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Research report

Excitotoxic hippocampal lesions disrupt allocentric spatial learning in mice: effects of strain and task demands

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Abstract

Spatial discrimination of ibotenic acid-lesioned C57BL/6 (B6) and DBA/2 (D2) mice was tested in two-choice water maze and plus maze tasks. B6 but not D2 mice learned the spatial discrimination in the water maze, but strains did not differ in learning a spatial discrimination in the plus maze paradigm. Ibotenic acid lesions of the hippocampus impaired percentage correct choices in the water maze spatial discrimination task in B6 but not in D2 mice, the latter of which may have been due to a floor effect. Furthermore, lesioned mice were more thigmotaxic, the distance travelled until a choice was made was longer and animals made more errors of omission. Despite the poor performance during water maze acquisition, lesioned animals, as well as sham-lesioned D2 mice, eventually acquired some place response in the water maze, as was evident when the location of the platform was reversed. However, hippocampus-lesioned mice of both strains were impaired when tested in the plus maze spatial discrimination task. Thus, ibotenic acid-induced lesions of the hippocampus impair acquisition of spatial discrimination in mice. These deficits were strain-dependent and likely comprise impaired accuracy as well as changes in non-mnemonic types of behaviour. Importantly, lesions in both strains impaired spatial learning, and whether a deficit was seen in mice of the D2 strain seemed to depend on the demands of the task.

Keywords: Hippocampus; Ibotenic acid; Lesion; Mouse; Spatial learning; Strain

1. Introduction

There is strong evidence supporting a role for the hippocampus in the mediation of spatial learning and memory in rats. Relatively little is known, however, about the role of the hippocampus in the mouse despite a growing number of studies, in particular with mutant mice, relating altered spatial performance with hippocampal function. However, it is problematic to infer a neural substrate for a certain cognitive process from murine behavioural performance in tasks which, in terms of neural substrates, have primarily been characterised in rats. Indeed, it has been demonstrated that at least some mouse strains perform poorly in certain spatial tasks when compared to rats, such as in the frequently employed circular water maze place navigation paradigm [27,44,46] and in radial maze delayed matching to position [27]. Mice, on the other hand, appear to perform as well as rats on a range of other maze tasks, such as in radial maze reference/working memory acquisition, reversal and retention, and delayed *non*-matching to position, using different degrees of complexity [27,46]. Even more pertinent, pre-training damage to the mouse hippocampus has been reported to spare or only partially affect some types of behaviour known to critically depend on hippocampal integrity in rats, such as contextual fear conditioning [11,16,19]. These examples clearly indicate that it is crucial to consider species differences when studying learning and memory in mice in tasks originally devised for testing rats.

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For example, we have recently adopted a water maze spatial discrimination task for testing mice [38]— a task which has been reported to be sensitive to hippocampal damage in rats [28]. This task allows a dissociation between errors of commission (i.e. choice accuracy) and errors of omission. Given that species differences in hippocampal function exist, however, it needs to be demonstrated that the mouse version of this task also depends on hippocampal integrity. Thus, one aim of the present experiments was to study the effects of hippocampal lesions on murine water maze spatial discrimination.

To add to the complexity of this situation, studies comparing the effects of hippocampal lesions in different mouse strains reported strain-dependent effects. For example, animals of the C57BL/6 strain (B6) are generally more susceptible to hippocampal damage than mice of the DBA/2 strain (D2) on tasks involving a spatial component [2,3,24,33,40]. Therefore, we also compared the effects of hippocampal lesions in B6 and D2 animals on spatial discrimination. In particular, a series of studies has shown that mice of the B6 strain perform superior to D2 mice on radial maze tasks if multiple choices between six or more arms are required [1,3–5,34], but not if the number of choices was limited to three [3], i.e. differences between these strains seem to depend, at least in part, on task complexity. Neuroanatomically and neurochemically, there is culminating evidence for hippocampal differences in these two mouse strains. More specifically, differences in spatial memory between the two strains have been reported to correlate with the size of the hippocampal intra- and infrapyramidal mossy fibre terminal fields [6,12,35,39]. Furthermore, a correlation between spatial performance and measures of the septohippocampal cholinergic system (cholinergic neuronal number, cholinergic fibre density, muscarinic M2 receptors) has been shown [39]. Finally, a comparison of different recombinant strains derived from C57BL/6 and DBA/2 demonstrated that hippocampal, but not cortical, protein kinase C (PKC) activity correlated with spatial learning [43]. The cholinergic septohippocampal projection has been suggested to modulate spatial memory (e.g. Refs. [36,37,39]), while PKC has been shown to be activated in a variety of learning and memory tasks [30,42].

In the present study, we compared the effects of hippocampal lesions in mice in two different spatial discrimination tasks, the two-choice spatial discrimination water maze paradigm and a two-choice spatial discrimination plus maze task. Both paradigms tax spatial reference memory, but differ in various aspects, such as task complexity [23], response requirement (swimming versus running) and the nature of the reinforcer (water escape versus food pellets).

However, with the exception of a few recent studies [11,18,19], the lesion techniques employed in studies on hippocampal lesions in mice were electrocoagulation or infusion of kainic acid [2,3,16,24,33,40], the former of which is known to destroy fibres of passage, while the latter has been criticised for causing distant lesions [21,22]. Therefore, the present study used the excitotoxin ibotenic acid which has been suggested to produce more localised hippocampal damage [23].

Thus, we compared the effects of ibotenic acid-induced lesions of the hippocampus in two mouse strains with known differences in spatial abilities in two spatial discrimination paradigms with different task demands.

2. Material and methods

2.1. Subjects

Three-month-old male C57BL/6N Crl BR (B6) and DBA/2N Crl BR (D2) mice were obtained from Charles River (Sulzfeld, Germany). Animals were housed individually and maintained on a 12:12-h light/dark cycle (lights on 07:00 h). All experiments were conducted during the light phase of the cycle. During testing in the plus maze, animals were food-deprived to 85% of their free feeding weights. Otherwise, food and water were available ad libitum. The experimental protocol was approved by the Ethical Committee on Animal Care and Use of the Government of Bavaria, Germany (AZ 211-2531-43/97).

2.2. Surgery

Mice were anaesthetised with isofluran, pre-treated with diazepam (10 mg/kg, i.p.) and atropine sulphate (0.04 mg, i.p.), and placed in a stereotaxic frame (TSE, Bad Homburg, Germany). Bilateral intrahippocampal infusions with ibotenic acid (15 μg/ml dissolved in phosphate-buffered saline, 0.1 M, pH 7.4; Sigma, Deisenhofen, Germany) were made over 2 min through a 5-μl Hamilton syringe connected via a tube to an infusion cannula (0.40 mm) mounted on the stereotaxic frame, at the following coordinates and volumes: (i) AP, −1.3 mm relative to bregma; L, ±1.2 mm lateral to the midline; V, 2.0 mm below the exposed dura mater, 0.10 μl; (ii) AP, −2.5 mm; L, ±2.5 mm; V, 2.2 mm, 0.15 μl; (iii) AP, −3.3 mm; L, ±3.1 mm; V, 4.1 mm, 0.15 μl [17]. The cannula was left in place for another 5 min prior to retraction. Sham-operated animals received vehicle injections.

2.3. Histology

After completion of the experiment all animals were sacrificed and their brains frozen on dry ice. Forty-μm thick sections were stained with Cresyl Violet in order to confirm correct placement of the lesion.

2.4. Experiment 1: water maze spatial discrimination

2.4.1. Apparatus

A circular swimming pool (80 cm in diameter, 30 cm high, white plastic) was filled to a depth of 20 cm with water (21 ± 1°C; rendered opaque by addition of a non-toxic dye). At the outside of the maze, eight start boxes ($10 \times 10 \times 26$ cm), also filled with water and fitted with sliding doors, were fixed, from which the animal could swim into the centre of the maze when the door was raised. Introduction of start boxes allowed tracking of the animal as soon as it entered the circular open field of the maze. Two identically looking circular platforms with white surface and dark grey rim were used (each 10 cm in diameter, protruding approximately 0.5 cm above the water surface). One platform was stable and provided support ('correct platform'), the other was floating and sank when a mouse tried to climb on it ('incorrect platform'). Performance was recorded by a video tracking system (TSE).

2.4.2. Testing

Animals were first habituated by placing them for 1 min on the stable platform in the middle of the pool. Subsequently, subjects were trained to choose between the two platforms [38] over six sessions (acquisition stage; ten trials per session, one session per day). On each trial, the stable platform remained in the same position (counterbalanced within groups), while the floating platform changed position from trial to trial in a pseudorandom manner (five possible positions). It was ensured that the spatial relationship between the platforms did not consistently reward turns into one direction, and that the distance between the start position and each of the two platforms was equal over the ten trials. A trial started by placing a mouse in one of six possible start boxes in pseudorandom sequence and the door was opened. All except the start positions in front of and opposite to the correct platform were used. Data recording started when the subject had left the start box. A trial terminated when a mouse climbed onto one of the two platforms or after 30 s. If a mouse climbed onto the stable platform within 30 s, it was allowed to stay there for another 10 s before it was returned to the holding cage. If an animal made an incorrect choice (climbing the incorrect platform) or after 30 s had lapsed, it was gently placed on the correct platform and allowed to stay there for 10 s before it was returned to the holding cage. Animals were trained in squads of four. ITIs were approximately 2–4 min and each session lasted approximately 30 min.

On day 7, the stable platform was moved to the opposite side of the water maze and the subjects were trained for another 6 days with the platform remaining in the new position (relocation stage).

2.5. Experiment 2: plus maze spatial discrimination

2.5.1. Apparatus

An eight-arm radial maze was used, with four out of eight arms being always closed. The maze was constructed of grey plastic. It was elevated 50 cm above floor level. Each arm (50×5 cm) extended from an octagonally shaped centre (15 cm in diameter) and was enclosed by side and end walls (15 cm high) made of clear perspex. At the end of each arm there was a food well (0.75 cm in diameter and 0.75 cm deep). Sliding doors made of clear perspex allowed to open and close each arm separately. Only four of the eight arms were used in this study, forming a cross. For the spatial discrimination task, two of the four arms (opposite to each other) served as choice arms, while the other two arms served as start arms.

2.5.2. Testing

Initially, mice received three habituation sessions, with ten trials per session. During each trial, a mouse was placed in the centre of the maze, the doors were raised and the animal was allowed to visit all four arms and to collect food pellets (20 mg each, Noyes, Lancaster, UK), which were scattered throughout the arms during session one and placed at the end of each arm during subsequent sessions. Each arm was closed after its exploration. A trial lasted until all four arms were explored or after one minute had lapsed.

Subsequently, spatial discrimination learning commenced. At the beginning of each trial, a food pellet was placed in one of the two choice arms ('correct arm') and the animal was placed in one of the two start arms. The correct arm remained always in the same position relative to spatial cues in the room (counterbalanced within groups), while the position of the start arm varied from trial to trial in pseudorandom sequence in order to prevent development of a turning strategy. Then three of the doors were raised, effectively forming a T-maze. A trial terminated when a mouse entered one of the two choice arms or after 90 s. Each animal was trained over six sessions (ten trials per session, one session per day). Between trials the maze was cleaned and rotated in order to minimise the use of olfactory intramaze cues.

On day 7, subjects had to make a forced choice into the previously baited arm. Thirty pellets were scattered throughout this arm and subjects were allowed to eat as much pellets as possible within 10 min (free feeding trial).

2.6. Behavioural measures

The accuracy measures described include percentage correct choices in the water maze and the plus maze. Errors of omission (water maze, number of failures to

choose a platform within the time limit of 30 s; plus maze, number of failures to choose an arm within the time limit of 90 s) were also counted in both experiments. Choice latency, distance travelled if a choice was made and swim speed were measured during water maze performance. If an error of omission was made, no latency, distance travelled or swim speed were scored. Furthermore, two measures of thigmotaxis, the relative time spent swimming and the relative distance travelled along the wall of the pool (as defined within a circular corridor of 10 cm), were calculated. In contrast to choice latency, distance travelled and speed, indices of thigmotaxis were calculated for all trials as thigmotaxis may be one factor leading to errors of omission. The difference in percentage correct choices between the last acquisition day and the first session with a changed platform position was calculated to indicate the severity of disruption induced by changing the spatial relationships of the stable platform. Finally, side bias was calculated in the plus maze task as the percentage of choices of the more preferred turning direction into the left or right maze arm, whereby it was considered irrelevant whether the left or the right choice was preferred by an animal.

In the water maze, a choice was made if an animal touched a platform with its forepaws or its snout. The occasional incident of brushing past the floating platform in passing was not considered a choice. In the plus maze, it was considered an arm entry if an animal entered a maze arm with all four paws within 90 s after starting the trial. Finally, the number of pellets eaten during the free feeding trial were scored.

2.7. Data analysis

Data were transformed as appropriate (arcsine after division by 100, all percentage measures; logarithmic, all latencies, distances and speed; square-root after addition of 0.5, errors of omission and number of pellets eaten) and analysed by MANOVA, including one-factor (strain or lesion) independent measures, a two-factor (strain × lesion; strain × session; lesion × session), and a three-factor (strain × lesion × session) mixed measure analysis, with session as the repeated measure.

3. Results

3.1. Histology

From 28 lesioned mice, 16 had to be excluded due to incomplete damage or damage of the overlying cortex, leaving seven B6 animals and five D2 animals with bilateral hippocampal lesions (B6 lesioned and D2 lesioned, respectively).

From those lesioned animals which were included in the study, area CA1 was completely and bilaterally destroyed in five out of seven B6 and in three out of five D2 mice. Parts of CA1 were spared unilaterally in the dorsal hippocampus in two B6 mice and two D2 animals, while CA1 was completely damaged on the contralateral site. CA1 was completely destroyed in the ventral hippocampus of all B6 mice, while one D2 animal showed complete CA1 lesion on one site of the hippocampus and incomplete damage of the contralateral CA1. Area CA2 was damaged bilaterally in all mice. Likewise, all lesioned mice showed complete and bilateral damage of area CA3 in the dorsal hippocampus. In D2 animals, area CA3 in the ventral hippocampus was also completely destroyed in all animals, while one out of seven B6 mice showed complete damage of ventral CA3 on one site, but only incomplete damage of CA3 in the contralateral hippocampus. Moreover, five of the seven lesioned B6 mice showed complete dentate gyrus lesions, but in two animals the dentate gyrus was mostly spared unilaterally, while the contralateral site was completely damaged. Finally, the ventral part of the dentate gyrus was only incompletely lesioned on one site in two D2 mice. Overall, lesion size was comparable between the two strains (Figs. 1 and 2).

Four sham-lesioned animals showed non-specific damage and were excluded from further analysis, leaving eight B6 and eight D2 control mice (B6 sham and D2 sham, respectively).

3.2. Experiment 1: water maze spatial discrimination

3.2.1. Acquisition

3.2.1.1. Accuracy.

(*i*) *Effects of strain*: there was a main effect of strain ($F_{1,24} = 6.72$, $P = 0.0016$) and a strain × session interaction ($F_{5,120} = 4.67$, $P = 0.001$) in the percentage correct choices made, with significant differences during sessions 3, 5 and 6. Overall, B6 mice reached higher levels of accuracy than D2 mice, which remained at chance level (Fig. 3).

(*ii*) *Effects of lesion*: although there was no overall effect of lesion on accuracy (lesion, $F_{1,24} = 2.43$, $P > 0.05$; lesion × session, $F_{5,120} = 1.81$, $P > 0.05$), MANOVA revealed a lesion × strain interaction ($F_{1,24} = 7.54$, $P = 0.011$): sham-lesioned B6 mice performed at higher accuracy level than B6 mice with hippocampal lesions (Fig. 3) or sham-lesioned D2 animals, while the two D2 groups performed near chance level and did not differ from each other (Fig. 3), and there was no significant difference between the two lesioned groups.

3.2.1.2. Choice latency and distance travelled.

(*i*) *Effects of strain*: overall, in D2 mice, choice latency was higher ($F_{1,24} = 20.72$, $P < 0.001$) and the

distance travelled until a choice was made was longer ($F_{1,24} = 22.82$, $P < 0.001$) than in B6 mice (Fig. 4A, B). Furthermore, there was a strain × session interaction, with significant differences during acquisition except for the first session (latency, $F_{5,120} = 4.10$, $P = 0.002$; distance, $F_{5,120} = 3.52$, $P = 0.005$).

(*ii*) *Effects of lesion*: choice latency ($F_{1,24} = 9.13$, $P = 0.006$) and distance ($F_{1,24} = 28.94$, $P < 0.001$) were increased as a result of hippocampal damage (Fig. 4A, B), but both effects were independent of strain or session (lesion × strain, $F_{1,24} < 0.01$ and $F_{1,24} = 0.19$ respectively; lesion × session, $F_{5,120} = 1.42$ and $F_{5,120} = 1.07$ respectively; all $P > 0.05$).

3.2.1.3. Swim speed. (*i*) *Effects of strain*: B6 animals swum faster than D2 mice (strain, $F_{1,24} = 45.02$, $P < 0.001$; strain × session, $F_{5,120} = 0.59$, $P > 0.05$; Fig. 4C). (*ii*) *Effects of lesion*: swim speed remained unaffected by the lesion in B6 animals, but was increased in D2 lesioned mice compared to sham-lesioned D2 animals (lesion, $F_{1,24} = 2.01$, $P > 0.05$; lesion × session, $F_{5,120} =$

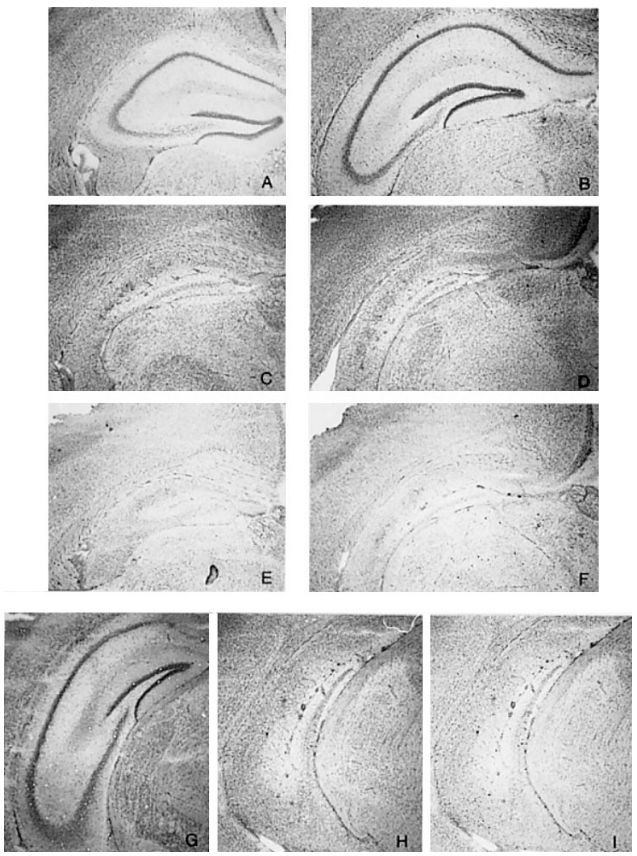

Fig. 1. Cresyl Violet staining of coronal sections from the hippocampi of a B6 sham mouse (A, B, G) and of two ibotenic acid-lesioned animals (B6, C, D, H; D2, E, F, I). Infusions of ibotenic acid caused extensive damage throughout the hippocampus (from rostral to caudal, A, C, E; B, D, F; G, H, I) without damaging surrounding brain areas. The size of the lesions was comparable between strains. Hippocampi from D2 sham animals (not shown) did not differ those of B6 sham mice in Cresyl Violet-stained sections.

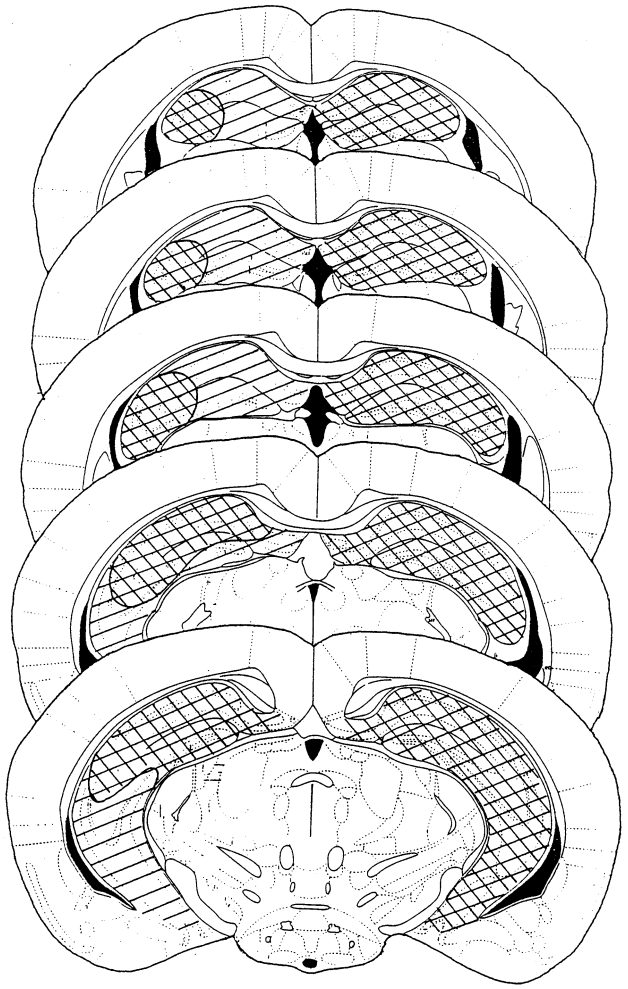

Fig. 2. Schematic illustrations of the extent of the lesions (smallest and largest lesion) in B6 mice included in the behavioural analysis (Figure re-drawn from Ref. [15]). The sizes of the smallest and the largest lesions in D2 mice were comparable.

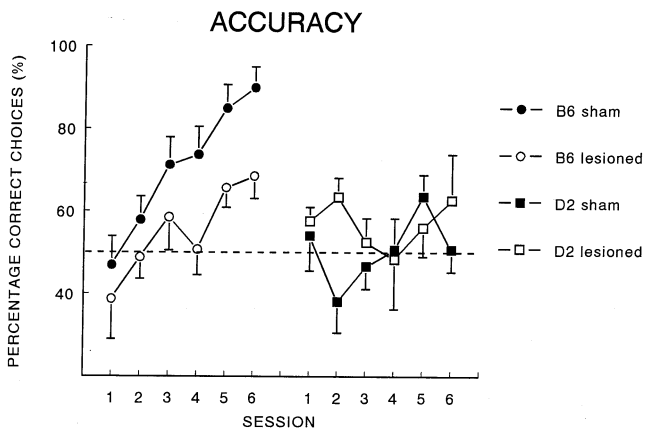

Fig. 3. Accuracy (percentage correct choices) during water maze spatial discrimination acquisition in B6 and D2 mice. The dotted lines represent chance performance. Data are expressed as mean values with error bars denoting S.E.M. values.

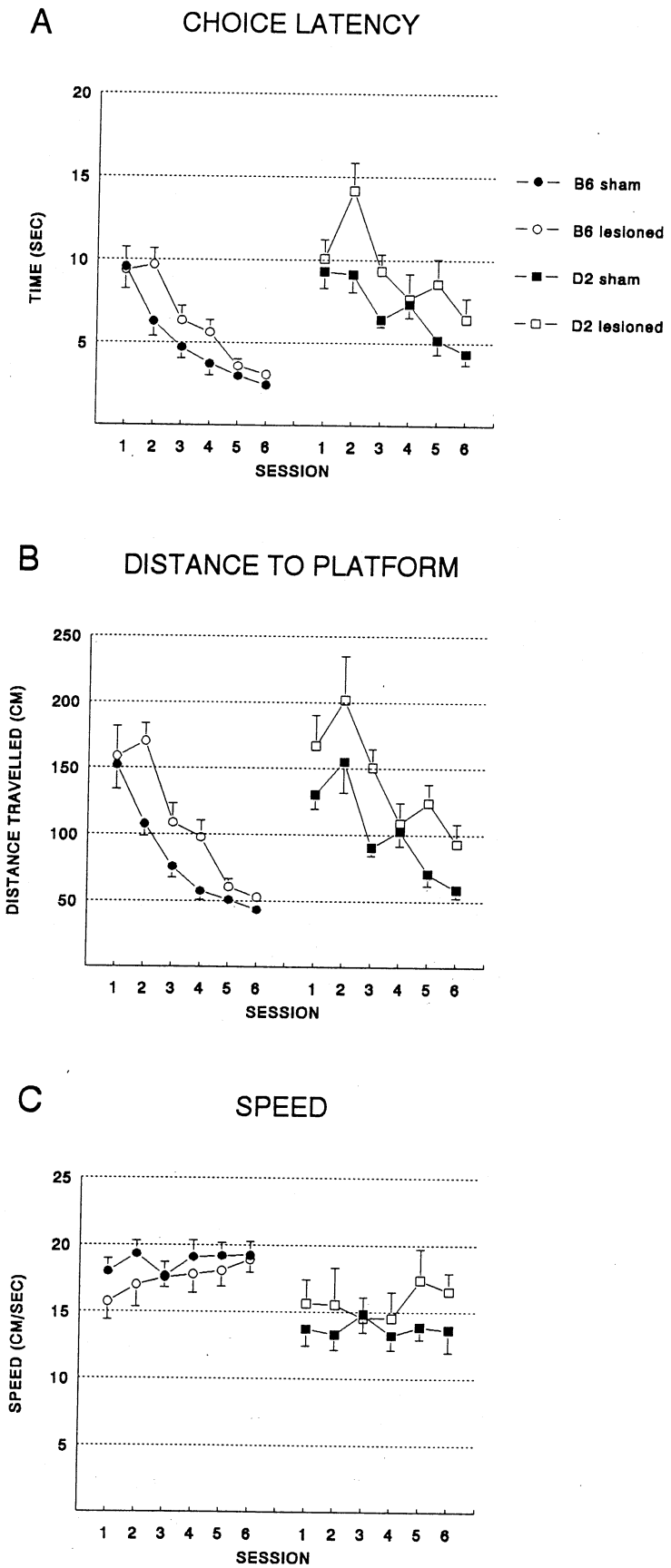

Fig. 4. Choice latency (A), distance travelled (B) and swim speed (C) during water maze spatial discrimination acquisition. Data are expressed as mean values with error bars denoting S.E.M. values.

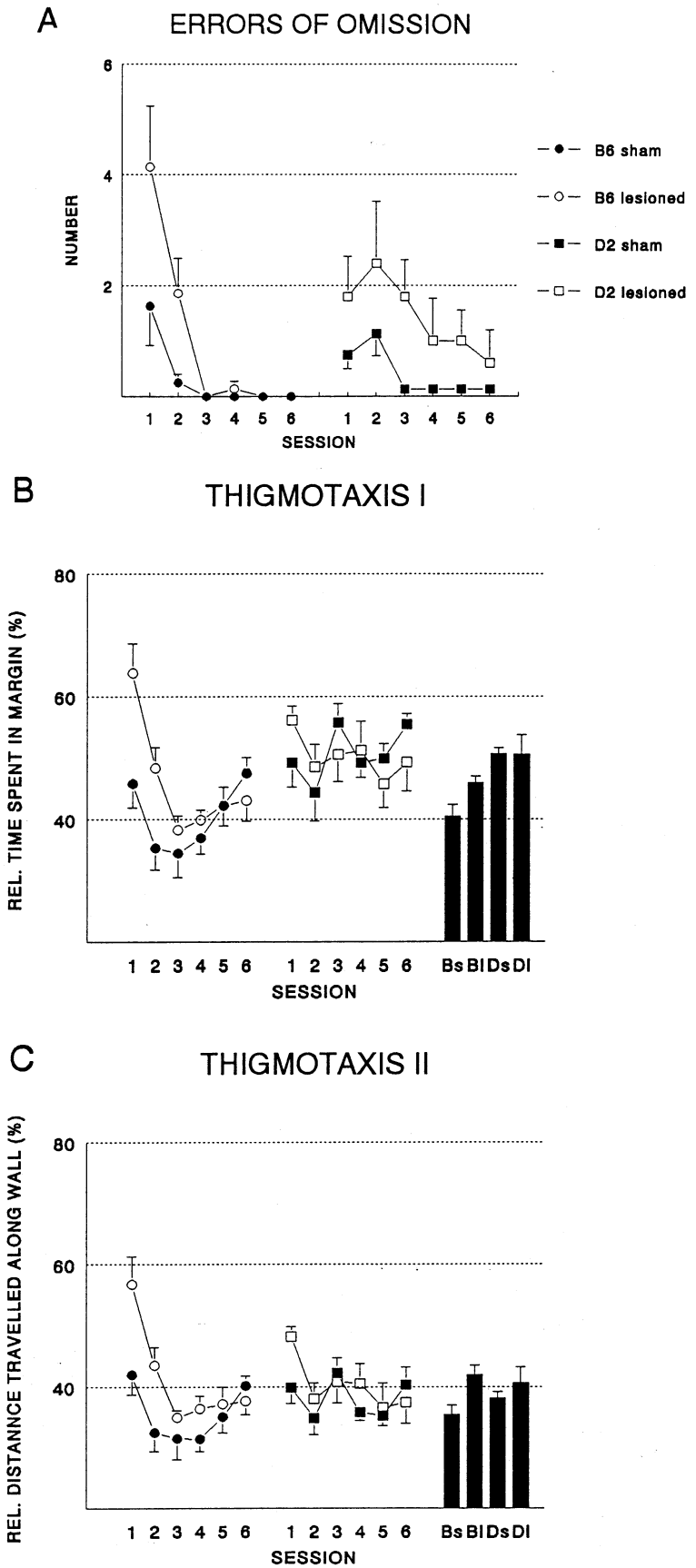

Fig. 5. Number of errors of omission (A), the relative time spent (B) and the relative distance travelled (C) in the margin of the pool during water maze spatial discrimination acquisition. Bars represent mean performance during acquisition. BS, B6 sham; Bl, B6 lesioned; Ds, D2 sham; Dl, D2 lesioned. Data are expressed as mean values with error bars denoting S.E.M. values.

0.69, $P > 0.05$; lesion × strain, $F_{1,24} = 7.19$, $P = 0.013$; Fig. 4C).

3.2.1.4. Errors of omission.

(*i*) *Effects of strain*: MANOVA showed that D2 mice made more errors of omission than B6 mice during sessions 3 and 5, but did not differ otherwise (strain, $F_{1,24} = 2.91$, $P > 0.05$; strain × session, $F_{5,120} = 4.48$, $P = 0.001$; Fig. 5A).

(*ii*) *Effects of lesion*: furthermore, lesioned mice made more errors of omission than sham-lesioned animals ($F_{1,24} = 15.24$, $P = 0.001$), an effect which did not differ over sessions ($F_{5,120} = 1.83$, $P > 0.05$; Fig. 5A). Although inspection of the data suggested that the two strains may have been differentially affected by the lesion and that the strain effect was primarily due to the lesioned D2 animals (Fig. 5A), there were no significant lesion × strain or three-way interactions ($F_{1,24} = 0.79$, $P > 0.05$; $F_{35,482} = 1.16$, $P > 0.05$; note that MANOVA failed to reveal any significant lesion × strain × session interactions during any stage of the experiment).

3.2.1.5. Thigmotaxis.

(*i*) *Effects of strain*: overall, D2 mice spent relatively more time than B6 mice in the periphery of the maze (strain, $F_{1,24} = 17.41$, $P < 0.001$; strain × session, $F_{5,120} = 3.64$, $P = 0.004$; Fig. 5B). However, the second measure of thigmotactic behaviour, the relative distance travelled along the wall, failed to reveal such clear cut results (strain, $F_{1,24} = 0.41$, $P > 0.05$), and the strain × session interaction revealed significant difference during session 3 only ($F_{5,120} = 3.52$, $P = 0.005$; Fig. 5C).

(*ii*) *Effects of lesion*: lesions of the hippocampus affected the relative time spent in the margin during the first session only (lesion, $F_{1,24} = 2.43$, $P > 0.05$; lesion × strain, $F_{1,24} = 2.68$, $P > 0.05$; lesion × session, $F_{5,120} = 4.98$, $P < 0.001$). However, lesions of the hippocampus significantly increased the relative distance travelled along the side walls of the maze during acquisition (lesion, $F_{1,24} = 6.49$, $P = 0.018$; lesion × session, $F_{5,120} = 4.60$, $P = 0.001$; Fig. 5C), while there was no lesion × strain interaction ($F_{1,24} = 1.06$, $P > 0.05$). It should be noted, however, that both measures of thigmotactic behaviour showed high variability over sessions, especially in D2 mice.

3.2.2. Platform relocation

3.2.2.1. Accuracy. Throughout this stage of the experiment, there were no significant effects of lesion, lesion × session, lesion × strain, or lesion × strain × session interactions (all $P > 0.05$). Thus, only the effects of strain will be considered further. Changing the position of the correct platform to the opposite site of the maze led to a decrease in percentage correct choices which was more pronounced in B6 animals ($F_{1,24} =$

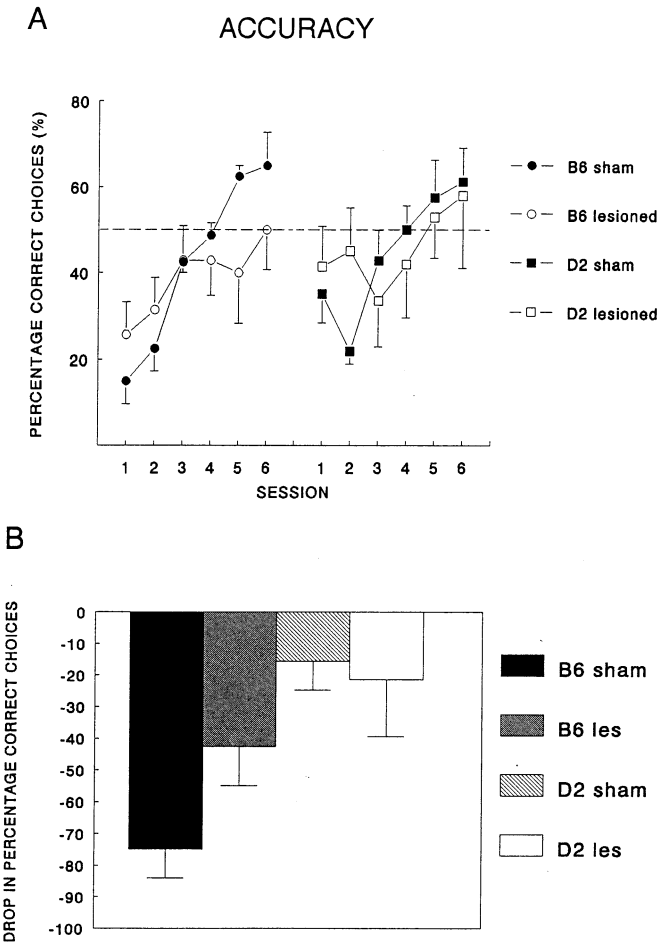

Fig. 6. Accuracy (percentage correct choices) during water maze spatial discrimination re-learning (A) and change in accuracy (percentage correct choices) during the transition from the last acquisition session to the first session where the platform was relocated (B). The dotted lines in (A) represent chance performance. Data are expressed as mean values with error bars denoting S.E.M. values. les, lesioned.

12.30, $P = 0.002$; Fig. 6B). However, over sessions there was no significant effect of strain (strain, $F_{1,24} = 0.74$, $P > 0.05$; strain × session, $F_{5,120} = 1.26$, $P > 0.05$; Fig. 6A).

3.2.2.2. Choice latency and distance travelled. However, D2 mice continued to show a higher choice latency than B6 animals (strain, $F_{1,24} = 10.51$, $P = 0.001$; strain × session, $F_{5,120} = 1.87$, $P > 0.05$; Fig. 7A) and travelled a longer distance until a choice was made (strain, $F_{1,24} = 10.51$, $P = 0.003$; strain × session, $F_{5,120} = 3.33$, $P = 0.008$; Fig. 7B).

3.2.2.3. Errors of omission. D2 animals also continued to make more errors of omission than B6 mice (strain, $F_{1,24} = 5.96$, $P = 0.022$; strain × session, $F_{5,120} = 2.98$, $P = 0.014$; Fig. 7E).

3.2.2.4. Thigmotaxis. Furthermore, D2 mice still spent more time than B6 mice in the outer margin of the

maze (strain, $F_{1,24} = 15.54$, $P = 0.001$; strain × session, $F_{5,120} = 1.87$, $P > 0.05$; Fig. 7C), but did not differ in the relative distance travelled along the side walls (strain, $F_{1,24} = 3.42$, $P > 0.05$; strain × session, $F_{5,120} = 0.29$, $P > 0.05$; Fig. 7D).

3.3. Experiment 2: plus maze spatial discrimination

3.3.1. Accuracy

(*i*) *Effects of strain*: the two strains did not differ in the percentage correct measure (strain: $F_{1,24} = 0.05$,

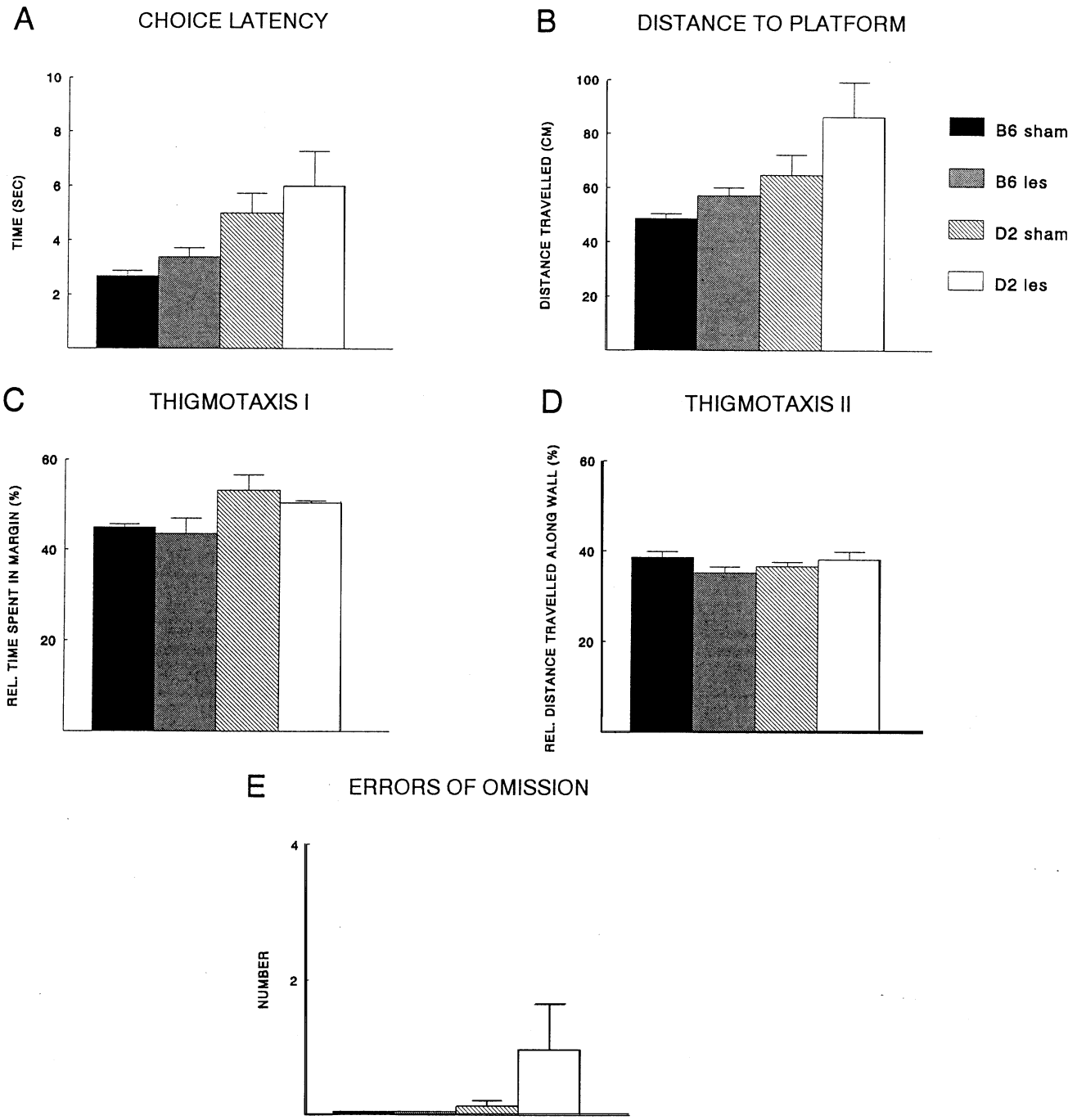

Fig. 7. Choice latency (A), distance travelled (B), number of errors of omission (E) and thigmotaxis (C, D) during water maze spatial discrimination re-learning. Data are expressed as mean values over all seasons with error bars denoting S.E.M. values. les, lesioned.

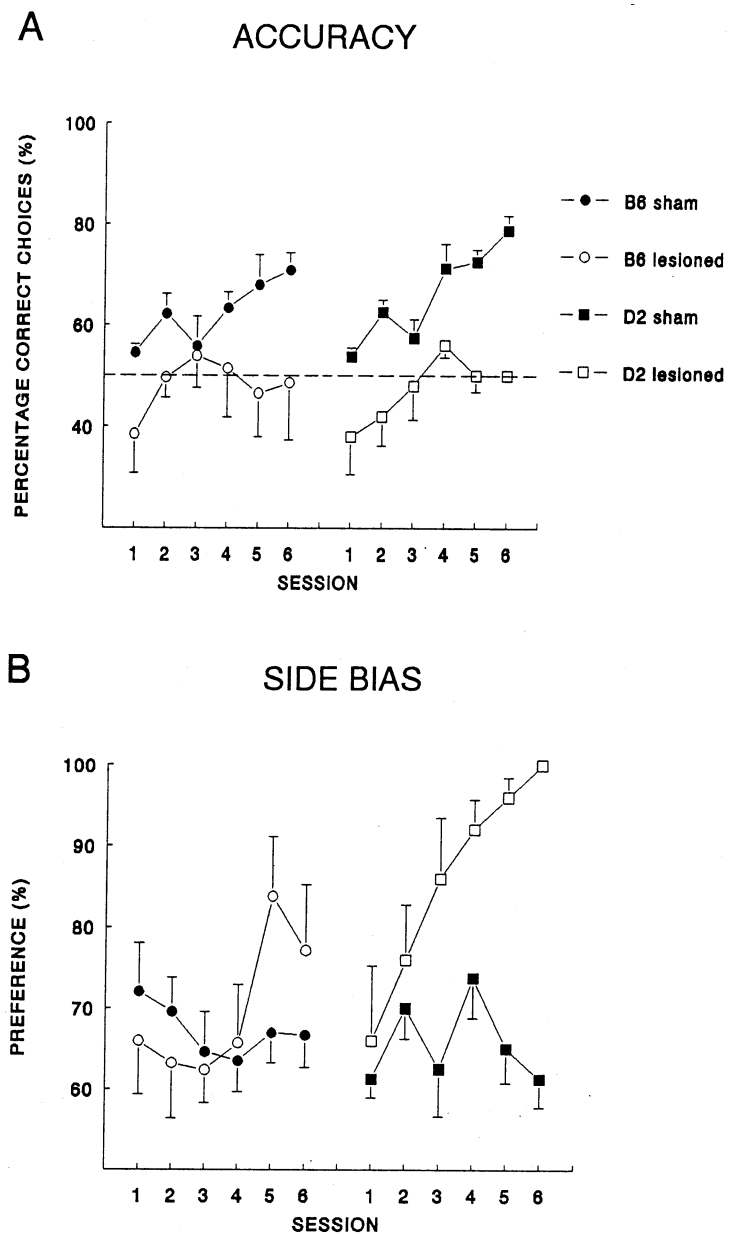

Fig. 8. Accuracy (percentage correct choices, A) and side bias (B) during plus maze spatial discrimination acquisition. The dotted lines in (A) represent chance performance. In (B), unbiased performance is represented by the x-axis (50%). Data are expressed as mean values with error bars denoting S.E.M. values. les, lesioned.

$P > 0.05$; strain × session: $F_{10,15} = 0.18$, $P > 0.05$), indicating that both B6 and D2 strains learned the task to a similar degree (Fig. 8A).

(*ii*) *Effects of lesion*: There was a main effect of lesion in percentage correct choices ($F_{1,24} = 19.00$, $P < 0.001$), but no lesion × session, lesion × strain, or lesion × strain × session interactions were observed (all $P > 0.05$; Fig. 8A).

3.3.2. Errors of omission

Groups did not differ in the number of omissions made. On average, groups made less than a total of two omissions per session (data not shown).

3.3.3. Side bias

(*i*) *Effects of strain*: overall, D2 mice had a stronger side bias than B6 animals ($F_{1,24} = 5.84$, $P = 0.024$).

(*ii*) *Effects of lesion*: likewise, lesions of the hippocampus increased side bias ($F_{1,24} = 18.33$, $P < 0.001$), and there was a significant lesion × strain interaction ($F_{1,24} = 7.56$, $P = 0.011$), with D2 lesioned animals being different from all the other groups, while B6 lesioned and sham-lesioned mice, and the two sham-lesioned groups did not differ from each other. This suggests that the effect of strain is primarily based on the performance of lesioned D2 animals (Fig. 8B).

3.3.4. Free feeding trial

(*i*) *Effects of strain*: food intake in D2 animals was increased relative to B6 mice ($F_{1,24} = 4.62$, $P = 0.042$).

(*ii*) *Effects of lesion*: however, the lesion did not affect food intake ($F_{1,24} = 1.27$, $P > 0.05$), and there was no lesion × strain interaction ($F_{1,24} = 0.01$, $P > 0.05$; number of pellets eaten (mean ± S.E.M.): B6 sham, 12.13 ± 1.9; D2 sham, 15.63 ± 1.24; B6 lesion, 14.0 ± 1.95; D2 lesion, 17.6 ± 1.33).

4. Discussion

4.1. Behavioural considerations

This study investigated the effects of ibotenic acid-induced hippocampal lesions in two strains of mice on spatial discrimination learning in a circular water maze and in a plus maze paradigm. Both tasks are spatial reference memory paradigms, and in both tasks animals had to choose between two alternatives. However, it appears to be pertinent for the following discussion to also consider the differences between these two paradigms and their relation to other frequently employed maze tasks, in particular water maze place navigation.

The water maze spatial discrimination task employed in the present study differs from the more frequently used place navigation paradigm in several aspects: first, we used visible instead of hidden platforms. Therefore, animals are not required to first learn that there is a platform at all. Second, two platforms were used, but only one provided support. This allowed a dissociation between errors of omission (no choice within the limited hold of 30 s) and errors of commission (choice of the incorrect platform). This distinction gains relevance from the fact that maze performance may be impaired for several reasons: animals may be unable to learn to navigate in the maze but, likewise, it is possible that other types of behaviour, such as impaired motor function or altered motivation, impair performance. Introduction of a choice reduces (but does not omit!) the influence of these confounding factors because a probability serves as the critical variable. However, an even more clear-cut dissociation of these different types of behaviour could be achieved by using paradigms which allow a dissociation between accuracy and bias, such as tasks which can be analysed according to the methods of signal detection theory [25,26].

Against these advantages of the two-choice task, it could be argued that place navigation is the more pure spatial mapping task, as it tests the ability of an animal to find the hidden platform solely in relation to other objects, while the two-choice task also involves a discrimination between two potential targets. Therefore, an accuracy deficit in this task could also reflect a difficulty of discrimination learning, rather than a spatial mapping deficit per se. However, it should be noted that rats with hippocampal damage can readily discriminate between two platforms differing in visual appearance, while spatial discrimination is impaired in the water maze two-choice task [28].

Moreover, the fact that the correct platform was left in a constant position, while the position of the incorrect platform was moved from trial to trial, opens the possibility that the animals were able to identify the correct platform by guidance towards one familiar cue or a set of cues. Clearly, this cue is not provided by the platform itself as performance dropped below chance level (less than 20% correct in B6 sham-lesioned mice, with 50% correct responses representing chance behaviour) when the position of the correct platform was relocated. This indicates that the animals did not merely discriminate the two platforms on the basis of their appearance or other signals unknown to the experimenter. However, at present we cannot rule out the possibility that the mice navigated by using only a restricted set of cues in terms of landmarks. Both location-specific (place) cells and landmark-related cells can be found in the hippocampus [20]. Nevertheless, even then the correct platform would be located in a stable spatial reference frame relative to the position of these landmarks. Moreover, animals were started from several different positions and, consequently, had to approach the two platforms from several different locations, using a theoretically unlimited number of different paths. This in turn may favour a place strategy over the use of landmarks, comparable to what is seen in the hidden platform (place navigation) task.

In the plus maze, animals had to make a left-right decision once they reached a choice point. This decision was supposed to be based on spatial cues surrounding the maze. However, the plus maze with its highly structured environment (two goal arms originating from the central choice area) makes it even more likely that animals use a restricted set of stimuli for discrimination in this task (i.e. those cues located directly behind the maze arms, viewed from the centre of the maze). Thus, besides the fact that the two tasks differ in the nature of the reinforcer and response-requirements, water maze and plus maze spatial discrimination seem to differ in complexity and hence in spatial demands. This suggestion receives further support by the finding that the two strains differed in learning the water maze discrimination, but performed at comparable level in the plus maze.

4.2. Strain effects

The finding that B6 but not D2 mice learned the spatial discrimination in the water maze is consistent with the idea that spatial abilities in B6 mice are better

than those of mice from the D2 strain [2,4,5,12–15,24,31–35,39–41,43]. Moreover, D2 mice swum slower than B6 animals and spent more time in the margin of the water maze, but did not differ in the relative distance travelled along the side wall. However, given that speed also differed between the two strains, it may be suggested that the time measure may have been confounded by speed, i.e. that the faster swimming animals spent relatively less time in the periphery. This interpretation receives support from the fact that lesions of the hippocampus (which had no overall effects on speed) also failed to affect the relative time spent in the margin with the exception of the first acquisition session, but showed altered thigmotaxis when the distance travelled in the margin was considered. Thus, the distance measure may be the more reliable index for thigmotactic behaviour. This in turn suggests that, in the present study, strain may not be a major factor contributing to thigmotaxis.

Interestingly, strains did not differ in learning a spatial discrimination in the plus maze task. However, as already discussed, the two tasks may differ in spatial demands. Further support for the possibility that spatial performance of the two strains depends on task complexity derives from a series of studies showing that mice of the B6 strain perform superior to D2 mice if multiple choices between six or more arms are required [1,2,4,5,33,34], but not if the number of choices is limited to three [3]— at the end, the plus maze is a radial arm maze offering just two choices. It should be noted that D2 mice perform similar to B6 animals or even better if simultaneous brightness or pattern discriminations are required [4,7–10,14,24,31]. In other words, D2 may perform similar to or even better than B6 mice on a range of tasks requiring non-spatial visual discriminations. This opens the possibility that D2 mice can also solve a maze task if the task allows for alternative strategies, such as a praxis strategy. The ability of D2 mice to solve the plus maze but not the water maze discrimination lends further support to the suggestion that water maze spatial discrimination depends on place navigation and possibly not on navigation by a restricted set of cues.

4.3. Effects of lesion

Ibotenic acid lesions of the hippocampus impaired percentage correct choices in the water maze spatial discrimination task in B6 but not in D2 mice, thus confirming earlier results in spatial acquisition, using less specific lesion techniques [2,3,16,24,33,40]. However, D2 sham-lesioned mice already performed at chance level with respect to percentage correct choices made, and therefore, a floor effect may have overshadowed a hippocampal deficit in D2 mice. Indeed, other parameters indicate that lesions of the hippocampus impair performance in both B6 and D2 mice during acquisition of the water maze spatial discrimination task: lesioned mice from both strains were thigmotaxic when the relative distance travelled along the side wall was considered, the distance travelled until a choice was made was longer in both groups with hippocampal lesions compared to sham-lesioned animals, and both lesioned groups also made more errors of omission. Thus, hippocampal lesions clearly impaired performance in the D2 strain. Consequently, the lack of the lesion to alter accuracy in the D2 strain could be due to the fact that the D2 sham animals already performed near chance level, or that hippocampal lesions have no effect on spatial learning in D2 mice. However, the latter possibility is unlikely, given that lesioned D2 mice were impaired in the plus maze spatial discrimination task.

Nevertheless, despite the poor performance during water maze acquisition, lesioned animals, as well as sham-lesioned D2 mice, eventually acquired some place response in the water maze. This was evident by the decrease in performance seen after changing the location of the platform. Interestingly, in rats, ibotenic acid lesions have been reported to impair postoperative acquisition of place navigation, while these lesions do not prevent eventual learning to control level [29]. Thus, hippocampal lesions do not prevent that some spatial learning takes place in either species. It is unlikely that this phenomenon simply reflects recovery, as the deficit in hippocampus-lesioned mice re-emerged when animals were tested in the plus maze spatial discrimination task. Whishaw [45] proposed that rats with hippocampal damage show an impairment in path integration, i.e. in a process of active monitoring of movements in space, rather than in place learning per se. If this would hold true for mice, it is possible that D2 mice as well as lesioned animals acquired a place response over time, but using different strategies compared to B6 sham-lesioned mice. However, the path integration hypothesis would also predict that hippocampal lesions would impair performance after platform relocation. Yet, it should be noted that, within the constraints of the six sessions scheduled during this experimental stage, B6 sham-lesioned animals recovered to just above chance level (65% correct choices), and it can be speculated that group differences might have re-emerged if more sessions would have been scheduled.

Alternatively, animals may have suffered from different degrees of interference from previously learned information. This is based on the assumption that animals which learned the original platform location best (group B6 sham) may have experienced more proactive interference than mice with weak memory for the previous platform position. As a consequence, B6 sham animals should be relatively more impaired and slow down re-learning the new position. However, the

slopes of the learning curves of B6 sham-lesioned mice, generated during acquisition and reversal, are very similar, rendering this possibility less likely.

In contrast to the inability of D2 sham-lesioned mice to learn the water maze task as well as B6 mice, accurate plus maze responding of the two sham-lesioned groups was comparable. Both groups with hippocampal lesions remained at chance level in this task, thus supporting our view that hippocampal lesions also impair spatial performance in the D2 strain, provided that the task is not too difficult to prevent learning in this strain. Changes in food intake are unlikely to contribute to the plus maze deficit in hippocampus-lesioned animals as food intake was not affected by the lesion during the free feeding trial. Interestingly, D2 mice with hippocampal lesions reverted to a response strategy in the plus maze, which was characterised by the development of a side bias over sessions. A strong side bias naturally would impair percentage correct choices. However, this bias developed over sessions, and therefore, it is more likely to develop in response to impaired accuracy rather than to be its cause. Taken together, this suggests that lesions of the hippocampus caused a substantial spatial deficit in both strains.

In summary, ibotenic acid-induced lesions of the hippocampus impair acquisition of two spatial discrimination tasks. These deficits were strain-dependent and likely comprise impaired accuracy as well as changes in non-mnemonic types of behaviour. Importantly, lesions in both strains impaired spatial learning, and whether a deficit was seen in mice of the D2 strain seemed to depend on the demands of the task.

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