Chapter 8

Chronic stress modulates the use of spatial and stimulus-response learning strategies in mice and man

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Published in *Neurobiology of Learning and Memory* (2008) 90: 495–503

Abstract

Acute stress modulates multiple memory systems in favor of caudate nucleus-dependent stimulus-response and at the expense of hippocampus-dependent spatial learning and memory. We examined in mice and humans whether chronic stress has similar consequences.

Male C57BL/6J mice that had been repeatedly exposed to rats ('rat stress') used in the circular hole board task significantly more often a stimulus-response strategy (33%) than control mice (0%). While velocity was increased, differences in latency to exit hole, distance moved or number of holes visited were not observed. Increased velocity and performance during retention trials one day later indicates altered emotionality and motivation to explore in rat stressed mice. Forty healthy young men and women were split into "high chronic stress" and "low chronic stress" groups based on their answers in a chronic stress questionnaire ("Trier Inventory of Chronic Stress"-TICS) and trained in a 2D task. A test trial immediately after training revealed that participants of the "high chronic stress" group used the S-R strategy significantly more often (94%) than participants of the "low chronic stress" group (52%). Verbal self-reports confirmed the strategy derived from participants' choice in the test-trial.

Learning performance was unaffected by the chronic stress level. We conclude that one consequence of chronic stress is the shift to more rigid stimulus-response learning, that is accompanied by changes in motivational factors in mice.

Introduction

Memory consists of multiple systems which differ regarding the processed kind of information, the performed operations and the underlying neural structure (Gabrieli 1998; Squire 2004a). "Cognitive" memory supports the acquisition of flexible, consciously accessible knowledge, such as the memory of your last birthday party, and is based on the medial temporal lobe, in particular the hippocampus (Scoville and Milner 1957; Eichenbaum 2004). "Habit" memory, on the other hand, processes simple stimulus-response (S-R) associations, such as "stop your car when the traffic lights are red". It is not necessarily accessible and relies on the caudate nucleus (Knowlton et al. 1996; Jog et al. 1999).

Hippocampus- and caudate-based systems work in parallel and process information simultaneously (Mizumori et al. 2004). The nature of interactions between these systems has been described as cooperative by some authors (Voermans et al. 2004) and competitive by others (Poldrack and Packard 2003) raising the question which factors coordinate their use. Recent findings suggested that stress plays a critical role in the modulation of multiple memory systems. Acute stress prior to training in a task that could be acquired by a hippocampus-based spatial and a caudate-based S-R strategy favored caudate-based learning both in rodents and humans (Kim et al. 2001; Packard and Wingard 2004; Schwabe et al. 2007). This stress-induced modulation of hippocampus-dependent and caudate-dependent systems is assumed to be mediated by the amygdala (Packard & Wingard, 2004). Effects of prolonged or repeated periods of stress on the modulation of caudate-dependent and hippocampus-dependent learning have not been studied yet. This however, would be particularly valuable since chronic stress has been related to psychiatric disorders such as depression (for a review: Willner 1997).

Chronic stress impairs hippocampus-dependent learning and memory (Bodnoff et al. 1995; Kleen et al. 2006). Non-hippocampal memory systems respond differently. Working memory was not affected after repeated restraint stress (Kleen et al. 2006), but fear memory was even strengthened following a prolonged stress period (Conrad et al. 1999). Interestingly, Wright and Conrad (Wright and Conrad 2005a) demonstrated in chronically stressed rats that salient intramaze cues prevented impaired performance in a spatial Y-maze task. We suggest that the introduction of intramaze cues allowed for S-R learning and thus, compensated for impairment of spatial functions. Consequently, we hypothesize that chronic stress modulates multiple memory systems in favor of caudate-based and at the expense of hippocampus-based learning.

To test this hypothesis, we used experimental designs that provide a single proximal and multiple distal cues for learning the task, i.e. allowing stimulus-response learning and spatial learning. Changing the position of the proximal cue in the last trial of the learning session revealed the used strategy in mice and humans. First, we examined in mice the effect of chronic stress (i.e. by repeatedly exposing the mouse to a rat, but separated by a partition) on the use of spatial and S-R learning strategies during the acquisition of a circular hole board task, followed by a retention test 24hrs later. Second, we examined in humans the influence of self reported chronic stress as assessed by the Trier Inventory of Chronic Stress (TICS) on the learning strategy used in a 2D spatial task in which the position of a win-field could be acquired by spatial and S-R strategies.

Materials and Methods

Mouse study

Animals

Male C57BL/6J mice (n = 24, 12 weeks old; purchased from Charles River, The Netherlands) were single-housed in a temperature- (21 ± 1°C) and humidity-controlled room on a 12-12h light-dark cycle (lights on at 0700h) with *ad libitum* access to food and water. Behavioral experiments were performed in the same room. Three times during the week before training started, mice were 'pretrained' to climb through an S-shaped tube into their home cage after weighing. Experiments were approved by the Local Committee for Animal Health, Ethics and Research of the University of Leiden. Animal care was conducted in accordance with the EC Council Directive of 24 November 1986 (86/009/EEC).

Experimental design

Five days prior to the beginning of the rat stress, general activity and exploratory behavior of mice were assessed on the circular hole board. Animals were randomly assigned to one of two conditions: control (n = 12) and 'rat stress' (n = 12; see below). Mice of the "rat stress" group were repeatedly exposed to a rat for 1 to 2hrs a day during 2 weeks. Seven days after the last rat exposure mice started with the circular hole board (CHB) task. Twenty-four hours after training retention performance was tested. Testing took place between 0800 and 1230h. One day later, mice were sacrificed between 0800 and 1000h. The experimenter was unaware of the previous treatment of the animals. Behavior

was recorded on videotape and analyzed by EthoVision 1.95 (Noldus Information and Technology BV, Wageningen, The Netherlands). This image analysis system sampled the position of an animal 12.5 times per second; to calculate the distance moved we chose for a minimal distance between samples of 3cm.

Rat stress paradigm

In nature, mice and rats avoid each other. Exposure to a rat is highly stressful for a mouse (Linthorst et al. 2000). In the first week, mice were exposed to male Wistar rats on 5 consecutive days (1-2h per day resulting in 9hrs in the first week). In the second week, mice were confronted with rats on Tuesday and Thursday for 1h. This time schedule was chosen to increase unpredictability and uncontrollability which are key stress components (Dickerson and Kemeny 2004). Rats were placed in a cage with a grid floor and Plexiglas walls on the top of two mouse cages which were covered by a grid. Thus, mice and rats could hear, see and smell, but not touch each other. During exposure to rats mice were kept in another cage than their home cage (but always the same cage for confrontation with rats) without food and water. The rat stress took place during the light phase (0700 to 1900h) in a room adjacent to the housing room. Previous studies using the same stress protocol showed that it induces reliable features of chronic stress expressed e.g., by reduced body weight, changes in corticosterone secretion and alteration in hippocampal corticosteroid receptor expression, strain-dependent alterations in learning and memory and motivation to explore (Grootendorst et al. 2001a; Grootendorst et al. 2001b). Mice of the control group (naïve) were housed in their home cage.

Learning task

Apparatus: The circular hole board (CHB) is a revolvable white Plexiglas plate (diameter: 110cm) with twelve holes (diameter: 5cm) at equal distance to each other, 10cm from the rim. It is situated 1m above the floor (see Figure 1A; light intensity at the level of the platform 120lux). Holes can be closed by a lid at a depth of 5cm. Whether a hole is open or not can be recognized by the mouse if it puts its head over the edge of the hole. If open, the hole provides access to the home cage of the mouse via an s-shaped 15cm long tunnel (diameter: 5cm). Since mice avoid open, illuminated areas, it is reasonable to assume that mice are motivated to leave the platform. Same as in landmark studies in the field (De Quervain et al. 1998; Winocur et al. 2005) numerous distal cues in the room allowed spatial orientation.

(**A**)

(**B**)

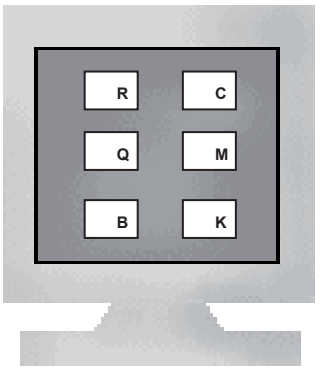

Figure 1
Apparatus used in the mouse **(A)** and human study **(B)**. Mice were trained to find an exit hole. They could use either a spatial (room cues) or a stimulus-response strategy (bottle). Relocation of the bottle in the test trial revealed the used strategy. In the human study, participants could identify the position a "win-field" with a spatial (right column, second row) or a stimulus-response (stimulus: letter M) strategy. Changing the arrangement of the letters in the test trial allowed revealed the employed strategy.

Procedure: At the beginning of each trial mice were placed in a cylinder (Plexiglas; 25cm high, 10cm in diameter) located at the centre of the CHB. After 5s the cylinder was lifted and mice could explore the board and exit through the open hole. There was just one open hole during training which was at the same location in all six training trials, next to a bottle (transparent 0.5 liter bottle filled with water; 22cm high, 5cm in diameter; placed at the rim of the board, see Figure 1A). Thus, the exit hole could be located via two strategies: mice could use cues in the room (spatial strategy) or they could use the bottle as a proximal cue (S-R strategy). If a mouse did not enter the exit hole within 120s the experimenter guided it there by a grid (20cm x 6cm). Six training trials were given (inter-trial interval: 15 min). This relatively low number of trials was chosen to avoid training to asymptotic performance which would promote the use of an S-R strategy (Packard &

McGaugh, 1996). Fifteen minutes after the last training trial a test trial (trial 7) revealed the strategy. In this test trial, the bottle was relocated next to the hole opposite to the position of the exit hole during training. Now, two exit holes were available: one next to the novel position of the bottle and one at the position of the exit hole during training. Leaving the CHB via the hole next to the bottle was classified as S-R strategy. Leaving the board through the hole in the old position was classified as spatial strategy. To avoid that behavior during the test trial could be biased by odor cues; the bedding of the home cage of one mouse was distributed over two cages each placed under one hole.

On the following day, three retention test trials were given which were exactly the same as the test trial. After each mouse, the board was wiped with 1% HAc solution to spread odor cues and turned clockwise until another hole was at the location of the exit.

Five days prior to the beginning of the rat stress, general activity and exploratory behavior of mice were assessed. All holes were closed (the bottle was at the location where it will be during training). After 5 min the hole next to the bottle was opened and the mouse was gently guided by a grid (20cm x 6cm) towards the exit hole. Mice did not show a bias for a certain location on the board during the exploration.

Thymus, Adrenals and Plasma Corticosterone

At the end of the experiment, mice were decapitated under basal resting conditions; thymus and adrenals were removed and weighed to verify the success of the stress protocol. Adrenal weights of three and thymus weights of two animals are missing. Furthermore, blood obtained via decapitation was collected individually in capillaries (coated with potassium-EDTA, Sarstedt, Germany) and stored frozen at -20°C. Plasma corticosterone concentrations were determined (in 10µl plasma) using commercially available radioimmunoassay kits with ^{125}I-corticosterone (MP Biomedicals Inc. Europe, Belgium; sensitivity 3ng/ml; intra-assay variability 7%).

Human study

Participants

Forty young healthy students (21 females, 19 males) aged between 20 and 32 years (mean: 23.9 yrs; SD = 2.7 yrs) participated in this study. Participants were recruited at the University of Trier and got paid a moderate monetary compensation. Exclusion criteria were checked in an initial interview and comprised current or chronic mental or substance use disorders, current physical disease as well as the use of medication

that affects central nervous and endocrine systems. All participants provided written informed consent.

Trier Inventory of Chronic Stress (TICS)

The Trier Inventory of Chronic Stress (TICS; Schulz and Schlotz 1999; Schulz et al. 2004) is a valid and reliable German 57-item questionnaire that was designed to measure 9 aspects of chronic stress: "work overload", "social overload", "pressure to succeed", "work discontent", "excessive work demand", "lack of social recognition", "social stresses", "social isolation" and "chronic concern". Items are descriptions of experiences such as "I have to finish too many things" and people are asked to specify on a 5-point rating scale ("never", "infrequent", "sometimes", "frequent", very frequent") how often they made the referring experience within the last 3 months. The time required to complete the TICS is 10 to 15 min.

"*High vs. low chronic stress":* To assess the effect of chronic stress, we calculated a chronic stress score by adding up the scores of the nine TICS scales. Next, we performed a median-split and assigned the participants with a chronic stress score higher than the median to the "high chronic stress" group and the participants with a chronic stress score lower than the median to the "low chronic stress" group. It is important to note that we tested healthy subjects and that the measured chronic stress scores were in a normal, non-pathological range. Our labels "low chronic stress" vs. "high chronic stress" refer to the median in the present study. They do not indicate low vs. high chronic stress in an absolute sense.

Learning task

Participants were presented six rectangles (6cm x 4cm) arranged in two columns on a customary 17" computer screen (Figure 1B). Each of the rectangles was marked by one letter: R,C,Q,M,B,K. Participants were told that one of these rectangles is a win-field and asked to click with the mouse cursor at the rectangle which they thought would be the win-field. Immediately thereafter, either a "win" or "blank" window popped up, serving as positive or negative feedback. Per trial one rectangle could be chosen. At the end of the experiment, participants received 50 Euro-Cent for each trial in which the win-field was found. The arrangement of the letters was the same in all 14 training trials. Participants were not informed that the win-field was always in the same position (marked by the letter M, right column middle). Thus, there were two possible strategies to identify the win-field: participants could learn the position of the win-field via the

association with the letter (S-R strategy) or they could use a spatial strategy, i.e. they could use the spatial location (right column, middle). Fourteen training trials were given (inter-trial interval: about 30s). Previous findings showed that the used learning strategy is a function of practice with participants using spatial learning at the beginning of a task and S-R learning after extensive practice (Iaria et al. 2003). We chose the number of training trials to assess participants' performance rather early in this process. Participants were classified as "learners" when they chose the correct field three times in a row and did not switch to another field in a subsequent trial. Trial 15 was the test trial - here, the six letters were rearranged. Choosing the field with the letter M in the novel position was classified as S-R strategy. Choosing the field in the position where the win-field had been during all training trials (second column, middle) was classified as place strategy. Trials 1 to 15 were performed within 8 to 10 min.

The experimental procedure was created with the help of the software E-prime (Psychological Software Tools, Inc.; Pittsburgh, USA). Behavioral analyses focused on reaction times and the chosen field in the test trial.

Verbal report

Subsequent to participants' choice in the test trial but before they received feedback, participants were asked (i) to indicate on a scale from 0 to 100 how certain they feel that the chosen field is the win-field (0 - "absolutely uncertain"; 100 - "absolutely certain") and (ii) to explain why they have decided for the chosen field.

Statistical analysis

Data were subjected to χ^2-test, mixed-design ANOVA or t-test, as appropriate. Reported p-values are two-tailed and $p < 0.05$ was accepted as significance. All calculations were done with the statistics software SPSS (version 14.0; SPSS Inc.).

Results

Chronic stress favors the use of stimulus-response learning strategies in mice

Learning strategy: Mice were repeatedly exposed to a rat over a period of 2 weeks, a procedure with long-lasting and profound effects on the stress responsive system and behavior of mice (Grootendorst et al. 2001a; Grootendorst et al. 2001b). One week after the last contact with a rat, mice were trained in six trials on a circular hole board (CHB)

to find an open hole providing access to the home cage. This hole was marked by a cue (a bottle) and could thus be located by caudate-dependent S-R *and* hippocampus-dependent spatial strategies (Figure 1A). Relocation of the cue to another hole in trial 7 (test trial) revealed the applied strategy. Control mice were housed in their homecage until behavioral testing started. They had been never exposed to rats. Groups differed significantly regarding the used learning strategy in the test trial ($\chi^2(1)=4.80$, $p < 0.03$; Figure 2). One third of the chronically stressed mice used an S-R strategy, while – in line with the findings of Kim and colleagues (2001) - all naïve control mice applied the spatial strategy.

Performance: Decreasing latencies and number of holes visited over trials indicated learning performance in both groups (latency: $F_{(5,110)}=8.37$, $p < 0.001$; number of holes visited: $F_{(5,110)}=4.04$, $p < 0.01$; Figure 3). The learning curve of the mice shows that no asymptote is reached which would be indicative for "extensive training". As shown in Figure 3, mice made on average 2-3 errors before selecting the correct hole in the last training trials. Nevertheless, search was not at all random as suggested by the fact that then proportion of time in which mice were in the correct quadrant of the CHB increased significantly over trials ($F_{(5,110)}=2.32$, $p < 0.05$). There were no group

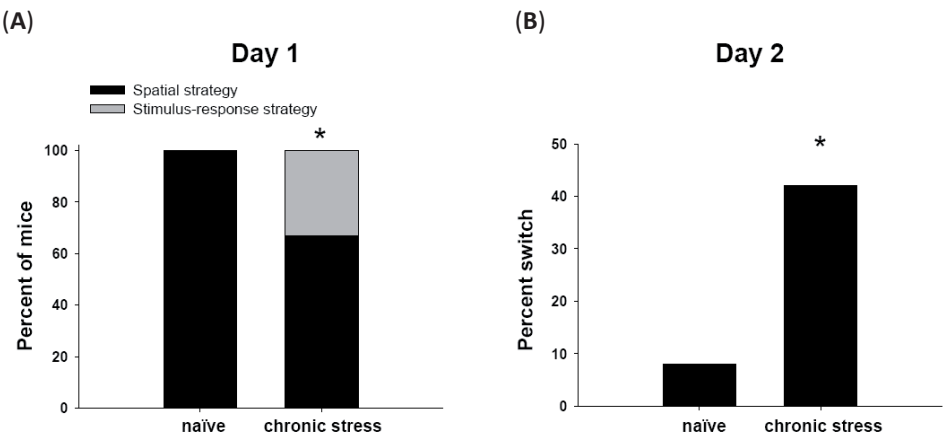

Figure 2

(A) Percent of chronically stressed and naive mice that used a spatial or stimulus-response strategy in the test trial on day 1. Chronic stress changed the used strategy towards more stimulus-response learning. **(B)** Percent of mice that chose a different hole in the first trial on day 2 than in the test trial on day 1. Behavior of chronically stressed mice was less predictable than that of controls. * $p \leq 0.05$.

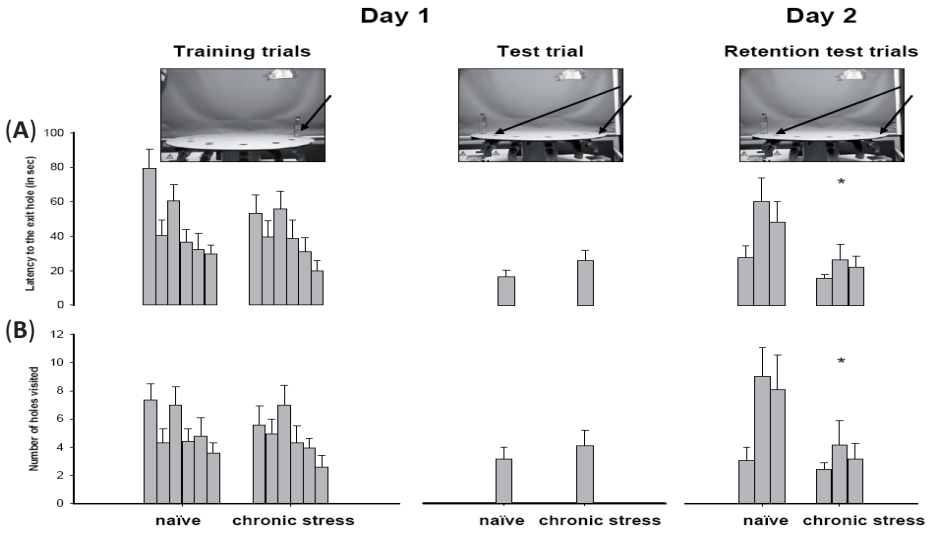

Figure 3

Mice: Latencies to the exit hole (A) and number of holes (B) visited during the six training trials and the test trial on day 1, and during the three retention trials on day 2. Chronic stress affected neither the latencies nor the number of holes visited on day 1 but reduced both parameters on day 2. Inset: circular hole board with the location of the bottle, arrows point at the exit hole(s). Data represent Mean ± S.E.M. * $p < 0.05$.

differences in the latency to the exit hole, neither during training ($F_{(1,22)}$=0.55, p = 0.47; group × trial: $F_{(5,110)}$=0.29, p = 0.91) nor in the test trial (t(22)=0.77, p = 0.57). Similarly, there was no effect of chronic stress on the number of holes visited during training ($F_{(1,22)}$=0.40, p = 0.53; group × trial: $F_{(5,110)}$=0.33, p = 0.89) or in the test trial (t(22)=0.66, p = 0.52). However, chronically stressed mice moved significantly faster during training than controls (velocity: $F_{(1,22)}$=5.37, p = 0.03). This pattern did not change when spatial learners of the chronic stress and control group were compared (all $F < 1.5$, all $p > 0.25$; except velocity: $F_{(1,17)}$=4.79, $p < 0.05$)

Interestingly, relocation of the cue in the test trial caused a decrease in latency in controls but an increase in chronically stressed mice underlining the rigidity and reduced flexibility of the behavior of chronically stressed mice (trial (t6, test trial) × group: $F_{(1,22)}$=4.58, $p < 0.04$; Table 1). A similar pattern was observed for velocities: while chronically stressed mice had decreasing velocities from trial 6 to the test trial, naïve mice increased velocity from trial 6 to the test trial (trial (t6, test trial) × group: $F_{(1,22)}$=5.49, p = 0.03; Table 1). Chronically stressed mice visited more holes after cue relocation in the

Table 1: Velocities and latencies to exit hole of naïve control and chronically stressed mice in the last training trial and the test trial. Controls had decreasing latencies and increasing velocity in response to cue relocation in the test trial; chronically stressed mice showed the opposite pattern (chronic stress × trial: velocity - $F_{(1,22)}$=4.58, p < 0.04; latency - $F_{(1,22)}$=5.49, p = 0.03; holes visited - $F_{(1,22)}$=1.11, n.s.). * Significantly lower than in the test trial (p < 0.05).

	Naïve		Chronic stress	
	Last training trial	Test trial	Last training trial	Test trial
Velocity (in cm/sec)	8.2 ± 0.5*	9.8 ± 0.6	9.8 ± 0.9	8.5 ± 0.6
Latency (in sec)	26.0 ± 5.8	16.4 ± 3.7	20.0 ± 5.3	29.6 ± 5.4
Holes visited	3.6 ± 0.7	3.1 ± 0.8	2.5 ± 0.8	4.1 ± 1.0

test trial than in the last training trial, whereas naïve mice tended to visit fewer holes in the test trial than in trial 6. However, the referring interaction effect failed to reach statistical significance (trial (t6, test trial) × group: $F_{(1,22)}$=1.11, p = 0.26; Table 1).

Retention performance: Twenty-four hours later, mice performed three trials. Two exits were available: one at the bottle (same as during test trial 7), the other at the position of the training trials 1-6. Both groups used mainly the hole at the position of the training trials to access their home cage. However, chronically stressed mice switched their strategy significantly more often from the test trial to the first trial on day 2 (42% chronically stressed vs. 8% naive mice: $\chi^2(1)$=3.56, p = 0.05; Figure 2B). A mixed-design ANOVA for the latencies to the exit hole revealed a significant group and trial effect. Both groups showed shorter latencies in the first than in the following trials ($F_{(2,44)}$=3.30, p = 0.05). Chronically stressed mice had shorter latencies than controls, especially in trials 2 and 3 ($F_{(1,22)}$=7.86, p = 0.01; Figure 3). The same pattern was found for distance moved and the number of holes visited (all p-values < 0.03). There was no trial effect on the animals' velocity ($F_{(2,44)}$=0.37, p = 0.69); like 24hrs before, chronically stressed mice moved significantly faster than controls ($F_{(1,22)}$=8.57, p < 0.01). When only spatial learners of the chronic stress group were considered, group differences remained unchanged (Fs > 5, p's < 0.05).

To assess basal exploratory behavior and locomotion, all mice had spent 5 min on the CHB (all holes closed), one week before the rat stress started. No group differences regarding the number of holes visited and the latency to the hole which provided access to the home cage in the training trials three weeks later were observed (both t-values < 1.04, and p's > 0.30).

Learning strategy and performance within the stressed group: Mice were classified as spatial and SR learners based on their performance in the test trial. Spatial

and S-R learners had similar latencies in trials 1 to 6 and in the test trial ($F_{(1,9)}$=0.02, p = 0.97). Over the three trials on day 2, S-R learners decreased their latencies to the exit hole, the distances walked and the numbers of holes visited, whereas these parameters increased in the spatial learners. Thus, spatial learners of the stress group showed the same performance pattern as spatial learners of the control group.

*Endocrine parameters (*Figure 4*):* More than one week after the last rat exposure, rat stressed mice had significantly enlarged adrenals (t(19)=2.31, p = 0.03); thymus weight was lower but did not differ significantly between rat stressed and control groups (M ± S.E.M. in mg; controls: 42.83 ± 2.22, chronic stress: 38.93 ± 2.67; t(21)=1.11, p = 0.27). Basal plasma corticosterone under resting conditions was significantly increased in the rat stressed group (t(22)=3.80, p = 0.001). The three parameters indicate the success of the chronic stress protocol.

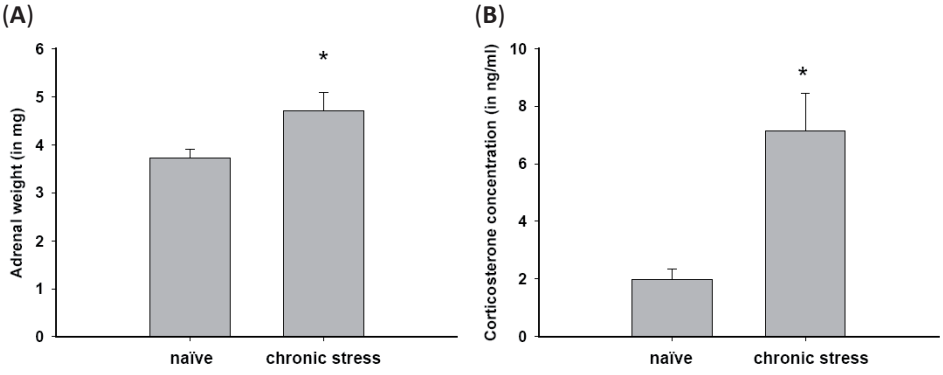

Figure 4

Chronic stress caused a significant increase in **(A)** adrenal weight and **(B)** plasma corticosterone suggesting that the use rat stress protocol was effective. * p < 0.05.

Chronic stress favors the use of stimulus-response learning strategies in humans

Chronic stress and learning strategy: Forty young healthy humans were given a questionnaire (Trier Inventory of Chronic Stress, TICS) measuring chronic stress and trained in a 2D spatial task. They had to locate the one win-field (marked by a cue) out of six (Figure 1B) in 14 trials using spatial or stimulus-based learning strategies. Relocation of the cue in the test trial (trial 15) revealed the applied strategy. Twenty-six participants (65 percent) used an S-R strategy, 9 (23 percent) employed a spatial strategy, 5 (12 percent) chose neither the S-R nor the spatial option ("non-learners").

Participants had been assigned to high vs. low chronic stress groups (n = 20 per group; "low chronic stress" – median: 435, range: 346 - 461; "high chronic stress" – median: 489, range: 463 - 579; Figure 5A). The number of non-learners did not differ between groups: two vs. three in the high vs. low chronic stress group. Importantly, "high chronic stress" changed the used learning strategy significantly ($\chi^2(1)=5.02$, p = 0.025; Figure 5B). Ninety-four percent (17 out of 18) of the learners in the "high chronic stress" group applied an S-R strategy in the test trial while the S-R strategy was used by 52 percent (9 out of 17) of the learners in the "low chronic stress" group.

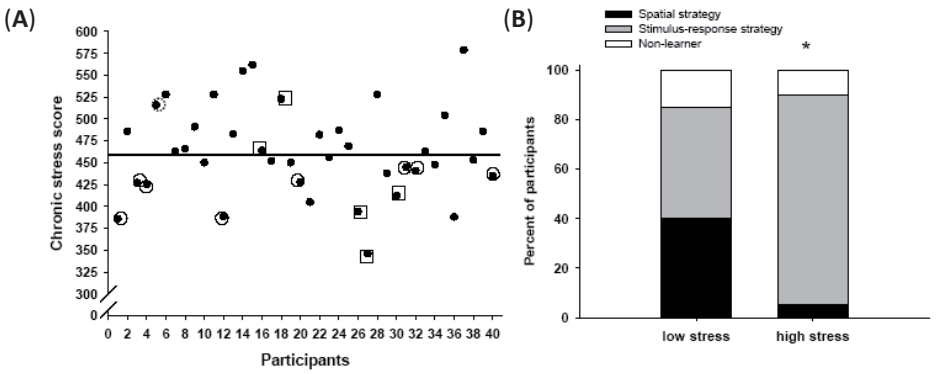

Figure 5

(A) Participants' chronic stress scores as measured by the Trier Inventory of Chronic stress (TICS). According to their chronic stress scores subjects were assigned to the "low chronic stress" and "high chronic stress" groups. The line shows the median. Circle – spatial learner in the "low chronic stress group"; Dotted circle – spatial learner in the "high chronic stress group"; Square – non-learners. **(B)** Percent of spatial, stimulus-response and non-learners in the high and low chronic stress groups. Significantly more participants of the "high chronic stress" group used of the stimulus-response strategy. * $p < 0.05$.

There was no effect of sex on the used strategy ($\chi^2(1)=0.47$, $p = 0.49$; ratio men to women in percent: spatial strategy – 42 to 58, S-R strategy: 56 to 44). Men and women were comparable with respect to their chronic stress scores ($t(38)=0.66$, $p = 0.52$; mean ± SEM: men – 460 ± 13, women – 470 ± 10).

Chronic stress and learning performance: A mixed design ANOVA on the reaction times during training revealed a significant time effect ($F_{(13,442)}=18.04$, $p < 0.001$), while there was neither an effect of chronic stress ($F_{(1,38)}=0.26$, $p = 0.61$) nor a time × chronic stress interaction ($F_{(13,442)}=0.80$, $p = 0.38$) indicating that the performance of high and low chronic stress groups improved similarly over trials. Reaction times increased from about 2 to 6s in the test trial, but were unaffected by chronic stress ($t(38)=0.11$, $p = 0.91$).

Spatial and S-R learners had comparable learning gradients (no main effect of the applied learning strategy ($F_{(1,33)}=0.45$, $p = 0.51$) nor an interaction of time and strategy ($F_{(2,52)}=0.98$, $p = 0.37$).

Verbal report: All participants that were classified as "learner" described the applied strategy in line with the chosen field. S-R learners reported that they used the stimulus (letter M) to identify the win-field; spatial learners described the use of the spatial arrangement (field in the second row of the right column). Non-learners stated that the position of the win-field was completely random and that there was no consistency. Interestingly, S-R learners tended to be more certain that the chosen field is the win-field than spatial learners (mean certainty: S-R 56%; spatial 44%; $t(33)=1.68$, $p = 0.11$).

Discussion

Our results showed that the experience of prolonged or repeated stress in mice and humans affects the learning strategy (S-R or spatial) used to acquire a task. (1) Repeated exposure to rats increased the use of an S-R strategy in mice. (2) Experiencing relatively high levels of stress within the three months prior to testing were associated with a significant change in the used learning strategy (derived from test trial performance and confirmed by subjects' verbal reports) towards more S-R learning in healthy young men and women. These effects refer to a change in the quality of learning.

Previous studies demonstrated that acute stress modulates multiple memory systems in rodents and humans in a manner which favors S-R over spatial learning and memory (Kim et al. 2001; Packard and Wingard 2004; Schwabe et al. 2007). Impairing

effects of chronic stress on hippocampus-dependent forms of learning and memory are well known (Bodnoff et al., 1995, Kleen et al., 2006, Wright & Conrad, 2005) and parallel changes in hippocampal plasticity (Bodnoff et al. 1995; McEwen 1999a; Conrad 2006). Indications that chronic stress affects learning strategies are derived from three studies (Grootendorst et al. 2001; Wright and Conrad 2005).

Grootendorst and colleagues (Grootendorst et al. 2001b) used the same paradigm of rat stress as we did and reported impaired spatial learning in the circular hole board task in 6 month old wild type mice with a C57BL/6J background. The training protocol of the circular hole board task covered several days, followed by a free exploration trial to detect search strategies. Remarkable was the shift to more perservative strategies, i.e., repeatedly return to the same hole, in the rat-stressed group. The same rat stress paradigm also impaired spatial learning in the Morris water maze together with a shift in search strategies from predominantly persistent in controls (60%) to concentric (58%) in rat-stressed mice (Grootendorst et al., 2001a). Both studies indicate that different learning strategies might have been used during training sessions, while the present study demonstrates that chronic stress indeed alters the learning strategy used to solve the task.

The findings of Wright and Conrad (Wright and Conrad 2005) pointed to an intriguing interaction of environmental conditions and task performance. Whereas chronically stressed rats were impaired in a Y-maze task which required the use of extramaze cues, i.e., hippocampus-dependent spatial learning, the introduction of intramaze cues eliminated the impairment. Thus, providing the use of more than one approach to solve the task allows switching to other problem-solving strategies. We conclude that their, like our task allowed for caudate-based stimulus-associated learning in addition to spatial learning, thereby rescuing performance (i.e., quantitative learning parameters). Our experimental setup clearly revealed the use of distinct learning strategies as a consequence of chronic stress.

Moreover, our data support the view of a non-competitive, cooperative interaction between memory systems (Voermans et al. 2004). It could be argued that chronic stress induced changes in the morphology of neurons decreases the functionality of the hippocampus (McKittrick et al. 2000; Fuchs et al. 2006), and therefore, the caudate nucleus might compensate for hippocampal impairment. This is not necessarily a case of the caudate “out-competing” the hippocampus but could be seen as the two systems working in parallel and one taking control when the other is dysfunctional.

Twenty-four hours after training, behavior of chronically stressed and control mice differed both qualitatively and quantitatively. Stressed mice behaved less

predictably than controls, in that they more often chose a different hole during the first trial of day 2 than on the test trial the day before. Whether this is due to chronic stress effects on memory consolidation or retrieval can not be decided here. To disentangle consolidation and retrieval effects, stress has to be administered either within a certain time window after learning or immediately prior to retention testing. Obviously, this is impossible in chronic stress studies. Next to differences in behavioral consistency, we obtained group differences in performance 24hrs after training. Now, stressed mice appear to perform "better", based on latencies and hole visits than mice of the control group. Does this indicate superior memory in chronically stressed animals? In our view, it does not. Memory effects would be expected especially in trial 1. Yet, group differences were absent in trial 1 but increased in the second and third trial. It is more likely and also suggested by others that chronic stress attenuates rodents' motivation to explore (Tejani-Butt et al. 1994; Conrad et al. 1999). We propose that performance 24hrs after training presents motivational rather than memory effects of chronic stress.

Moreover, chronically stressed mice moved significantly faster than controls which might suggest higher emotionality after chronic stress. Long-lasting effects of repeated stress on predominantly fear-related behavior and characteristic exploration patterns have been found in rodents (Grootendorst et al. 2001b; Wood et al. 2008) and humans (Armony et al. 2005). Importantly, others describe these manifestations of enhanced emotionality in relation to stress-induced structural alterations in hippocampus and amygdala. While chronic stress induces dendritic atrophy and debranching in hippocampal neurons, it enhances dendritic arborization and synaptic connectivity in the amygdala (Vyas et al. 2002; Mitra et al. 2005). Interestingly, the amygdala has been assigned a critical role in acute stress effects on memory functions (Kim et al. 2001; Roozendaal 2002) and in the "emotional" modulation of spatial and S-R learning (Packard and Wingard 2004). Intra-amygdala infusions of anxiogenic drugs were sufficient to switch learning strategies form predominant spatial to more S-R learning in rats. It is tempting to speculate that the amygdala plays also a critical role in the observed modulation of spatial and S-R learning by chronic stress.

Corroborating previous rodent and human studies we obtained no differences in quantitative learning parameters between spatial and S-R learners during task acquisition, neither in humans nor in mice (Kim et al. 2001; Schwabe et al. 2007). However, 24hrs later S-R learners showed decreasing latencies, number of holes visited and distances moved over the three trials on day 2, whereas all these parameters were increased in the spatial learners in the stress group - same as in spatial learners in the control group. If longer latencies in the second and third trial are indicative for motivation to explore

which in turn is - as argued above - attenuated by chronic stress, then the differences between spatial and S-R learners on day 2 might be interpreted as indication of a higher chronic stress level in S-R learners.

A challenging question derives from the fact that a certain percentage of the tested population of both species is resistant or vulnerable to the effects of stress. Here, the contribution of an epigenetic predisposition could be tested in animals experiencing discrete early life events like maternal care (Meaney et al. 2007). Additionally, assessing the degree of emotionality which is known to modulate cognitive performance (Packard and Wingard 2004; Brinks et al. 2007a) could contribute to the understanding of a resistant or vulnerable phenotype.

Chronic stress has been frequently associated with “depressive-like” symptoms (for reviews: Willner 1997a; Blackburn-Munro and Blackburn-Munro 2001). Here, the focus was primarily on emotional and motivational factors. Several authors showed that chronic stress contributes to anhedonia (the core symptom of the melancholic subtype of major depression) expressed e.g., as reduced sucrose consumption and preference or reduced sexual behavior in rats (Konkle et al. 2003; Gronli et al. 2005). In the present study, we demonstrate that chronic stress leads to a shift from elaborate “cognitive” to rather rigid “habit” learning. Comparable cognitive dysfunctions were observed in depressive patients. For instance, Harvey and colleagues (Harvey et al. 2004) as well as Purcell and colleagues (Purcell et al. 1997) report deficits in mental set shifting in patients with depression. We suggest that cognitive rigidity, here expressed by the S-R learning strategy, is an important factor in the etiopathogenesis of depression.

Finally, some limitations of the present study have to be addressed. The human task we used here is relatively simple and it is rather unlikely that it is dependent on the hippocampus *per se.* Memory for a single location is primarily a function of the parahippoacmpal cortex (Duzel et al. 2003). Alternatively, choosing of the win-field could be done using a simple S-R strategy without making use of any external landmarks. Thus, task difficulty might be an even more contributing factor rather than the fact that a task is hippocampus-dependent or not. Furthermore, we compared in the present study effects of experimentally induced chronic stress (mice) and self-reported stress (humans) which might raise questions regarding the comparability of the chronic stress effects in mice and man. This is a problem hardly to solve because chronic stress cannot be induced experimentally in humans, for obvious reasons.

Moreover, it is important to note that we did not examine effects of severe, pathological stress. Human subjects were healthy. Chronic stress levels were rather moderate. We stressed mice for 11hrs over a period of two weeks. In line with the study

of Grootendorst et al. (2001a) this resulted in increased basal corticosterone secretion indicative for an effective stress procedure. One of the very few studies that varied the duration of chronic stress found a biphasic effect on performance in a radial maze task. While 21 days of stress resulted in memory impairments, 13 days of stress did not impair but even enhanced memory performance (Luine 2002). It is likely that our "rat stress" paradigm belongs to the category of rather mild chronic stress that still allows adaptation and prevents performance impairment. Extending the stress period in mice and testing a patients suffering from a stress-related disease will provide answers to the more detrimental effects of chronic stress. Initially, chronic stress-induced changes should be viewed as signs of an adaptive response, yet the potential for damage and pathology is increased.

So far, research on memory effects of chronic stress predominantly focused on quantitative parameters such as the number of items remembered in humans and latencies to a goal in animals, i.e. *how much* is learned. The present findings show clearly that chronic stress affects the quality of learning; i.e. which memory system is involved in the process of learning, *how* an individual learns. Independent of the used memory system, quantitative parameters may remain unchanged and thus veil the actual effects of stress on learning and memory. The use of S-R instead of spatial strategies appear to be a first signal of the impact of chronic stress in a vulnerable individual, while the level of performance can still be maintained, as long as the environment remains stable (such as during the training trials in the present studies) and alternative approaches are allowed.

Acknowledgements

This work was part of the Trier-Leiden IRTG program, supported by grants DFG GRK 1389/1, NWO DN 95-420 and KNAW Dr. J.L. Dobberke Stichting 8848. We gratefully acknowledge the assistance of Maaike van der Mark in the animal study.