Research paper

Acta Neurobiol Exp 2017, 77: 323–336

ACTA NEUROBIOLOGIÆ EXPERIMENTALIS

Effects of different delayed exercise regimens on cognitive performance in fimbria-fornix transected rats

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Studies have shown that exercise can positively influence cognitive performance after brain injury. This study investigated the effects of different exercise regimens on allocentric place learning after fimbria-fornix (FF) transection. One hundred and sixteen pre-shaped rats were subjected either to a mechanical transection of the FF or control sham surgery and divided into following groups: i) no exercise (NE), ii) voluntary exercise in a running wheel (RW), iii) forced swimming exercise administered as interval training of short (3x5 min) duration (FS-SI), iv) forced swimming exercise administered as interval training of long (3x15 min) duration (FS-LI), v) forced swimming exercise administered as one session of short (5 min) duration (FS-SS), and vi) forced swimming exercise administered as one session of long (15 min) duration (FS-LS). The exercise was initiated 21 days post-surgery. Subsequently, all animals were administered 28 acquisition sessions in an 8-arm radial maze. Both sham operated and lesioned animals showed a significant learning response, however, the lesion induced a marked and lasting impairment, which was not alleviated neither by voluntary nor forced (spaced or one-session only) exercise regimens. Exercise regimens had no effect on the place learning of control sham animals. We conclude that the lesion location as well as factors related to the exercise- and cognitive testing protocols can profoundly influence the potential of exercise as a general recovery-promoting method.

Key words: voluntary exercise, forced exercise, animal model, brain injury, cognitive performance, hippocampus

INTRODUCTION

Physical exercise is a potent factor in supporting cognitive health throughout life (Gomez-Pinilla and Hillman 2013, Hillman et al. 2008). Studies of healthy humans have found positive effects of exercise on cognitive measures in both children, younger adults, and elderly populations (see e.g. Guiney and Machado 2013, Hopkins et al. 2012, Khan and Hillman 2014). Furthermore, findings from human studies suggest a beneficial effect of physical exercise regarding prevention of age-related cognitive impairment and potentially even development of Alzheimer's disease (Erickson et al. 2011, Laurin et al. 2001, Lindsay et al. 2002). There is a paucity of data regarding the cognitive recovery effects of exercise in brain injury rehabilitation (but see El-Tamawy et al. 2014, Grealy et al. 1999, Mossberg 2012) and further research regarding the use of exercise in the treatment of human neurological conditions is urgently needed (McDonnell et al. 2011).

Animal studies have found effects of exercise on a long range of neurobiological measures. Exercise has been found to promote neurogenesis, synaptic efficacy and expression of molecules involved in learning and memory (Farmer et al. 2004, Nishijima et al. 2013, Vaynman et al. 2003, 2004). In particular, exercise has been shown to upregulate growth factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) (Berchtold et al. 2010, Cetinkaya et al. 2013, Ding et al. 2011, Gomez-Pinilla et al. 1997, Soya et al. 2007, Uysal et al. 2015).

Animal studies examining the effects of exercise on molecular and/or neural parameters are far more plentiful than studies examining cognitive outcome measures. In a recent systematic review, Wogensen et al. (2015) found only 22 publications examining cognitive effects of post-injury exercise with a somewhat inconsistent outcome picture. Evidence from animal research has made it clear that the effects of post-in-

Received 12 July 2016, accepted 24 October 2017

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jury exercise on cognition are not straightforward and depend on several factors, such as timing, dose, intensity, and administration schedule of a particular exercise program.

Most research studies examining the cognitive recovery effects of exercise after brain injury initiate exercise in the early post-injury phases (Wogensen et al. 2015). Studies examining the effects of delayed (post-acute) exercise on cognitive recovery are surprisingly scarce even though the delayed regimens represent the closest parallel to the clinical situation. Nevertheless, delaying post-injury exercise to 14 days post-injury or later has been associated with cognitive enhancement on measures of spatial learning and retention (e.g. Gram et al. 2016, Piao et al. 2013, Winocur et al. 2012, Wong-Goodrich et al. 2010). Delaying the start of exercise to approx. 4 month post-injury has been associated with both an improvement and with no effects depending on the cognitive task (Clark et al. 2008). In the current study, we employed a delayed exercise regimen (21 days post-injury) as we have previously found positive cognitive recovery effects in our own laboratory using this regimen (Gram et al. 2016).

Another question is whether exercise should be administered in a voluntary or forced fashion. We recently reviewed the current status of publications regarding the effects of voluntary and forced exercise on post-injury cognition. We found a quite mixed outcome picture, which appeared to depend on many variables including the timing of exercise and intensity parameters (Wogensen et al. 2015). Studies examining molecular and neural parameters have shown that voluntary exercise can increase hippocampal neurogenesis, cell proliferation and survival, as well as BDNF levels (Ehninger and Kempermann 2003, Griesbach et al. 2009, Jin et al. 2010, Luo et al. 2007, Piao et al. 2013, van Praag et al. 1999). Forced exercise can positively influence hippocampal neurogenesis and suppress apoptosis across models of brain injury (Itoh et al. 2011a, 2011b, Ji et al. 2014, Kim et al. 2010, 2014, Sim et al. 2004, Zhang et al. 2013). Forced exercise has also been shown to lead to increased levels of glucocorticoids as well as BDNF suppression (Griesbach et al. 2012, Ke et al. 2011). Moreover, brain injury itself can lead to a heightened stress response (Griesbach et al., 2012). A study by Greenwood et al. (2013) suggests that the stress component of exercise is not a critical factor in conferring the positive effects of exercise. This assumption is supported by results showing that, although inherently different, both types of exercise can lead to cognitive benefits in the healthy brain (Alomari et al. 2013).

Whether or not exercise will enhance recovery is also a question of exercise intensity and duration (Ploughman et al. 2005, 2007, Shen et al. 2013). Several studies indicate that low-to-moderate intensity (compared to high intensity) forced exercise increases hippocampal BDNF and has a positive impact on cognitive functioning after acquired brain injury (Chen et al. 2006, Shen et al. 2013, Shih et al, 2013). A recent systematic review investigating the effects of exercise on recovery after stroke in animal models (Austin et al. 2014) reports that early-initiated (24–48 h post-stroke) moderate intensity forced exercise is most effective at reducing lesion size, protecting perilesional tissue against oxidative damage and inflammation – at least short term (4 weeks). Such findings challenge the dominant view on intensity – that higher levels of intensity more efficiently promote neural reorganization and cognitive recovery in the injured brain (see e.g. Kleim and Jones 2008). Finally, the optimal administration schedule remains to be clarified: is exercise more effective if administered in shorter or longer intervals several times daily compared to once daily over a longer period of time? There are reports of cognitive recovery effects after post-injury exercise durations ranging from 1 day to 4 months (Wogensen et al. 2015). However, the evidence is sparse and very few studies include different administration schedules or examine the effects of varying exercise intensity (see e.g. Shen et al. 2013, Shih et al. 2013).

In the current study, we sought to examine the effects of short-term voluntary and forced exercise on cognitive recovery after brain injury. Furthermore, we examined the effects of different administration schedules, thus targeting both exercise frequency and duration, of (forced) swimming exercise. As the cognitive task, we employed an allocentric place learning task in an 8-arm radial maze. The injury consisted of transection of the fimbria-fornix (FF) fiber bundle, which causes neurochemical and neurophysiological alterations as well as neurodegeneration of the implicated structures (Cain et al. 2006, Gaskin and White 2007, Ginsberg and Martin 1998, 2002, Ginsberg et al. 1999, Oddie et al. 2002). The FF-transection represents a location-specific axotomy that prevents a normal functioning of the hippocampus and septum. In functional terms, the transection is associated with profound deficits in allocentric navigation (e.g. de Bruin et al. 2001, Herrera-Morales et al. 2007, Mogensen et al. 2004a, Parslow et al. 2005, Rogers and Kesner 2006) and the impairment of the current task as well as its recovery after various interventions have been documented in previous studies (Malá et al. 2005, 2007, 2008). The type of injury and task was therefore chosen in order to further elucidate the potential – and limitations – of exercise as a therapeutic agent. We examined the effects of two different types of exercise: i) effects of voluntary running exercise, administered as free access to wheel

running for 6 hours/day for 5 consecutive days, and ii) effects of forced swimming exercise, administered as either spaced interval training or as daily sessions. The forced swimming exercise was, furthermore, administered either as short (5 min) or long (15 min) spaced (interval) training (for 5 consecutive days), or as single daily sessions of 5 or 15 min duration (for 15 consecutive days). In all cases, the exercise was initiated 21 days post-injury as delayed starting points.

The literature is clearly contradictory regarding how timing, stress and intensity influence the therapeutic effects of exercise on the cognitive consequenses of TBI. We did, however, hypothesize that one or more of the currently applied exercise regimens would lead to a significantly enhanced recovery of the addressed task acquisition. In order to be able to evaluate the potential effects of exercise in normal animals as well as in the FF transected groups we included sham operated groups as well. The inclusion of the sham groups also allowed an evaluation of the level of task impairment caused by the FF lesion.

The ability of FF transections to impair allocentric place learning tasks has been documented in numerous studies (e.g. Cain et al. 2006, de Bruin et al. 2001, Gram et al. 2015, Malá et al. 2008, 2012, 2013, Mogensen et al. 2004a, 2004b). Additionally, two studies that previously studied recovery of allocentric place learning have shown that the FF transection-induced deficit can be ameliorated either by applying a delayed (21-days) restraint procedure or a delayed (21-days) exercise regimen post-injury (Gram et al. 2016, Gram et al. 2015).

It may correctly be argued that other TBI models – e.g. the Controlled Cortical Impact (CCI) (e.g. Bolkvadze and Pitkänen 2012) or Fluid Percussion Injury (FPI) (e.g. Frey et al. 2009, Wahab et al. 2015) models – have a higher ecologic validity than the FF transection. But as argued elsewhere (Mogensen and Malá 2017) the use of a restricted and well defined lesion such as the FF transection comes with a number of benefits regarding the study of for instance the neurocognitive mechanisms of potential therapeutic interventions. It may be added that fornix and hippocampal atrophies are often part of the neuropathological profile of TBI (Bigler et al. 1997, Christidi et al. 2011, Gale et al. 1993, Tate and Bigler 2000, Yallampalli et al. 2013).

As nocturnal animals, rats are most active during the night. In order to support this natural activation pattern and avoid potential stressors of being activated during their time of rest, all exercise training and cognitive testing took place during the animals' dark phase. Lights were either turned off or reduced in all experimental rooms (the latter in cases where some light was needed in order for the animals or the human experimenters to visually orientate themselves) in all phases of the experiment to promote natural movement and activity in the animals.

METHODS

Subjects and experimental groups

One hundred and sixteen experimentally naïve, adult, male *Wistar* rats with an initial body weight of approx. 300 g served as experimental subjects. The animals were housed in pairs in Makrolon type 3 cages with elevated lid – allowing rearing in the cage – under controlled temperature (22 ± 2°C) and humidity (50 ± 5%). A reversed 12-hour light/dark cycle was maintained in the animal quarters (lights on at 7: 00 PM) and all experimental procedures took place during the animals' dark phase. During the exercise period the animals were given commercial rat chow *ad libitum*. During behavioral procedures in the maze, the animals were fed once daily after training/testing and were maintained at approximately 85% of their *ad libitum* body weight. Water was always available *ad libitum*.

The animals were randomly divided into 12 experimental groups:

1. Sham surgery group subjected to no exercise (n=9, Sham/NE)
2. Sham surgery group subjected to voluntary exercise in a running wheel for 5 consecutive days (n=14, Sham/RW)
3. Sham surgery group subjected to forced swimming exercise administered as interval training of short (3x5 min) duration for 5 consecutive days (n=9, Sham/FS-SI)
4. Sham surgery group subjected to forced swimming exercise administered as interval training of long (3x15 min) duration for 5 consecutive days (n=9, Sham/FS-LI)
5. Sham surgery group subjected to forced swimming exercise administered as one session of short (5 min) duration for 15 consecutive days (n=9, Sham/FS-SS)
6. Sham surgery group subjected to forced swimming exercise administered as one session of long (15 min) duration for 15 consecutive days (n=9, Sham/FS-LS)
7. Fimbria-fornix transected group subjected to no exercise (n=9, FF/NE)
8. Fimbria-fornix transected group subjected to voluntary exercise in a running wheel for 5 consecutive days (n=12, FF/RW)
9. Fimbria-fornix transected group subjected to forced swimming exercise administered as interval training of short (3x5 min) duration for 5 consecutive days (n=9, FF/FS-SI)
10. Fimbria-fornix transected group subjected to forced swimming exercise administered as interval training of

long (3x15 min) duration for 5 consecutive days (n=10, FF/FS-LI)
11. Fimbria fornix transected group subjected to forced swimming exercise administered as one session of short (5 min) duration for 15 consecutive days (n=9, FF/FS-SS)
12. Fimbria fornix transected group subjected to forced swimming exercise administered as one session of long (15 min) duration for 15 consecutive days (n=8, FF/FS-LS)

All experiments were performed in accordance with the guidelines of the Danish Animal Experimentation Act and the European Council Directive of 22 September 2010 (2010/63/EU). The behavioral training and testing were performed by experimenters who were blinded as to which experimental group the animals belonged.

Running wheels

The voluntary exercise took place in ENV-042 Activity wheels with Modular Holding Cage for rat (MED Associates Inc., USA). The holding cage – from which the animals could freely access the running wheel – was 17.8 cm in height, 16 cm wide and 20.3 cm in depth and equipped with bedding. The wheel was 35.6 cm in diameter and 11 cm in width with a free wheeling drag of approximately 12 g. The floor of the holding cage was bedded with wood wool. The number of wheel revolutions per every five minutes was recorded by computer software (Ellegaard Systems, A/S, Denmark). The mean number of revolutions was calculated for each period of six hours/day.

Forced swimming

The forced swimming exercise took place in a rectangular water tank measuring 103 cm × 114 cm × 60 cm (l × w × d) made of hard white plastic. Inside the water tank, 4 mm thick acrylic walls were mounted, dividing the water tank into five equal swim lanes measuring 82 cm × 19.5 cm × 40.5 cm (l × w × d). The water tank was filled with 18.5 ± 1°C warm water at a depth of approximately 30 cm. At the distal end outside of each lane, water pumps (Selekta, max. pumping ability: 4200 l/h) were attached. The pumps were connected to two-head galvanized ion pipes (diameter 3.5 cm) mounted inside the distal end of each lane allowing a smooth water current in each lane just below the water surface. In order to escape the water, the animal had to reach and mount the iron pipe. The pipes were equipped with stainless steel valves controlled by handles which allowed adjustment of the water current. The water in the lanes was changed every 1–2 days throughout the exercise period.

Eight-arm radial maze

All behavioral training and testing was performed in an open, grey, one-unit 8-arm radial maze with 3 cm high walls and 11.7 cm wide corridors. The eight arms radiated equidistantly from a circular central area with a diameter of 50.0 cm. Each arm was 60.0 cm long, and at the end of the arm a circular food well (diameter, 4.8 cm; depth, 2.3 cm) contained reinforcements in the form of 45 mg food pellets (Precision Food Pellets, Campden Instruments, Campden, U.K.). The maze was placed approximately 100 cm above the floor in the centre of room with dimmed light. The mean light intensity measured at the centre of the apparatus was 33.93 lx and 27.71 lx (resolution 0.1) at the end part of the arms. The light intensity was measured by a light meter (Extech Instruments, EA31). The room was equipped with a multitude of two- and three-dimensional distal cues, and no other animals were present during training and testing.

PROCEDURES

Voluntary exercise in running wheels

Rats in the exercise groups were placed in the running wheel apparatus during their dark period, as this is their most active period. The lights were off in the experimental room during running exercise. There was only one animal in each running wheel in order to ensure unlimited access to the wheel during each exercise session. While in the apparatus they had *ad libitum* access to water. Preoperatively – and before introduction to the 8-arm radial maze – the animals were habituated to the running wheels for two hours/day for three consecutive days. Postoperatively, experimental groups with access to the running wheels were exercising six hours/day for five consecutive days.

Forced swimming exercise

Prior to the cognitive training and surgery, the animals randomly selected for the forced swimming exercise were habituated 10 min/day for three consecutive days to swimming in the lanes against a mild current (pump setting at approx. 2000 l/h).

The swimming procedure started 21 days post-surgery. Rats in the swimming groups were placed in the apparatus during their dark period, as this is their most active period. Only one lighting source was turned on in the experimental room during the swimming exercise to ensure that the human experimenters could orientate themselves during the training. Each animal was placed

in a lane in the opposite end of the water current outflow and swam against the current until reaching and mounting the iron pipe surface. The animals were then lifted back to the start end of the lane and the procedure was repeated until they had swum the predetermined amount of time. All groups swam against a strong current at a pump setting of approx. 4000 l/h. Animals subjected to forced swimming exercise administered as interval training of short duration (FS-SI) were given five min of swimming three times/daily for five consecutive days, with a break of 30-60 min between swimming sessions. Animals subjected to forced swimming exercise administered as interval training of long duration (FS-LI) were given 15 min of swimming three times/daily for five consecutive days, with a break of 90-120 min between swimming sessions. Animals subjected to forced swimming exercise administered as one session of short duration (FS-SS) were given five min of swimming once a day for 15 consecutive days. Animals subjected to forced swimming exercise administered as one session of long duration were given 15 min of swimming once a day for 15 consecutive days. After each swim trial the animals were gently dried with a towel and placed under a low-noise dryer at a distance of approx. 30 cm until dry.

Allocentric place learning task

Preoperatively, all animals were habituated to the maze and shaped. The habituation lasted for two sessions of 16 min duration allowing the animal undisturbed exploration of the maze. During the first habituation session, 45 mg reinforcement pellets were scattered all over the maze. On the second habituation session, the reinforcement pellets were present only in and around the food wells located in the distal ends of the arms. On the third session, the shaping procedure was initiated. During shaping, 15 trials (runs) were given per (daily) session, and the start arm of each trial was randomly selected. The reinforcement pellets were present in the food wells of all arms but the start arm, and the animal was released from the distal end of the start arm. After reaching the end of any of the response arms, the animal was allowed to eat the four reinforcement pellets. Subsequently, the animal was picked up and the next trial initiated. The shaping procedures continued until all animals promptly (within less than 10 sec) entered one of the response arms when released. Thereafter, surgery was performed. The preoperative performance of the animals on each shaping session was carefully monitored and recorded. This performance was – together with the animals' body weight – used during the randomization process of the animals into the experimental groups in order to minimize bias in the experimental group allocation.

Animals that had gone through the interval swimming, the running wheel exercise, and the sedentary animals resumed the behavioral procedures in the 8-arm radial maze on postoperative day 26. Animals that had gone through the non-interval swimming resumed the behavioral procedures in the maze on postoperative day 37. On the first three postoperative sessions, the animals were reshaped. On the fourth post-operative session, training and testing of the place learning task was initiated. All animals were given 15 trials (one session) per day for 28 consecutive days. After having completed a trial, the animal was transferred to a holding cage until the next trial (a pause of approximately one min duration). One arm (defined according to its spatial lo-

Table I. Schematic representation of the experimental procedures

Experimental phase	Duration	Experimental group	Feeding
Habituation to exercise apparatus	3 days	All exercise groups	*Ad libitum*
Habituation to maze	2 days	All	Food deprivation
Shaping	19 days	All	Food deprivation
Surgery	30 min.	All	*Ad libitum*
Post-surgery break	21 days	All	*Ad libitum*
Exercise	5 days	Sham or FF: RW, FS-SI, FS-LI	*Ad libitum*
Exercise	15 days	Sham or FF: FS-SS, FS-LS	*Ad libitum*
Reshaping	3 days	Sham or FF: NE, RW, FS-SI, FS-LI	Food deprivation
Place learning task	28 days	Sham or FF: NE, RW, FS-SI, FS-LI	Food deprivation
Reshaping	3 days	Sham or FF: FS-SS, FS-LS	Food deprivation
Place learning task	28 days	Sham or FF: FS-SS, FS-LS	Food deprivation

cation within the experimental room) was defined as the goal arm. The remaining seven arms served as start arms and were used in a randomized order. To limit the animals' use of intramaze cues for the task solution, the maze was rotated daily before the start of testing. When entering the goal arm, the animal was allowed to reach the food well and consume four reinforcement pellets. If an incorrect arm was entered, the animal was picked up before reaching the food well. From each session, two parameters were recorded: the number of total errors and the number of distal errors (defined as all errors but those to the two arms adjacent to the goal arm). Additionally, the parameters 'summed total errors' and 'summed distal errors' were calculated for each animal across all sessions. See Table I for an overview of the experimental procedures.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 20.0. The histological data were analysed using Kruskal-Wallis non-parametric test. Data regarding the amount of exercise across the groups allowed access to running wheels (Sham/RW, FF/RW) were analysed using a mixed design, repeated measures analysis of variance (ANOVA) comparing the distance (measured in m) the animals ran in running wheels on day 1, 2, 3, 4 and 5. Data regarding the body weight change was calculated as group average on the first and last day of exercise administration, respectively, and analysed by one-way ANOVA to identify potential group effects. Additionally, using pairwise comparisons we compared the body weight changes between the first and last day of exercise within individual groups. Parameters reflecting the overall acquisition of the place learning task ('summed total errors' and 'summed distal errors') were analysed using two-way analysis of variance (ANOVA) with subsequent post-hoc tests regarding group comparisons. In order to identify the overall lesion effect and acquisition curve over time, a mixed design, repeated-measures analysis of variance (ANOVA) was applied for analyses of acquisition sessions divided into seven groups of four sessions (session 1–4, 5–8, 9–12, 13–16, 17–20, 21–24 and 25–28) regarding the mean values of 'number of total errors' and 'number of distal errors'. In case of violated sphericity Greenhouse-Geisser or Huynh-Feldt correction was applied. In order to identify the specific effects of the exercise procedures in the lesioned and control operated sham groups, separate repeated-measures ANOVAs (using similar session intervals) were performed for sham and fimbria-fornix transected groups, respectively. If the analysis of variance revealed significant differences, Bonferroni post-hoc test was used to examine differences between the individual groups. All significances are two-tailed. Significance level was defined as $P<0.05$.

Surgery

Surgery (which lasted approximately 30 min per animal) was performed with the aid of a surgical microscope under clean conditions. The animals were anesthetized with medetomidine ('Dexdomitor', 0.5 mg/kg) and ketamine ('Ketaminol', 75 mg/kg) and subsequently given atropine (0.3 mg/kg) – all via i.p. injections. The incision site was shaved, iodized and a topical analgesic (Lidocain) was administered on the skin.

Detailed descriptions of the surgical procedures have been published previously (e.g. Malá et al. 2005, Mogensen et al. 2004b, Mogensen et al. 2005). Bilateral transections of the fimbria-fornix were performed stereotaxically using a wire-knife. The coordinates were calculated with the aid of a stereotaxic atlas (Paxinos and Watson 1986). At a point 1.1 mm posteriorly to bregma and 1.2 mm laterally to the sagittal suture, a hole was drilled in the skull. The guiding cannula of the wire-knife was lowered to a position 3.2 mm ventrally to the dura and the knife was extended laterally to a length of 1.6 mm. After extension of the knife, the wire-knife was lowered to a position 5.0 mm ventrally to the dura and left in this position for 1 min. Subsequently, the (still extended) wire-knife was raised to a position 3.2 mm ventrally to the dura. Then the knife was drawn into the guiding cannula and the instrument was rotated 180°. The knife was then re-extended to a length of 1.6 mm medially and lowered to a position 5.0 mm ventrally to the dura (and left in this position for 1 min). The extended wire-knife was then returned to the position 3.2 mm ventrally to the dura. Finally, the knife was again drawn into the guiding cannula and withdrawn from the brain. Identical procedures were performed in both hemispheres. The control sham operation consisted of similar procedures regarding the anaesthesia and the opening of the scalp, but no brain trauma was induced.

Immediately following surgery all animals were given analgesic treatment ('Rimadyl Vet', 5 mg/kg) and sterile saline (2 ml per animal) via i.p. injections. The animals' eyes were lubricated with a protective salve (Neutral Ophtha) to prevent drying. The animals were put in clean cages and kept under heating lamps until fully out of anesthesia. Analgesic treatment was repeated post-surgically for a minimum of 48 hours, and continued if necessary. The animals were given commercial rat chow soaked in water the week following

surgery to ease feeding during recovery and optimize water intake.

Histology

After completion of the behavioral testing, all animals were deeply anesthetized and transcardially perfused with 10% sucrose in saline followed by a 4% buffered formaldehyde in saline solution. Subsequently, the brains were removed and stored at 4°C in a 10% formalin in saline solution. The brains were cut horizontally on a vibratome at 50 µm. The Cresyl violet-stained sections were examined under a microscope (Leica DMD 108, Leica Microsystems), to verify the locus of the lesion and to quantify the size of lesion. The proportion of fimbria-fornix fibers that remained intact was estimated and quantified using the microscope associated software (Leica DMD 108, Leica Microsystems). The measurements were obtained from two levels representing the dorsal and ventral edge of the fimbria-fornix fiber bundle: level 1 (dorsal: interaural 6.40; bregma -3.60) and level 2 (ventral: interaural 5.72; bregma -4.28) (Paxinos and Watson 1986). The obtained values were averaged in the sham and lesioned animals, respectively, and percentage of the intact fibers in the FF-transected animals was calculated relative to sham operated control animals. The values were subsequently analysed statistically.

RESULTS

Anatomy

The histological examination revealed that in all of the lesioned animals, the fimbria-fornix rostral to the dorsal hippocampus had been almost completely transected – only a minor portion of the fibers remained intact (ranging from 0 to 23%). The size of lesion in the six fimbria-fornix transected groups (FF/NE, FF/RW, FF/FS-SI, FF/FS-LI, FF/FS-SS, FF/FS-LS) was estimated and compared using the independent samples Kruskal-Wallis test. The analysis revealed that the lesion sizes did not differ (P=0.127) across the groups. In all animals, the fimbria and dorsal fornix were damaged at the level of the ventral hippocampal commissure (anteroposterior distance to bregma -1.30 mm to -1.40 mm). In a few animals, the damage extended ventrally into the subfornical organ, dorsally into the ventral part of corpus callosum, and laterally into the dorsomedial neostriatum. Fig. 1 schematically summarizes the extent of the lesion in each of the experimental groups and shows that it did not differ across groups.

Voluntary exercise in running wheels

A mixed design, repeated measures ANOVA of running distances on day 1, 2, 3, 4, and 5 showed that the two groups subjected to running wheel exercise (Sham/RW, FF/RW) performed significantly differently over the course of the five running days ($F_{(4, 96)}$=3.226; P<0.05) and there was a significant interaction between time × surgery ($F_{(4, 96)}$=2.419; P=0.054). Between-subjects analysis did not reveal a significant effect of surgery ($F_{(1, 24)}$=3.882; P=0.06) with the FF/RW group showing a tendency to run

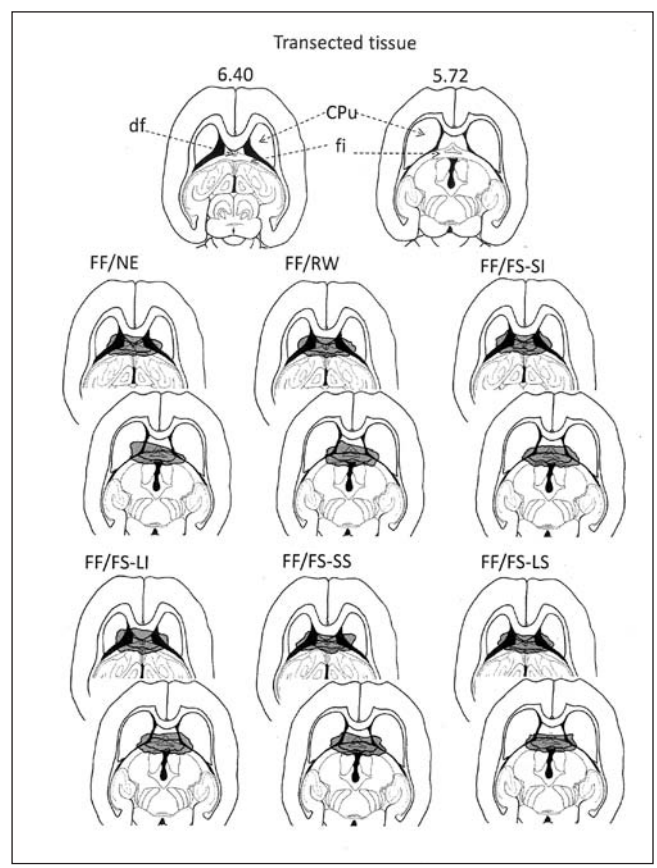

Fig. 1. Schematic diagram of the fimbria-fornix transections. The grey area indicates the lesioned area. The outer line corresponds to the area lesioned in at least one animal. The inner line corresponds to the area transected in all animals. Illustrations of the six groups that were subjected to the fimbria-fornix transection: the group not subjected to exercise (FF/NE), the group subjected to voluntary exercise in running wheels (FF/RW), the group subjected to forced swimming exercise administered as interval training of short duration (FF/FS-SI), the group subjected to forced swimming exercise administered as interval training of long duration (FF/FS-LI), the group subjected to forced swimming exercise administered as one daily session of short duration (FF/FS-SS), and the group subjected to forced swimming exercise administered as one daily session of long duration (FF/FS-LS) (for more information see Methods and Results). The diagrams show horizontal levels 6.40 and 5.72 in front of the interaural (IA) line (Paxinos and Watson, 1986). Abbreviations: df-dorsal fornix; fi-fimbria; CPu-caudate putamen.

slightly more (330.11 m/day) than the Sham/RW group (221.87 m/day) (see Fig. 2).

Body weight

There were no significant differences in body weights between the individual groups on the first day ($F_{(11, 52)}$=1.565; P=0.137) or the last day of exercise administration ($F_{(11, 52)}$=1.862; P=0.067) confirming that the animals across the experimental groups were of equal size and equally fit during the exercise period. Pairwise comparisons examining the body weight changes from the beginning of the exercise period to its end revealed significant differences only in the Sham/FS-LS and FF/NE groups, in both cases a weight increase by the end of the exercise period. The rest of the groups maintained a stable body weight (See Fig. 3).

Allocentric place learning task

Analysis of the parameter 'summed total errors' revealed a pronounced lesion effect ($F_{(1, 104)}$=461.44; P<0.001) but no effect of exercise ($F_{(5, 104)}$=0.48; P=0.791) or interaction between lesion and exercise ($F_{(5, 104)}$=1.44; P=0.215). The analysis of 'summed distal errors' yielded a similar picture with a clear lesion effect ($F_{(1, 104)}$=243.96; P<0.001), but no exercise ($F_{(5, 104)}$=0.55; P=0.736) or lesion × exercise effect ($F_{(5, 104)}$=1.4; P=0.232). In order words, FF animals showed a marked impairment on these two parameters (See Figs 4A and 4B).

The entire course of place learning was analysed by a mixed design, repeated measures ANOVA applied to acquisition sessions intervals 1-4, 5-8, 9-12, 13-16, 17-20, 21-24 and 25-28. Regarding the parameter 'number of total errors' there was a significant effect of time ($F_{(2.541, 264.217)}$=379.38; P<0.001) and time × surgery ($F_{(2.541, 264.217)}$=107.13; P<0.001), but no effect of time × exercise ($F_{(12.703, 264.217)}$=1.59; P=0.091) or time × surgery × exercise ($F_{(12.703, 264.217)}$=1.01; P=0.444). Between-subjects analysis revealed a clear lesion effect ($F_{(1, 104)}$=461.44; P<0.001), but no effect of exercise ($F_{(5, 104)}$=0.48; P=0.791) and no interaction between surgery and exercise ($F_{(5, 104)}$=1.44; P=0.215). Regarding the 'number of distal errors', there was again a significant effect of time ($F_{(2.981, 309.981)}$=370.3; P<0.001), a significant interaction time × surgery ($F_{(2.981, 309.981)}$=62.04; P<0.001), a significant interaction time × exercise ($F_{(14.903, 309.981)}$=1.77; P<0.05), but no significant interaction time × surgery × exercise ($F_{(14.903, 309.981)}$=1.07; P=0.3388). Between subject analysis confirmed the lesion effect ($F_{(1, 104)}$=243.96; P<0.001), but not effect of exercise ($F_{(5, 104)}$=0.55; P=0.736) or interaction lesion × exercise ($F_{(5, 104)}$=1.4; P=0.232) (See Figs 5A and 5B).

Attempting to identify the specific effects of exercise separately in the sham and lesioned groups respectively, the repeated measures ANOVAs using similar session intervals were performed for the sham and fimbria-fornix transected groups, respectively. In the sham groups, the animals showed a significant learning response both on the parameter 'number of total errors' ($F_{(2.534, 134.406)}$=570.2; P<0.001) and the parameter 'number of distal errors' ($F_{(2.702, 143.187)}$=428.49; P<0.001), but no interaction time × exercise ($F_{(12.670, 134.406)}$=1.25; P=0.251 for 'number of total errors'; $F_{(13.508, 143.187)}$=1.146; P=0.325 for 'number of distal errors) and no effects of exercise regimen ($F_{(5, 53)}$=0.77; P=0.573 for 'number of total errors', and $F_{(5, 53)}$=0.79; P=0.561 for 'number of distal errors). Similarly, the lesioned animals

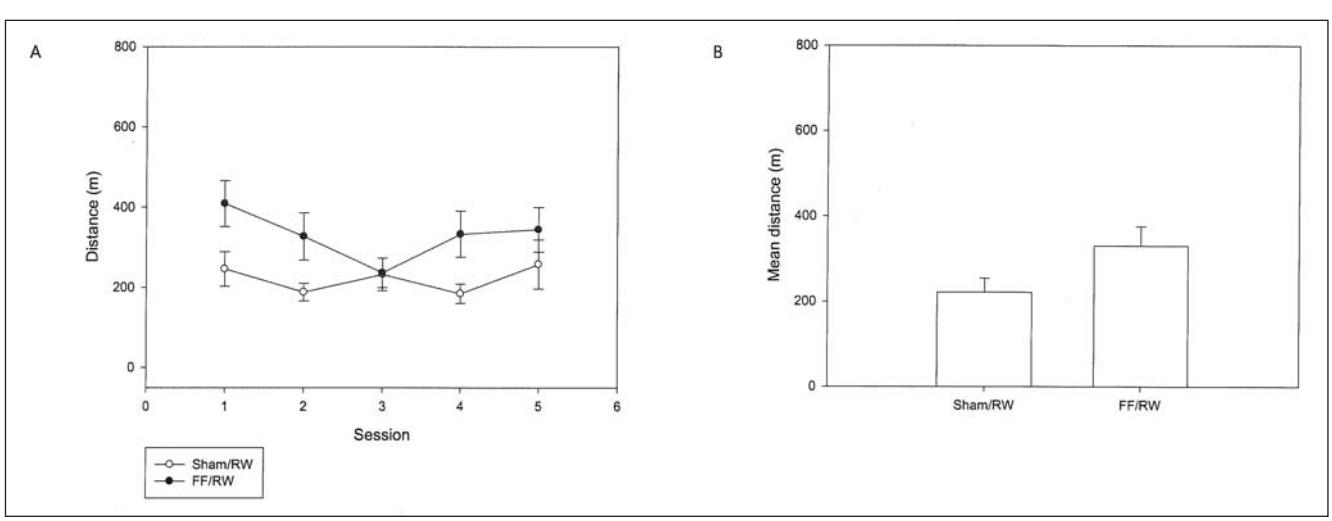

Fig. 2. A) Running distances (in meters) of groups exercised in running wheels. Open circle: Sham/RW; black circle: FF/RW. B) Total running distances (in meters) of groups exercised in running wheels. All values are given as means with SEM.

showed a significant learning response on both parameters ($F_{(1.893,\ 96.549)}$=74.114; P<0.001 'number of total errors', $F_{(2.639,\ 134.614)}$=118.58; P<0.001 for 'number of distal errors'), but no interaction time × exercise ($F_{(9.466,\ 96.549)}$=1.29; P=0.249 for 'number of total errors'; $F_{(13.197,\ 134.614)}$=1.52; P=0.118 for 'number of distal errors) and no effects of exercise regimen ($F_{(5,\ 51)}$=1.08; P=0.384 for 'number of total errors', and $F_{(5,\ 51)}$=1.03; P=0.413 for 'number of distal errors). Given these insignificant results no additional *post hoc* comparisons were performed.

DISCUSSION

The present study evaluated the effects of different exercise regimens (voluntary, forced spaced interval training, and forced one session only) on the acquisition of an allocentric place learning task after mechanical transection of the fimbria-fornix. All animals showed a significant learning response. The lesion produced a marked impairment in all injured groups, which remained relatively stable regardless of the exercise regimen to which the animals were exposed. Thus, neither the voluntary nor forced exercise regimens enhanced the posttraumatic task acquisition. It may be argued that exposure to forced exercise is a stressful event, however, as previously noted (see Greenwood et al. 2013), stress per definition does not have to hinder the positive effects of exercise and may, under certain circumstances, even promote posttraumatic cognitive recovery (Gram et al. 2015, Malá et al. 2008). Several studies report a positive impact of post-injury exercise on spatial acquisition paradigms (Cechetti et al. 2012, Griesbach et al. 2004b, Kim et al. 2010, Piao et al. 2013, Shen et al. 2013, Shih et al. 2013, Sim et al. 2004). There are also studies reporting detrimental or no effects of exercise on spatial acquisition measures (Clark et al. 2008, Griesbach et al. 2004a, Hicks et al. 1998, Luo et al. 2007, Piao et al. 2013, Song et al. 2012). These outcome differences can be attributed to a wide array of methodological differences between studies and illustrate the complexity of using exercise as a tool to enhance post-injury cognition (Wogensen et al. 2015).

In the final phase of the place learning period, the group subjected to forced exercise in short intervals (FS-SI) began to show a marked trend towards an improved acquisition of the task compared to all other lesioned groups. Had we continued the acquisition period, it is possible that this group would have reached a level of superior performance. This suggests that interval training employing shorter bouts of exercise can potentially support cognitive recovery. Further studies are needed to resolve the issue of whether certain exercise regimens are more beneficial than others given the type of brain lesion and characteristics of the cognitive task.

In the present study, several parameters may account for the lack of cognition-enhancing exercise effects. Presently, however, we are not able to identify the critical combination of factors. At least part of the explanation of these effects lies either in the application of the injury model or in the methodology. The injury to the fimbria-fornix and thereby to the entire hippocampal formation has negatively intervened with a range of reorganizational processes that likely underlie the cognitive improvements seen in other brain injury models. The applied lesion has selectively affected a neural system that is critical for allocentric place learning and its significance has been documented in numerous studies (e.g. Cain et al. 2006, de Bruin et al. 2001, Gram et al. 2015, Malá et al. 2008, 2012, 2013, Mogensen et al. 2004a, 2004b). Interestingly, two studies that previously studied recovery of allocentric place learning have shown that the transection-induced deficit can be ameliorated either by applying a delayed (21-days) restraint procedure or a delayed (21-days) exercise regimen post-injury (Gram et al. 2015, 2016). The applied lesion does, however, also disrupt a brain region involved in exercise-induced plastic reorganization processes, such as enhanced expression of synaptic molecules, increase in neurotrophic factors, and increased neurogenesis (Berchtold et al. 2010, Farmer et al. 2004, Griesbach et al. 2004a, 2004b, 2009, Kleim et al. 2003, Vaynman et al. 2003, 2004, Yau et al. 2012).

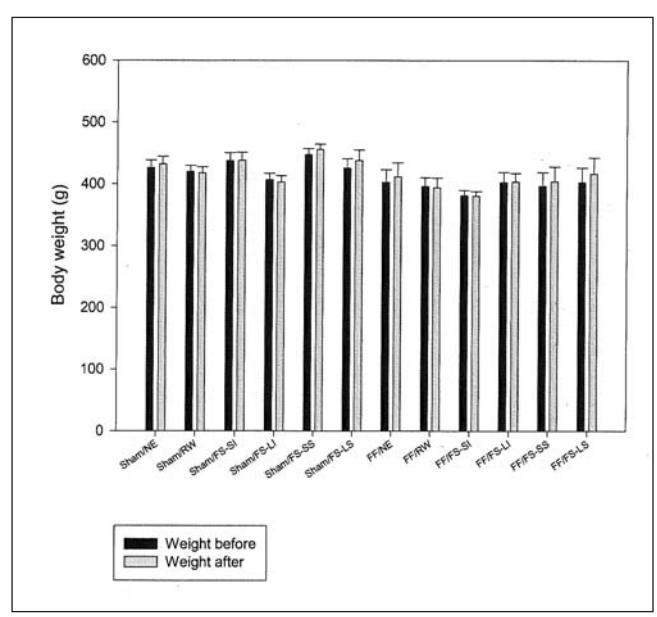

Fig. 3. Animals' body weights (in grams) on the first day (black bars) and last day (grey bars) of exercise. All values are given as means with SEM.

Examining the acquisition speed in this and our previous studies carried out in the same setup (Malá et al. 2005, 2007, 2008) reveals that the lesioned animals in the current experiment acquired the task more slowly. The speed of acquisition of the sham control groups is comparable to that seen previously. One methodological difference between this and our previous studies is a different light intensity in the experimental room. In our previous studies, the animals were handled and tested in full light during the light phase. In the present study, we changed the light-dark cycle so that the animals were handled and tested in the dark phase (during which rats are most active). Therefore, the lights in the experimental room were dimmed. The mean light intensity measured at the center of the 8-arm radial maze in this experiment was 33.93 lx and 27.71 lx (resolution 0.1) at the end of the arms. The mean light intensity in our previous studies with full light was 402.5 lx in the center of the maze and 329.63 lx (resolution 1.0) at the end of the arms (Malá et al. 2005, 2007, 2008). In general, there is a paucity of studies that evaluate the effects of different lighting on the spatial learning and memory of rodents. One laboratory (Hodges et al. 1991a, 1991b) showed that dim light led to an increased number of errors in a working memory and reference memory task in a radial maze in intact animals and in animals with lesions of the nucleus basalis and medial septum, but not in animals with lesion of the hippocampus. The fact that we have seen a slower task acquisition in the FF transected groups in the current study partly corroborates these findings and may be linked to the septal neurodegeneration after FF transections (Ginsberg and Martin 1998). It has been suggested by Pirchl et al. (2010) that the lighting in the experimental room affects performance even in healthy animals. They found that spatial memory performance in an 8-arm radial maze was impaired in female rats when the task was performed in dimmed blue and red light – however, this was not the case in male rats. In another study (Markus et al. 1994), in vivo cell recordings in male rats showed that firing rates of CA1 place field cells in a forced choice task in an 8-arm radial maze differed depending on whether the animals performed the task in light or darkness. In the dark, the animals made more errors, which was linked to a graded loss of place cell specificity and reliability. The question of whether light intensity (as well as light color and animal gender) has an influence on spatial tasks is currently unresolved. However, it would appear that the saliency of the visual cues directly affect the animals' task solution abilities. The neural system crucially implicated in saliency appears to be the basal forebrain cholinergic system (Kilgard 2003). Engaging this system is critical for efficient place learning (Chang and Gold 2003, Khakpai et al. 2012). Collaboration between the medial septum and hippocampal sub-regions via the fimbria-fornix is important for acquisition of spatial information (Okada and Okaichi 2010) and impaired cholinergic function may disrupt the cortical reorganization mediating functional recovery after brain injury (Conner et al. 2005). More research in needed in order to further inform us about the behavioral consequences of variations in cue saliency and the underlying brain processes at play.

While the present study did not find significant effects of any of the included exercise regimes, the FF transections led to highly significant impairments of the acquisition of the allocentric place learning task. This is in agreement with our previous results (e.g. Malá et al. 2005, 2007, 2008) and in general with other results pointing to a significant role of the rodent FF and

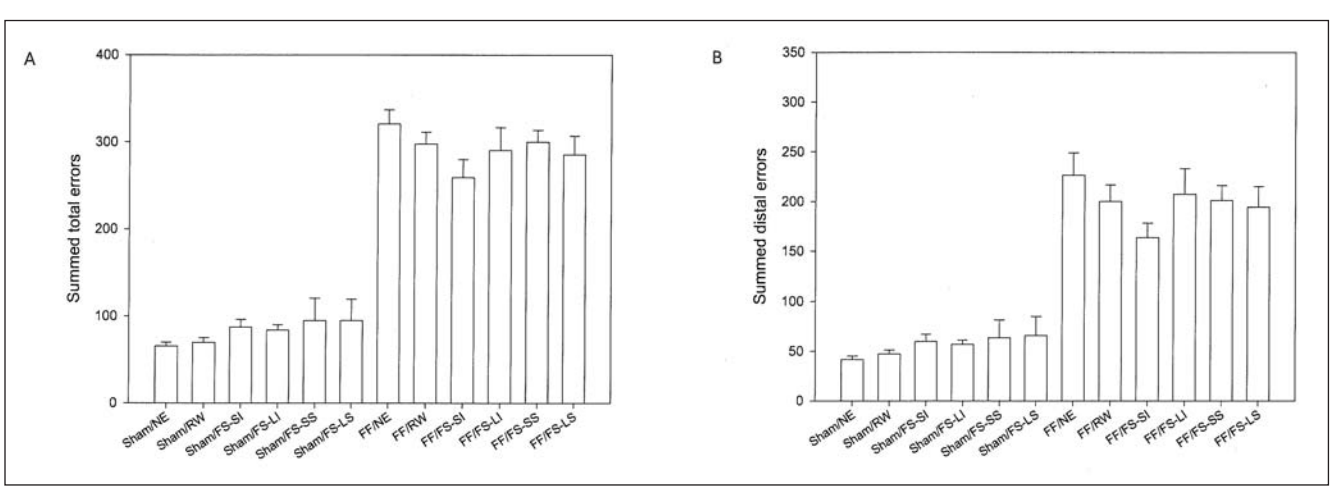

Fig. 4. A) Summed total errors across all 28 acquisition sessions. B) Summed distal errors across all 28 acquisition sessions. All values are given as means with SEM.

other hippocampal regions in the mediation of tasks including an either allocentric or egocentric spatial component (e.g. Mogensen et al. 2004a, Mogensen et al. 2005, Aggleton et al. 1986, O'Keefe and Nadel, 1978). In humans and non-human primates the available results paint a somewhat mixed picture – but also indicating that hippocampal regions (including FF) participate in mediation of spatial orientation.

Relatively few studies have addressed the consequences of selective lesions of FF in human patients. When such lesions do occur the primary consequences seem to be anterograde and retrograde amnesia mainly affecting episodic/autobiographical memory (Poreh et al. 2006, Gilboa et al. 2006). While such results do not explicitly point to a role of the human FF in mediation of spatial orientation it can be argued that spatial information processing is an integral and essential factor in both the acquisition and retention of episodic memory (e.g. Moscovitch et al. 2006). Additional data does, indeed, support a significant role of the human hippocampal system in mediation of spatial orientation and related memory and cognition in general (e.g. Baumann and Mattingley 2014, Kaplan et al. 2014, Boccia et al. 2014, Ekstrom et al. 2014, Hartley et al. 2014).

In non-human primates transection of FF impairs the fast acquisition of a conditional visuospatial task (Kwok and Buckley 2010a) – but not the long-term retention of such a task (Kwok and Buckley 2010b). Maybe surprisingly, such monkeys produce fewer perseverative errors during the early stages of learning in at least some visuospatial tasks (Kwok and Buckley 2009) – a result that is, however, interpreted by the authors as a reflection of a lower likelihood to adopt spatial strategies (and consequently reflecting impaired spatial information processing). Parker and Gaffan (1997) found the performance of an object-in-place task to be impaired by lesions of FF in monkeys and concluded that the spatial component of the task may have been the critical factor leading to the impaired task performance after FF lesions (as well as after lesions of the mammillary bodies). Impairments of exploration in FF lesioned monkeys also seem mainly to reflect disturbances of spatial functions rather than for instance basic locomotion (Kwok and Buckley 2006).

CONCLUSIONS

The tested exercise regimens - regardless of whether they were administered in a forced or a voluntary manner and as a one session or a spaced exercise - were not associated with a cognitive improvement after lesion to the fimbria-fornix.

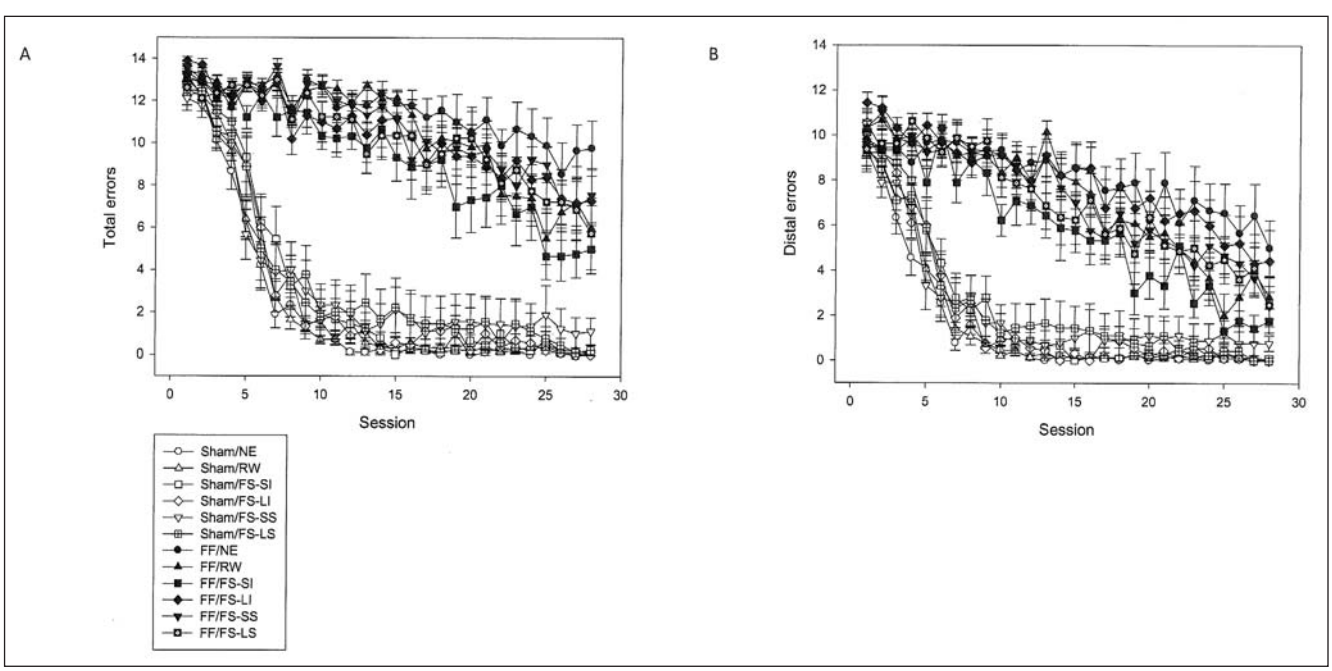

Fig. 5. A) Acquisition curve displaying the number of total errors during allocentric place learning over 28 consecutive days. B) Acquisition curve displaying the number of distal errors during allocentric place learning over 28 consecutive days. Open circle: Sham/NE; open upturned triangle: Sham/RW; open square: Sham/FS-SI; open rhombus: Sham/FS-LI; open downturned triangle: Sham/FS-SS; open crossed square: Sham/FS-LS; black circle: FF/NE; black upturned triangle: FF/RW; black square: FF/FS-SI; black rhombus: FF/FS-LI; black downturned triangle: FF/FS-SS; black crossed square: FF/FS-LS. All values are given as means with SEM.

ACKNOWLEDGEMENTS

The present study was supported by a grant from the Danish Council for Independent Research. The authors wish to thank Nicoline Balslev Bechmann, Sara Fleischer, Line-Muriel de Ginet, Brian Erik Hansen, Jan Hedegaard, Simone Horwitz, Svend Heini Winther Johnsen, Anna Lindstrøm Nielsen, Rasmus Rydbirk, Peter Schack-Nielsen, Marie Svenningsen, Katrine Sværke and Johanne Asperud Thomsen for their assistance with behavioral training and testing.

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