A new touchscreen test of pattern separation: effect of hippocampal lesions

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Researchers are becoming increasingly interested in the role of the hippocampus in pattern separation, a process which keeps items distinct in memory. In this study, we develop and test a new automated touchscreen-based method for studying pattern separation in rodents. Rats were trained to discriminate locations on a computer screen that varied in their similarity, that is, their distance apart on the screen. Animals with lesions of the dorsal hippocampus were impaired when the locations discriminated were close together but not when they were far apart, indicating impaired pattern separation. This test provides an automated test of pattern separation, which adds to an expanding battery of cognitive tests that can be carried out using the touchscreen testing method. *NeuroReport* **20:881–885 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

NeuroReport 2009, **20**:881–885

Keywords: dorsal hippocampus, high throughput, pattern separation, spatial learning

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Received 13 March 2009 **accepted** 1 April 2009

Introduction

It is well established that the hippocampus has an essential role in memory [1–4]. Recently, attention has turned to more specific functions of the hippocampus. One such function is pattern separation, which can be described as the processing of two or more sets of similar inputs such that they are represented in a way that keeps them separate and distinct in memory [5,6]. Computational modelling has explored how pattern separation functions within the context of the complex circuitry of the hippocampus [5,6], and empirical evidence for the involvement of the hippocampus in pattern separation comes from a number of physiological experiments [7,8]. Kesner and his colleagues [9,10] have shown that the hippocampus, and specifically the dentate gyrus, is necessary for spatial pattern separation.

Thus, the stage is set for further exploration of the structures and mechanisms underlying pattern separation – for example, the receptors involved [11,12] and the role of neurogenesis in pattern separation [13]. To achieve this aim, it is essential that we have behavioural tests that are efficient and accurate at our disposal. Efficiency, accuracy and objectivity of behavioural tasks can be greatly increased through automation. Therefore, in this study, we introduce a new, automated task for studying spatial pattern separation. This task embeds indices of pattern-separation ability within a two-choice spatial discrimination learning task. Rats with excitotoxic lesions of the dorsal hippocampus were tested on this paradigm to explore the sensitivity of the task to hippocampal manipulations. A deficit in pattern separation would be revealed by an impairment in the ability to learn to discriminate similar, but not dissimilar, locations on the computer screen.

Methods

Animals

Twenty male Lister hooded rats (Harlan UK, subdivision of Harlan Laboratories Inc., Indianapolis, Indiana, USA), housed in pairs and kept on a reverse day/night light cycle (lights on 19 : 00–7 : 00 h) were used for this study. Rats were maintained on ad libitum food (standard rat chow; Purina Mills, St. Louis, Missouri, USA) and water before surgery and during the week after surgery. When training began, rats were food deprived to 85% of their free-feeding weight. All behavioural testing occurred during the rats' dark phase. All experimentation was conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986.

Apparatus

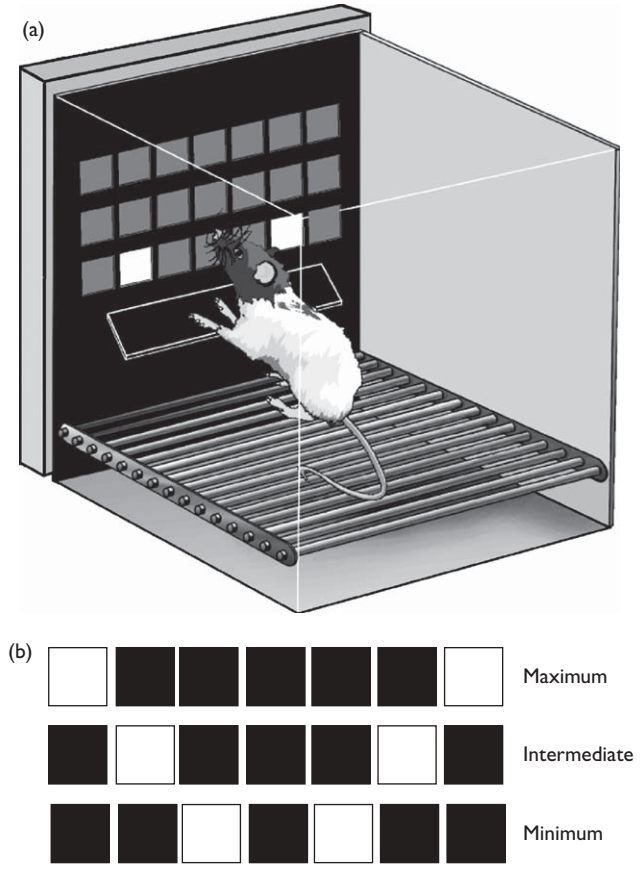
Animals were trained using an automated touchscreen apparatus [14,15]. The description of the apparatus used can be found in Refs [16,17]. Briefly, the apparatus consisted of an operant chamber (Med Associates, Vermont, USA; height=23 cm, width=30 cm, depth=25 cm) with clear Perspex walls and a metal frame, and a floor consisting of metal bars spaced about 1 cm apart. The floor of the operant chamber was mounted around 3 cm above a waste receptacle lined with paper inserts. A touch-sensitive liquid crystal display monitor was

0959-4965 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/WNR.0b013e32832c5eb2

mounted at one end of the chamber. The monitor was covered by a black Perspex 'mask' (38 × 28 cm) which had 35 open response windows (2 × 2 cm, equally spaced 1 cm apart) arranged in an array of five rows by seven columns. Only the bottom row was used in the present experiment to display one of the three different stimulus arrays, each consisting of two white squares (2 × 2 cm) presented within two response windows, thus denoting two locations. The two locations were separated by either one, three or five empty squares to create three separation conditions: minimum, intermediate and maximum (see Fig. 1 for apparatus and stimulus arrays). The mask was attached to the screen leaving around a 0.5 cm gap so that it would not trigger the touchscreen. A spring-hinged 'shelf' (6 × 20.5 cm) was attached 15 cm above the grid floor. This shelf was at a 90° angle to the mask and encouraged the rats to pause, rear-up and look at the stimuli before responding.

A food magazine served by a pellet dispenser and equipped with a light and infrared beam to detect rats' head entries into the magazine was located on the wall opposite to the touchscreen. A house light (3 W) and a tone generator were situated above the food magazine. Each setup was placed inside a sound-attenuating chamber and ventilated by a small fan. Each box and touchscreen computer screen was controlled by an IBM computer running custom software written by one of us (A.C. Mar) in Visual Basic 6.0 (Microsoft Corp., Redmond, Washington, USA).

Fig. 1



(a) Operant chamber with the intermediate separation array displayed as it would be during a trial; (b) showing the three possible stimulus arrays. Rats were trained initially using the intermediate stimulus array, followed by probe sessions on the maximum and minimum conditions. After surgery, rats were tested on all conditions.

Surgery

The surgical procedure used here is adapted from Jarrard [18]. When criterion performance on the behavioural task had been reached by all animals, they were divided into two groups of equal prelesion performance; animals in one group were given dorsal hippocampal lesions (DHPC group) and animals in the other group were given sham surgery. Anaesthesia was maintained throughout surgery using 100 ml/kg Avertin [10 *g* of 2,2,2-tribromoethanol (Sigma-Aldrich Ltd., Gillingham, Dorset, UK) in 5 *g* of tertiary amyl alcohol, diluted in a solution of 40 ml of ethanol and 450 ml of PBS]. After induction of anaesthesia, animals were placed into a stereotaxic frame (David Kopf Instruments, Tujunga, California, USA) with the incisor bar set at −3.3 mm. A craniotomy was performed to expose the relevant coordinates for injection into the hippocampus. Ibotenic acid (0.9M, Sigma-Aldrich Ltd., Gillingham, Dorset, UK) was then injected through a bevelled microsyringe at a rate of 0.1 µl/min [0.1 µl at coordinates: anterior–posterior (AP) = −2.4, medial–lateral (ML) = −1, dorsal–ventral (DV) = −3.4; AP = −3, ML = −3, DV = −3; AP = −4, ML = −3.7, DV = −3; AP = −5.7, ML = −4.1, DV = −3.8; and 0.05 µl at coordinates: AP = −3, ML = −1.4, DV = −3.4; AP = −3, ML = −1.4, DV = −2.6; AP = −4, ML = −2.6, DV = −3.3; AP = −4, ML = −2.6, DV = −2.3; AP = −4.9, ML = −3.9, DV = −3.5; a total of nine injections bilaterally]. Rats in the sham control lesion group were subjected to exactly the same surgery, but no injections were made. AP and ML coordinates were calculated relative to bregma and DV coordinates were calculated relative to dura. Immediately after surgery, animals were given glucose-saline subcutaneously and placed on a heat pad in a cage in a darkened room and allowed to recover with free access to food and water. No seizures were observed, as all animals remained anaesthetized for several hours postsurgery. One sham animal died postoperatively leaving group sizes of nine sham control rats and 10 DHPC.

Histology

When all behavioural testing was completed, animals were anaesthetized with Dolethal (Vétoquinol UK Ltd., Buckingham, Bucks, UK) (2 ml, intraperitoneally) and transcardially perfused with 100 ml of PBS, followed by 250 ml of 4% paraformaldehyde. Brains were then removed and further fixed in paraformaldehyde at 4°C for a minimum of 24 h. Before cutting, samples were

soaked in 20% sucrose in PBS for 24 h. Sections of 60 µm were cut using a microtome. Every fifth section was mounted on a gelatin-coated glass slide and then stained with cresyl violet. Both DHPC and sham control brains were examined under a light microscope to assess the extent of damage.

Behavioural pretraining

At the start of training, animals were given one session of combined habituation and Pavlovian training. In this session, stimuli (white squares) were displayed on the screen in any position, one at a time, for a period of 30 s. Food pellets (0.045 *g* Purified Rodent Tablet, Testdiet, Richmond, Indiana, USA) were dispensed automatically following stimulus offset, accompanied by a tone and illumination of the magazine light. However, if during stimulus display the animal made a nose-poke in the response window in which the stimulus was displayed then three pellets were dispensed. This session ended when the rat had received 100 pellets or when 60 min had elapsed, whichever came first. After this session, there were three stages of training. In the first stage, on a given trial, one stimulus was displayed on the screen in any position on the bottom row. If the rat responded to the correct position, it received a food pellet accompanied by the light and tone; there were no consequences for responding to any other unlit location. The intertrial interval was 20 s. Animals were required to complete 100 trials in 60 min to move to the next stage. The second stage was the same as the first stage except that animals were required to initiate trials by nose-poking the illuminated magazine. Initiation became available after an intertrial interval of 20 s. Again the criterion was completion of 100 trials in 60 min. The third stage was the same as the second stage, with the exception that touching any of the nonilluminated squares on the screen triggered a time-out period in which the house light was extinguished for 5 s. In this stage, the animals were required to get 95 correct out of 100 trials within 45 min. Touching the illuminated square was scored as correct; responding elsewhere was scored as incorrect. As soon as animals reached criterion on this stage of pretraining, they moved onto spatial discrimination training.

Spatial discrimination training

During spatial discrimination training, positions 2 and 6 (intermediate separation; Fig. 1) were illuminated and one was designated as correct (counterbalanced across animals). Rats were rewarded for touching the correct location; touching the incorrect location resulted in a 5-s time-out period. If the animal touched the correct location on nine out of 10 trials (calculated on a rolling basis) then the correct and incorrect locations were reversed. The purpose of this was to allow the rat to complete multiple discriminations per session to facilitate learning. Rats were given 100 trials per session. The intertrial interval was initially 10 s; however, after a week of training this was decreased to 3 s. Each day the starting correct location was changed, so if on day 1 location 2 was correct at the start, on day 2 location 6 would be correct at the start, irrespective of the number of discriminations completed on day 1. The criterion for completion of spatial discrimination training was to perform six or more discriminations per session for two consecutive sessions.

Spatial discrimination testing – prelesion

Once they achieved criterion on the discrimination training in the intermediate separation condition, rats were given four probe sessions; two at maximum separation and two at minimum separation. The order in which these sessions were given and the starting correct side was counterbalanced, such that each rat received one session at each separation starting with the left side correct and one session at each separation starting with the right side correct. On these sessions, animals were given up to 60 min to perform two discriminations (one initial discrimination and one reversal).

Spatial discrimination testing – postlesion

After they had recovered from surgery, animals were given eight sessions of each of the three separations (maximum, intermediate and minimum) for a total of 24 sessions. The order in which these sessions were given, and the starting correct side, was counterbalanced such that rats received sessions of all separations starting on both sides.

Data collection and statistical analysis

For all sessions, data were recorded automatically by the touchscreen system. For initial discrimination training, the number of discriminations per session was recorded. For the test sessions, the mean number of trials taken to complete the discriminations was recorded.

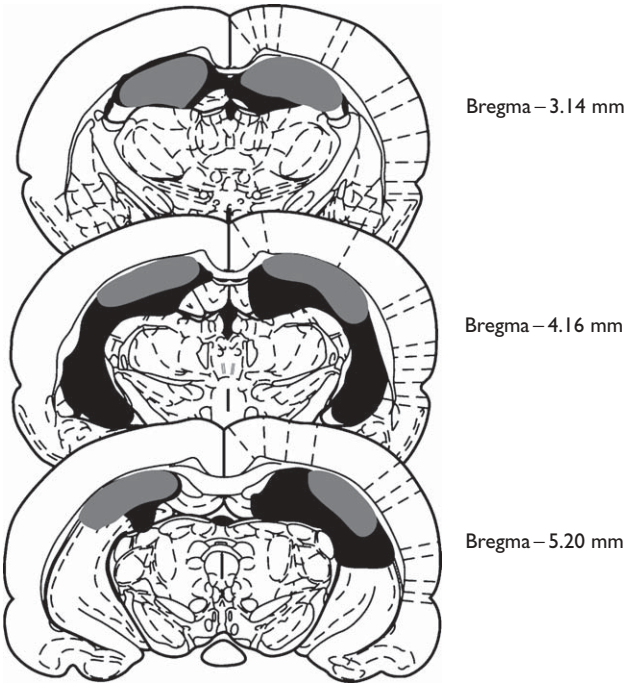
These data were analysed with repeated-measures analysis of variance (ANOVA), and where appropriate, post-hoc independent-samples *t*-tests using Bonferroni correction for multiple comparisons, using SPSS (SPSS Inc., Chicago, Illinois, USA). Mean trials to complete the discriminations were used as the dependent variable, with lesion as the between-subjects factor and separation as the within-subjects factor.

Results

Histology

Reconstructions of dorsal hippocampal lesions are shown in Fig. 2 (adapted from Ref. [19]). Three animals were excluded from the DHPC group because of excessive sparing of the hippocampus. Of the remaining seven, three exhibited incidental cortical damage outside of the dorsal hippocampus bilaterally. Analysis of the data excluding these three animals revealed an identical pattern to that obtained when all animals were included

Fig. 2



The extent of damage to the hippocampus in the dorsal hippocampal lesion (DHPC) group showing the largest (black) and smallest (grey) lesions. Adapted from Ref. [19].

(a significant lesion × separation interaction; see below); therefore, all seven animals were included in all analyses throughout. Only the largest lesion extended to a minor extent into the ventral hippocampus; the six other lesions were restricted to the dorsal region.

Discrimination training

All rats completed criterion performance of six or more reversals per session for two consecutive sessions. On average, this took 22.5 ± 2.50 sessions (mean ± SEM).

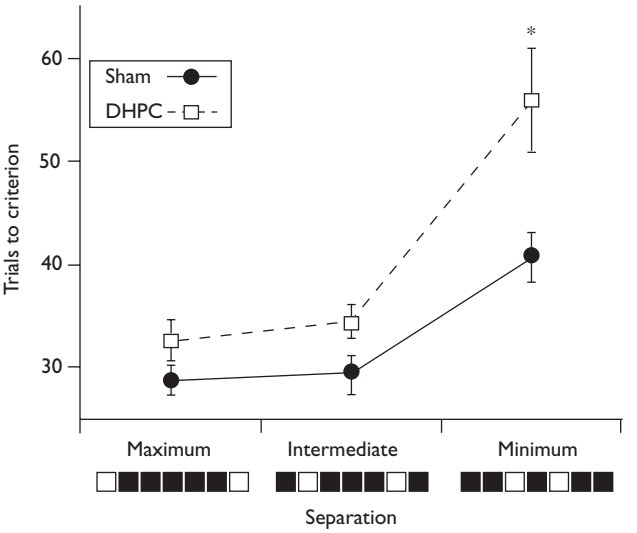
Prelesion performance

Rats were divided into two groups for analysis, those who were to receive lesions of the dorsal hippocampus, and those who were to receive sham control operations. Repeated-measures ANOVA with within-subjects factor of separation (with two levels of separation, minimum and maximum), and between-subjects factor of later lesion group showed a significant effect of separation [$F(1,14) = 13.94$, $P < 0.005$], but no effect of later lesion group [$F(1,14) = 2.24$, $P > 0.1$] and no interaction ($F > 1$).

Postlesion performance

The postlesion data are shown in Fig. 3. Repeated-measures ANOVA with a within-subjects factor of separation and a between-subjects factor of lesion revealed a significant effect of separation [$F(2,28) = 42.45$, $P < 0.001$], a significant effect of lesion [$F(1,14) = 9.63$, $P < 0.01$] and a significant lesion × separation interaction [$F(2,28) = 3.95$, $P < 0.05$]. Post-hoc t-tests using Bonferroni correction revealed a significant difference between the sham control and DHPC group on minimum separation [$t(14) = 2.97$, $P < 0.01$], a trend toward a difference on intermediate separation [$t(14) = 2.20$, $P = 0.05$], and no difference on maximum separation [$t(14) = 1.13$, $P = 0.28$].

Fig. 3



Performance of the dorsal hippocampal lesion (DHPC) and sham groups during eight postlesion probe sessions on each of the three separations (total of 24 sessions). A clear difference in performance can be seen at the minimum separation, but not at either the maximum or intermediate separations. *$P < 0.01$.

Analysis of the four rats without cortical damage using a similar repeated-measures ANOVA revealed an identical pattern to that obtained when all rats were included, that is, a significant effect of separation [$F(2,22) = 43.65$, $P < 0.001$] and of lesion [$F(1,11) = 8.92$, $P < 0.05$] and a significant lesion × separation interaction [$F(2,22) = 4.63$, $P < 0.05$]. This finding indicates that incidental cortical damage cannot account for the effects of the hippocampal lesion on pattern separation.

Discussion

In this study, we developed and tested a new task for studying spatial pattern separation in the rodent. The results indicate that rats with lesions of the dorsal hippocampus were impaired at spatial discrimination learning when the locations to be discriminated were close together but not when they were far apart, indicating an impairment in pattern separation. Indeed, preliminary results from our lab indicate that the task is exquisitely sensitive to hippocampal dysfunction; a subtle manipulation of the hippocampus involving knock-down of neurogenesis in the dentate gyrus of mice, resulting

in only about a 10% decrease in total cell numbers in that area, produces a separation-dependent impairment on this task strikingly similar to that reported in this study [13].

This separation-dependent impairment after dorsal hippocampal lesions is consistent with that found in other studies in rats using different methodology. For example, Gilbert *et al*., using a delayed nonmatching-to-sample task in a maze-based paradigm, have found separation-dependent deficits after complete hippocampal [9] or selective dentate gyrus [10] damage. Although such maze-based methods can be effective, the present method offers additional advantages which may be attractive to researchers. For example, the necessity for locomotion such as running or swimming is minimized, making the touchscreen method particularly suitable for use in disease models with motor deterioration [20]. The method also minimizes experimenter bias during testing and allows for high throughput, in the sense that a large number of animals can be tested simultaneously. Finally, a number of tests of learning, memory, attention, impulsivity and compulsivity are already available for this system [14,17,20–24]. These various tests of cognition can be compared in an unconfounded manner, as all the tests feature the same kinds of stimuli, responses, rewards and testing environment. Finally, the method allows the presentation to rodents of tasks that are similar in all important respects to those used in human test batteries, allowing effective translation from the laboratory to the clinic [25].

Conclusion

The task described above provides a novel automated method for studying pattern separation in rodents. This task is impaired by dorsal hippocampal lesions in a separation-dependent manner, showing that the dorsal hippocampus is necessary for learning to distinguish similar spatial locations. This finding is consistent with other tests of pattern separation that have been used in rodents and offers additional advantages.

Acknowledgements

The authors thank Michal T. Kucewicz, Madeleine M. Wilson and Rebecca L. Collins for their help in initial testing, and John C. Talpos for assistance with figures. This work was supported by a grant from the Alzheimer's Research Trust (L.M.S. and T.J.B.), and a 3-year MRC-funded PhD studentship at The University of Cambridge, MRC/Wellcome Trust Behavioural and Clinical Neurosciences Institute (S.M.M.).

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