The effects of a reminder of underwater trauma on behaviour and memory-related mechanisms in the rat dentate gyrus

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Abstract

Intrusive re-experiencing is a core symptom in post-traumatic stress disorder (PTSD), often triggered by contextual cues associated with the trauma. It is not yet clear if intrusive re-experiencing is only the result, or whether it may contribute to the establishment of PTSD following acute stress. This study aimed at examining the impact of an underwater trauma (UWT) reminder on anxiety-like behaviour and on neuronal activity and plasticity in the hippocampus and the amygdala. Sprague–Dawley rats were exposed to UWT and 24 h later were re-exposed to the context. The effects on behaviour, activation of the amygdala (BLA) and dentate gyrus (DG), and on long-term potentiation (LTP) and local circuit activity (frequency-dependent inhibition (FDI) and paired-pulse inhibition (PPI)) in the DG were assessed. The exposure to UWT by itself resulted in increased anxiety behaviour in the open field, together with increased PPI. Upon exposure to the UWT reminder, an additional increase in anxiety was also observed in the EPM and in FDI. Moreover, reminder exposure resulted in impaired DG LTP and a significant BLA extracellular-signal-regulated kinases (ERK) 2 activation. In conclusion, these observed effects of exposure to a trauma reminder, following the exposure to the initial trauma, might be associated with the progression of trauma-related pathologies and the development of related disorders.

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Introduction

Life-threatening experiences trigger a severe stress response, the effects of which on anxiety, learning and memory and brain functioning has long been recognized. Moreover, traumatic events can be associated with surrounding contextual elements (i.e. sounds, smells, locations) which can later elicit trauma-like manifestations (Bower and Sivers, 1998; Elzinga and Bremner, 2002; Ehlers et al., 2004). Intrusive re-experiencing is a core symptom of post-traumatic stress disorder (PTSD) and can take various forms, including intrusive images, nightmares, distress and physiological reactions. Often, these are triggered by re-exposure to contextual cues associated with the trauma. However, it is unclear whether such responses have unique associated neuropathology that may be addressed in relevance to PTSD treatment (Armario et al., 2008).

The underwater trauma (UWT) has been proposed as a model of a brief, traumatic experience, with ethological relevance to experimental animals (Richter-Levin, 1998). The model involves a sudden and brief restraint underwater. It has been demonstrated that exposure of rats to UWT results in increased anxiety behaviour (Richter-Levin, 1998; Cohen et al., 2004), context-specific spatial memory deficits (Richter-Levin, 1998; Wang et al., 2000) and impairment in long-term potentiation induction in the dentate gyrus (DG) that is correlated with memory deficits found in the Morris water maze (Wang et al., 2000). Exposing animals to reminder cues of the UWT may serve as an effective platform to elucidate neural mechanisms associated with the behavioural responses to that reminder cue.

The hippocampus and amygdala are known for their involvement in interactions between stress and memory. The hippocampus plays a key role in the neuroendocrine regulation of the stress response (Kim and Diamond, 2002), and stress exposure impairs hippocampal-dependent memory in both humans and animals (Lupien and McEwen, 1997). The amygdala is involved in emotional modulation of memory, partly through the modulation of hippocampal functions (McGaugh, 2002, 2004). The activation of the amygdala was found to correlate with the intensity of the stressful experience, and the basolateral nucleus (BLA) is activated in PTSD patients.
exposed to reminder cues (Liberzon et al., 1999). However, it is not yet clear if intrusive re-experiencing is only the result or whether it may contribute to the establishment of PTSD following acute stress.

Here we analysed the effects of exposure to a trauma reminder by employing behavioural assessments (elevated plus maze and open field) that aim to evaluate the emotional state of the animal (Pellow et al., 1985; Hiroi and Neumaier, 2006). In addition, the effects of trauma reminder on neural activity and plasticity were also evaluated. Long term potentiation (LTP) of synaptic transmission is the most studied neurophysiological model for learning and memory processes in the mammalian nervous system, and is a sensitive readout for stress and memory interactions. Indeed, stress was found to suppress LTP in the cornus ammonis 1(CA1) area of the hippocampus, but to have a differential effect in the DG. In fact, depending on the stress paradigm being used, stress can impair (Shors and Dryver, 1994; Akirav and Richter-Levin, 1999), enhance (Kavushansky et al., 2001) LTP in the DG.

Another level of processing that might be relevant to memory formation is local circuit activity, which involves interactions between local, mostly inhibitory GABAergic neurons, and pyramidal or granular principle cells in the hippocampus and cortex (Freund and Antal, 1988; Freund and Buzsaki, 1996). Local circuit activity in the hippocampus has been proposed to participate in regulating memory processes (Maroun and Richter-Levin, 2002) and it was demonstrated that a stressful experience may lead to alterations in local circuit activity in the DG (Yarom et al., 2008). It is, therefore, conceivable that local circuit activity would be affected by the UWT reminder.

Moreover, given the modulatory role of the BLA on hippocampal functioning (McCaugh, 2002, 2004), BLA activation could be expected to be triggered by exposure to UWT reminder. Therefore, in the current study we examined the impact of exposure to UWT reminder on (1) anxiety behaviour, measured with the elevated plus maze and open field tests, (2) neuronal activity and plasticity in the DG, as well as local circuit activity within this area, and (3) neuronal activation of the BLA and the DG by quantification of extracellular-signal-regulated kinases (ERK) 2 activation.

Methods and materials

Animals

Experiments were performed using male Sprague-Dawley rats (~60 d old, 250–350 g, Harlan Laboratories, Israel). Rats were housed four per cage (22±2 °C; light–dark cycle: 12/12 h, lights on at 07:00 hours) with water and food ad libitum. Animals were allowed 5 d of acclimation to the vivarium before behavioural manipulations began. All experimental procedures were performed between 08:00 hours and 17:00 hours, adhered to the NIH Guide for the care and use of laboratory animals and were approved by the University of Haifa ethical committee.

Experimental design

Rats were randomly assigned to one of the experimental groups:

1. Underwater trauma+reminder (‘UWT+R’) – Rats exposed to swim trials, underwater trauma and reminder.
2. Underwater trauma (‘UWT’) – Rats exposed to swim trials and underwater trauma, without reminder.
3. Swim+reminder (‘swim+R’) – Rats exposed to swim trials and to the reminder.
4. Swim (‘swim’) – Rats exposed only to swim trials.
5. ‘Naïve’ – Rats not exposed to swim trials, underwater trauma, or the reminder.

Rats were given swim trials for five consecutive days, one trial per day. Each day, rats were habituated to the room for 1 min, and then placed in a plastic tank (diameter 40 cm, height 45 cm) that contained water (30 cm deep) at 22±1 °C and allowed to swim for 1 min.

On day 6, ‘UWT’ rats were exposed to the UWT while ‘swim’ rats were exposed to an additional swim trial. For the UWT, rats were given 1 min habituation to the room, followed by 30 s of free swimming in a plastic tank and then they were held underwater for an additional 30 s using a metal net (20×20×15 cm).

On day 7, half of the rats from the ‘UWT’ and ‘swim’ groups were exposed to the reminder, while the other halves were left in their home cage. The reminder consisted of 30 s of swimming in the same tank used for the swim trials and the UWT. Following the reminder exposure, ‘swim+R’ and ‘UWT+R’ rats returned to their home cages for 30 min and were then taken to behavioural tests, to electrophysiological recordings or were decapitated for biochemical analysis. Different sets of animals were used for each experiment.

‘Swim’ and ‘UWT’ rats were tested 24 h following the last exposure (i.e. UWT or swimming). Naïve rats were not exposed to any of the aforementioned treatments (swimming, UWT or reminder) and were taken directly from their home cage.

Behavioural assessment

Open field test

The open field consisted of a dimly-lit, ventilated, sound-attenuated Plexiglas box (50×50×38 cm), divided into 25 equal squares of 10×10 cm each. Following 5 min of habituation to the room, the rat was placed at one corner of the open field facing the wall, and allowed to explore the arena for 5 min while its behaviour was videotaped. Each session was analysed offline by an experimenter blind to animal groups.
Elevated plus maze test

The elevated plus maze was placed 70 cm above the floor and consisted of two open arms and two closed arms (with 30 cm high walls and no roof), arranged in a way that similar arms are opposite to each other. Following 5 min of habituation to the room, the animal was placed in the centre of the maze, facing an open arm, and allowed to explore the arena for 5 min, while its behaviour was video-taped. Each session was analysed offline by an experimenter blind to animal groups.

Electrophysiology

Surgery

Rats were anesthetized (40% urethane +5% chloral hydrate, 0.5 ml/100 g, i.p.) and mounted on a stereotaxic frame (Stoelting, USA). Body temperature was monitored and maintained at 37 °C±0.5 by a regulated heating pad. The scalp was incised and retracted, and the head position was adjusted to place bregma and lambda in the same horizontal plane. Small burr holes were drilled in the skull for the placement of electrodes. A 125 μm coated wire reference electrode was affixed to the skull in the area overlapping the nasal sinus. A recording glass electrode (tip diameter, 2-5 μm; 2M NaCl) was positioned in the DG granular cell layer (4.0 mm AP, 2.5 mm ML and 3.2–3.7 mm DV from dura). The dorsal/ventral location of recording and stimulating electrodes was adjusted to maximize the amplitude of evoked field potentials.

Stimulating and recording procedures

DG field potentials evoked by a single pulse to the PP (0.1 ms rectangular monophasic pulses delivered at 0.1 Hz.), were amplified (+100) (AM systems), digitized at 10 kHz (CED) and stored on a PC hard drive for off-line analysis (Spike 2 software). Baseline responses were established by means of stimulation intensity sufficient to elicit a response representing 30–50% of the maximal amplitude of the evoked-field potential. After positioning the electrodes, the rat was left undisturbed for 20 min before commencing the experiment. Measurements of input–output response were made to determine the stimulation intensity for the baseline response. The following recording protocol was then conducted: first baseline (1st baseline) recorded for 30 min, immediately followed by local circuit protocols of frequency dependent inhibition (FDI) and paired-pulse inhibition (PPI) (in that order, see below). After that, a second baseline (2nd baseline) was recorded for 20 min to serve as pre-theta-burst stimulation (TBS) values. This was followed by a TBS and LTP measurements for 30 min.

Local circuit activity

Frequency dependent inhibition (FDI) index. FDI was determined as described previously (Sloviter, 1991; Rosenblum et al., 1999). Briefly, 10 baseline pulses were delivered to the PP at 0.1 Hz, followed by 10 pulses delivered at 1 Hz. The population spike (PS) amplitude of the 0.1 Hz stimulation was averaged and compared to that of PP stimulation at 1 Hz. The averaged response to 0.1 Hz stimulation was set as 100%, and the averaged responses at 1 Hz are expressed as the percent change of the response at 0.1 Hz. (FDI index, e.g. Fig. 2(a)).

Paired-pulse inhibition (PPI) index. Guided by earlier studies (Andersen et al., 1966; Richter-Levin and Segal, 1991; Sloviter, 1991), paired-pulse inhibition (PPI) was measured by applying five pairs of two constant stimuli to the PP at inter-stimuli interval of 15 ms. The PS amplitude of the response to the second stimulus was compared to the PS amplitude of the response to the first stimulus. The response to the first stimulus was set as 100%, and the net mediated inhibition measured by paired pulse protocol is expressed as the percent change of the PS amplitude of the second response compared to the PS amplitude of the first one (PPI index, e.g. Fig. 2(b)).

LTP induction. TBS of the PP was used to induce LTP. The TBS protocol included three sets of 10 trains each, each train consisting of 10 pulses at 100 Hz, at baseline stimulation intensity (inter-train interval: 200 ms; inter-set interval: 1 min). LTP was measured as the difference in EPSP slope before and 30 min after TBS.

Western blot

Thirty minutes following exposure to the reminder rats were decapitated, their brains were removed and snap-frozen using dry ice powder. Coronal slices, 2 mm thick, containing the relevant regions, were dissected according to the atlas of Paxinos and Watson (1998). Dorsal DG and BLA regions were incised bilaterally with a sterile 2 mm spatula. Tissues were collected into 1.5 ml eppendorf tubes, frozen in liquid nitrogen and stored at −80 °C until further use.

Tissues were homogenized in a glass Teflon homogenizer in 300 μl of ice-cold urea lysis buffer (1 mM EDTA, 0.5% Triton X, 6 M Urea, 100 μM PMSF) with freshly added protease and phosphatase inhibitors (0.1 mM sodium orthovanadate, 1 lg/ml leupeptine, 1.6 lg/ml aprotinin, 5 μM NaF, and 1 lg/ml protease inhibitor cocktail P2714; all reagents: Sigma, USA). 10 μg of each sample were subjected to 10% SDS-PAGE and transferred to nitrocellulose membrane. After Ponceau staining (Sigma, USA), blots were blocked for unspecific binding and incubated with primary antibodies: anti-ERK1/2 (p44/42 MAP kinase) and anti-p-ERK2 (phospho-p44/42 MAP kinase; Thr202/Tyr204), both diluted to 1:1000.
(Cell Signaling, USA), in TBST at 4°C overnight. Signals were detected with horseradish-peroxidase coupled secondary anti-rabbit antibody (1:10,000, Cell Signaling) and EZ-ECL chemiluminescence substrate (Amersham, USA). Quantification was performed with a CCD camera (XR6 BioRad) and Quantity One 1-D Analysis software. Phosphorylation levels were calculated as the optical density (OD) ratio between the phosphorylated (phospho-ERK2) and the non-phosphorylated (ERK2) and normalized to the ‘naïve’ group values. Only exposures that were in the linear range of the ECL reaction were used for quantification analysis, with data being presented as the ratio of phospho-ERK2/total ERK2 in the sample, while p-ERK1 was not quantified.

**Statistical analysis**

The results are expressed as means±SEM. Data was analysed with SPSS 15, using one-way, or repeated measures, analysis of variance (ANOVA). All post-hoc comparisons were made using the least significant difference multiple comparison test.

**Results**

**Behavioural effects of UWT reminder**

**Open field test**

One way ANOVA indicated a significant main effect for the different experimental conditions on activity and time spent in the centre of the arena (Fig. 1(a, b), p<0.001, see legends for detailed statistical analysis). Further post-hoc comparisons indicated that while ‘UWT’ rats were less active in the centre of the arena compared to ‘naïve’ and ‘swim’ rats and spent less time in the centre of the arena compared only to ‘naïve’ rats, ‘UWT+R’ rats spent less time and were less active in the centre of the arena, compared to all other groups (Fig. 1(a, b)).

In addition, one-way ANOVA did not reveal any significant difference in the total activity (total number of line crossing) in the open field between the different groups (F4,55=1.457, n.s., data not shown).

**Elevated plus maze test**

One way ANOVA indicated a significant main effect for the different experimental conditions on number of entries and time spent in the open arms of the arena (Fig. 1(c, d, p<0.001 and p<0.01, respectively). Further post-hoc comparisons indicated that ‘UWT+R’ rats entered less frequently and spent less time in the open arms of the arena compared to all other groups (Fig. 1(c, d)).

In addition, one-way ANOVA did not reveal any significant difference in the total activity (total number of line crossing) in the elevated plus maze between the different groups (F4,55=1.052, n.s., data not shown).

**Effects of UWT reminder on local circuit activity and LTP induction in the DG**

One-way ANOVA did not reveal any significant difference in stimulus intensities applied to all groups (Table 1, n.s.). Comparison between the groups using ANOVA with repeated measures for the time points before the application of local circuit protocols (‘1st baseline’) did not reveal any significant difference in PS amplitude or in EPSP slope (Table 2, n.s.).

**The effects UWT reminder on local circuit activity in the DG**

**Frequency-dependent inhibition.** Altering the frequency of the stimulation to the PP from 0.1 to 1.0 Hz resulted in a significant reduction of the PS amplitude in all groups, as indicated by the FDI index (Fig. 2(c), one-way ANOVA, p<0.01). Further post-hoc comparisons indicated that ‘UWT+R’ rats showed stronger inhibition of PS amplitude compared to all the other groups (Fig. 2(c)).

**Paired-pulse inhibition.** One way ANOVA revealed a significant reduction of the PS amplitude of the response to the second stimulus compared to the response to the first stimulus at a 15 ms interval in all groups, as indicated by the PPI index (Fig. 2(d), p<0.001). Further post-hoc comparisons indicated that compared to all other groups, both ‘UWT’ and ‘UWT+R’ rats exhibited stronger inhibition of PS amplitude as was expressed by the low PPI index (Fig. 2(d)).

**The effect of exposure to the reminder on LTP induction in the DG.** Comparison between the groups using ANOVA with repeated measures for time points before the application of TBS (‘2nd baseline’) did not reveal any significant difference in PS amplitude or in EPSP slope (Table 3, n.s.).

Comparison between the different groups using ANOVA with repeated measures for time points after the application of TBS did not reveal any significant difference in PS amplitude potentiation (Fig. 3(a), n.s.).

Comparison between the different groups using ANOVA with repeated measures for time points after the application of TBS revealed a significant effect for the different experimental conditions on EPSP slope potentiation (Fig. 3(b), p<0.05). Further post-hoc comparisons indicated that ‘UWT+R’ rats exhibited reduced potentiation of EPSP slope, compared to all the other groups (Fig. 3(b)).

**The effect of the exposure to the UWT reminder on ERK2 activation in the DG and the BLA**

**BLA ERK2 activation**

One way ANOVA indicated a significant main effect for the different experimental conditions on ERK2 activation (pERK2 as a percentage of total ERK2 expression) (Fig. 4(c), F4,40=4.18, p<0.01). Further post-hoc
comparisons indicated that ‘UWT+R’ rats showed higher ERK2 activation compared to all other groups (Fig. 4(c)).

DG ERK2 activation

One-way ANOVA did not reveal any significant difference between the groups in ERK2 activation (Fig. 4(b), pERK2 as a percentage of total ERK2 expression, n.s.).

Discussion

The objectives of this work were to further validate the UWT as a model for studying re-experiencing symptoms and to characterize the consequences of exposure to a trauma reminder.

Exposure of rats to swim sessions had only minor effects on activity in the open field, and no effects on activity in the EPM, on LTP induction or on local circuit properties in the DG. Similarly, these animals did not exhibit significant changes in ERK2 activation, whether in the BLA or in the DG. In contrast, in accordance with previous studies (Richter-Levin, 1998; Wang et al., 2000; Cohen et al., 2009), undergoing the traumatic experience of UWT resulted in altered behavioural and electrophysiological responses even 24 h after the exposure to the trauma. However, re-exposure to a trauma reminder resulted in additional alterations, not seen in UWT exposed animals tested without a reminder cue, including altered FDI index, reduced potentiation of the EPSP.
Overall, these data demonstrate that re-exposure to a trauma-associated reminder 24 h after the trauma itself elicits specific, reminder-related responses, as was suggested before (Liberzon et al., 1999).

Table 1. Stimulation intensities

<table>
<thead>
<tr>
<th>Group</th>
<th>Intensity (mA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>1.51±0.17</td>
</tr>
<tr>
<td>Swim</td>
<td>1.35±0.14</td>
</tr>
<tr>
<td>Swim+R</td>
<td>1.55±0.19</td>
</tr>
<tr>
<td>UWT</td>
<td>1.48±0.17</td>
</tr>
<tr>
<td>UWT+R</td>
<td>1.53±0.11</td>
</tr>
</tbody>
</table>

This table summarizes the mean stimulation intensities applied to the different groups (One way ANOVA, F_{4,35}=0.24, n.s.).

Table 2. First baseline

<table>
<thead>
<tr>
<th>Group</th>
<th>PS amplitude (mV)</th>
<th>EPSP slope (mV/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>3.83±0.31</td>
<td>5556.02±418.72</td>
</tr>
<tr>
<td>Swim</td>
<td>2.70±0.18</td>
<td>6272.27±445.52</td>
</tr>
<tr>
<td>Swim+R</td>
<td>3.87±0.38</td>
<td>5798.73±347.97</td>
</tr>
<tr>
<td>UWT</td>
<td>2.40±0.18</td>
<td>6423.65±648.16</td>
</tr>
<tr>
<td>UWT+R</td>
<td>3.28±0.42</td>
<td>5768.86±374.22</td>
</tr>
</tbody>
</table>

This table summarizes the averaged PS amplitude and EPSP slope baselines. No significant difference was observed between groups (One way ANOVA, F_{20,118}=1.10, ns; F_{20,118}=1.21, n.s., respectively).

Fig. 2. Effects of UWT reminder on local circuit activity in the DG. (a) Representative field potential response of DG granule cells to stimulation of the PP at 0.1 Hz (left) or at 1.0 Hz (right). Altering the frequency of stimulation from 0.1 to 1.0 Hz resulted in suppression of the PS amplitude. (b) Representative field potential responses to double stimulation of the PP at 15 ms interval. Applying two constant stimuli to the PP at inter-stimuli interval of 15 ms resulted in suppression of the PS amplitude of the second response. (c) Frequency dependent inhibition: FDI was measured by comparing field potential response of DG granule cells to stimulation of the PP at 0.1 Hz and 1.0. Altering the frequency of stimulation resulted in a reduction of the PS amplitude expressed as FDI index. The exposure to UWT reminder increases frequency-dependent inhibition compared to all groups, as is indicated by the decrease of the FDI index (one way ANOVA: F_{4,35}=4.14, p<0.01; post-hoc test: ***p<0.001 compared to naïve; **p<0.01 compared to swim; #p<0.01 compared to swim+R, p<0.05 compared to UWT). (d) Paired pulse inhibition: applying paired pulse stimulation to the PP in 15 ms interval results in a reduction of the PS amplitude expressed as PPI index. The exposure to UWT with or without the reminder increases paired pulse inhibition compared to all other groups as is indicated by the decrease of the PPI index (one way ANOVA: F_{4,35}=5.89, p<0.001; post-hoc test: ***p<0.001 compared to naïve; **p<0.01 compared to swim; #p<0.01 compared to swim+R).

Undergoing UWT results in reduced exploration in the open field even 24 h after the trauma compared to ‘swim’ and ‘naïve’ groups. However, exposure to the reminder...
further reduced exploration in the open field, and resulted in significantly enhanced anxiety behaviour also in the EPM, suggesting that the anxiety state triggered by the trauma was further exacerbated by the reminder. It was previously demonstrated that the trauma has long-lasting effects on anxiety-related behaviour, since 3 wk after the trauma, rats still exhibited reduced exploration of the open arms in the EPM (Richter-Levin, 1998; Sood et al., 2013). However, in those cases enhanced anxiety behaviour was also observed in the context of the trauma. Manifestations triggered by exposure to trauma reminder are core symptoms of PTSD and are suggested to involve conditioning mechanisms, while manifestations occurring without reminders are likely to reflect generalized anxiety (Pitman et al., 2012). Thus, the present data points to the ability of the reminder context to elicit anxiety-like behaviour, but also to exacerbate a generalized anxiety state already induced by the trauma that occurred 24 h earlier. It may thus be that re-experiencing as a result of exposure to reminders of the trauma contributes to the development of PTSD from acute stress (Yehuda, 2004).

It was previously demonstrated that EPSP-LTP was reduced 30 min following UWT (Wang et al., 2000). In the current study, 24 h after the trauma, UWT rats exhibited EPSP-LTP similar to ‘swim’ and ‘naïve’ rats. This finding could be taken to suggest that the impairment in DG-LTP observed immediately following the trauma is recovered with time. However, the fact that exposure to a reminder cue, which by itself had no effect on LTP, could suppress EPSP-LTP in UWT exposed animals, indicates that the exposure to the UWT induces a form of metaplasticity, which when triggered by a reminder cue results in suppressed synaptic plasticity even long after the exposure to the trauma.

GABAergic neurons locally affect activity and plasticity of DG principal cells through inhibitory mechanisms. This local circuit activity has been suggested...
to be relevant for learning and memory formation (Maroun and Richter-Levin, 2002). Inhibitory neurons are heterogeneous, consisting of several subpopulations distinguished in their function. For example, with the use of particular electrophysiological protocols it is possible to reflect more aspects of feedback or feedforward inhibition. FDI is suggested to reflect more feedforward inhibition (Maroun and Richter-Levin, 2002), while paired pulse stimulation to the PP at an inter stimulus interval of 15 ms reflects more feedback inhibitory effects.

We have previously demonstrated that local circuit activity is sensitive to stress (Yarom et al., 2008). Here we further demonstrate that local circuit activity is a sensitive measure that could differentiate between effects of exposure to a trauma and effects of exposure to a trauma reminder within the acute stress response time frame. UWT resulted in enhancement of PPI (stronger feedback inhibition), regardless of whether or not the reminder was presented. In contrast, FDI was only enhanced following re-exposure to the reminder. The reduction in PPI index was thus not specific to re-exposure to the reminder, since rats that underwent the trauma, but were not re-exposed to the water 24 h later, still exhibited a reduction in PPI. This suggests a long lasting effect of the trauma itself on feedback inhibition processes in the DG, without further effect of the reminder, either because this specific local circuit activity is not modulated by traumatic memory retrieval or because of a floor effect. In contrast, the enhancement in FDI was specific to re-exposure to the trauma reminder, indicating that mechanisms specific to the retrieval of the traumatic experience take place in the DG at a 24 h time point.

It was proposed that feedforward and feedback inhibition are mediated by different interneurons population. Feedforward inhibition was suggested to engage chandelier or axo-axonic interneurons, while feedback inhibition may involve interneurons targeting the soma of principal cells. Difference in effects of the reminder on FDI and PPI suggests that retrieval of trauma-related memories differentially involves subpopulations of interneurons in the DG. Traumatic memories are characterized by strong and rather inflexible emotional memories. It was previously suggested that an increase in FDI...
could participate in reducing behavioural flexibility (Yarom et al., 2008), as shown for instance in aged animals (Maroun and Richter-Levin, 2002). It may be that durable alteration in PPI, and alteration in FDI upon re-exposure to trauma-associated context, are early functional features which could underlie the later development of traumatic memories.

The present protocol was found to be associated with activation of the BLA when animals were re-exposed to the reminder of the trauma. This selective activation of the BLA in response to the reminder cue suggests that the effects of the reminder are mediated at least in part by the amygdala. The BLA is known to modulate hippocampal activity and plasticity (Vouimba and Richter-Levin, 2005) and hippocampus-dependent learning (McGaugh, 2004). More specifically, it was demonstrated that BLA activation modulates DG plasticity differentially depending on stimulation intensity and timing between BLA priming and DG recording. Indeed, weak BLA priming has been shown to enhance LTP (population spike and EPSP slope) in the DG, while stronger priming impaired it (Li and Richter-Levin, 2012). These data are in agreement with the effects of stress on DG neuronal plasticity, ranging from impairment to no effect to enhancement, depending on stress parameter, thus strengthening the possibility that it is the BLA which mediates these modulatory effects of stress exposure. A recent study showed that uncontrollable stress resulted in a decrease of ERK expression in the DG, while lesion of the BLA prevented this reduction (Jeon et al., 2012), further supporting the notion that under emotional conditions, the BLA modulates stress-induced alterations in the DG through the modulation of hippocampal ERK signaling. At the same time, it has also been shown that the ERK signaling pathway within the BLA is involved in the stress effect on LTP in CA1 area (Yang et al., 2008). It is thus suggested that reminder-induced retrieval of traumatic memories elicits BLA activation, which could in turn participate in the specific modulation of DG activity and plasticity, resulting in stronger feedforward inhibition in the GABAergic local circuit and a reduction in EPSP slope following theta burst stimulation.

The current results differentiate between neural mechanisms associated with the effects of exposure to a trauma and those associated with re-exposure to a trauma reminder. Furthermore, these differential neural mechanisms, found in the DG, strengthen the notion that under emotional conditions the DG assumes a more significant role in determining the outcome with respect to hippocampal functioning than was appreciated before. We demonstrate here specific activity and plasticity mechanisms within the DG following exposure to a trauma reminder. DG involvement in the processing of such memories could represent the first steps toward the development of pathological memories related to the trauma and the development of PTSD.

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Statement of Intrest

None.

References


