Psicothema 2009. Vol. 21, nº 3, pp. 471-474
www.psicothema.com

ISSN 0214 - 9915 CODEN PSOTEG

Lidocaine, tetrodotoxin and their effect on consolidation of spatial memory

José Manuel Cimadevilla, Francisco López, Luz Nieto, Mª Jesús Aguirre and Rubén Fernández
Universidad de Almería

This study was aimed at comparing the effect of unilateral hippocampal inactivation with tetrodotoxin (TTX) and lidocaine on spatial memory consolidation. Both drugs block voltage-dependent sodium channels. However, TTX and lidocaine differ in the duration of their effects, with maximum TTX effect between 30 min and 120 min, washing out in 24 hours. Lidocaine maximum effect occurs 20-30 minutes after administration. Our experimental subjects, twenty-four 3-month-old Wistar rats, were unilaterally implanted with stainless-steel cannulae aimed at the right dorsal hippocampus. Animals received four daily trials for 5 consecutive days. Control injections of 1 µl saline, or inactivating injections of 5ng of TTX in 1 µl saline or lidocaine (2%) in 1 µl were made through a guide cannula 1 minute after the last trial from day 1 to day 4. Results showed that the groups that received TTX or lidocaine did not differ but were impaired regarding controls, suggesting that short-term consolidation processes can account for the memory impairment observed here.

Lidocaína, tetrodotoxina y su efecto sobre la consolidación de la memoria espacial. El objetivo de este estudio ha sido comparar el efecto de la inactivación unilateral del hipocampo con tetrodotoxina (TTX) o lidocaína sobre la consolidación de la memoria espacial. Ambas drogas bloquean los canales de sodio dependientes de voltaje, pero difieren en la duración de sus efectos, mostrando la tetrodotoxina su máxima actividad entre 30 y 120 minutos, eliminándose completamente a las 24 horas. En cambio, el máximo efecto de la lidocaína acontece entre 20-30 minutos tras su administración. Nuestros sujetos experimentales fueron canulados unilateralmente sobre hipocampo dorsal. Los animales recibieron cuatro ensayos diarios durante 5 días consecutivos. Las inyecciones control de 1 µl de un compuesto salino, o inyecciones inactivadoras de 5ng de TTX en 1 µl de salino o lidocaína (2%) en 1 µl de salino, se aplicaron a través de una cánula guía 1 minuto después del último ensayo desde los días 1 al 4. Los resultados mostraron que los grupos que recibieron TTX o lidocaína no difieren entre ellos en sus efectos, pero en cambio resultaron perjudicados en comparación con el grupo control, lo que sugiere que los procesos de consolidación a corto plazo pueden explicar la alteración mnésica que se observa en este trabajo.

To study the neural basis of behaviour, scientists apply different kinds of techniques. By using temporal inactivating methods, neuroscientists can control not only which brain structures are involved in certain behaviour, but also the temporal demands of this brain area (Lorenzini, Baldi, Bucherelli, Sacchetti, & Tassoni, 1999). TTX and lidocaine are widely used drugs in behavioural studies to transiently inactivate brain structures (Cimadevilla, Miranda, López, & Arias, 2005; Fenton & Bures, 1993; Zhuravin & Bures, 1991). TTX is a specific voltage-dependent sodium channel blocker whose maximum effect begins between 30 and 120 minutes after its administration and lasts more than 3 hours and disappears within 24 hours. This period would be enough to alter both short-term and long-term consolidation processes. On the other hand, lidocaine is also a specific voltage-dependent sodium channel blocker, but its effect is shorter than TTX. Hence, its maximum effect begins mostly immediately after administration, lasting about 10-20 minutes and disappearing between 30 and 120 minutes (Pereira de Vasconcelos, Klur, Muller, Cosquer, López, Certa, & Cassel, 2006).

Hippocampal lesions produce disastrous consequences in human memory, since an intact hippocampus is needed to form declarative memories (Scoville & Milner, 1957; Squire & Zola, 1996). The hippocampal formation is also required in all mammals to form memories about space (Cimadevilla, Conejo, Miranda, & Arias, 2004; O'Keefe & Dostrovsky, 1971; Morris, Garrud, Rawlins, & O'Keefe, 1982; Hartley et al., 2007).

During the last few years, several reports have proved that unilateral hippocampal inactivation impairs several kinds of memories, including spatial memory in the Morris water maze and other spatial tasks as well (Cimadevilla, Wesierska, Fenton, & Bures, 2001; Cimadevilla et al., 2005). In addition, different memory processes were reported to be altered after unilateral

Fecha recepción: 27-3-08 • Fecha aceptación: 11-1-09
Correspondencia: José Manuel Cimadevilla
Facultad de Psicología
Universidad de Almería
04120 Almería (Spain)
E-mail: jcimadev@ual.es

hippocampal interventions. Hence, acquisition, retrieval and consolidation phases were impaired after blocking one hippocampus (Cimadevilla, et al., 2005; Fenton & Bures, 1993; Lorenzini, Baldi, Bucherelli, Sacchetti, & Tassoni, 1996; Lorenzini, Baldi, Bucherelli, Sacchetti, & Tassoni, 1997).

Consolidation refers to the progressive post-acquisition stabilization of long-term memory, as well as the memory phase during which such presumed stabilization take place (Dudai, 2002). Unilateral hippocampal inactivation with TTX, following spatial training in the Morris water maze, was reported to affect consolidation processes (Cimadevilla et al., 2005). However, since TTX effect lasts so long, it is not possible to determine whether the impairment is produced by interfering short-term or long-term consolidation processes. This question can be addresses by using lidocaine instead of TTX. Lidocaine blockade is shorter than TTX, and the comparison of both drugs can help to understand how TTX impairs behaviour.

Methods

Subjects

Twenty eight three-month-old male Wistar rats (275-380 g.) obtained from the breeding colony of the University of Granada, Spain, were used in this study. Subjects were housed in pairs in plastic cages with food and water available ad libitum in a room with artificial light (lights on: 08:00-20:00) and with a constant temperature of 20-21°C. They were distributed randomly into three groups: Tetrodotoxin (n= 6); lidocaine (n= 10) and saline (n= 11). The work was conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) for the care and use of laboratory animals.

Surgery

Rats were anaesthetized with ketamine (50 mg/kg i.p.) and xylazine, (20 mg/kg i.m.) and placed in a Kopf (Tujunga, CA) sterotaxic frame. All subjects were implanted unilaterally with stainless-steel cannulae (25 ga) aimed at the right dorsal hippocampus (3.5 behind Bregma, 2.5 lateral and 1 below dura, according to the atlas of Paxinos and Watson (1986). Cannulae and anchor screws (2 per subject) were encased in dental acrylic.

Apparatus

Behavioural training was conducted in the Morris water maze. It consisted of a circular pool (150 cm-diameter x 40 cm-deep) made of fibreglass painted black and placed on a metal platform 35 cm high, in the centre of a room (3 m × 3.5 m) with several landmarks (in the North a covered window, in the South a white wall, in the West a computer and several posters, and a door in the East). Two lamps located in the South and West indirectly illumined the whole room. A black round platform (10 cm in diameter) occupied the centre of one of the four virtual quadrants into which the pool was divided to assess the animals' behaviour. It was located 30 cm from the wall of the pool. The temperature of the water was maintained between 22ºC and 23ºC. The experiment was recorded using a tracking system (Noldus, Netherlands) that provided information about the tracks displayed by the animals. The latency to the platform grouped by days and the time spent in each virtual quadrant of the pool were measured.

Procedure

All subjects were handled for three days during 5 min/day. The behavioural testing was run between 10:00 a.m. and 19:00 p.m during 5 consecutive days. On day 1 the platform was visible. During days 2, 3, 4 and 5 the platform remained 2 cm under the water.

Animals received four daily trials. A trial consisted of releasing the animal randomly from one of four compass locations around the pool (North, South, East, West) and allowing it to swim until it either climbed onto the hidden platform or until 60 seconds had elapsed. In this case, it was placed on the platform for 15s. During the inter-trial interval the animals waited inside a black bucket.

On days 1, 2, 3, and 4, one minute after the last trial, subjects received 1 microliter of a saline compound or 5ng of TTX in 1 microliter of saline or 1 microliter of lidocaine (2%). The animal was gently restrained by hand and an internal cannula (32 ga) was inserted into the guide cannula so that it protruded 2 mm into the hippocampal target. The injection solution was delivered for 90 sec using a Hamilton syringe connected to the internal cannula with a short piece of polyethylene tubing.

Histology

Rats were killed by decapitation. Brains were frozen and thirty micrometer histological slices were extracted, stained with cresyl violet and the position occupied by the cannulae was verified to correspond to the dorsal hippocampus. Two animals from TTX group and 1 subject from lidocaine group were discarded.

Results

A one-way ANOVA applied to the latencies to the visual platform on day 1 showed that all groups performed the same ($F_{2,21}= 0.36$, $p>0.05$) (figure 1).

However, when we analysed the latencies to the platform during days 2, 3, 4 and 5, differences between groups appeared. A two-way ANOVA (groups x days, with repeated measures in the last factor) revealed a main effect of groups ($F_{2,21}= 4.69$, $p<0,05$), days ($F_{3,63}= 27.4$, $p<0.001$) but no interaction ($F_{6,63}= 0.72$, $p>0.05$). Post hoc test (Newman-Keuls test) revealed that both

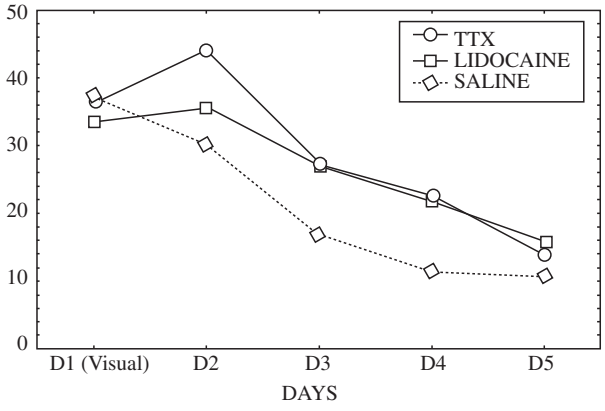

Figure 1. *Mean latency to the platform during the five training days. During day 1 the platform was visible. Days 2, 3, 4 and 5 the platform remained 2 cm below the water. Differences appeared during non-visual training (days 2 to 5) between treated groups and controls, $p<0.05$*

treatment groups were impaired regarding controls ($p<0.05$). Moreover, the latencies decreased on days 2, 3 and 4 in comparison with day 1 as well as on day 4 compared with days 2 and 3 ($p<0.05$) (figure 1).

The mean latency to the platform on the first trial of days 2, 3 and 4 was analysed separately (figure 2). This procedure allows to measure the effect of our treatments on retrieval, avoiding the interference of new learning during trials 2, 3 and 4. A one-way ANOVA revealed statistical differences between groups ($F2,21= 10.74$, $p<0.001$). Post hoc Newman Keuls test showed that subjects that received lidocaine and tetrodotoxin were impaired regarding controls ($p<0.05$).

Discussion

This study shows that consolidation processes in the Morris water maze were equally impaired by posttraining administration of lidocaine or tetrodotoxin. Both drugs altered consolidation in comparison with a control group (see figure 1). Any treatment that blocks consolidation has to be visible through the retrieval process. In this study, latencies to reach the hidden platform show the poor learning memory in the task.

In addition, the analysis of the latency of the first trial of days 2, 3 and 4 probably reflects the treatment effect more accurately, since subjects do not have the opportunity to re-learn the task in one trial, a measure used before (Cimadevilla et al., 2005). The analysis of this data confirmed the results obtained from the joint analysis of the latencies of all the trials, i.e. lidocaine and TTX did not differ but were impaired regarding controls (see figure 2). We presume that, since both hippocampi were available for encoding, and because procedural learning, known to be independent of hippocampal integrity, was not disrupted, subjects improved their performance in the remaining trials of the session (trials 2, 3 and 4).

These results from TTX group match those data obtained before (Cimadevilla et al., 2005). Hence, unilateral hippocampal treatment with TTX is sufficient to impair performance in the Morris water maze. A higher number of subjects in the TTX group will be not justified since the same procedure was already displayed before (Cimadevilla et al., 2005).

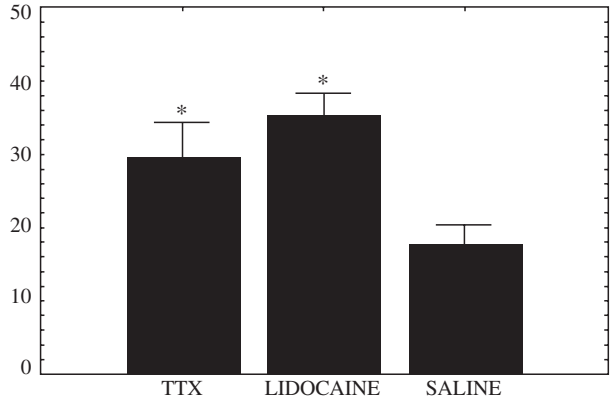

Figure 2. *Mean latency to the platform during the first trial of days 2, 3 and 4. Note that both treated groups (lidocaine and tetrodotoxin) were impaired in comparison to those animals that received a saline compound. Mean + SEM. * $p<0.05$*

Moreover, memory formation is generally divided into two phases or stages: an RNA synthesis-independent phase lasting from minutes to 3 hours approximately (called short-term memory), and an RNA synthesis-dependent phase which can last days or even weeks (called long-term memory). It was suggested that the time around the training and immediately after (1-3 hours) transcription factors and immediate early genes are being expresses, whereas after the initial 3 hours, the structural genes are being expressed and their products are involved in the morphological changes required for LTM (Muller Igaz, Vianna, Medina, & Izquierdo, 2002). Since lidocaine and TTX did not differ, we can assure that the alteration of performance after unilateral TTX inactivation can be explained by interfering with short-term consolidation processes. We can not exclude a possible interference between TTX and those molecular processes that take place 3 hours after acquisition. Probably, the behavioural effect would be inappreciable, since TTX is also altering STM.

It is interesting to point out that unilateral hippocampal interventions impaired acquisition, retrieval, as well as consolidation of memories in the Morris water maze, and the degree of alteration seems not to depend on unilateral or bilateral treatments. As showed before, unilateral and bilateral inactivations did not differ on consolidation (Cimadevilla, Miranda, López, & Arias, 2008). This agrees with previous studies that reported that there is a minimum amount of hippocampal tissue required for an adequate performance in spatial memory tasks (De la Hoz, Moser, & Morris, 2005; Moser & Moser, 1998).

It was stressed before that hippocampal blockade with TTX can alter mobility in rats (Pereira de Vasconcelos et al., 2006). In this experiment, hippocampal treatment was applied after subject's execution, so during training animals were free of any drug. Taking this into account, the results obtained in this experiment can not be considered to be modified by side-effects of inactivation drugs on motor and/or motivational aspects of the tasks.

Unilateral hippocampal inactivation was also reported to alter spatial memory in other tasks (Cimadevilla et al., 2007; Wesierska et al., 2005). However, consolidation was not altered after unilateral hippocampal inactivation in other hippocampal dependent tasks like the passive avoidance task (Cimadevilla et al., 2007; Lorenzini, Baldi, Bucherelli, Sacchetti, & Tassoni, 1996). This finding can reflect the different demands of those tasks and the participation of additional brain structures in this passive place avoidance.

In conclusion, unilateral hippocampal treatment with TTX or lidocaine causes a deep impairment in consolidation, what can be viewed as an interference with short term consolidation processes that take place in the initial phases of memory formation.

Acknowledgements

This study was supported by SEJ2005-05067/PSIC from MEC (Spain). We thank Nobel Perdu for help with English.

References

Cimadevilla, J.M., Conejo, N., Miranda, R., & Arias, J.L. (2004). Sex differences in the Morris water maze in young rats: Temporal dimensions. *Psicothema*, *16*, 611-614.

Cimadevilla, J.M., Miranda, R., López, L., & Arias, J.L. (2005). Partial unilateral inactivation of the dorsal hippocampus impairs spatial memory in the MWM. *Cognitive Brain Research*, *25*, 741-746.

Cimadevilla, J.M., Méndez, M., Méndez-López, M., & Arias, J.L. (2007). Unilateral hippocampal blockade reveals that one hippocampus is sufficient for learning a passive avoidance task. *Journal of Neuroscience Research*, *85*, 1138-1142.

Cimadevilla, J.M., Wesierska, M., Fenton, A.A., & Bures, J. (2001). Inactivating one hippocampus impairs avoidance of a stable room-defined place during dissociation of arena cues from room cues by rotation of the arena. *PNAS*, *98*, 3531-3536.

Cimadevilla, J.M., Miranda, R., López, L., & Arias, J.L. (2008). Bilateral and unilateral hippocampal inactivation did not differ in their effect on consolidation processes in the MWM. *International Journal of Neuroscience, 118*, 619-626.

De Hoz, L., Moser, E.I., & Morris, R.G.M. (2005). Spatial learning with unilateral and bilateral hippocampal networks. *European Journal of Neuroscience*, *22*, 745-754.

Dudai, Y. (2002). *Memory from A to Z keywords, concepts and beyond*. Oxford: Oxford University Press.

Fenton, A.A., & Bures, J. (1993). Place navigation in rats with unilateral tetrodotoxin inactivation of the dorsal hippocampus: Place but not procedural learning can be lateralized to one hippocampus. *Behavioral Neuroscience*, *107*, 552-564.

Hartley, T., Bird, C.M., Chan, D., Cipolotti, L., Husain, M., Vargha-Khadem, F., & Burgess, N. (2007). The hippocampus is required for short-term topographical memory in humans. *Hippocampus*, *17*, 34-48.

Lorenzini, C.A., Baldi, E., Bucherelli, C., Sacchetti, B., & Tassoni, G. (1996). Role of dorsal hippocampus in acquisition, consolidation and retrieval of rat's passive avoidance response: A tetrodotoxin functional inactivation study. *Brain Research*, *730*, 32-39.

Lorenzini, C.A., Baldi, E., Bucherelli, C., Sacchetti, B., & Tassoni, G. (1997). Role of ventral hippocampus in acquisition, consolidation and retrieval of rat's passive avoidance response memory trace. *Brain Research*, *768*, 242-248.

Lorenzini, C.A., Baldi, E., Bucherelli, C., Sacchetti, B., & Tassoni, G. Neural topography and chronology of memory consolidation: A review of functional inactivation findings. *Neurobiology of Learning and Memory*, *71*, 1-18.

Morris, R.G.M., Garrud, P., Rawlins, J.N.P., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, *297*, 681-683.

Muller Igaz, L., Vianna, M., Medina, J.H., & Izquierdo, I. (2002). Two time periods of hippocampal mRNA síntesis are required for memory consolidation of fear-motivated learning. *The Journal of Neuroscience*, *22*, 6781-6789.

O'Keefe, J., & Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Research*, *34*, 171-175.

Paxinos, G., & Watson, Ch. (1986). *The rat brain in Stereotaxic Coordinates*. San Diego: Academic Press.

Pereira de Vasconcelos, A., Klur, S., Muller, C., Cosquer, B., López, J., Certa, U., & Cassel, J.C. (2006). Reversible inactivation of the dorsal hippocampus by tetrodotoxin or lidocaine: A comparative study on cerebral functional activity and motor coordination in teh rat. *Neuroscience*, *141*, 1649-1663.

Scoville, W., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery and Psychiatry*, *20*, 11-21.

Squire, L.R., & Zola, S.M. (1996). Structure and function of declarative and nondeclarative memory systems. *PNAS*, *93*, 13515-13522.

Wesierska, M., Dockery, C., & Fenton, A.A. (2005). Beyond memory, navigation and inhibition: Behavioral evidence for hippocampus-dependent cognitive coordination in the rat. *The Journal of Neuroscience*, *25*, 2413-2419.

Zhuravin, I.A., & Bures, J. (1991). Extent of the tetrodotoxin induced blockade examined by pupillary paralysis elicited by intracerebral injection of the drug. *Experimental Brain Research*, *83*, 687-690.