Stress, emotion and cognition : role of mineralo- and glucocorticoid receptors

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Chapter 5

Corticosterone facilitates extinction of fear memory in BALB/c mice but strengthens cue related fear in C57BL/6 mice

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Accepted in Experimental neurology

ABSTRACT

Corticosterone, the naturally occurring glucocorticoid of rodents is secreted in response to stressors and is known for its facilitating, but also detrimental effects on emotional learning and memory. The large variability in the action of the stress hormone on processing of emotional memories is postulated to depend on genetic background and the spatio-temporal domain in which the hormone operates. To address this hypothesis, mice of two strains with distinct corticosterone secretory patterns and behavioural phenotype (BALB/c and C57BL/6J mice) were treated with corticosterone (250 µg/kg, i.p.), either 5 minutes before or directly after acquisition in a fear conditioning task. As the paradigm allowed assessing in one experimental procedure both context- and cue-related fear behaviour, we were able to detect generalization and specificity of fear. BALB/c showed generalized strong fear memory, while C57BL/6J mice discriminated between freezing during context-and cue episodes. Corticosterone had opposite effects on fear memory depending on the mouse strain and time of injection. Corticosterone *after* acquisition did not affect C57BL/6J mice, but destabilized consolidation and facilitated extinction in BALB/c. Corticosterone *5 min before* acquisition strengthened stress-associated signals: BALB/c no longer showed lower fear memory, while C57BL/6J mice displayed increased fear memory and impaired extinction in cue episodes. We propose that corticosterone-induced facilitation of fear memory in C57BL/6J mice can be used to study the development of fear memories, corticosterone administration in BALB/c mice rather presents a model to examine treatment. We conclude that genetic background and time of corticosterone action are modifiers of fear memory with interesting translational implications for anxiety-related diseases.

INTRODUCTION

Emotional experiences are remembered very well. However, the strength of emotional memory varies between individuals. Good memory of a salient experience has the advantage to facilitate adaptation to similar situations in the future. However, when memory for emotional events becomes too strong and also unpredictable, pathologies such as post traumatic stress disorder (PTSD), panic and anxiety disorders might develop.

Individuals suffering from PTSD show abnormal cognitive-emotional interactions. This implies that specific situations may lead to re-emergence (retrieval) of intrusive, unwanted memory of a traumatic event together with extreme emotions related to fear. Recent clinical trials have shown that treatment with glucocorticoids can have a beneficial effect on established PTSD [1] and specific phobias [2]. It is known for decades that glucocorticoids modulate fear memories [3-10]. For a rational treatment of anxiety disorders it is therefore essential to understand how glucocorticoids contribute to the formation and extinction of emotional memories.

The present study is focused on the interplay of glucocorticoids with memory formation and extinction of a traumatic event. BALB/c and C57BL/6J mice have a distinctly different stress neuroendocrinological and behavioural phenotype. During fear conditioning BALB/c mice display a much higher stress responsivity and emotionality than C57BL/6J mice [11;12]. Hence these two mouse strains will be used to examine the role of corticosterone in individual differences in processing of fearful information.

Pavlovian fear conditioning provides one of the best rodent models to study cognitive processes related to fear. Fear conditioning studies classically consist of the pairing of a conditioned stimulus (CS) with an aversive unconditioned stimulus (US; mostly electric footshock), which mainly induces freezing as a conditioned fear response. Different neural mechanisms seem to be involved depending on whether the CS is a relatively simple stimulus or cue, such as a tone or light (unimodal), or the context (multimodal) in which the US is delivered. Lesion experiments showed that the amygdala is necessary for both types of conditioning, whereas the hippocampus is predominantly required for contextual conditioning [13;14].

Our recently developed fear conditioning paradigm allows the assessment of both context and cue related fear-memory processes in one experimental procedure. Using this paradigm we recently found that BALB/c mice show strong fear-responses to context and cue (i.e., generalization), while C57BL/6J mice display specific fear memory towards the predictive conditioned stimulus, the cue [11]. Remarkably, BALB/c mice have a twofold higher corticosterone response after conditioning and retrieval of fear memory than C57BL/6J mice

[11]. Based on these results, we hypothesized that additional corticosterone treatment prior to acquisition and consolidation of fear memories will result in altered fear-related memory formation and thus, retrieval and extinction patterns of fear behaviour. For this purpose corticosterone was administered either 5 minutes before or directly after acquisition. We expect that the timing of the corticosterone treatment in relation to acquisition and consolidation will affect subsequent retention of behaviour in a strain dependent fashion.

MATERIAL AND METHODS

Animals

Twelve week old male BALB/c (n=40) and C57BL/6J mice (n=36) from Charles River (Maastricht, The Netherlands) were housed individually with sawdust bedding, water and food *ad libitum*, at 20^0C with controlled humidity under a 12 h: 12 h light/dark cycle (lights on at 07.00 a.m.) for at least one week. All experiments were approved by the committee on Animal Health and Care from Leiden University, The Netherlands and performed in strict compliance with the EEC recommendations for the care and use of laboratory animals.

Pain sensitivity

We included an experiment to determine possible differences in the pain threshold between BALB/c and C57BL/6J mice. A separate group of mice (n=8/strain) were subjected to a tail flick protocol that included placing the last two cm the tail in water with a constant temperature of 55^0C [15]. Tailflick latencies of three subsequent trials per mouse were determined with a cut-off latency of 12 sec. The experiment was performed between 09.00 and 10.00 hrs. Tailflick latencies were in the range of 1.32 to 4.18 sec and similar in BALB/c and C57BL/6J mice (data not shown, $F(1,47)1.192$, $p=0.281$), indicative for comparable pain thresholds between strains.

Corticosterone dose and time of injection

Corticosterone (corticosterone-HBC complex, Sigma, The Netherlands) was dissolved in physiological saline on the day of the experiment and injected intraperitoneally (i.p.) with a dose of 250 µg/kg bodyweight in a volume of 0.2 ml. The vehicle (saline) was injected in a corresponding volume of 0.2 ml. A pilot experiment (data not shown) using several corticosterone doses showed that the 250 µg/kg bodyweight dose increases corticosterone concentration of C57BL/6J mice to the level of BALB/c mice when exposed to our fear conditioning procedure [11].

BALB/c (n=16) and C57BL/6J mice (n=14) were injected with corticosterone or vehicle at 5 minutes before the start of the acquisition on testing day 1. We expected that this treatment would affect both acquisition and consolidation processes. To selectively influence the consolidation process, BALB/c (n=16) and C57BL/6J mice (n=14) were injected with corticosterone or vehicle directly after acquisition on testing day 1.

Fear conditioning

Apparatus

The fear conditioning chamber was made of black Plexiglas (25x 25 x 35 cm high) covered by a transparent rim (3 cm width). A speaker was fixed into one wall (25 cm high) and connected to a tone generator (70 dB). The floor consisted of stainless steel bars (5 mm in diameter, spaced 0.5 cm apart) connected to a shock generator. Hereunder was a tray with paper tissues to collect faeces and urine of the mice. A white light source (260 lux) and a camera connected to a video recorder were fixed 20 cm above the conditioning chamber.

A radio produced 20 dB of background noise and the light intensity of the experimental room was 90 lux. After each animal, the chamber was cleaned with tap water and the tissues were replaced.

Procedure

The fear conditioning paradigm allowed differentiating between context and context/cue related behavioural responses in the same setting. Training (day 1) involved 3 minutes of baseline recording, followed by 6 light/tone (CS) + shock (US) pairings with an episode of one minute. Pairings consisted of the cue (i.e., a combined light (260 lux) and tone exposure (70dB)) for 20 seconds and an electric footshock (0.4 mA) during the last two seconds of the cue. Mice were returned to their homecage 2 minutes after the last pairing. At 48 and 72 hrs after conditioning (days 3 and 4, respectively), the same experimental procedure was repeated in absence of shocks to test for memory and extinction of the conditioned fear response. The procedure lasted 12 minutes per mouse/day and was performed between 8.00 a.m. and 13.00 p.m. in an experimental room adjacent to the housing room.

Behavioural assessment

Freezing behaviour was recorded as parameter of fear behaviour. Freezing is defined as immobility of the body including the head devoid of any interaction with the environment. According to Morgan and colleagues, we started and finished behavioural registration with the first and sixth cue presentation during memory and extinction testing [16]. To determine the behavioural structure,

freezing and behaviours such as scanning, grooming, sitting, rearing, stretched attends, jumping and walking were subjected to a Principal Component Analysis (PCA). All behaviours were scored with a semi automatic scoring program (The Observer 4.1, Noldus, Wageningen, The Netherlands) from the video tape.

Statistical analysis

Differences in tailflick latency between BALB/c and C57BL/6J mice were determined by one-way-ANOVA.

Fear conditioning data are presented as mean $\pm$ SEM percentage of freezing during context and cue episodes of the whole session and for each context and cue episode. For acquisition, pre- and post acquisition treatment groups were analysed using General Linear Model (GLM) to determine treatment (naive, saline, corticosterone), strain (BALB/c and C57BL/6J) and time (progression over separate episodes) effects over context or cue episodes. GLM analyses per treatment group (pre- or post acquisition) was used to determine main effects of treatment (corticosterone, saline), strain (BALB/c, C57BL/6J) and day (days 1, 3 and 4) for averaged freezing behaviour in context and cue episodes. If main effects were present, subsequent GLM analyses on context ***or*** cue induced freezing behaviour were performed to determine treatment, strain and day effects. Progression of context ***or*** cue induced freezing behaviour per testing day was also determined with GLM, if adequate, followed by post-hoc LSD test.

Principal Component Analysis (PCA) was performed over all behavioural data. Kaiser normalisation was used on behaviours with communalities over 0.68, i.e., more than 68% of variation is explained by the factors extracted. Factors with an Eigenvalue over 1 were included in the results. A subsequent two-way ANOVA on factor loadings was performed to determine the significance of treatment and strain differences. $P \leq 0.05$ was accepted as level of significance.

RESULTS

BALB/c and C57BL/6J mice were trained in a fear conditioning paradigm in which a novel environment (context) and a light-tone stimulus (cue) were paired with a footshock. Corticosterone had been injected either 5 min before or directly after acquisition. Forty-eight hours later (day 3), re-exposure to the context and cue paradigm (without shock) elicited significant fear responses indicating retrieval of a learned association between this environment and the aversive footshock stimulus. Another 24 hrs later (day 4), mice were re-exposed to the same conditions to study extinction of the conditioned fear responses. Data are presented in the sequence of the phases of memory: acquisition, memory retrieval and extinction in relation to corticosterone treatment. We

found strain, treatment and time of treatment dependent effects on freezing behaviour.

Fear conditioning: Acquisition (Day 1, figures 1-4)

Comparing the percentage of freezing during the alternating cue and context episodes revealed that treatment prior to fear conditioning changed the freezing responses depending on the mouse strain (interaction: strain x treatment, F(2,53) 4.77, p=0.012; Figures 1 and 2). Both strains increased freezing over time although with different patterns of freezing in cue and context episodes (F(11,583) 4.613, p=0.0001) and treatment (F(22,583) 2.125, p=0.002). Strain effects (no injection, Figures 3 and 4): Already naïve BALB/c and C57BL/6J mice responded with a different freezing pattern to fear conditioning (strain F(1.27) 11.846, p=0.002). Freezing increased in both strains during consecutive cue/shock pairings and intermittent context periods, albeit with a different pattern (F(11,297) 4.083, p=0.0001). While freezing during context was comparable between strains, BALB/c mice were more active during cue periods than the C57BL/6J mice (i.e, more freezing in C57BL/6J mice during cue episodes F(1,27) 31.321, p=0.0001). Treatment effects within strains (compare Day 1, Figures 1 and 3 and Figures 2 and 4): Injection of either vehicle or corticosterone before conditioning increased freezing in BALB/c mice (F(2,29) 6.467, p=0.005; steeper increase F(22,319) 2.725, p=0.0001; and more freezing during cue and context episodes (cue F(2,29) 6.994 p=0.003; context F(2,29) 3.571, p=0.041)). Injections prior to conditioning did not affect freezing in C57BL/6J mice. Treatment effects between strains (Figures 1A ,2A vs 1B, 2B): Due to the injection procedure BALB/c mice displayed more freezing to context than C57BL/6J mice (F(1,26) 4.753, p=0.038). Total amount of freezing during cue episodes was comparable between strains, but showed a different time course (F(5,130) 3.016, p=0.013).

Memory retrieval and extinction overall: strain dependency and time of corticosterone treatment

Corticosterone treatment resulted in a strain dependent effect (interaction strain x treatment F(1,51) 8.120, p=0.006). In addition, time of treatment (before or after acquisition) differentially influenced the freezing to cue and context during the retrieval and extinction tests on days 3 and 4 (interaction time x treatment F(1,51) 8.220, p=0.006). In both strains, freezing responses were altered by corticosterone (BALB/c: treatment F(1,28) 7.304, p=0.012; interaction time x treatment F(1,28) 4.531, p=0.042; C57BL/6J: interaction time x treatment F(1,23) 3.850, p=0.05).

Memory retrieval and extinction: Treatment prior to acquisition (figures 1 and 2)

Overall analysis of freezing on days 3 and 4 revealed an interaction of strain x treatment (F(1,56) 4.178, p=0.046). Increased total amount of freezing on day 3 indicated the retrieval of fear memory.

Overall, BALB/c displayed more freezing during context than C57BL/6J mice (F(2,25) 7.127, p=0.004), while C57BL/6J mice froze more during cue episodes (F(2,25) 13.147, p<0.0001, Figure 1). Depending on the strain, vehicle and corticosterone differentially altered cue-related freezing (strain x treatment F(2,25) 6.056, p=0.007): cue freezing was initially not affected and later on decreased in BALB/c mice, while it was increased in C57BL/6J mice (Figure 1).

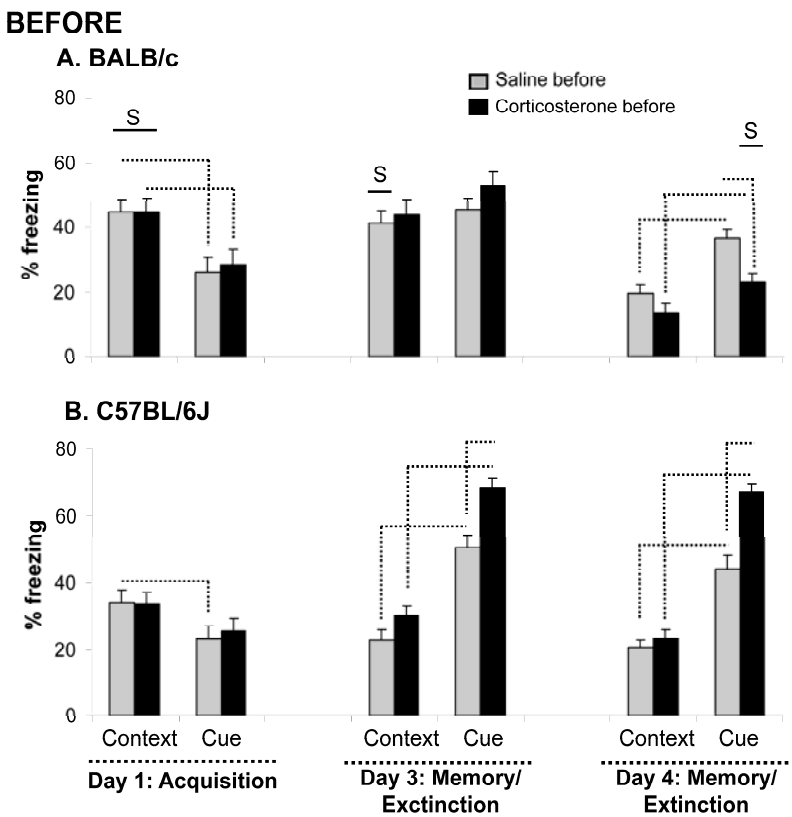

Figure 1. Treatment before acquisition. Percentage of freezing for context only or additional cue intervals of BALB/c mice (A) and C57BL/6J mice (B) injected i.p. with corticosterone (black bars) or saline (grey bars). P<0.05, dotted lines: within strain effects and, S: between strain effect determined with ANOVA.

Vehicle injection: Context and cue induced freezing progressed differently in BALB/c and C57BL/6J mice over days (context: F(2,52) 7.392, p=0.0001, cue: F(2,52) 12.023, p<0.0001, Figure 1). BALB/c mice decreased their freezing during context from day 3 to 4 (Figure 1), while freezing was generally lower and did not differ between days in C57BL/6J mice. Freezing during cue increased in both strains from day 1 to 3, remained high in C57BL/6J mice on day 4, but decreased in BALB/c mice.

Corticosterone had distinct effects on freezing during cue, but not during context episodes, in both strains (interaction of strain x treatment (F(2,52) 5.081, p=0.01). Compared to the vehicle treated C57BL/6J mice, C57BL/6J mice of the corticosterone group had increased freezing during cue episodes on days 3 and 4 (Figure 1). In contrast, cue induced freezing of BALB/c mice did not differ on day 3, but dropped significantly on day 4.

Freezing to alternating context and cue conditions within a session (figure 2)

Analyzing the freezing pattern of alternating cue and context episodes provides additional information on the progression of distinct strain specific behavioural responses: are mice able to show different degrees of freezing to context and cue? These data were analyzed for days 3 and 4.

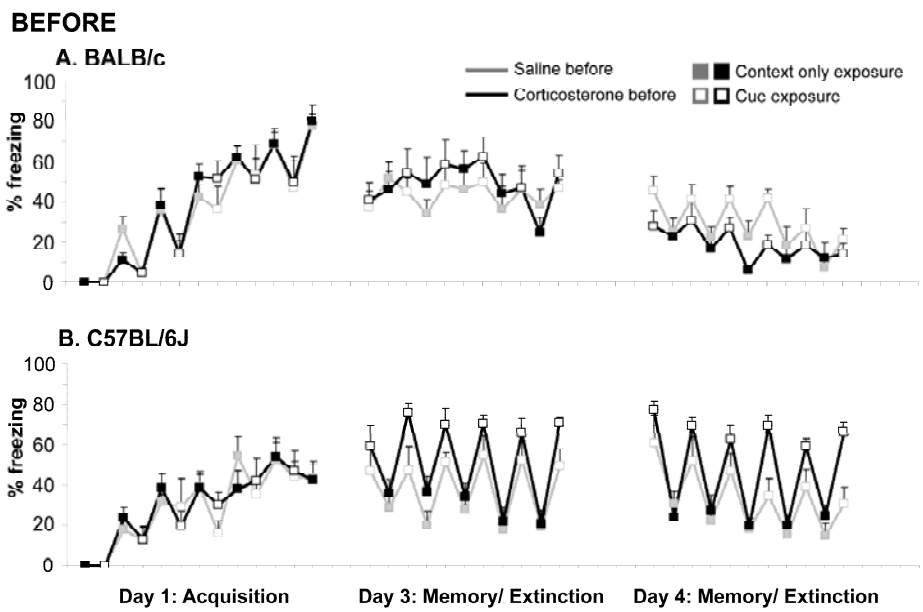

Figure 2. Treatment before acquisition. Freezing behaviour of BALB/c (A) and C57BL6J mice (B) during the three testing days injected i.p. with saline (gray) or 250 µg/kg corticosterone (black). Closed markers indicate context intervals alternating with open markers representing cue intervals. Note that C57BL/6J mice distinctly switch between freezing during context to cue intervals.

Day 3: ANOVA revealed a significant interaction of cue-context x strain (F(10,260) 3.492, p=0.0001). BALB/c mice did not discriminate between freezing to context and cue episodes throughout the session, independent of treatment. In contrast, C57BL/6J mice showed a strong alternating pattern of cue-context freezing (F(10,120) 12.865, p=0.0001), also independent of treatment.
Day 4: The significant interaction of cue-context x strain F(10,260) 4,194, p=0.0001, was complemented by an interaction cue-context x strain x treatment F(10,260) 2.470, p=0.008.
While the differentiation between freezing to context and cue was rather small in BALB/c mice (independent of treatment), it was clearly expressed in C57BL/6J mice and distinctly different in the corticosterone group (interaction cue-context x treatment F(10,120) 3.144, p=0.001).

Memory retrieval and extinction: Treatment immediately after acquisition (figures 3 and 4)
Overall analysis of freezing on days 3 and 4 revealed main effects of strain (F(1,54) 14.615, p=0.0001), treatment (F(1,54) 7.105, p=0.010) and an interaction of strain x treatment (F(1,54) 4.314 p=0.043). Both mouse strains freeze more on day 3 than day 1, indicating the retrieval of fear memory.
Vehicle injection: From day 3 to 4, freezing during context decreased more in BALB/c than in C57BL/6J mice (F(2,50) 4.956, p=0.011, figure 3). BALB/c froze less during cue episodes than C57BL/6J mice, already on day 3 (cue F(1,27) 5.696, p=0.025). Cue-related freezing further decreased in BALB/c on day 4, but remained at the same high level in C57BL/6J on both days (F(2,50) 3.744, p=0.031).
Corticosterone resulted in less freezing to context and cue in BALB/c mice on day 3 (F(1,25) 6.596 p=0.017, figure 3), which further decreased on day 4 (strain (F(1,25) 31.622, p=0.0001). Corticosterone and vehicle-treated C57BL/6J mice showed comparably strong freezing responses to context and cue (interaction strain x treatment F(1,25) 4.346, p= 0.047).
Freezing to alternating context and cue conditions within a session (figure 4)
BALB/c and C57BL/6J mice showed different responses to the alternating cue and context conditions.
Day 3: ANOVA revealed a significant main effect of treatment (F(1,25) 6.596, p=0.017) and interaction of cue-context x strain F(10,250) 2.439, p=0.009 and cue-context x treatment F(10,250) 2.056, p=0.029. BALB/c mice did not discriminate between freezing to context and cue throughout the session; however, when treated with corticosterone, freezing declined in the course of the session. In contrast, C57BL/6J mice showed an alternating pattern of more

cue than context freezing (F(10,110) 6.330, p=0.009) at the end of the session, which was independent of treatment.
Day 4: The significant interaction of cue-context x strain F(10,250) 3.711, p=0.0001 indicated the little differentiation of BALB/c mice between freezing to context or cue episodes and the fast decrease of freezing during the session. C57BL/6J mice again differentiate between freezing to cue (more) and context (less) episodes (F(10,110) 16.000, p=0.0001). Corticosterone treated C57BL/6J mice remain responding with high freezing to the cue throughout the session, while freezing decreases in vehicle-injected mice (interaction cue-context x treatment (F(1,110) 2.361, p=0.041).

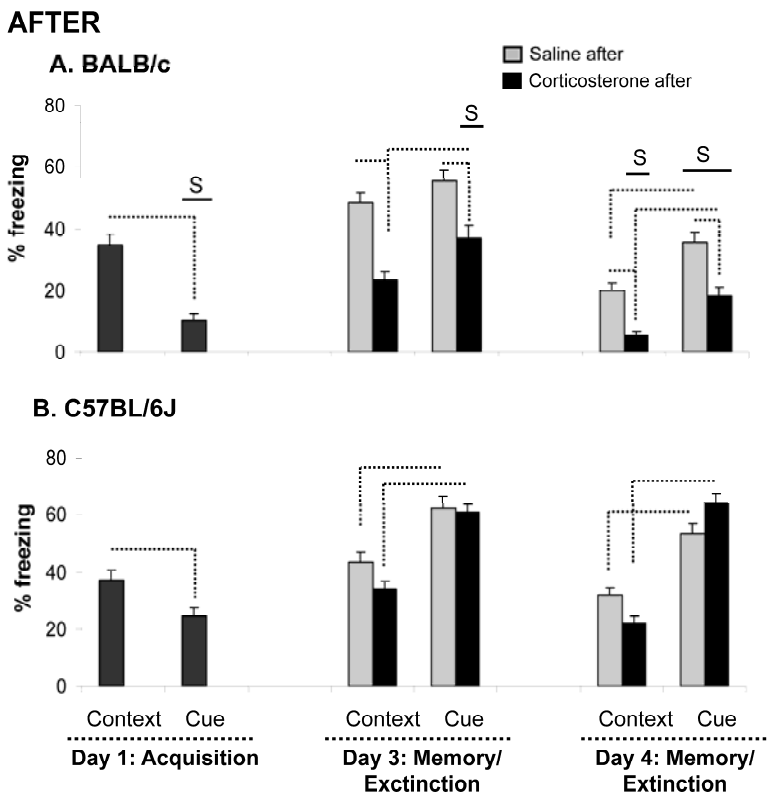

Figure 3. Treatment after acquisition. Percentage of freezing for context only or additional cue intervals of BALB/c mice (A) and C57BL/6J mice (B) injected i.p. with corticosterone (black bars) or saline (grey bars). P<0.05, dotted lines: within strain effects and, S: between strain effect determined with ANOVA.

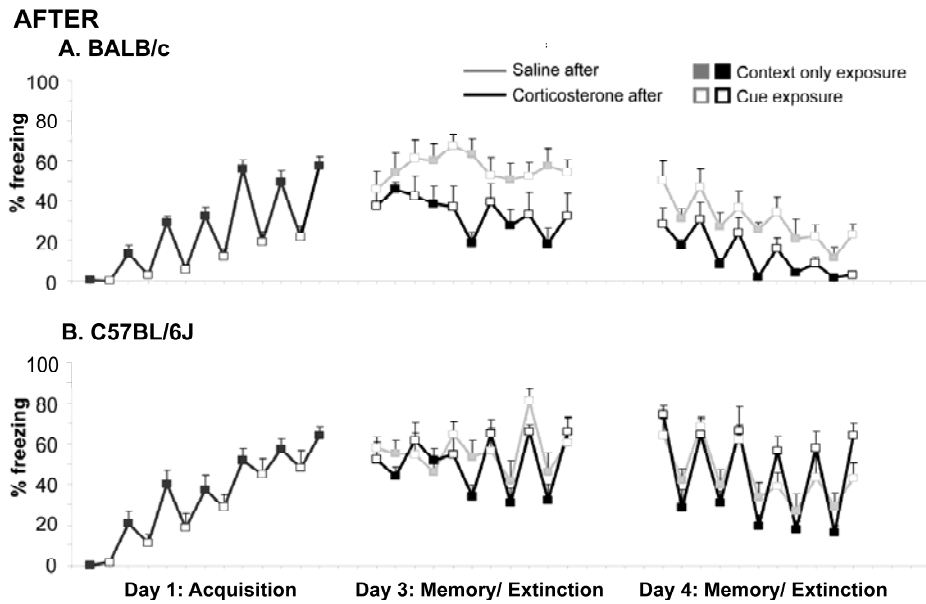

Figure 4. Treatment after acquisition. Freezing behaviour of BALB/c (A) and C57BL/6J mice (B) during the three testing days when injected with saline (grey line) or corticosterone (black line). The dark grey line on day 1 represents pooled data of mice that received treatment later on during the experiment. Closed boxes indicates context intervals, open boxes indicates additional cue intervals.

PCA analysis

PCA analysis resulted in the extraction of one factor explaining 76.3% of the variance. This factor included the behaviours freezing (factor loading: -0.917), sitting (factor loading: 0.877) and walking (factor loading: 0.825), indicating fear or immobility behaviour during all testing days. Further ANOVA's on factor loadings revealed a significant treatment effect in post-acquisition treated BALB/c mice ($F(1,527)$ 63.126, $p<0.0001$) and pre-acquisition treated C57BL/6J mice ($F(1,461)$ 7.936, $p=0.005$).

In addition, PCA analysis showed distinct strain specific fear behaviour when treated with saline post-acquisition ($F(1,461)$ 9.348, $p=0.002$), or pre- and post-acquisition corticosterone ($F(1,527)$ 17.102, $p<0.0001$ and $F(1,494)$ 87.563, $p<0.0001$ respectively). However, pre-treatment of saline diminished strain differences between BALB/c and C57BL/6J mice ($F(1,461)$ 0.127, $p=0.721$), likely reflecting the injection effect during acquisition.

DISCUSSION

Our results demonstrate distinct strain-dependent differences in the acquisition, consolidation, retrieval and extinction of fear memories. The highly stress sensitive and emotional BALB/c mice generalize their fear memory, which is expressed by similar amounts of freezing during context and cue episodes (Figures 1 and 3, day 3: saline). In contrast, the less stress sensitive and less emotional C57BL/6J mice exhibit more freezing during cue than context episodes. C57BL/6J mice specifically identified the cue as predictor of the aversive experience. Corticosterone has opposite effects on fear memory depending on the mouse strain and the time of injection. In C57BL/6J mice, pre-acquisition corticosterone enhances cue fear memory and prevents cue extinction. In BALB/c mice however, post-acquisition corticosterone destabilizes consolidation of fear memory, allowing faster extinction. Remarkably, pre-acquisition corticosterone counteracts this weak retrieval on day 3, while showing similar fast extinction as post-acquisition treated BALB/c mice one day later. These data identify genetic background and time of corticosterone application as modifiers of fear memory, a finding with interesting translational implications for PTSD and other anxiety disorders.

BALB/c and C57BL/6J mice show different context vs. cue related fear acquisition and memory

The fear conditioning paradigm uses six pairings of combined auditory and visual stimulus stimuli (i.e, the cue) with aversive shocks alternating with "context only" episodes. Freezing as fear response to the environment where the aversive shock has been received (i.e., the context) is related to hippocampal information processing while the cue-related fear response is controlled by the amygdala [13;14]. In support and extension of our previous findings [11], BALB/c and C57BL/6J mice display different patterns of fear acquisition and memory in the alternating context and cue episodes.

During acquisition, C57BL/6J mice display more freezing during cue episodes that precede and predict the shock than BALB/c mice. BALB/c mice are more active during this cue and freeze relatively more during the intermittent context episodes. Thus, C57BL/6J mice respond rather to discrete (cue) than more complex stimuli. In line with this reasoning, we have previously shown in an appetitive learning task that C57BL/6J mice preferentially use a visual stimulus driven learning strategy compared to the predominant spatial and emotionally biased learning, which is favoured by BALB/c mice [12]. These findings indicate a remarkable strain-dependent behavioural performance and cognitive processing. It possibly reflects the active (increased activity, escape) coping style

displayed by BALB/c as opposed to the passive coping style (freezing) of C57BL/6J related to fear. These distinct behavioural strategies are likely to affect later consolidation and thus contribute to memory formation.
Indeed, BALB/c and C57BL/6J mice also show distinct fear memory. While C57BL/6J mice display higher cue (70%) than context (about 20-30%) related freezing during memory testing, BALB/c mice generalize freezing over context and cue during memory testing on day 3. These differences in cue and context related fear memory between BALB/c and C57BL/6J mice forward strain-specific abilities of identifying the cue as predictive stimulus for the aversive experience and most likely represent the strain-specific contribution of hippocampus and amygdala to fear memory.
Besides generalized fear memory, BALB/c mice display a strong extinction of contextual and cued fear memory on day 4. Similar improved extinction of fear memory of BALB/c mice compared to four inbred mouse strains has been reported in another paradigm [17]. This facilitated extinction of fear behaviour has been ascribed to corticosterone [18;19]. We propose that the high post-retrieval corticosterone concentrations we observed in BALB/c mice [11] are causally related to the facilitated extinction of fear memory. Indeed, Cai et al [3] also reported the same results with post-retrieval injections of corticosterone, which will be discussed below.

Fast non-genomic effects of corticosterone during acquisition
Injections *before* and *after* acquisition further differentiate subsequent fear conditioning effects between strains as well as the spatio-temporal action of corticosterone. Pre- and postacquisition treatments are expected to influence the consolidation, but corticosterone treatment *before* is the only one to have an effect on acquisition. An important observation is the apparent absence of corticosterone-induced behavioural effects during acquisition. This might lead to the idea that corticosterone treatment is ineffective and thus, devoid of fast non-genomic effects [20;21]. However, when comparing the effects of corticosterone treatment *before* and *after* acquisition on later memory/extinction testing on day 3, another argument becomes more likely. For BALB/c mice, corticosterone treatment *before* has little effect on fear memory while corticosterone treatment *after* acquisition has a strong impairing effect on fear memory. For C57BL/6J mice, corticosterone *before* clearly increases fear memory, specifically for the cue, while corticosterone after acquisition does not have such clear effect. In fact, the timing of corticosterone action just differs by 17 minutes. It is therefore (1) more likely that the difference in fear memory / extinction between corticosterone treatment *before* and *after* originates from a difference in corticosterone levels and its action during acquisition, and thus, (2) to conclude that corticosterone treatment before

acquisition does have fast non-genomic effects on the acquisition process, most likely via the low affinity membrane bound mineralocorticoïd receptor (Karst et al 2005; Joels et al. 2008). In the case of BALB/c mice, these fast effects seem to diminish the effect of later high corticosterone levels during consolidation, i.e., counteract the destabilized consolidation and weak retrieval. In C57BL/6J mice, high corticosterone during acquisition potentiates fear memory for the cue.

Long-term corticosterone actions differ between strains: corticosterone treatment increases cue memory in C57BL/6J mice, but decreases cue and context fear memory in BALB/c mice

There is an intriguing dual action of corticosteroids: they facilitate memory consolidation, but behavioural responses that are of no more relevance are extinguished [8;9;22]. Using a forced extinction paradigm in a one step-through inhibitory avoidance test, this effect appeared to be specific for corticosterone [8]. In the present study, we report the strain-dependency of this dual action of corticosterone: the already shortly discussed augmented cue fear memory in C57BL/6J mice and less fear memory in BALB/c mice. The observed increase in cued fear memory in C57BL/6J mice likely reflects a well known facilitating effect of increased glucocorticoid receptor (GR) activation seen in other tasks using this mouse strain [12;23;24]. In BALB/c mice, post-acquisition corticosterone treatment does not affect freezing in the first cue and context episodes on day 3, but reduces freezing in the later episodes of retention testing on that day, suggesting that consolidation is less stable due to corticosterone treatment. The observed corticosterone-induced decrease in fear memory and thus improved extinction corresponds to other studies in which corticosterone facilitates extinction in an appetitive operant conditioning task [9]. Interestingly, post-retrieval injections of corticosterone in C57BL/6J mice [3] also results in enhanced extinction of freezing. We may assume that the high endogenous post-retrieval corticosterone concentrations, as reported by Brinks et al., 2008, modify subsequent memory reconsolidation and extinction processes.

Studying strain- and time-dependent effects, we did not address the issue of possible dose-dependent effects of corticosterone. Fear of BALB/c and C57BL/6J mice, with and without corticosterone, does not reflect a linear gradient that is characteristic for fear memories (Sandi, Pinelo-Nava, 2007). Post-retrieval injected corticosterone, that supposedly modifies re-consolidation of fear memory also nicely follows a linear dose-response relationship and impairs extinctions (0.3 mg up to 10 mg/kg corticosterone; Cai et al 2006). In the present study, corticosterone underlined the strain-dependent fear behaviour: it strengthened the already existing strong distinction between context- and cue-related fear in C57BL/6J mice and destabilized memory and facilitated extinction in BALB/c mice. It seems unlikely, that further increasing the dose of

corticosterone in C57BL/6J mice would result in processing of fear comparable to the high stress sensitive, high corticosterone secreting BALB/c mouse.
Rey and colleagues [25] provide mechanistic data on how corticosterone could decrease fear memory in BALB/c mice. While corticosterone is known to enhance LTP, the cellular mechanism believed to underlie learning and memory, in the hippocampal CA1 area of C57BL/6J mice [26], moderate and high doses of corticosterone decrease the spike amplitude in hippocampal slices of BALB/c mice. This decrease could lower the number of action potentials, therefore impair LTP and in parallel decrease (fear) memory [17;27;28].

Molecular mechanisms contributing to fear acquisition and memory
Distinct HPA reactivity of BALB/c and C57BL/6J mice and thus corticosterone levels, would likely contribute to distinct corticosterone related molecular mechanisms in the hippocampus and amygdala. For example, Yilmazer-Hanke and colleagues [29] found strain differences in corticosterone related NMDA and GABA receptor expression in the amygdala. The NMDA receptor in the amygdala, which facilitates the magnitude of contextual fear [30] seems to be higher expressed in BALB/c than C57BL/6J mice. In addition, GABA receptors, which are more abundant in the amygdala of C57BL/6J mice, specifically affect fear expression to conditioning stimuli during acquisition [31] and memory testing [32].
The sympathic nervous system, in collaboration with the glucocorticoid stress system, is also involved in fear related memory formation [33]. Hu and collegues [34] have shown that increased norepinephrine function in the amygdala lowers threshold for LTP and thus providing a molecular mechanism for the well known enhancing effect of emotion on learning and memory. In contrast, Maroun and Akirav [35] reported that increased arousal via activation of noradrenergic receptors in the amygdala is detrimental for the consolidation processes. This discrepancy suggests that emotional load or norandrenergic activity can both facilitate and impair cognitive functioning.
BALB/c mice, which are highly emotional and display higher amygdala beta-adrenoceptor expression [36] and noradrenergic activity [37] compared to C57BL/6J mice, display less stable consolidation or earlier onset of extinction than C57BL/6J mice. This might suggest that very high emotional or noradrenergic involvement in the BALB/c mice, and less emotional and noradrenergic involvement in C57BL/6J mice contributes to distinct fear related memory and extinction processes.

Timing of corticosterone treatment is important in revealing its effects on fear behaviour

As underlined by principal component analysis (PCA), pre-acquisition corticosterone mainly affects retention of fear behaviour in C57BL/6J mice, while post acquisition corticosterone predominantly affects retention of fear behaviour in BALB/c mice. Relevance of timing has been shown for the corticosterone effects on LTP [26;38]. In these studies, corticosterone facilitated LTP when given in the same time domain as the tetanus and then, even regulated beta-adrenergic modulation of LTP. We not only show that this timing effect has a cognitive functionality, but that it also differs between mouse strains.

Why corticosterone would affect fear behaviour in different time domains in BALB/c and C57BL/6J relies most likely on the background of the neuroendocrine and behavioural phenotype [12], and thus the aforementioned differences in fast corticosterone actions.

Conclusion

Conditioning of fear and testing of fear memory in an alternating cue and context set-up proves to be a promising approach towards a mouse model for PTSD and anxiety disorders. Strain-specific formation and extinction of fear memories, the importance of timing of corticosterone actions in BALB/c and C57BL/6J mice present a tool to study specific aspects of stress-related psychiatric disorders. (1) C57BL/6J mice might serve to address the strengthening of emotional memory related to certain cues under influence of stress and stress hormones, and thus the development of PTSD, while (2) BALB/c mice might serve as model to study strong context-related, rather generalized fear and the process of how stress hormones decrease fear memories, as also observed in successful treatment of PTSD and patients with phobias. (3) The neuroendocrine and behavioural phenotype of both strains (Brinks et al. 2007) is promising for the identification of biomarkers that are predictive for vulnerability or resilience to stress-related anxiety disorders.

ACKNOWLEDGEMENTS

This study was supported by the Netherlands Organization of Scientific Research NWO-Cognition 051.02.10, NWO-Aspasia 015.01.076 grant and the Trier-Leiden IRTG program supported by NWO DN 95-429. We thank Maarten Koper for his practical help and Harm Krugers and Olof Wiegert, University of Amsterdam for scientific discussions.

Reference List

1. A. Aerni, R.Traber, C.Hock, B.Roozendaal, G.Schelling, A.Papassotiropoulos, R.M.Nitsch, U.Schnyder, and D.J.de Quervain, Low-dose cortisol for symptoms of posttraumatic stress disorder, Am. J. Psychiatry 161 (2004) 1488-1490.

2. L.M. Soravia, M.Heinrichs, A.Aerni, C.Maroni, G.Schelling, U.Ehlert, B.Roozendaal, and D.J.de Quervain, Glucocorticoids reduce phobic fear in humans, Proc. Natl. Acad. Sci. U. S. A 103 (2006) 5585-5590.

3. W.H. Cai, J.Blundell, J.Han, R.W.Greene, and C.M.Powell, Postreactivation glucocorticoids impair recall of established fear memory, J. Neurosci. 26 (2006) 9560-9566.

4. M.I. Cordero, N.D.Kruyt, J.J.Merino, and C.Sandi, Glucocorticoid involvement in memory formation in a rat model for traumatic memory, Stress. 5 (2002) 73-79.

5. M.I. Cordero, C.Venero, N.D.Kruyt, and C.Sandi, Prior exposure to a single stress session facilitates subsequent contextual fear conditioning in rats. Evidence for a role of corticosterone, Horm. Behav. 44 (2003) 338-345.

6. V. Rau, J.P.DeCola, and M.S.Fanselow, Stress-induced enhancement of fear learning: an animal model of posttraumatic stress disorder, Neurosci. Biobehav. Rev. 29 (2005) 1207-1223.

7. B.L. Thompson, K.Erickson, J.Schulkin, and J.B.Rosen, Corticosterone facilitates retention of contextually conditioned fear and increases CRH mRNA expression in the amygdala, Behav. Brain Res. 149 (2004) 209-215.

8. B. Bohus and E.R.de Kloet, Adrenal steroids and extinction behavior: antagonism by progesterone, deoxycorticosterone and dexamethasone of a specific effect of corticosterone, Life Sci. 28 (1981) 433-440.

9. J. Micheau, C.Destrade, and B.Soumireu-Mourat, [Posttrial injections of corticosterone in dorsal hippocampus of the BALB/c mouse facilitate extinction of appetitive operant conditioning in the Skinner box], C. R. Seances Acad. Sci. III 294 (1982) 1109-1112.

10. B. Roozendaal, E.L.Hahn, S.V.Nathan, D.J.de Quervain, and J.L.McGaugh, Glucocorticoid effects on memory retrieval require concurrent noradrenergic activity in the hippocampus and basolateral amygdala, J. Neurosci. 24 (2004) 8161-8169.

11. V.Brinks, E.R.de Kloet, and M.S.Oitzl, Strain specific fear behaviour and glucocorticoid response to aversive events: modelling PTSD in mice. In: 2008, pp. 241-248.

12. V. Brinks, M.Van der Mark, E.R.de Kloet, and M.S.Oitzl, Emotion and cognition in high and low stress sensitive mouse strains: a combined neuroendocrine and behavioral study in BALB/c and C57BL/6J mice, Front. Behav. Neurosci (2007).

13. R.G. Phillips and J.E.LeDoux, Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning, Behav. Neurosci. 106 (1992) 274-285.

14. N.R. Selden, B.J.Everitt, L.E.Jarrard, and T.W.Robbins, Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues, Neuroscience 42 (1991) 335-350.

15. E.J. Bilsky, C.E.Inturrisi, W.Sadee, V.J.Hruby, and F.Porreca, Competitive and non-competitive NMDA antagonists block the development of antinociceptive tolerance to morphine, but not to selective mu or delta opioid agonists in mice, Pain 68 (1996) 229-237.

16. M.A. Morgan, J.Schulkin, and J.E.LeDoux, Ventral medial prefrontal cortex and emotional perseveration: the memory for prior extinction training, Behav. Brain Res. 146 (2003) 121-130.

17. L.A. Schimanski and P.V.Nguyen, Mouse models of impaired fear memory exhibit deficits in amygdalar LTP, Hippocampus 15 (2005) 502-517.

18. B. Bohus, The medial thalamus and the opposite effect of corticosteroids and adrenocorticotrophic hormone on avoidance extinction in the rat, Acta Physiol Acad. Sci. Hung. 38 (1970) 217-223.

19. B. Bohus, J.Grubits, and K.Lissak, Influence of cortisone on heart rate during fear extinction in the rat, Acta Physiol Acad. Sci. Hung. 37 (1970) 265-272.

20. E. Mikics, M.R.Kruk, and J.Haller, Genomic and non-genomic effects of glucocorticoids on aggressive behavior in male rats, Psychoneuroendocrinology 29 (2004) 618-635.

21. E. Mikics, B.Barsy, B.Barsvari, and J.Haller, Behavioral specificity of non-genomic glucocorticoid effects in rats: effects on risk assessment in the elevated plus-maze and the open-field, Horm. Behav. 48 (2005) 152-162.

22. M.S. Oitzl and E.R.de Kloet, Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning, Behav. Neurosci. 106 (1992) 62-71.

23. S. Cabib, C.Castellano, F.R.Patacchioli, G.Cigliana, L.Angelucci, and S.Puglisi-Allegra, Opposite strain-dependent effects of post-training corticosterone in a passive avoidance task in mice: role of dopamine, Brain Res. 729 (1996) 110-118.

24. C. Weiss, E.Sametsky, A.Sasse, J.Spiess, and J.F.Disterhoft, Acute stress facilitates trace eyeblink conditioning in C57BL/6 male mice and increases the excitability of their CA1 pyramidal neurons, Learn. Mem. 12 (2005) 138-143.

25. M. Rey, E.Carlier, and B.Soumireu-Mourat, Effects of corticosterone on hippocampal slice electrophysiology in normal and adrenalectomized BALB/c mice, Neuroendocrinology 46 (1987) 424-429.

26. O. Wiegert, M.Joels, and H.Krugers, Timing is essential for rapid effects of corticosterone on synaptic potentiation in the mouse hippocampus, Learn. Mem. 13 (2006) 110-113.

27. E. Thiels, N.N.Urban, G.R.Gonzalez-Burgos, B.I.Kanterewicz, G.Barrionuevo, C.T.Chu, T.D.Oury, and E.Klann, Impairment of long-term potentiation and associative memory in mice that overexpress extracellular superoxide dismutase, J. Neurosci. 20 (2000) 7631-7639.

28. J.L. Zhou, T.N.Shatskikh, X.Liu, and G.L.Holmes, Impaired single cell firing and long-term potentiation parallels memory impairment following recurrent seizures, Eur. J. Neurosci. 25 (2007) 3667-3677.

29. D.M. Yilmazer-Hanke, T.Roskoden, K.Zilles, and H.Schwegler, Anxiety-related behavior and densities of glutamate, GABAA, acetylcholine and serotonin receptors in the amygdala of seven inbred mouse strains, Behav. Brain Res. 145 (2003) 145-159.

30. P. Matus-Amat, E.A.Higgins, D.Sprunger, K.Wright-Hardesty, and J.W.Rudy, The role of dorsal hippocampus and basolateral amygdala NMDA receptors in the acquisition and retrieval of context and contextual fear memories, Behav. Neurosci 121 (2007) 721-731.

31. O. Stork, H.Yamanaka, S.Stork, N.Kume, and K.Obata, Altered conditioned fear behavior in glutamate decarboxylase 65 null mutant mice, Genes Brain Behav. 2 (2003) 65-70.

32. M. Van Nobelen and L.Kokkinidis, Amygdaloid GABA, not glutamate neurotransmission or mRNA transcription controls footshock-associated fear arousal in the acoustic startle paradigm, Neuroscience 137 (2006) 707-716.

33. B. Roozendaal, G.K.Hui, I.R.Hui, D.J.Berlau, J.L.McGaugh, and N.M.Weinberger, Basolateral amygdala noradrenergic activity mediates corticosterone-induced enhancement of auditory fear conditioning, Neurobiol. Learn. Mem. 86 (2006) 249-255.

34. H. Hu, E.Real, K.Takamiya, M.G.Kang, J.Ledoux, R.L.Huganir, and R.Malinow, Emotion enhances learning via norepinephrine regulation of AMPA-receptor trafficking, Cell 131 (2007) 160-173.

35. M. Maroun and I.Akirav, Arousal and stress effects on consolidation and reconsolidation of recognition memory, Neuropsychopharmacology 33 (2008) 394-405.

36. N.N. Dyaglo and G.T.Shishkina, Genetic differences in the synthesis and reception of noradrenaline in the mouse brain and behavior in a novel environment, Neurosci Behav. Physiol 30 (2000) 327-330.

37. B. Tannenbaum and H.Anisman, Impact of chronic intermittent challenges in stressor-susceptible and resilient strains of mice, Biol. Psychiatry 53 (2003) 292-303.

38. Z. Pu, H.J.Krugers, and M.Joels, Corticosterone time-dependently modulates beta-adrenergic effects on long-term potentiation in the hippocampal dentate gyrus, Learn. Mem. 14 (2007) 359-367.