participate in two sessions in the open field, with the same interval between sessions as the sham operated animals.

Twenty-two Wistar rats were used. The mean weight of rats at the start of the experiments was 250 ± 19 g. The 22 animals were tested to assess their exploratory capacity in open field during two 15-minute sessions. In the first session, the animals were distributed into two groups of 11 animals each. One unmanipulated control group (CO-1) and another group to be later sham operated (PRE-S group).

The open field consisted in a circular enclosure of polymetacrylate 1.2 m diameter with walls 40 cm high. All the animals started from the same point on the edge of the field. The animals exploratory behaviour was recorded by a video camera connected up to a computer equipped with the program Ethovision Pro (Noldus Information Technology, Netherlands). The area of the open field was divided virtually into three concentric rings: outer, middle and inner, of 0.2, 0.2 and 0.4 m, respectively. The variables

evaluated were: distance travelled (cm), velocity (cm/s) and the time the animals spent in the outer, middle and inner region. The total distance and velocity were used to measure the general locomotor activity and the time the animals spent in the different rings as an index of anxiety (Weiss & Greeberg, 1998). Afterwards, the 11 PRE-S animals were submitted to a sham operation according to the method described above. During the postoperative period the animals were kept in individual cages after which they were grouped together in cages of 4 and 3 animals until the end of the 40-day postoperative period. The animals were then required to perform the second experiment, the open field test (SHAM group) under the same conditions and using the same equipment as in the previous test. The CO-1 animals also remained in cages of groups of 4 and 3 animals for 40 days. After this, they were placed again in the open field to study their behaviour (group CO-2).

Experiment 2. Spatial orientation learning: Morris Circular Pool

The circular pool designed by Morris (Morris, 1981; 1984) also called the Morris Water Maze (MWM), is one of the experimental models most used to study spatial memory. The pool functions as an open field in which the rats train to escape from the water by swimming to reach a small platform. In the MWM, the position of the platform can be changed, and the exit can also be altered in each trial, so the animals cannot use motor strategies to reach the platform. Different experimental procedures can be used with elements in the pool. The simplest correspond to training the animal with a hidden or visible platform in a fixed position during a series of trials. Use of a visible platform implies that the animal will use intra-maze cues to reach it, while with the hidden platform it will use extra-maze cues, permitting spatial reference memory to be evaluated.

Eighteen Wistar rats were used. The mean weight of rats at the start of the experiments was 259 ± 13 g. The animals were divided into two groups: CONTROL (n=10) and SHAM (n=8). The sham operation was carried out using the method described above and the animals carried out the learning task after a postoperative period of 35 days.

The spatial learning task was run between 10:00h and 14:00h for 6 consecutive days. On day 1, the animals were habituated to the task and received 4 trials while a visible platform was located in the centre of the pool. On the following 5 days, the subjects received 4 acquisition trials while the platform was hidden and located in the

centre of the Northern quadrant of the pool. Each trial consisted in randomly releasing the subject from one of four compass locations around the pool North (D= escape), South (C=opposite), East (A= right, West (B= left) and allowing it to swim until it either climbed onto the hidden platform or 60 seconds had elapsed. The time to locate it was recorded. If an animal failed to find the goal within 60 seconds it was placed there for 20 s. During the inter-trial interval, the animals were placed inside a black bucket for 30 s. A probe test was run daily during the 5 days of training immediately after the fourth trial for each animal. During the probe trial (Stewart & Morris, 1993) the hidden platform was removed, the rat released from the opposite quadrant and allowed to search for the platform for 25 s. Immediately after the probe test, the animals received an additional trial with the hidden platform located in the same place as before to avoid the possible interference of a daily probe test. The quadrants consisted of imaginary divisions of equal area made by the computer program Ethovision Pro (Noldus Information Technology, Netherlands). The variables studied were escape latencies and swimming time in each quadrant during the recall task.

Experiment 3. Associative Learning: Two-way Active Avoidance

Two-way active avoidance was used as a test of associative learning. Active avoidance is an instrumental conditioning resulting from the association of an EC and an EI (Clincke & Werbrouck, 1993) Animals must learn to avoid a harmful stimulus, in this case an electric charge, by triggering a specific motor response that consists in displacement to another compartment, after learning the association between EC (sound) and EI (shock).

Thirty Wistar rats were used. The mean weight of rats at the start of the experiments was 261 ± 23 g. The animals were divided into three groups for a first learning process: unmanipulated controls (CO-1, n=10), unmanipulated controls to be later sham operated (PRE-S, n=10) and sham operated animals (SHAM group). The sham operation was carried out as described previously and the postoperative period was 40 days (15 days in individual cages and 25 days in cages of 4 animals).

Active avoidance testing was conducted in a two-way automated shuttle-box (LE-916, Letica, Barcelona, Spain). The box was made of perspex (50 X 24 X 23 cm) and divided into two compartments separated by a door. Each compartment had an independent electrified floor made from stainless-steel bars. A light and sound source could be used jointly or separately in each of the compartments. The shuttle-box was

enclosed in a sound-attenuating box ventilated by an extractor fan. The learning program carried out in the box was controlled by an associated module (LI-2706, Letica, Barcelona, Spain).

For associative conditioning of avoidance the animals were submitted to a daily session of 50 trials for 5 days, from 9-13 hours. The conditioned stimulus (CS) consisted of an 80 dB tone and 1.4 KHz for 4 s. The unconditioned stimulus (US) was a 0.6 mA electrical footshock presented for 4 s. Appearance of the US was contiguous with the end of the CS. The inter-trial interval was 30 s. Before starting the first session (day 1) the animals had a habituation period of 5 minutes for free exploration. The variables recorded in each of the sessions were number of avoidances, number of escapes and number of crossings between compartments during the inter-trial interval.

After the first learning session, PRE-S animals were sham-operated, carried out according to the procedure described in experiment 1, with a postoperative period of 40 days. After this time, the animals were once again submitted to an active avoidance task with the same previously described protocol. The animals in this second session were called POST-S and their respective controls, the CO-1 group of the first session, was called CO-2.

Experiment 4. Determination of Plasma Levels of Testosterone and Corticosterone

The final objective of this experiment was to determine whether the sham operation causes physiological differences in the plasma levels of testosterone and corticosterone. These two variables were chosen for different reasons. We decided to test for plasma testosterone levels because in a previous study, our group had observed a degree of testicular atrophy in sham operated animals compared to control animals or those without a cerebral sham lesion (López et al., 1997). Plasma corticosterone levels were determined because of the important role of this hormone in stress, since the sham operation can be considered to be a highly stressful situation for the animals.

Eighteen Wistar rats were used. The mean weight of rats at the start of the experiments was 220 ± 16 g. The animals were divided into two groups: CONTROL (*n*=9) and SHAM (n=9). The sham operation was carried out according to the protocol described previously and the animals were evaluated after 40 postoperative days. The blood sample was taken, in both groups, between 12 and 13 h to avoid circadian variations in this hormone (De Boer & Van der Gugten, 1987).

Plasma corticosterone was measured using a radioimmunoassay kit from DRG Instruments, Germany. The sensitivity of the assay was 25 ng/ml and the intra-assay coefficient of variation was 9.32 %. The sample was assayed in duplicate. A similar procedure was used to determine plasma testosterone, with a kit of DRG Instruments (Germany). The sensitivity in this case was 0.1 ng/ml and the intra-assay coefficient of variation was 10.43 %. All samples in each assay were measured on the same day.

Data Analysis

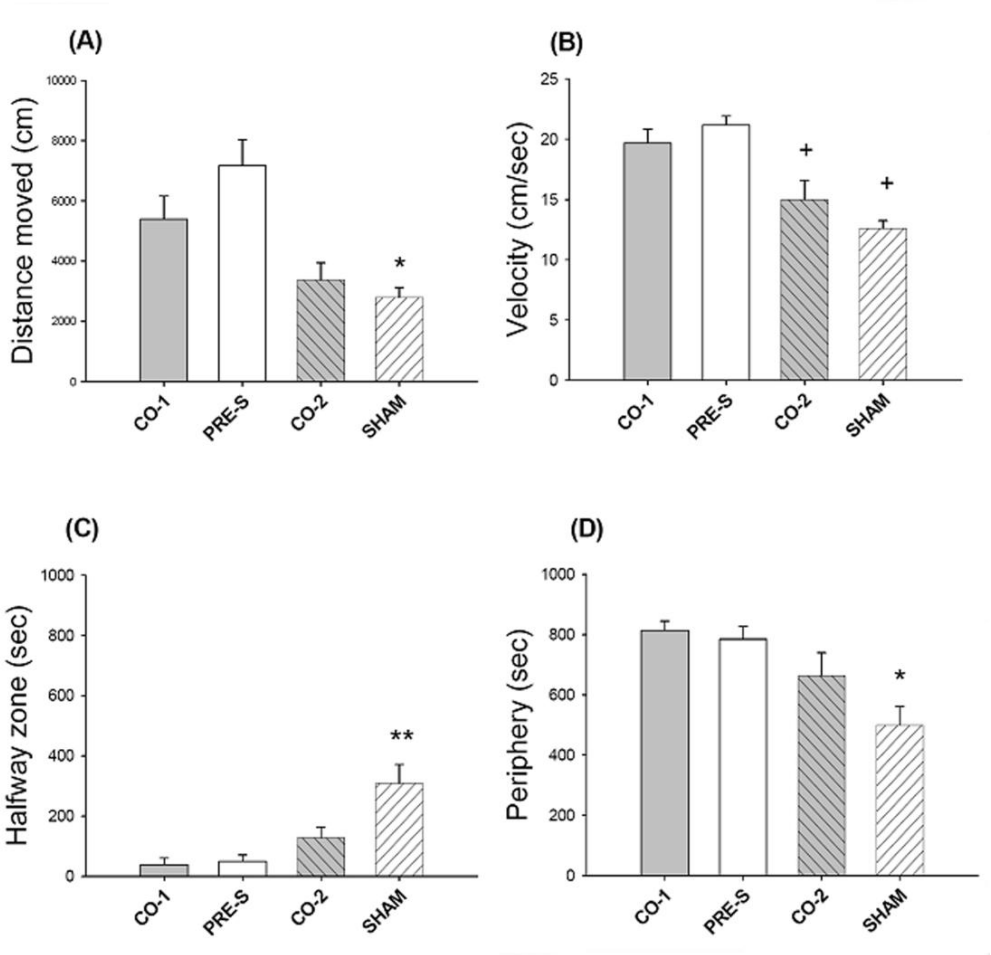
All data were analyzed in the Sigma-Stat 3.2 program (Systat, Richmond, USA) and were expressed as mean±SEM. The results were considered as statistically significant if $p<0.05$.

Results

Experiment 1. Open Field

ANOVA was applied for each of the variables selected. The results can be observed in Figure 1. The graph depicting the time the animals stayed in the inner region has been omitted, since the ANOVA revealed no differences between the groups, $F(3,40)=2.39$, *ns*. Regarding the distances swam, differences were observed between the groups, $F(3,40) = 9.071$, $p<0.001$, and application of Tukey's test revealed significant differences between the SHAM group and the PRE-S group ($p<0.001$) and CO-1 ($p<0.05$) (Figure 1A). ANOVA also demonstrated differences in the velocities of the different groups $F(3,40)=12.96$, $p<0.001$, with significant differences between both the SHAM group and the CO-2 group compared to the PRE-S ($p<0.001$, Tukey test) and CO-1 groups ($p<0.05$, Tukey test) respectively (Figure 1B). Differences were observed between the times animals from the different groups spent in the middle region, $F(3,40)=10.29$, $p<0.001$. Tukey's test revealed differences between the SHAM group and the PRE-S groups ($p<0.01$), CO-1 ($p<0.001$) and CO-2 ($p<0.05$) (Figure 1C). Finally, the ANOVA applied to time spent in the outer region also revealed group differences, $F(3,40)=6.57$, $p<0.001$, with the SHAM group presenting differences compared to the PRE-S group ($p<0.01$) and CO-1 ($p<0.01$) (Fig. 1d). It is noteworthy, that no statistically significant differences were obtained between groups CO-1 and PRE- S for any of the variables evaluated.

Figure 1



Experiment 2. Spatial orientation learning: Morris Circular Pool.

The latencies to arrive at the hidden platform were analysed by a two-way repeated measures ANOVA, with one between-subjects variable (group) and one within-subjects variable (day), and post hoc Tukey test were carried out (SigmaStat 3.0.1, Spss Inc, USA). The SHAM group found the hidden platform as quickly as the CONTROL. ANOVA showed no differences between groups, $F(1,16)=1.491$; *ns*, between days, $F(4,64)=7.697$; $p<0.001$ (Figure 2), and no significant interaction term, $F(4,64)=1.06$; *ns*). No-platform probe tests were analysed daily by using a repeated measures one-way ANOVA. This was done by studying the time spent by each group in the four virtual quadrants of the pool. Both groups missed the location of the platform on day 1, $F(3,36)=1.842$; *ns*, and $F(3,28)=3.163$; $p<0.05$, for control and sham group respectively. The CONTROL group displayed a more accurate searching pattern on day

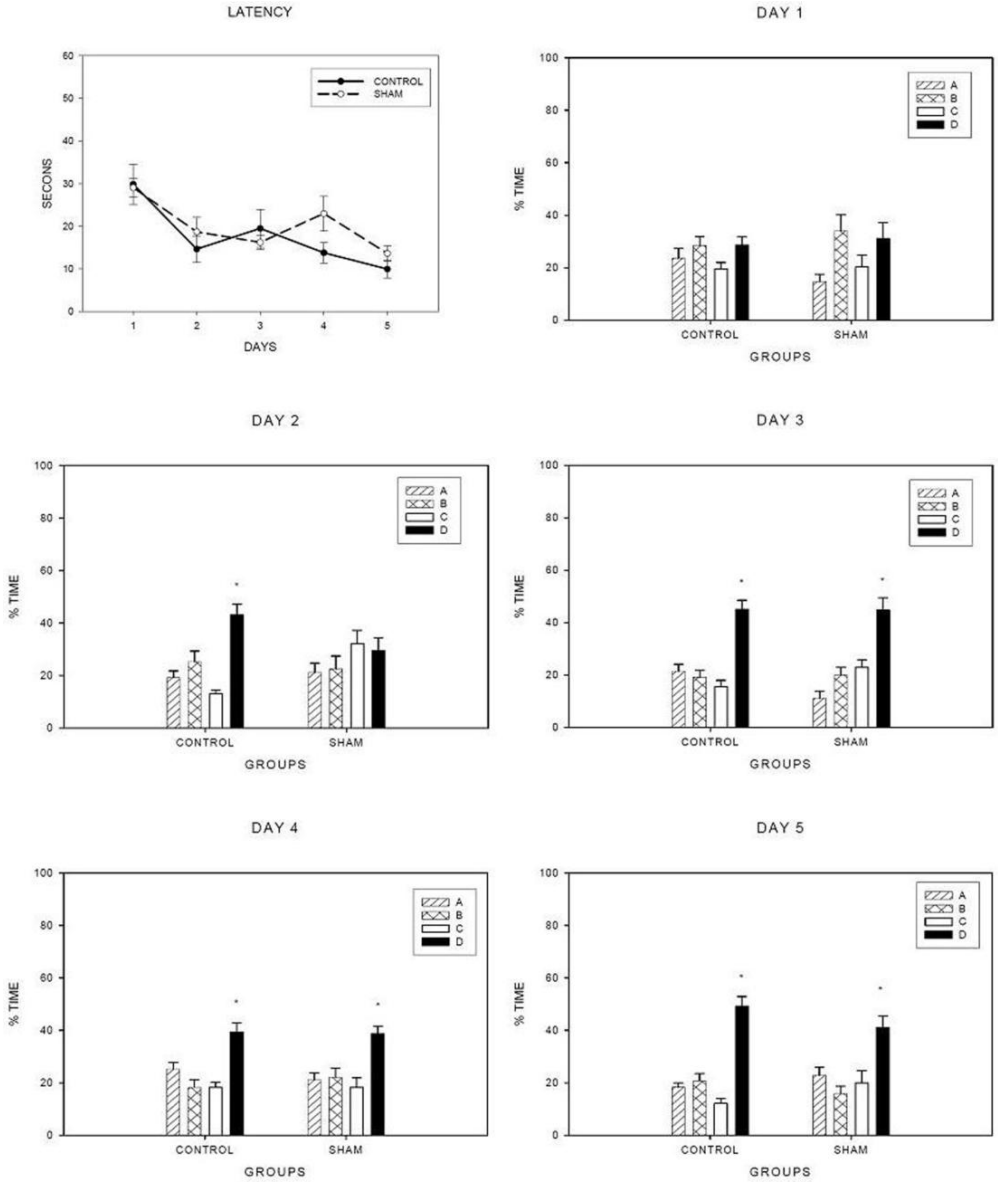
2, $F(3,36)=16.537$; $p<0.001$, showing a bias towards the location occupied by the platform during training compared to other quadrants. The SHAM group did not show biased searching on day 2, $F(3,28)=1.306$; *ns.* ANOVA revealed a preference on days 3, 4 and 5 for the escape quadrant in CONTROL, $F(3,36)=22.586$; $p<0.001$, $F(3,36)=12.877$; $p<0.001$, $F(3,36)=38.848$; $p<0.001$, and SHAM animals, $F(3,28)=18.037$; $p<0.001$, $F(3,28)=8.177$; $p<0.001$, $F(3,28)=8.689$; $p<0.001$.

Experiment 3. Associative Learning: Two-way Active Avoidance.

Differences between groups in the step-through for each of the variables were analyzed by a two-way repeated measures ANOVA, with one between-subjects variable (group) and one within-subjects variable (day), and a post hoc Tukey test was carried out (SigmaStat 3.0.1, Spss Inc, USA).

Sham, Presham and Control-1 groups. Avoidances (Figure 3A): ANOVA showed that the variable Group was significant, $F(2,27)=4.486$; $p<0.05$). The variable days, $F(4,108)=53.098$; $p<0.001$, and the interaction, $F(8,108)=3.699$; $p<0.001$, were also significant. Post hoc comparison between groups revealed that, in general, PRE-S did more avoidances than SHAM ($p<0.05$). On day 3, CO-1 group did more avoidances than SHAM ($p<0.05$), on day 4 SHAM group made fewer escapes than PRE-S ($p<0.01$) and CO-1 ($p<0.05$), and on day 5 SHAM group made fewer escapes than PRE-S ($p<0.001$) and CO-1 ($p<0.01$). % Escapes (Figure 3B): ANOVA showed that the variable Group was significant, $F(2,27)=5.933$; $p<0.01$, and the interaction, $F(8,108)=2.236$; $p<0.05$) was also significant. A post hoc comparison between groups revealed that PRE-S and CO-1 made more escapes than SHAM, ($p<0.01$) and ($p<0.05$) respectively. Intertrial crossings (Figure 3C): ANOVA showed no significant differences between variables.

Figure 2

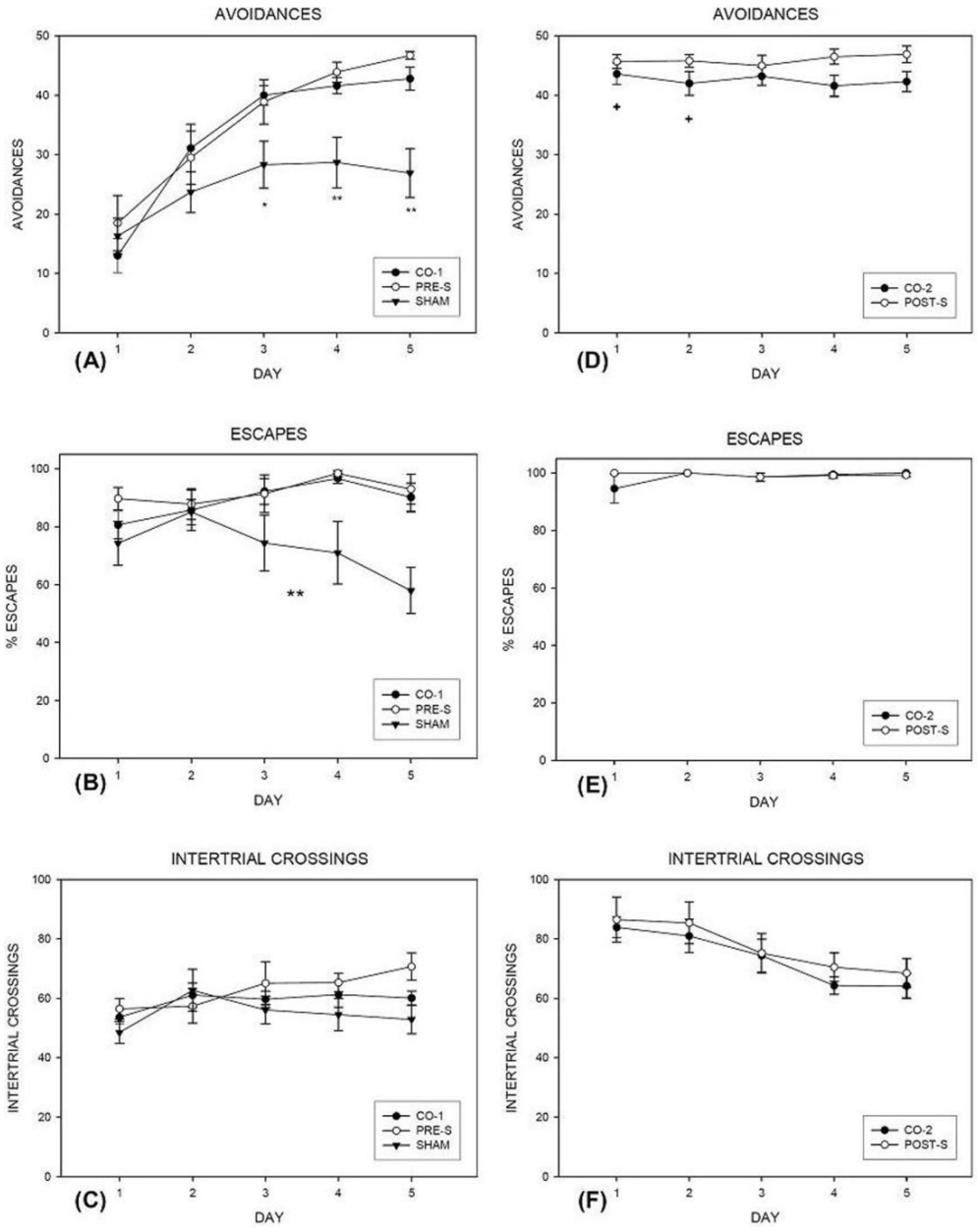


Postsham and Control-2. Avoidances (Figure 3D): ANOVA showed no significant differences between groups. % Escapes (Figure 3E): ANOVA showed no significant differences between variables. Intertrial crossings (Figure 3F): ANOVA showed no significant differences between variables.

Presham, Postsham, Control-1 and Control-2 groups. Differences between groups in the step-through for each of the variables were analyzed on each day by a two-way repeated measures ANOVA, with one between-subjects variable (group) and one within-subjects variable (TEST), and a post hoc Tukey test was carried out.

Avoidances: ANOVA showed that the variable test was significant on day 1, $F(1,18)=118.563$; $p<0.001$, and 2, $F(1,18)=22.412$; $p<0.001$ (Figure 3A-D), groups presented more avoidances in the second test.

Figure 3

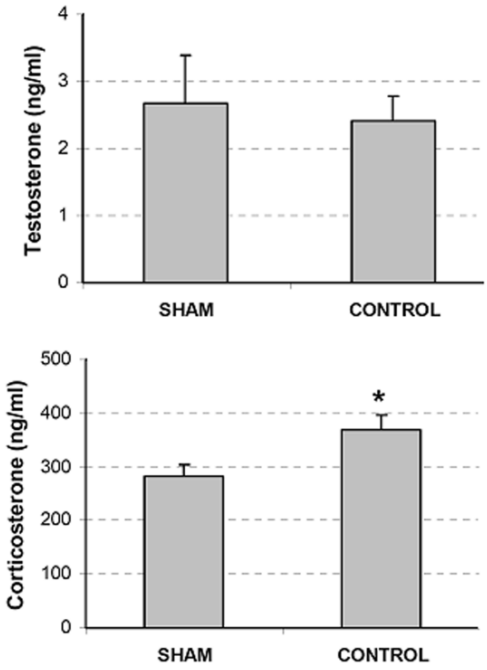


Experiment 4. Determination of Plasma Levels of testosterone and corticosterone.

The results, analysed by the Student's t-test revealed no significant differences in the plasma testosterone in the SHAM and the CONTROL groups, $t(16)= 0.319$; *ns*,

(Figure 4). However, there were statistically significant differences in the corticosterone levels in both groups, $t(16)= -2.56$; $p<0.05$, (Figure 4).

Figure 4



Discussion

Our results seem to confirm that the sham operation can cause some changes in the animals in postoperative periods of longer than 30 days. Minimal changes would perhaps be expected in some physiological variables. However, the surprising findings in this study are the changes in the sham-operated animals' capacity for associative learning compared to unmanipulated controls.

From the results of the open field test (Experiment 1), sham operated animals presented minimum differences compared to controls. In the first open field session, no differences were observed for any of the variables (distance swam, velocity and the time spent in the different regions) between the control group (CO-1) and the pre-sham operated group (PRE-S) (Figure 1). In the second open field session, after the sham operation and the postoperative period, the velocity and the distance decreased to a similar extent in both the SHAM and control groups (CO-2). This suggests that both

groups remember the experimental situation that is no longer a novel situation, in spite of this session taking place 40 days after the first session. In this second session it is interesting that the SHAM group seems to be less anxious than its corresponding control (CO-2). Although the control animals spend longer exploring the middle region of the open field than in the first session, this behaviour is more pronounced in the SHAM group (Figure 1C). On the contrary, animals in the SHAM group spend less time in the outer region than they did in the first session (Figure 1D).

Experiment 2 evaluates the animals' ability for spatial orientation using the circular Morris pool. Although the escape latencies decreased over the learning period, there were no differences between the groups. However, the lack of any significant differences in the latencies does not reflect that the animals have the same capacity for learning spatial orientation, which we observe from data for the recall test (Figure 2). We can, therefore, observe that the CONTROL group presents learning on the second day of the experiment, since these animals spent significantly longer swimming in the quadrant containing the escape platform than in the other quadrants. This learning in only 8 trials in the pools is not achieved by the SHAM group, which requires one more day to consolidate it (Figure 2), reflecting a slower acquisition of the tasks. It must be noted that after learning the orientation task, both groups remember the location for the remaining days, as can be observed in the transfer task on days 3, 4 and 5 for the SHAM animals, and days 2, 3, 4 and 5 for the CONTROL group.

In Experiment 3, the sham operated and the control group present clearly different performances. The learning curve for the avoidance task reflects that sham operated animals have a poor acquisition, and do not reach the criteria established to consider the learning as correct at any time (80% of avoidances in the 50 daily trials) as can be observed in fig. 3a. In this same experiment, control animals (group CO-1) achieve 80% at day 3, 83.2% at day 4 and 85.6% at day 5, while the control group that will later receive the sham operation (PRE-S group) presents 77.8% at day 3, 87.8% at day 4 and 93.4% at day 5. In contrast, the SHAM group presents 56.6% avoidances at day 3, 57.4% at day 4 and 53.8% at day 5. This poor performance cannot be due to the presence of freezing, since the graph of intertrial crossings presents no differences between the groups, and the current used to produce the shock is not sufficient to cause freezing either (Figure 3C). Even SHAM animals present a degree of disorientation in the task over the learning days, since, in addition to the learning curve stabilising after

the third day, the percentage of escapes diminishes after day 2 (Figure 3B), revealing differences between the CO-1 and PRE-S groups.

The most interesting finding from Experiment 3 was that animals in the PRE-S group receiving the sham operation after the avoidance learning procedure, still remembered this task correctly after 40 postoperative days. As can be observed in Figure 3D, both the POST-S and the CO-2 group present a percentage of avoidance higher than 83% over the 5 days of the task. The POST-S group did not present any differences compared to the CO-2 group either in relation to percentages of escape or intertrial crossings (Figures 3E-3F).

These data seem to suggest that the sham operation does not affect memory, since after acquiring the task, this is not affected by the surgical intervention. This is clearly observed in the active avoidance learning task. It also seems to be confirmed by the open field experiment, since the reduced distance and velocity in the second session confirms that the experimental situation has been remembered. However, sham operated animals do seem to find it more difficult to acquire the active avoidance task. In this test, we do not observe delayed acquisition, as occurred in the Morris water maze, but instead a poorer performance, with no increase in learning over the days of the experiment. SHAM animals appeared to be incapable of correctly associating the conditioned and unconditioned stimulus, resulting in them not being able to avoid the EI after presentation of the EC, as occurs in controls and pre-sham animals.

Finally, experiment 4 revealed significant differences in the corticosterone levels of sham operated animals compared to controls. There is a significant reduction in this hormone in SHAM animals, whereas this does not occur with testosterone (Figure 4). This may be caused by the surgical intervention, since levels of this hormone can decline as a consequence of stress (Yehuda & Antelman, 1993; Yehuda, Southwick, Nussbaum, Wahby, Giller, & Mason, 1990). On the other hand, lower levels of corticosterone can affect active avoidance learning, since the association between ACTH and avoidance behaviour was established some time ago in the shuttle box (Levine, 1971). At the same time, other researchers have observed that rats with lower corticosterone levels show less avoidance behaviour than animals with higher values (Brush, 2003). Finally, other authors have shown the effects of corticosterone administration on learning and memory such as aversive taste conditioning (McEwen & Sapolsky, 1995).

In conclusion, our results seem to indicate that the sham operation used in rats as a control of portocaval shunt causes both physiological and behavioural alterations, which must be studied in greater depth. These changes, apart from being secondary to a phenomenon of hepato-intestinal ischaemia-revascularisation (Hall, Smith, Harding, Pierro & Eaton, 2005), may also be secondary to anaesthetic or surgical techniques, in other words, to the laparotomy performed. At the same time, methodologically it seems necessary to reconsider what would constitute an appropriate control group for portacaval shunt, or, at least, introduce in the research control groups of sham operated animals and also of unmanipulated control animals.

Acknowledgements

We would like to thank Piedad Burgos and Begoña Valdes for their technical assistance. This research was supported by grants from current Spanish Ministry of Science and Innovation and FEDER SEJ2007-63506 and Fundación Mutua Madrileña AP/6977-2009.

References

Abraldes, J.G., Pasarín, M., & García-Pagán, J.C. (2006). Animal models of portal hypertension. *World Journal of Gastroeneterology, 12,* 6577-6584.

Benoist, S., Sarkis, R., Baudrimont, M., Delelo, R., Robert, A., Vaubourdolle, M., Balladur, P., Calmus, Y., Capeau, J., & Nordlinger, B. (2000). A reversible model of acute hepatic failure by temporary hepatic ischemia in the pig. *The Journal of Surgical Research, 88,* 63-69.

Bhatnagar, A., & Majumdar, S. (2003). Animal models of hepatic encephalopathy. *Indian Journal of Gastroenterology, 22*(Suppl 2), S33-36.

Blei, A.T., & Córdoba, J. (2001). Hepatic encephalopathy. *The American Journal of Gastroeneterology, 96*, 1968-1976.

Blei, A.T., Omary, R., & Butterworth, R.F. (1992). Animal models of hepatic encephalopathies. In A. Bouton, B. Baker & R.F. Butterworth (Eds.),

Animal models of neurological disease II. Neuromethod (Volume 2, pp. 183-222). New Jersey: The Humana Press Clifton.

Brush, F.R. (2003). The Syracuse strains, selectively bred for differences in active avoidance learning, may be models of genetic differences in trait and state anxiety. *Stress, 6*, 77-85.

Butterworth, R.F. (2003). Pathogenesis of hepatic encephalopathy: New insights from neuroimaging and molecular studies. *Journal of Hepatology, 39*, 278-285.

Chamuleau, R.A. (1996). Animal models of hepatic encephalopathy. *Seminars in Liver Disease, 16*, 265–270.

Clincke, G.H.C., & Werbrouck, L. (1993). Two-way active avoidance. In A. Sahgal (Ed.), *Behavioural neuroscience* (Volume I, pp. 71-79). New York: Oxford University Press.

De Boer, S.F., & Van der Gugten, J. (1987). Daily variations in plasma noradrenaline, adrenaline and corticosterone concentrations in rats. *Physiology & Behavior, 40*, 323-328.

Diéguez, B., Aller, M.A., Nava, M.P., Palma, M.D., Arias, J.L., Lopez, L., & Arias, J. (2002). Chronic portal hypertension in the rat by triple-portal stenosing ligation. *Journal of Investigative Surgery, 15*, 329-336.

Ferenci, P., Lockwood, A., Mullen, K., Tarter, R., Weissenborn, K., & Blei, A.T. (2002). Hepatic encephalopathy. Definition, nomenclature, diagnosis, and quantification. Final report of the Working Party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology, 35*, 716–721.

Filipponi, F., Fabbri, L.P., Marsili, M., Falcini, F., Benassai, C., Nucera, M., & Romagnoli, P. (2001). A new surgical model of acute liver failure in the pig: Experimental procedure and analysis of liver injury. *European Surgical Research, 23*, 58-64.

Filipponi, F., & Mosca, F. (2001). Animal models of fulminate hepatic failure: need to test liver support devices. *Digestive and Liver Disease, 33*, 607-613.

Hall, N.J., Smith, V.V., Harding, B., Pierro, A., & Eaton, S. (2005). Intestinal ischemia-reperfusion injury does not lead to acute central nervous system damage. *The Journal of Surgical Research, 129*, 288-291.

Hazell, A.S., & Butterworth, R.F. (1999). Hepatic encephalopathy: An update of pathophysiologic mechanisms. *Proceedings of the Society for Experimental Biology and Medicine, 222*, 99-112.

Jover, R., Madaria, E., Felipo, V., Rodrigo, R., Candela, A., & Compan, A. (2005). Animal models in the study of episodic hepatic encephalopathy in cirrhosis. *Metabolic Brain Disease, 20*, 399-408.

Kelley, A.E. (1993). Locomotor activity and exploration. In A. Sahgal (Ed.), *Behavioural neuroscience* (Volume II, pp. 1-21). New York: Oxford University Press.

Kountouras, J., Billing, B.H., & Scheuer, P.J. (1984). Prolonged bile duct obstruction: A new experimental model for cirrhosis in the rat. *British Journal of Experimental Pathology, 65*, 305-311.

Laleman, W., Elst, I.V., Zeegers, M., Servaes, R., Libbrecht, L., Roskams, T., Fevery, J., & Nevens, F. (2006). A stable model of cirrhotic portal hypertension in the rat: thioacetamide revisited. *European Journal of Clinical Investigation, 36*, 242–249.

Levine, S. (1971). Stress and behaviour. *Scientific American, 224*, 26-31.

Li, X., Benjamin, I.S., & Alexander, B. (2002). Reproducible production of thioacetamide-induced macronodular cirrhosis in the rat with no mortality. *Journal of Hepatology, 36*, 488-493.

López, L., Begega, A., Burgos, P., Santín, L.J., Lorente, A., Arias, J., & Arias, J.L. (1997). Portacaval shunt control animals: Physiological consequences derived from the sham operation. *Laboratory Animals, 31*, 225-230.

McEwen, B.S., & Sapolsky, R.M. Stress and cognitive function. (1995). *Current Opinion in Neurobiology, 5*, 205–216.

Montagnese, S., Amodio, P., & Morgan, M.Y. (2004). Methods for diagnosing hepatic encephalopathy in patiens with cirrhosis: A multidimensional approach. *Metabolic Brain Disease, 19*, 281-312.

Morris, R.G.M. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. Journal of Neuroscience Methods, 11, 47-60.

Morris, R.G.M. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation, 12*, 239-260.

Ong, J.P., & Mullen, K.D. (2001). Hepatic encephalopathy. *European Journal of Gastroenterology & Hepatology, 13*, 325-334.

Quero, J.C., & Herrerías, J.M. (2006). Diagnostic methods in hepatic encephalopathy. *Clinica Chimica Acta, 365*, 1-8.

Stewart, C.A., & Morris, R.G.M. (1993). The Watermaze. In A. Sahgal (Ed.), *Behavioural neuroscience* (Volume I, pp. 107-122). New York: Oxford University Press.

Terblanche, J., & Hickman, R. (1991). Animal models of fulminant hepatic failure. *Digestive Diseases and Sciences, 36*, 770-774.

Weiss, E., & Greenberg, G. (1998). Open-field procedures. In G. Greenberg & M.M. Haraway (Eds.), *Comparative psychology: A handbook* (pp. 357-263). New York: Garland.

Yehuda, R., & Antelman, S.M. (1993). Criteria for rationally evaluating animal of posttraumatic stress disorder. *Biological Psychiatry, 33*, 479-486.

Yehuda, R., Southwick, S., Nussbaum, G., Wahby, V., Giller, E., & Mason, J. (1990). Low urinary cortisol excretion in patients with posttraumatic stress disorder. *The Journal of Nervous and Mental Disease, 178*, 366-369.