

available at www.sciencedirect.comwww.elsevier.com/locate/brainres**BRAIN
RESEARCH****Research Report****Therapeutic effects of a restraint procedure on posttraumatic place learning in fimbria-fornix transected rats****Hana Malá, María Rodríguez Castro, Julia Knippel, Peter Jes Køhler, Pia Lassen, Jesper Mogensen****The Unit for Cognitive Neuroscience, Department of Psychology, University of Copenhagen, Oester Farimagsgade 5A, building 10, DK-1353 Copenhagen K, Denmark***ARTICLE INFO***Article history:*

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ABSTRACT

Restraint procedures have been shown to influence the neural processes in the brain (dendritic changes or changes in the expression of neurotrophins, etc.) as well as to alter the behavioural performance. While many report deleterious effects of this procedure in normal animals, there are also indications of positive effects in the context of brain injury. In order to address the issue from the perspective of functional posttraumatic recovery, we studied 6 experimental groups of rats—3 groups undergoing a fimbria-fornix transection, and 3 groups remaining neurally intact. Within the lesioned and intact groups, respectively, one group of animals was subjected to an 8-day long restraint procedure (2 h daily) that ended immediately prior to the infliction of trauma; another group was subjected to the same procedure starting immediately after the infliction of trauma; and one group was not subjected to the restraint procedure at all. After a brief period of postoperative pause, the animals were tested on their acquisition of an 8-arm radial maze based place learning task and the effects of the restraint procedure on the task acquisition were evaluated. The results show that within the neurally intact groups, the administration of this procedure had no effect at all. However, the lesioned groups that were subjected to the restraint procedure showed significantly improved acquisition of the studied task compared to the lesioned animals that did not undergo the restraint procedure. The improved task performance suggests a therapeutic effect of this manipulation on the functional recovery after a mechanical trauma.

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1. Introduction

Every type of injury to the brain initiates a multitude of neural processes that are likely to influence the posttraumatic plastic reorganization of the brain—and thereby potentially the outcome of subsequent rehabilitation training. Posttraumatic functional outcome can be influenced by, for instance, the immediate pretraumatic and/or posttraumatic activity within endocrine (Grasso et al., 2004; Stein, 2005), neurotransmitter

(Barbay et al., 2006; M'Harzi et al., 1988) and neurotrophic (Radecki et al., 2005) systems—all of which may respond to various types of intense activity and/or experience (e.g. Harvey et al., 2006; Murakami et al., 2005; Shansky et al., 2006; Vaynman et al., 2004).

This study utilized the restraint procedure as experimental paradigm. During such a procedure the animal is placed in a wire mesh restrainer, plastic tube or box that effectively restrains its mobility. The level of immobilization varies from a complete

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immobilization to allowing a level of e.g. head or limb movement. The classical paradigm represents studies that focus on stress-related aspects and the consequences of stressful events on either organic and/or behavioural variables. Within the neuroscientific field, many diverse effects of restraint have been reported, ranging from morphological changes especially in the hippocampus, prefrontal cortex and amygdala (Brown et al., 2005; Conrad et al., 1999; Cook and Wellman, 2004; Jackson and Moghaddam, 2006; Liston et al., 2006; Luine et al., 1996; Magarinos and McEwen, 1995; Magarinos et al., 1997; Radley et al., 2006; Vyas et al., 2002; Watanabe et al., 1992); changes in protein expression and synthesis in different brain regions (Adlard et al., 2004; Dagnino-Subiabre et al., 2006; Givalois et al., 2001; Marmigere et al., 2003; Murakami et al., 2005; Nibuya et al., 1999; Rage et al., 2002; Scaccianoce et al., 2004; Smith et al., 1995); to effects of restraint procedure on cell proliferation and neurogenesis (Bain et al., 2004; Lee et al., 2006; Pham et al., 2003; Trneckova et al., 2006).

A great variety of cognitive/behavioural tasks is affected by the administration of restraint procedure. The level of the immobilization-induced changes regarding the performance appears to depend on the task and the stress intensity. Learning and memory that are sensitive to hippocampal lesions have repeatedly been demonstrated to be impaired by exposure to restraint (Abidin et al., 2004; Conrad et al., 1996, 2004; Kitraki et al., 2004; Luine et al., 1994b; Sandi et al., 2003; Wright and Conrad, 2005). The amount of restraint in the mentioned studies was 6 h/day for 21 consecutive days. Beck and Luine (1999) found that object recognition in an open field was impaired by such amount of chronic stress, but the degree of impairment could be attenuated by food deprivation. Several studies report impairments of spatial performance in water mazes (Abidin et al., 2004; Kitraki et al., 2004; Radecki et al., 2005; Sandi et al., 2003). Here, however, the amount of stress varied between 7 and 21 days. Additionally, restraint-induced functional impairments have been demonstrated on tasks normally impaired by lesions of the prefrontal cortex (Liston et al., 2006; Shansky et al., 2006).

The impairments induced by restraint procedure have been shown to be reversible (Bowman, 2005; Bowman et al., 2002; Luine et al., 1994a) and the literature also provides examples of studies reporting that behavioural performance stayed unaf-

fected by chronic stress or became even enhanced (Bowman et al., 2001, 2003; Luine, 2002; Luine et al., 1996; Shors et al., 1992). Such effects have been primarily shown in female rats and therefore, it has been suggested that they are at least partly associated with the moderating effects of gonadal hormones (estrogen in particular) (Bowman et al., 2001).

In the present study we investigated the effects of the restraint procedure in the context of a brain injury. The neural trauma consisted in a mechanical transection of the fimbria-fornix fibre bundle, which renders the hippocampus dysfunctional and deprives the rest of the brain of the majority of its hippocampal input (Ginsberg and Martin, 1998, 2002; Lahtinen et al., 1993). During the restraint procedure, we placed the animal into a tightly fitting box, which however did allow a small degree of head and neck movement, as well as limb movement. The tail was not restrained at all. In this way, the procedure represented a combination of a stressful experience and a potential motor activation (motivated by the attempts to escape the restraint box). The effects of the restraint procedure were evaluated on acquisition of an allocentric place learning task in an 8-arm radial maze, a task that has been found to be impaired by transection of fimbria-fornix (Malá et al., 2005). Although the immobilization-induced effects on learning were examined both in normal, control operated and lesioned animals, the primary purpose of the present study was to investigate these effects on posttraumatic task acquisition. Therefore, we chose – in separate groups – to administer the restraint procedure (2 h daily for 8 days) for the last week prior to and during the first week after the trauma infliction, respectively. This schedule meant that in some of the experimental groups, there was a considerable delay between the restraint exposure and the actual learning situation spanning 1 to 3 weeks (for details see Experimental procedures and Table 1).

2. Results

2.1. Anatomy

Histological examination of the fimbria-fornix transected brains established that all lesioned animals had transections of the major portion of the fimbria-fornix, although a minor portion of

Table 1

Summary of the experimental design

The procedures were identical for the sham operated control groups and the fimbria-fornix transected groups, respectively, but differed with respect to the administration of the restraint procedure.

Group	Sham/No Restraint	Sham/PRE-OP Restraint	Sham/POST-OP Restraint
	FF/No Restraint	FF/PRE-OP Restraint	FF/POST-OP Restraint
Day 1 to 8	Food deprivation	Food deprivation	Food deprivation
9 to 10	Habituation	Habituation	Habituation
11 to 30	Shaping	Shaping	Shaping
31 to 38	No handling	Restraint	No handling
38 to 40	Surgery	Surgery	Surgery
40 to 47	No handling	No handling	Restraint
48 to 57	Food deprivation	Food deprivation	Food deprivation
58 to 60	Re-shaping	Re-shaping	Re-shaping
61 to 90	Acquisition training	Acquisition training	Acquisition training

the fibres of this bundle remained intact. Fig. 1 shows that there were only minor variations between the extents of lesion in individual animals. Likewise, the lesions of the fimbria-fornix transected groups that were subjected to the restraint procedure (either preoperatively or postoperatively) were of similar extent as the lesions of the fimbria-fornix transected group not exposed to restraint procedure.

2.2. Changes in body weights

The results regarding changes in body weights are illustrated in Fig. 2. The initial analysis of the changes in body weight by MANOVA revealed no significant effects of neither lesion ($F=0.716$; $p=0.401$) nor interaction between the lesion and the exposure to restraint procedure ($F=1.739$; $p=0.193$). The only significant effects were seen on the parameter restraint procedure ($F=13.665$; $p<0.001$). The comparison of mean percentage values reflecting the change in body weights within the

Restraint and No Restraint subjects revealed significant differences ($p<0.001$).

2.3. Behaviour

The behavioural results are illustrated in Fig. 3. The overall MANOVA for all 30 sessions revealed significant effects of lesion ($F=1891.7$ and $F=840.9$ for total number of errors, and the number of distal errors, respectively, in both cases $p<0.001$), the restraint procedure ($F=43.1$ and $F=31.3$ for total number of errors, and the number of distal errors, respectively, in both cases $p<0.001$), session number ($F=87.2$; $p<0.001$ for total number of errors and $F=80.9$; $p<0.001$ for the number of distal errors), and interaction between lesion and restraint procedure on both the behavioural parameters ($F=31.3$; $p<0.001$ for total number of errors, and $F=21.8$; $p<0.001$ for the number of distal errors). t-test comparisons between the Sham/No Restraint group to the FF/No Restraint group, the

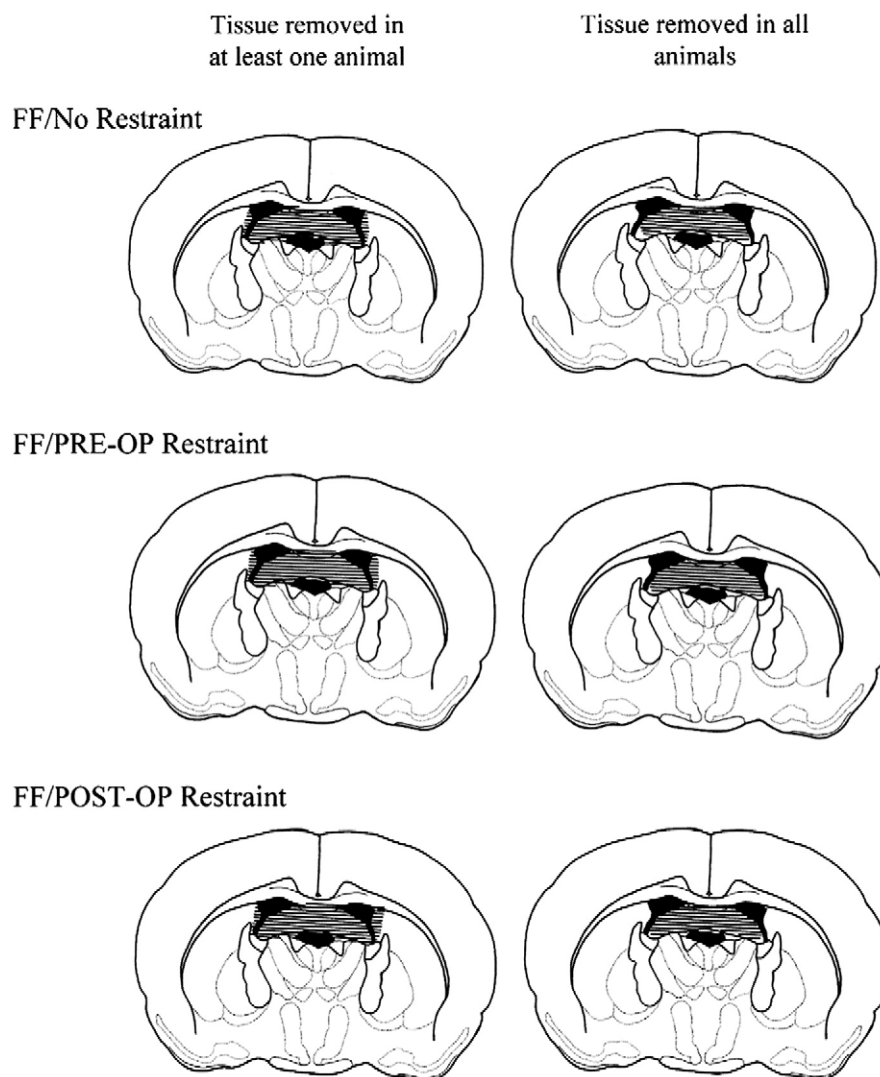


Fig. 1 – Illustrations of the lesions of the three groups that were subjected to the fimbria-fornix transection: the lesioned group not exposed to any restraint procedure (FF/No Restraint), the lesioned group restrained preoperatively (FF/PRE-OP Restraint), and the lesioned group restrained postoperatively (FF/POST-OP Restraint). The horizontal stripes indicate the lesioned area. The diagrams show level 7.70 mm in front of the interaural line (Paxinos and Watson, 1986).

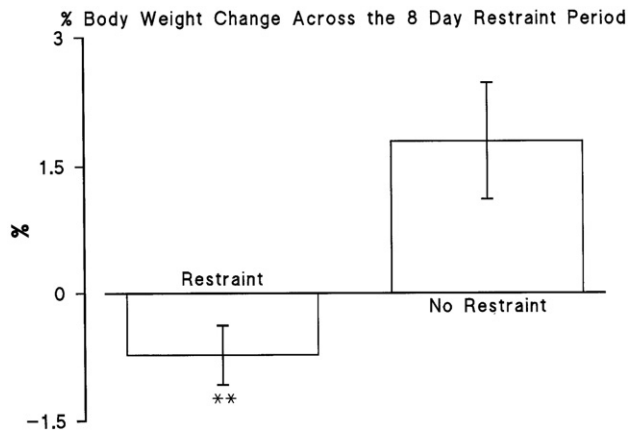


Fig. 2 – Percentage change in body weight during the administration of the restraint procedure. ‘Restraint’ includes all animals that were subjected to the restraint procedure, ‘No Restraint’ includes all animals not subjected to the restraint procedure. Values are given as means with S.E.M. **: significantly ($p < 0.01$) different from the No Restraint group. The ‘0’ level represents the body weight prior to restraint procedure. Negative values indicate weight loss, while positive values indicate weight gain.

Sham/PRE-OP Restraint group to the FF/PRE-OP Restraint group and the Sham/POST-OP Restraint group to the FF/POST-OP Restraint group all reached $p < 0.001$ level of significance with respect to both examined parameters.

Separate analysis was performed for the first ten sessions (sessions 1–10) and the last ten sessions (sessions 21–30). MANOVA during the first 10 sessions showed significant effects of lesion ($F = 617.2$; $p < 0.001$ for total number of errors and $F = 299.9$; $p < 0.001$ for the number of distal errors), restraint procedure ($F = 4.7$; $p < 0.01$ for the number of total errors and $F = 7.4$; $p < 0.001$ for the number of distal errors), and session number ($F = 54.5$; $p < 0.001$ for the total number of errors, and $F = 42.01$; $p < 0.001$ for the number of distal errors). Furthermore, there was a significant interaction between the session number and lesion ($F = 34.9$; $p < 0.001$ regarding the total number of errors, and $F = 16.9$; $p < 0.001$ regarding the number of distal errors) and a significant interaction between the lesion and the restraint procedure on the parameter number of distal errors ($F = 3.4$; $p < 0.05$). The group comparisons uncovered expected lesion effects between the individual groups of fimbria-fornix transected animals and their respective sham operated controls — the lesioned groups demonstrated a significantly ($p < 0.001$) impaired task performance reflected in higher number of both the total and the distal errors. MANOVA performed for the last ten sessions (sessions 21–30) revealed significant effects of lesion on both the behavioural parameters ($F = 272.5$; $p < 0.001$ for total number of errors and $F = 108.0$; $p < 0.001$ for the number of distal errors), restraint procedure ($F = 29.4$; $p < 0.001$ for the number of total errors and $F = 31.1$; $p < 0.001$ for the number of distal errors), and session number ($F = 2.2$; $p < 0.05$ for the total number of errors). Furthermore, there was a significant interaction between the session number and lesion ($F = 2.3$; $p < 0.05$ on the total number of errors) and a significant interaction between the lesion and

the restraint procedure ($F = 31.1$; $p < 0.001$ regarding the parameter total number of errors and $F = 32.0$; $p < 0.001$ regarding the parameter number of distal errors).

Administration of the restraint procedure for 8 consecutive days either preoperatively or postoperatively had no significant effects in the sham operated control groups (regardless of whether analyzing all 30 sessions or first and last 10 sessions, respectively). *t*-test comparison examining the effect of pre-operative restraint stress relative to No Restraint across 30 sessions reached levels of $p = 0.693$ for total number of errors, $p = 0.702$ for the number of distal errors, while the administration of the postoperative restraint led to levels of $p = 0.549$ for the total number of errors and $p = 0.529$ for the number of distal errors. Likewise, there was no significant effect when comparing the preoperative restraint to the postoperative restraint in

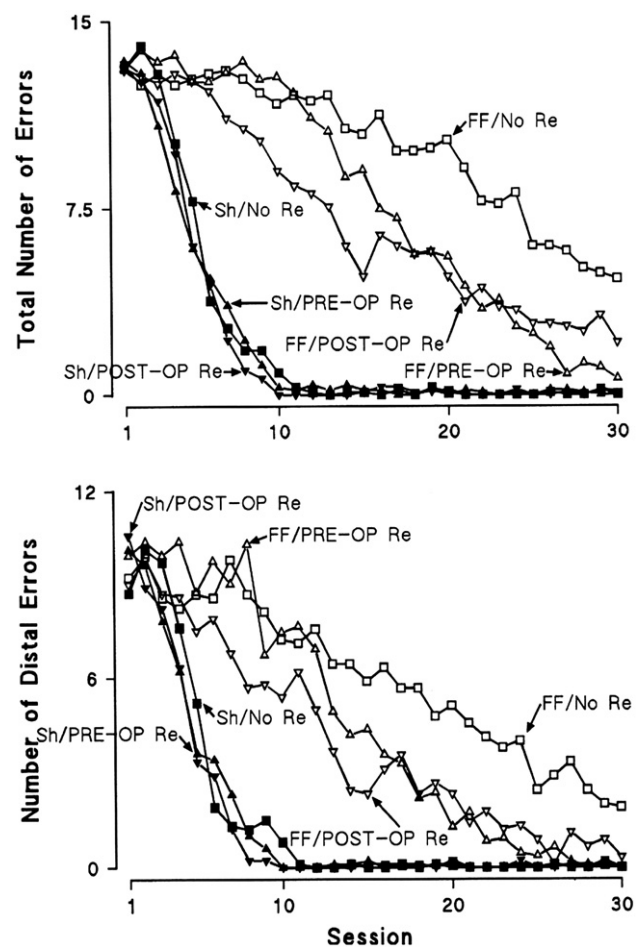


Fig. 3 – Performance of the six experimental groups during the 30 sessions of task acquisition. Black symbols: the task acquisition of the sham operated control groups; open symbols: the task acquisition of the fimbria-fornix transected groups. Black square: Sham/No Restraint; black upwards pointing triangle: Sham/PRE-OP Restraint; and the black downwards pointing triangle: Sham/POST-OP Restraint. Open square: FF/No Restraint; open upwards pointing triangle: FF/PRE-OP Restraint; and the open downwards pointing triangle: FF/POST-OP Restraint. Significant group differences are given in the Results section. Values are given as means.

the sham operated controls ($p=0.828$ for the number of total errors and $p=0.801$ for the number of distal errors).

Administration of the restraint procedure did, however, have significant effects in the fimbria-fornix lesioned groups. For both the studied behavioural parameters significant ($p<0.001$) differences were found (using *t*-tests) between on the one hand the FF/No Restraint group and on the other hand both the FF/PRE-OP Restraint group and the FF/POST-OP Restraint group. The FF/PRE-OP Restraint and the FF/POST-OP Restraint groups did not differ significantly (*t*-test comparisons between these groups showed levels of $p=0.156$ for the total number of errors and $p=0.133$ for the number of distal errors).

t-tests demonstrated that for the first 10 sessions the FF/PRE-OP Restraint group did not differ significantly from the FF/No Restraint group. The FF/POST-OP Restraint group did, however, differ significantly ($p<0.01$) from the FF/No Restraint group on both parameters. Additionally, the FF/POST-OP Restraint and FF/PRE-OP Restraint groups showed significantly ($p<0.001$) different performance on both behavioural parameters on these initial 10 sessions.

For the last 10 sessions, *t*-tests demonstrated significant ($p<0.001$) differences on both parameters between on the one hand the FF/No Restraint group and on the other hand both the FF/PRE-OP Restraint group and the FF/POST-OP Restraint group. A comparison of the FF/PRE-OP Restraint group to the FF/POST-OP Restraint group demonstrated a significant ($p<0.05$) difference on the parameter number of distal errors, while these groups did not differ significantly with respect to the total number of errors on these final 10 sessions ($p=0.071$).

3. Discussion

3.1. Body weights

Administration of the restraint procedure is associated with a reduction in body weight gains (Fig. 2). These reductions are present irrespectively of whether the individual is subjected to a brain lesion and whether the exposure to the restraint procedure takes place pretraumatically or post-traumatically. Our data are consistent with the data obtained in other studies (Bowman et al., 2006; Magarinos and McEwen, 1995; Watanabe et al., 1992) as well as with the study by Bowman et al. (2001) showing the most pronounced effects of restraint-induced reductions in weight gains during the first 7 days of chronic immobilization.

3.2. Behaviour

Fig. 3 shows the acquisition curves of the individual groups. The performance of all of the fimbria-fornix transected groups was significantly impaired compared to their respective sham operated control groups. The impaired acquisition of an allocentric spatial task by the fimbria-fornix transected groups is in accordance with results obtained in previous studies (de Bruin et al., 2001; Malá et al., 2005; Mogensén et al., 1995, 2004a) and was thus expected. This is also why we chose to apply this experimental paradigm in the current study in order to investigate the effects of the restraint procedure in the context of such a mechanical brain injury.

Exposure to the restraint procedure had no significant effects in the sham operated control groups. Since restraint procedure has previously been shown to impair spatial learning and memory (Abidin et al., 2004; Conrad et al., 1996; Kitraki et al., 2004; Sandi et al., 2003), one could ask why no impairment was observed in the sham operated controls. The most likely explanation is that there was a substantial delay between the last day of the restraint exposure and the initiation of the acquisition training (this pause lasted 21 day in the PRE-OP Restraint group and 12 days in the POST-OP Restraint group). Usually, the restraint-induced effects are evaluated immediately or shortly after the exposure to this procedure (e.g. Bowman et al., 2006). It is therefore likely that this delay led to the normalization of the behaviour in the restraint-exposed animals, which is why we do not see any significant effects in the sham control groups. Furthermore, the most frequently utilized stress paradigm is an administration of the restraint procedure for 6 h daily during 21 days — a schedule that is substantially longer than the one applied in the current study.

The most interesting results of the current study are the effects of the restraint procedure in the fimbria-fornix transected groups. There was a significant effect of the exposure to the preoperative and postoperative restraint in the lesioned animals on both the behavioural parameters (the total number of errors and the number of distal errors). In both cases, the animals subjected to the restraint procedure demonstrated a greater reduction in the total number of errors and in the number of distal errors compared to the lesioned, not restrained group (FF/No Restraint), and showed a faster post-traumatic acquisition of this allocentric place learning task. Hence, both parameters reflect actual improvement of the behavioural performance of the restraint-exposed groups. When comparing the overall behavioural performance of the lesioned, preoperatively restrained group (FF/PRE-OP Restraint) to the performance of the lesioned, postoperatively restrained group (FF/POST-OP Restraint), no significant effects were found. In other words, it appears that exposure to the preoperative restraint as well as the exposure to the post-operative restraint enhanced the posttraumatic acquisition of the presently utilized task.

Analysis performed separately for the first ten sessions (sessions 1–10) showed significant effects of lesion, restraint procedure, and session number on both parameters. The group comparisons uncovered expected lesion effects between the individual groups of fimbria-fornix transected animals and their respective sham operated controls which reflected in higher number of both the total and the distal errors. Interestingly, already during the initial phase of the task acquisition, significant effects of the restraint procedure were seen in the lesioned animals. While the performance of the preoperatively restrained fimbria-fornix transected group was not statistically different from that of the lesioned, not restrained animals, the performance of the fimbria-fornix transected and postoperatively restrained group was significantly enhanced. These animals demonstrated already during these first sessions a lower number of total as well as distal errors, and thus started acquiring the task faster compared to the lesioned group not subjected to the restraint procedure. Comparing the effects of the preoperative restraint with the effects of the postoperative

restraint in the lesioned groups revealed that the postoperatively restrained animals had significantly lower number of total as well as distal errors.

Likewise, analysis of the last ten sessions (sessions 21–30) revealed significant effects of lesion, restraint procedure, and session number on both of the behavioural parameters. Not surprisingly, the lesion effects were still pronounced even during the last ten sessions. Comparing the behavioural performance of the fimbria-fornix transected groups (across the restraint groups) with the performance of the sham operated animals revealed that the lesioned groups had significantly higher number of both total and distal errors. However, there was still a significant performance-enhancing effect of the preoperative and postoperative restraint procedure, respectively, in the lesioned animals compared to the lesioned, not restrained animals. The animals exposed to the preoperative or postoperative restraint, had significantly lower number of total as well as distal errors. A direct comparison of the effects of preoperative restraint with the effects of postoperative restraint in the fimbria-fornix operated groups did not reveal a significant effect on the parameter total number of errors ($p=0.071$), however the lesioned, postoperatively restrained group had a tendency to have a slightly higher number of errors. This tendency reached significance ($p<0.05$) in the number of distal errors.

3.2.1. *The therapeutic effects of the restraint procedure*

The present study demonstrated – in contradiction to what could have been expected based on the literature – an enhanced posttraumatic performance on an allocentric place learning task. The exposure to 2 h of a restraint procedure for 8 days either prior to or after the infliction of brain injury led to an improved acquisition of the task in the lesioned groups. Hence, the administration of the restraint had a recovery-enhancing effect.

The studies examining the behavioural effects of restraint procedure have more often than not demonstrated functional impairments in the restraint-exposed individuals (Abidin et al., 2004; Conrad et al., 1996, 2004; Kitraki et al., 2004; Luine et al., 1994a; Sandi et al., 2003). However, it needs to be remembered that the absolute majority of these studies has been performed on non-lesioned animals. The presence of brain injury dramatically changes the homeostasis of the entire system, leading to activation of various endogenous pathways (including gene expression) as well as to lesion-induced remodelling of the neural network in attempt to cope with the altered situation (Carmichael, 2006; Carmichael et al., 2005; Dancause et al., 2005; Li and Carmichael, 2006). The relationship between the processes facilitating the neural degeneration and the processes promoting neural repair and recovery is intricately intertwined. The morphological changes that take place in response to brain injury (Bramlett and Dietrich, 2002, 2004; Bramlett et al., 1997; Corbett et al., 2006; Grady et al., 2003; Smith et al., 1997) as well as in response to the restraint stress (Brown et al., 2005; Jackson and Moghaddam, 2006; Liston et al., 2006; Magarinos and McEwen, 1995; Magarinos et al., 1997; Radley et al., 2006; Watanabe et al., 1992) in combination might have provided a more optimal tuning of the neural system for the posttraumatic learning, thus mediating the functional recovery.

The recovery-enhancing changes in neural remodelling of the brain injured individuals induced by the restraint procedure might have included modifications in the gene expression of the neurotrophic factors, in particular factors of the neurotrophine family: brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF). Both up- and down-regulation of BDNF in various brain regions have been demonstrated in response to stress (Franklin and Perrot-Sinal, 2006; Givalois et al., 2001; Marmigere et al., 2003; Murakami et al., 2005; Rage et al., 2002; Scaccianoce et al., 2000; Smith et al., 1995). Interestingly, Radecki et al. (2005) showed that an infusion of BDNF can protect against the immobilization-induced impairments on a water maze based place learning task. At the same time, neurotrophines have been shown to protect against neural degeneration (Canudas et al., 2005) and against apoptotic cell-death following brain injury by inhibiting caspase 3 activation (Kim and Zhao, 2005).

Behavioural stimulation has a direct impact on the neural circuitry and its timing is crucial (Biernaskie et al., 2004; Briones et al., 2006). Early start of a rehabilitative training may lead to an improved functional outcome as well as a reduction of neurodegenerative events (Lippert-Grüner et al., 2007b; Maegele et al., 2005). The latter studies utilized an experimental early rehabilitation model combining an enriched environment, multisensory stimulation and motor training after traumatic brain injury, and demonstrated that such behavioural stimulation was associated with reduced CNS lesion volume and enhanced neuromotor functioning tested up to 30 days post-injury (Lippert-Grüner et al., 2007a). Although the treatment schedule as well as the general experimental design utilized in the current study differed drastically from the one utilized in the above mentioned research, one could imagine that the exposure to the restraint procedure shared at least some of the (potentially crucial) aspects of the early stimulation, thus enhancing the posttraumatic functioning.

It has become an established fact that exposure to stressful experience in general leads to an activation of the catecholaminergic pathways (Anisman and Zacharko, 1986, 1990; Cuadra et al., 1999), and in particular to the activation of central dopaminergic systems (Blanc et al., 1980; Cuadra et al., 1999; Deutch et al., 1985; Gresch et al., 1994; Orsini et al., 2002; Puglisi-Allegra et al., 1991; Ventura et al., 2001). Stress has been shown to have differential effects on the dopaminergic activation in the mesocortical and mesolimbic areas (Abercrombie et al., 1989) with repeatedly reported increase in the medial prefrontal cortex (Abercrombie et al., 1989; Beck and Luine, 1999; Cuadra et al., 1999; Smith et al., 2006). The increased dopaminergic efflux can potentially have beneficial implications in the context of a mechanical injury to the fimbria-fornix. We have previously found that the alternative neural substrate mediating the acquisition of an allocentric place learning task posttraumatically is highly dependent on catecholaminergic, predominantly dopaminergic, contributions—mainly within the prefrontal cortex (Mogensen et al., 2002, 2004a, 2007; Wörtwein et al., 1995). Since the administration of restraint procedure has been shown to elevate the dopamine levels in the prefrontal cortex, it is tempting to speculate that the beneficial effects of restraint seen in the current study were, at least partly, due to the enhanced availability of dopamine in this part of the brain. Such an

explanation would account for the restraint-induced effects in both preoperatively and postoperatively restrained lesion groups. Additionally, it can be mentioned that there are indications that dopaminergic agonists might be used as a pharmacological treatment to supplement rehabilitative therapy following brain injury (Barbay et al., 2006; M'Harzi et al., 1988).

The current data do not offer a further clarification of the obtained results and thus, a more detailed explanation of the restraint-induced therapeutic effects will have to await further experimentation. Only few studies can be cited in support of our results. Luine et al. (1996) showed that chronic stress can lead to a significant enhancement of performance on a spatial working memory task in an 8-arm radial maze 10–13 days post-stress. However, these results were obtained in neurally intact females. Bisagno et al. (2004) showed also in female rats that chronic stress can have a therapeutic effect on object recognition impairments induced by chronic amphetamine treatment and can counteract the anxiogenic effects of amphetamine on spontaneous exploration in an open field. Future studies will hopefully shed light onto the mechanisms mediating the beneficial effects of the currently applied procedure.

4. Experimental procedures

4.1. Subjects and experimental groups

All experimental procedures were approved by the Danish National Review Committee for the use of Animal Subjects ("Dyreforsøgstilsynet"). All procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Every attempt was made to minimize animal suffering and use as few animals as possible.

Fifty-eight experimentally naive, male Wistar albino rats weighing approximately 300 g at the beginning of the experiment served as subjects. The animals were housed two per cage under controlled conditions of temperature ($22 \pm 2^\circ\text{C}$) and humidity ($50 \pm 5\%$). The animal quarters were kept on a 12 h light cycle (on at 7.00 am). Water was always available. All experimental procedures were performed during the light phase. The animals were fed commercial rat chow once daily after training and were maintained at approximately 85% of their ad libitum body weights. The rats were randomly divided into six experimental groups:

Sham surgery group not subjected to any restraint procedure (Sham/No Restraint) ($n=10$),

Sham surgery group subjected to an 8-day long restraint procedure (2 h daily) immediately prior to the surgery (Sham/PRE-OP Restraint) ($n=10$),

Sham surgery group subjected to an 8-day long restraint procedure (2 h daily) immediately after the surgery (Sham/POST-OP Restraint) ($n=9$),

Fimbria-fornix transected group not subjected to any restraint procedure (FF/No Restraint) ($n=9$).

Fimbria-fornix transected group subjected to an 8-day long restraint procedure (2 h daily) immediately prior to the surgery (FF/PRE-OP Restraint) ($n=11$), and

Fimbria-fornix transected group subjected to an 8-day long restraint procedure (2 h daily) immediately after the surgery (FF/POST-OP Restraint) ($n=9$).

4.2. Apparatus

4.2.1. 8-arm radial maze

All training and testing was performed in an open, black, one-unit 8-arm radial maze with 2.7 cm high walls and 9.2 cm wide corridors. The 8 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 the reinforcements in the form of 45 mg food pellets (Precision Food Pellets of Campden Instruments, England). The maze was placed in the middle of a well-lit room, in which no other animals were present during training and testing, and in which a multitude of two- as well as three-dimensional distal cues were available.

4.2.2. Restraint box

Locally manufactured restraint boxes made out of hard cardboard were used for the restraint procedure. The boxes allowed complete immobilization of the animals and had the following dimensions: length 17 cm; height 5 cm; and width 5 cm. The boxes were equipped with a closing lid and appropriate openings allowing the animal to breath and move the tail freely. The lid was adjustable which allowed adjustments according to differences in body weight. Once the animal was secured in the restraint box, it was placed in a ventilated room separate from the animal quarters. Control animals were left undisturbed in their cages during the administration of the restraint procedure. In order to monitor the overall effects of the restraint procedure, all animals were weighed immediately prior to every administration of the restraint procedure. Percentage change in body weight (net change in weight across the administration of the restraint procedure $\times 100$ /body weight at the beginning of the restraint procedure) was calculated for each animal.

4.3. Behavioural procedures

Preoperatively, all animals were habituated to the maze and then shaped. The habituation lasted for two sessions. Each session allowed the rats 25 min of undisturbed exploration of the maze. During the first habituation session, 45 mg reinforcement pellets were scattered all over the maze, whereas in the second habituation session, the reinforcement pellets were present only in and around the food wells. On the third session, the shaping procedure was initiated. During shaping, 15 trials (runs) were given per session (daily), 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 end of the start arm. After reaching the end of any of the response arms, the animal was allowed freely to eat four reinforcement pellets. Subsequently, the animal was picked up and the next trial was initiated. The shaping procedures continued until all animals promptly (within less than 10 s) entered one of the response arms when released. During shaping, animals were kept food deprived to approximately 85% of their ad libitum body weight. During administration of the restraint procedures and the

posttraumatic pause, food and water were freely available. After completion of shaping, one of the lesioned groups (FF/PRE-OP Restraint) and one of the control operated groups (Sham/PRE-OP Restraint) underwent 2 h of daily restraint procedure lasting for 8 consecutive days. The restraint procedure was administered during the interval 4 to 6 pm except the eighth restraint session in the PRE-OP Restraint groups and the first session in the POST-OP Restraint groups that were timed according to surgery. The last restraint session of the PRE-OP Restraint groups took place immediately prior to surgery. After the completion of the surgical procedures, one of the lesioned groups (FF/POST-OP Restraint) and one of the control operated groups (Sham/POST-OP Restraint) were subjected to the restraint procedure (2 h daily for 8 consecutive days). The first post-surgical restraint session took place immediately after the effects of anaesthesia ceased. Immediately following the last restraint session of the POST-OP Restraint groups, both restrained and non-restrained subjects were placed on food deprivation. Initially, a 7-day period allowed the animals to reach the 85% deprivation level. Subsequently, the 8-arm radial maze-related procedures were initiated. During the first three sessions, the animals were briefly reshaped. On the fourth session, the acquisition training and testing of the place learning task began. All animals were given one daily place learning session on 30 consecutive days. At each acquisition session, 15 trials (runs) were given and 4 reinforcement pellets were present in all arms except the start arm. One arm (defined according to its spatial location within the experimental room) was defined as the goal arm for all trials throughout the task acquisition period. The remaining seven arms served as (for each trial randomly selected) start arms. To avoid the possibility of successful task solution being based on local (intra-maze) cues, we rotated the maze between sessions (in such a way that only the spatial position but not the intra-maze identity of the goal arm remained constant between sessions). When entering the goal arm, the rat was allowed to reach the food well and consume the four reinforcement pellets. If, however, an incorrect arm was entered, the animal was picked up before reaching the food well and returned to a holding cage where it remained until the next trial. After a correct trial, the animal was likewise transferred to a holding cage until the next trial. The inter-trial periods were of approximately 1 min duration. From each trial, two parameters were recorded: the total number of errors and the number of 'distal' errors—defined as all errors but those to the two arms adjacent to the goal arm.

4.4. Surgery

Surgery lasting approximately 30 min per animal was performed with the aid of a surgical microscope under clean but non-sterile conditions. The animals were anaesthetized by intraperitoneal injection of Dormitor Vet (0.12 mg/kg body weight) and Ketamine (Ketaminol Vet) (18 mg/kg body weight). Additionally, every animal was administered 1% Atropin sulphate (0.9 mg/kg body weight). Bilateral transections of the fimbria-fornix were performed stereotactically using a wire-knife. Detailed descriptions of the surgical procedures have previously been published (e.g., [Mogensen et al., 2004a,b, 2005](#)).

4.5. Histology

After completion of the behavioural testing, all animals were deeply anaesthetized and transcardially perfused with saline, followed by 10% formalin in saline solution. After perfusion, the brains were removed from the skull and allowed to sink at 4 °C in a 10% formalin in saline solution containing 20% sucrose. The brains were cut horizontally at 40 µm on a vibratome. The Nissl-stained sections were examined with the help of a microfiche reader, and the locus and size of lesion were verified.

4.6. Statistical analysis

All behavioural data were originally analyzed using a multivariate analysis of variance (MANOVA) in order to identify potential interactions among the independent variables and the association with dependent variables. The two parameters (total number of errors and the number of 'distal' errors) from all acquisition sessions were analyzed in this manner. If the analysis of variance allowed so, Student's *t*-tests for independent samples were applied to examine significant differences between individual groups. Furthermore, similar but separate analyses were performed for the first 10 acquisition sessions (sessions 1–10) and the last 10 acquisition sessions (sessions 21–30), including multivariate analysis with tests of between-subjects effects. The differences in the performance of individual groups were examined by applying Student's *t*-test for independent measures in those cases where MANOVA permitted doing so. The data regarding the body weight changes during the period of restraint were initially analyzed by using MANOVA in order to test effects and potential interactions of the lesion and restraint procedure. Since no significant effects of lesion or the interaction between lesion and the restraint procedure were found, the body weight data were pooled across experimental groups according to whether the animals underwent the restraint procedure or not. The percentage change in body weight was calculated as the net change in weight (the body weight just prior to the first administration of the restraint procedure minus the body weight on the last day of the administration of the restraint procedure $\times 100$ /body weight at the first day of the exposure to the restraint procedure). The mean percentage values were compared applying the Student's *t*-test. All significances are given two-tailed. Effects were considered statistically significant if $p < 0.05$.

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