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

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Chapter 4

Strain specific fear behaviour and glucocorticoid response to aversive events: modelling PTSD in mice

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ABSTRACT

Post Traumatic Stress Disorder (PTSD) is a stress related disease that has large individual vulnerability. It can develop weeks or even months after a traumatic stressful experience and is characterised by intrusive persistent memories of the traumatic event and changes in the glucocorticoid stress system. Here, we works towards an animal model for PTSD using a modified fear conditioning paradigm in which we can (i) follow learning/acquisition of the negative event by measuring scanning and freezing behaviour, (ii) test memory/retrieval processes for both context and cue after a delay of 24 hrs, (iii) measure corticosterone as endocrine stress parameters before and in response to conditioning and (iv) show the influence of the genetic background on acquisition and retrieval of the negative event. By using two mouse strains, with distinct stress system markers (BALB/c and C57BL/6J) we expect our results to be more representative for the individual vulnerability to stress-related disorders.

BALB/c mice have high fear behaviour with corresponding corticosterone response indicative for a generalised fear response. They display strong fear acquisition/learning, but also strong memory for the negative event. In contrast, C57BL/6J mice display lower fear behaviour during learning, but very strong memory for the cue. Concerning "PTSD like" symptomatology, C57BL/6J mice seem to be more vulnerable to cue specific "flashbacks", while BALB/c mice are suitable for studying generalised fear memory. Fear-extinction paradigms should reveal the capacity to extinguish fear memory.

INTRODUCTION

Post Traumatic Stress Disorder (PTSD) has a clear-cut stress-related onset and genetic components that determine its occurrence. PTSD can develop in the course of weeks or even months after the traumatic event has taken place. Behavioural characteristics are intrusive persistent memories of the trauma, avoidance behaviour and hyperarousal. Besides behavioural symptomatology, also changes in endocrine systems of HPA-axis activity, the glucocorticoid related part of the stress system are present. People suffering from PTSD are reported to have low basal cortisol levels, increased sensitivity to stress and glucocorticoid negative feedback [1]. Furthermore, the volume of the hippocampus, a brain target of glucocorticoid stress hormones, is lower in PTSD patients, indicating a period of strong or prolonged exposure to high stress-hormone concentrations [2;3]. This makes the stress system / HPA-axis activity a key player in PTSD research.

More and more biological data on PTSD patients is published [4-7]. At present, only animal research can provide clues to uncover the molecular mechanisms. But, like all other psychiatric disorders, animal models will never cover all aspects of PTSD. The parameter (component) of strengthened memory for the adverse event offers a central access for animal research focussing on fear conditioning and its molecular mechanisms.

In fear conditioning, an unexpected, for the mouse even of unknown quality, aversive stimulus such as an electric shock (unconditioned stimulus UCS), is given once or several times in association with a non aversive stimulus (cue; conditioned stimulus CS) such as a light and/or tone, in a distinct environment (context). This is the well-known Pavlovian conditioning paradigm. The animal will remember the association between the announcing cue and aversive stimulus but also the surrounding in which the aversive stimulus was given. Thus, placing the animal in the same context and/or turning on the light/tone that was previously associated with an electric foot shock, will evoke a fear response expressed in mice and rats as scanning and freezing behaviour (conditioned response, CR). We defined scanning as immobility of the body, while the head is moving horizontally from side to side. The animal is still actively interacting with its environment. We defined freezing as immobility of the body and head and is devoid of interaction with the environment. Although scanning and freezing are interdependent, they express a different quality of fear. With automatic scoring, both scanning and freezing are measured as immobility behaviour.

The present experiment works towards an animal model for PTSD in which we can (i) follow learning/acquisition of the negative event by measuring scanning and freezing behaviour, (ii) test memory/retrieval processes for both context

and cue after a delay of 24 hrs, (iii) measure corticosterone as endocrine stress parameters before and in response to conditioning and (iv) show the influence of the genetic background on acquisition and retrieval of the negative event. By using two mouse strains, we expect our results to be more representative for the individual vulnerability to stress-related disorders.

MATERIAL AND METHODS

Male BALB/c and C57BL/6J mice (n=8 per group; 3-month-old) were subjected to a specific fear conditioning paradigm that allowed to differentiate context and context/cue related responses in the same setting. This included 10 light/tone+shock pairings with a one minute interval on day 1. Pairings were as follows; light (260 lux) and tone (70dB) were given simultaneously for 20 seconds of which an additional shock (0.4 mA) was administrated at the last two seconds. Scanning and freezing was measured when the animals were placed in the setting (Figure 1, point 1) and during the 1-min intervals after light/tone+shock pairings (Figure 1, points 2-11). Memory of this association, expressed as scanning and freezing was estimated 24 hrs later on day 2. Then, mice were returned to the same box: 3 min exposure to the setting (context only) was followed by 2 min of light/tone exposure (context/cue) and ended with 2 min exposure to the setting (context only). Plasma corticosterone was estimated at several time points: on the day before conditioning, after conditioning and after retention testing (see Table 1). General Linear Model-Repeated Measures was used to test for significant progression of scanning and freezing over conditioning intervals on day 1. Students T-test for independent variables was used to compare percentage scanning and freezing for context and context/cue between strains on day 2. Furthermore, Students t-test statistics were used to compare corticosterone concentrations of the two strains at each time point and to basal corticosterone values before the experiment started.

RESULTS

Figure 1A and B presents the percentages of freezing and scanning in both strains on days 1 and 2.

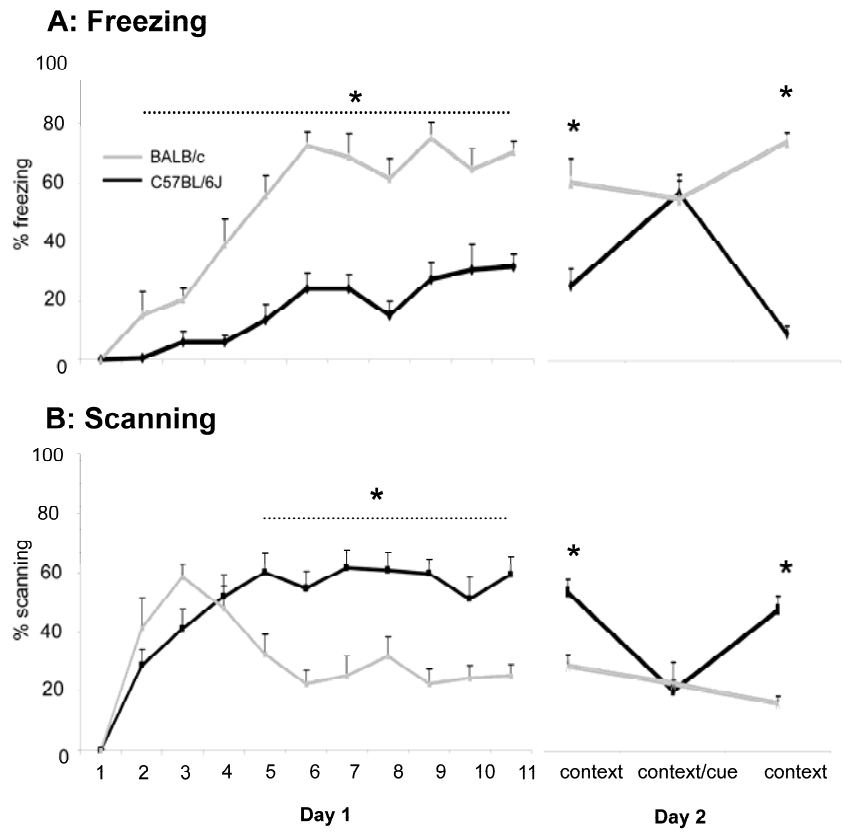

Figure 1. A. Percentage freezing of C57BL/6 (black line) and BALB/c mice (grey line). B. Percentage scanning of C57BL/6 (black line) and BALB/c mice (grey line). Day 1: Acquisition; time point 1 represents scanning and freezing during the first minute in the setting; time points 2-11 represent scanning and freezing in the 1-min intervals between the 10 light/tone+shock pairings. Day 2: memory/retrieval; scanning and freezing during context (3min), context/cue (2 min) and context (2 min) exposure is presented. Data are presented as mean (± SEM) percentage of behaviour. Horizontal lines and asterisks indicate significant differences between groups. Significance was accepted at $p < 0.05$.

On day 1, when the light/tone+shock pairings took place (conditioning), the percentage of freezing progressively increased for both C57BL/6J and BALB/c mice ($F(10,140)$ 25.710, $p=0.000$), albeit to a different degree ($F(10,140)$ 4.860, $p=0.000$). BALB/c mice displayed faster increase in freezing resulting in a plateau at approximately 70%, while freezing in C57BL/6J mice reached approximately 30-40%. Also scanning behaviour increased in both mouse strains ($F(10,140)$ 12.279, $p=0.000$) to different degrees ($F(10,140)$6.662 $p=0.000$). Group differences for scanning and freezing appear at separate time points: distinct

scanning behaviour starts at interval 5, while freezing percentages differ from interval 2 onwards. Also, there is a strain-dependent main effect for freezing and scanning: C57BL/6J mice display high scanning and lower freezing behaviour, while BALB/c mice have high freezing and low scanning behaviour. Interestingly, total immobility measured by scanning and freezing together is the same for C57BL/6J and BALB/c mice.

On day 2, BALB/c mice displayed more freezing compared to C57BL/6J mice when first exposed to the context ($F(1,14)$ 10.551, $p=0.001$). For both strains, this percentage of freezing is comparable to the last freezing response on day 1. Next, the light/tone cue was presented. This resulted in a comparable amount of freezing in C57BL/6J and BALB/c mice ($F(1,14)$ 12.921, $p= 0.857$). Subsequently switching off the cue (and thus only exposure to the context) again separated the strains ($F(1,14)$ 12.988, $p=0.000$). C57BL/6J mice reduced their freezing while BALB/c mice even increased their freezing response to the context.

Scanning of BALB/c mice was lower when first exposed to the setting compared to C57BL/6J ($F(1,14)$ 9.873, $p=0.008$), however in both strains comparable to the last scanning data on day 1. When presenting the light/tone cue, C57BL/6J and BALB/c mice displayed similar low percentage of scanning ($F(1,14)$ 13.688, $p=0.689$). Differentiation between strains occurred again when the cue was turned off ($F(1,14)$ 9.930, $p=0.000$).

Plasma corticosterone concentrations mirrored the behavioural response of C57BL/6J and BALB/c mice for acquisition and retrieval of fear memories (see table 1). At 30 and 60 minutes after onset of testing on day 1, corticosterone concentrations were twofold higher in BALB/c compared to C57BL/6J mice (30 min: $F(1,6)$ 5.761, $p= 0.000$, 60 min: $F(1,6)$ 5.111, $p= 0.002$). On day 2, corticosterone concentrations of BALB/c mice were increased compared to C57BL/6J mice at 30 min ($F(1,6)$ 4.972, $p= 0.027$), but returned to comparable low levels at 60 min. Undisturbed basal morning resting corticosterone concentrations were comparable between strains ($F(1,14)$ 10.589, $p= 0.483$) and significantly lower than all 30 and 60 min data.

		Day 1. Acquisition		**Day 2. Retrieval**	
	Basal	**30 min**	**60 min**	**30 min**	**60 min**
C57BL/6J	11.2 ± 2.4	172.5 ± 10.0*	94.9 ± 15.5*	84.0 ± 9.6*	50.4 ± 12.5
BALB/c	9.0 ± 1.3	370.4 ± 12.6*	189.0 ± 10.0*	137.4 ± 15.7*	54.3 ± 8.8

Table 1. Plasma concentrations of corticosterone in ng/ml (mean ± SEM) of C57Bl/6J and BALB/c mice measured on the day before conditioning (basal morning values), day 1 (30 and 60 min after the start of conditioning) and day 2 (30 and 60 min after start of retention test). Corticosterone assay was performed with the use of a commercially available radio immune assay kits (MP Biomedicals Inc., Calif., USA). Data is represented as mean ± SEM. Significant differences: all 30 and 60 min samples compared to basal concentrations, * C57BL/6 versus BALB/c mice. Significance was accepted at $p < 0.05$.

DISCUSSION

The two mouse strains used in this study show distinctly different fear responses during conditioning. BALB/c mice display the fastest increase and highest plateau in freezing, while scanning is the main fear response in C57BL/6J mice. During retrieval on day 2, both strains have the same anticipatory amount of freezing and scanning to the cue, but differ in their response to context. BALB/c mice lack the discrimination between context and cue, showing a comparable amount of freezing to context and cue, which indicates a generalised and even potentiated fear response. C57BL/6J mice clearly differentiate between context and cue by adapting the ratio of scanning and freezing accordingly. Only few fear conditioning studies have been performed with C57BL/6 and BALB/c mice together. In one study, mice were subjected to two cue-shock pairings and tested for context and cued fear memory expressed as immobility only. With this method, generalised freezing was observed in C57BL/6J, but not BALB/c mice [8]. Another study showed that BALB/c mice have a memory impairment for the aversive event when exposed to the cue only [9]. Apparently, severity of the conditioning protocol (number of shocks) and type of memory testing (separate or combined context and cue-retrieval) are important factors influencing fear behaviour. Our study is the first to measure scanning and freezing behaviour separately, and has of advantage that differences between strains (or treatments) are more pronounced. Measuring total immobility (scanning and freezing), which was similar for C57BL/6J and BALB/c mice, would not have been effective in showing differences in fear behaviour this experiment.

Our model therefore allows a differentiation between different qualities of fear: the more active fear behaviour expressed by scanning and the rather passive fear behaviour indicated by freezing. Recognizing the light/tone stimulus as threat and freezing in anticipation of the negative event can be considered as an adaptive response. Increased scanning in the context indicates a more active coping strategy that might allow possibilities to escape the expected aversive event [10].

The endocrine data after acquisition and retention test shows that BALB/c mice are more corticosterone responsive to our fear conditioning paradigm compared to C57BL/6J mice. Here, corticosterone concentrations might be indicative for behavioural responses to and in anticipation of the negative event. Furthermore, the increased corticosterone concentration after conditioning likely facilitates the consolidation process [11].

Different brain areas are involved in context and cue fear conditioning. The role of the hippocampus is more in the spatial aspect of conditioning, i.e., associating the environment/context with the shock. The amygdala is involved

in the association between cue and shock [12]. Knowing this, we may conclude that for C57BL/6J and BALB/c mice, the hippocampus and amygdala differentially contribute to learning and memory processes involved in fear conditioning. Furthermore, the amygdala and hippocampus are both targets for corticosterone action, so their functionality in this fear conditioning paradigm could be modulated by different corticosterone concentrations [13].

Our data shows that consolidation and possibly retrieval processes are different for C57BL/6J and BALB/c mice. While C57BL/6J mice display distinct fear responses to context and additional cue, BALB/c mice show generalised fear to both. This raises the question whether extinction processes would also be different between these strains. People with PTSD have recurring intrusive memories for the negative event that do not extinguish. So studying this process in C57BL/6J and BALB/c mice should be the next step in modelling PTSD in mice.

The different fear behaviours, possibly influenced by differential contributions of the amygdala and hippocampus, and corticosterone response in the C57BL/6J and BALB/c mice can be explained by genetic make-up but also by differences in environmental factors such as maternal care [14]. BALB/c dams display low maternal care behaviour (nursing, licking and grooming) compared to C57BL/6 dams. Cross fostering BALB/c pups with C57BL/6J dams resulted in reduced anxiety behaviour and basal corticosterone concentrations of the BALB/c mice in later life, showing the role of early life events and a differentially organized stress system for the phenotype. This also corresponds to the fact that vulnerability for PTSD in humans is influenced by traumatic early life events [15].

The behavioural and endocrine data shows that our fear conditioning model can be used to follow acquisition of a negative event, but also to test context and context/cue retrieval processes. A distinct genetic background and early life programming seem to be important for the acquisition and memory of fear. With this model we can therefore mimic some PTSD symptoms in mice.

Conclusion

The BALB/c mice show high fear behaviour with corresponding corticosterone response indicative for a generalised fear response. They display strong fear acquisition/learning, but also strong memory for the negative event. C57BL/6J mice have lower fear behaviour during learning, but very strong memory for the cue. Concerning “PTSD like” symptomatology, C57Bl/6J mice seem to be more vulnerable to cue specific “flashbacks”, while BALB/c mice are suitable for studying generalised fear memory. Fear-extinction paradigms should reveal the capacity to extinguish.

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