TRAPping Trauma: Distinct Trauma Memory Encoding and Retrieval in the Dentate Gyrus Lies at the Root of Resiliency to Posttraumatic Stress Disorder

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Posttraumatic stress disorder (PTSD) is a psychiatric disorder that can develop after exposure to trauma. Although over 80% of the population will be exposed to a traumatic event in their lifetime, only 7-9% will actually develop PTSD. The neural processes underlying susceptibility to this disorder are not yet known, however, PTSD symptomatology directs towards overgeneralisation of the traumatic memory. Considering its role in pattern separation, the dentate gyrus (DG) is considered to be involved in overgeneralisation of trauma-related memories. Here, we exposed the TRAP transgenic mouse line to an established PTSD-induction model, known to induce PTSD-like symptoms in part of the mice, whereas others are resilient. In these mice we compared the neuronal activity associated with the encoding and retrieval of the trauma memory in the dentate gyrus of PTSD-susceptible and resilient mice to investigate whether structural changes in neuronal activity during these memory processes are linked to PTSD. Although we did not find any neuronal differences between PTSD-like and resilient mice, we did find distinct encoding and retrieval levels in the suprapyramidal region of the ventral and dorsal hippocampus in resilient mice in comparison to controls. Together, these data suggest a link between neuronal cell activity in the DG and display resilient behavior after trauma.

Keywords: posttraumatic stress disorder, susceptibility, overgeneralisation, dentate gyrus, pattern separation, tdTomato, memory, hippocampus, neuronal activity

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Posttraumatic stress disorder (PTSD) is a psychiatric disease that can develop after exposure to a severely traumatic event. Although over 80% (Breslau et al., 1998) of the population will be exposed to one or more traumatic events in their lifetime, only 7-9% will actually develop PTSD (Kessler et al., 2005a; Kessler, Chui, Demler, & Walters, 2005b; Hinton & Lewis-Fernández, 2011). Patients affected by PTSD suffer from re-experiencing the trauma in recurrent memories, distressing dreams, flashbacks, and experience prolonged psychological distress in response to cues that remind them of the traumatic event. Additionally, trauma-related increases in arousal and reactivity are observed in these patients as reflected by their risky behavior, hypervigilance, heightened startle reactions, and insomnia (American Psychiatric Association, 2013). The functional consequences of PTSD, besides personal social and physical impairment, are substantial economic costs and high levels of medical utilization (Arnow, Hart, Hayward, Dea, & Taylor, 2000).

Over-generalisation of the memories of the trauma to safe contexts has been suggested to underlie this disorder and implicates dysfunctional hippocampal processing in PTSD (Weeden, Roberts, Kamm, & Kesner, 2015; Zou et al., 2016; Tamminga, Southcott, Sacco, Wagner, & Ghose, 2012; Astur et al., 2006; Kitayama, Vaccarino, Kutner, Weiss, & Bremner, 2005). The hippocampus is the dominant brain structure involved in memory encoding and consolidation. The hippocampus is divided into the dentate gyrus (DG) and hippocampus proper (CA1/CA3) (Amaral & Witter, 1989; Andersen, Bliss, & Skrede, 1971). During episodic events, an abundant amount of sensory information enters the hippocampus via the entorhinal cortex (EC). Given the large amount of sensory information, and the limited number of DG cells the EC projects to, this information is coherently filtered (Fyhn, Molden, Witter, Moser, & Moser, 2004; Hales et al., 2014; Wilson, Watanabe, Milner, & Ainge, 2013) (Fig. 1). The filtered information first reaches the DG (Kaczkurkin et al., 2016), which is considered the first step in memory production, and ensuring memory specificity by "pattern separation". Pattern separation entails the alteration of patterns of input, to induce sparser and less overlapping nodes so that similar experiences can be stored as different entities (Kesner, 2007; Rolls, 2016; Myers & Scharfman, 2009; Amaral, Scharfman, & Lavenex, 2007; Schmidt, Marrone, & Markus, 2012; Gilbert, Kesner, & Lee, 2001). The advantage of pattern separation is the reduction in nodes necessary to represent

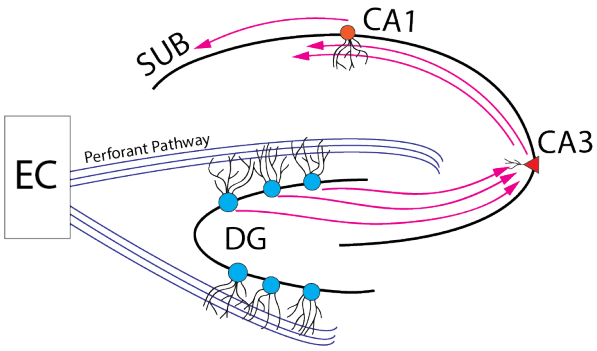

Fig. 1. Schematic representation of the hippocampus. The entorhinal cortex performant projections to, for example, the molecular layer of the dentate gyrus, which in turn project to the CA3 of the hippocampal formation. [EC = Entorhinal cortex, SUB = subicular cortex, DG = dentate gyrus, CA3 = hippocampal formation region CA3, CA1 = hippocampal formation region CA1].

distinct memories, facilitating the appropriate storage of distinct memories. Based on both human and rodent studies, it is proposed that inaccurate activation of cells in the DG during pattern separation is implicated in overgeneralisation of memories and may be observed in PTSD (Lissek & van Meurs, 2015; Kheirbek, Klemenhagen, Sahay, & Hen, 2012; Kaczkurkin et al., 2016; Bakker, Kirwan, Miller, & Stark, 2008)

Three structures are manifested within the DG; the molecular layer, the principal cell layer (granule cell layer), and the polymorphic region (hilus). Axons derived from the EC cross the molecular layer to terminate at the principal cell layer. The principal cell layer predominantly contains packed granule cells and inhibitory pyramidal basket cells. These cells innervate the CA3 via the polymorphic region for subsequent memory processing and storage (Amaral, Scharfman, & Lavenex, 2007) (Fig. 2). Granule cell activity in the suprapyramidal blade seems to be higher than in the infrapyramidal blade, suggesting a functional distinction between the two blades of the DG. More specifically, overall expression in the infrapyramidal blade does not seem to change despite of changes in environment (Chawla et al., 2005; Ramirez-Amaya, Marrone, Gage, Worley, & Barnes, 2006). Thus, the suprapyramidal blade seems to be more involved in pattern separation when exposed to different environments (Satvat, Schmidt, Argraves, Marrone, & Markus, 2011) and this has supposedly to do with the greater dendritic length of the granule cells in the suprapyramidal blades (Claiborne,

Amaral, & Cowan, 1990; Desmond & Levy, 1982), as well as with the many projections it receives in comparison to the infrapyramidal blade (Wyss, Swanson, & Cowan, 1979). The granule cells also have a bidirectional connection with the mossy cells in the hilar region of the DG. Although these cells innervate each other by excitation and inhibition, activity patterns show a distinction in function (Goodsmith et al., 2017). While granule cells seem to be involved in sparse coding to facilitate pattern separation, mossy cells appear to be more important for spatial memory encoding (Neunuebel & Knierim, 2012).

Another contributing factor in memory formation are the transmitter γ-aminobutyric acid (GABA) inhibitory interneurons in the granule cell layer and the hilar region. Somatostatin-expressing interneurons (SOM) in the dentate gyrus control granule cell formations during memory encoding. Activation of the DG SOM interneurons associated in the hilar-perforant pathway results in long-term depression, while activation of the DG SOM interneurons associated in the hilar region provide long term potentiation, both important for memory processes (Yuan et al., 2017). Also, innervation of SOM by granule cell activity inhibits retrieval of contextual memory (Stefanelli, Bertollini, Lüscher, Muller, & Mendez, 2016). Further, interneurons are generally known to modulate behaviours like anxiety, fear, and memory (Cho, Deisseroth, & Bolshakov, 2013; Donato, Rompani, & Caroni, 2013; Wolff et al., 2014). Dysfunction of these interneurons can have serious implications for psychiatric disorders (Marín, 2012). This was shown for the dominantly available perisomatic interneuron parvalbumin (PV), for which expression levels in the DG appear to be critical in proper regulation of anxiety and fear (Zou et al., 2016). However, it still remains unknown whether the pre-existing individual differences in the number of PV expressing interneurons are involved in regulating cell activity related to pattern separation and if aberrant function may indeed lead to susceptibility to PTSD.

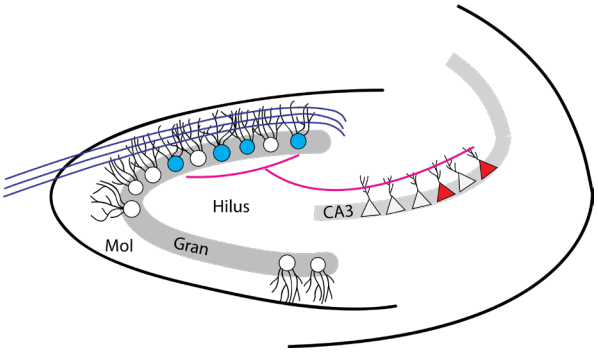

Fig. 2. Schematic representation of pattern separation in the DG. The memory representation enters via the molecular layer towards the neurons in the principal cell layer. From there information travels via the hilus towards the hippocampal formation CA3 region, where fewer nodes are activated and, thus, ensures an efficient transfer of spatial memory information. [Mol = molecular layer, Gran = granule cell layer (principal cell layer), Hilus = polymorphic region, CA3 = hippocampal formation CA3].

Important to note is also the dissociation in hippocampal function along the dorsoventral axis. Whereas the dorsal hippocampus is more involved in spatial memory (Klur et al., 2009; Pothuizen, Zhang, Jongen-Rêlo, Feldon, & Yee, 2004), the ventral hippocampus appears responsible for emotional memory processes (Henke, 1990). This is not surprising, given that the most prominent projections from the dorsal hippocampus are to the cingulate cortices; regions primarily involved in cognitive processing of spatial information and memory processing. Meanwhile, the ventral CA1 has a major bidirectional connectivity with amygdalar nuclei which receives sensory information and in turn innervates the bed nuclei of the stria terminalis (BNST) which are both regions that have strong emotional components (for a full review on dorsal and ventral distinctions, see Fanselow & Dong, 2010).

Here, we sought to understand how neuronal activation in the DG is associated with the encoding and retrieval of trauma-related memories (compared to neutral ones), and how this activity may confer vulnerability to PTSD. To study traumatic memory processing in the DG in a well-controlled and detailed, cell-specific manner, we utilized a specific transgenic mouse line, the so-called target recombination in active population (TRAP) mice (Guenthner, Miyamichi, Yang, Heller, & Luo, 2013), combined with an established mouse model for PTSD (Lebow et al., 2012). We compared neuronal activity during trauma encoding and retrieval in both the ventral and dorsal DG and tried to link this to the later development of PTSD-like symptoms. We expected that either during encoding or retrieving, neuronal activity levels in the suprapyramidal blade and the hilar region of the DG in the dorsal hippocampus are differently regulated in PTSD-like mice, because of their role in pattern separation of spatial memory. We also

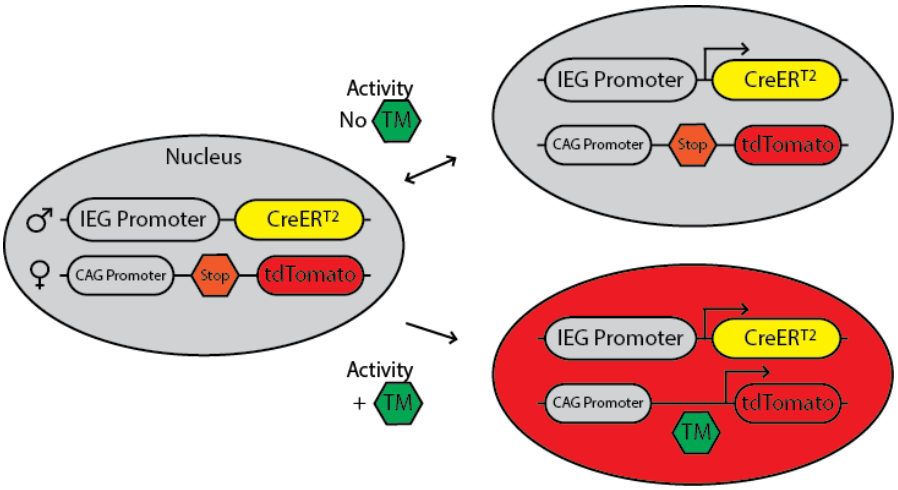

Fig. 3. A heterozygous male knock-in mouse CreER^{T2} from the activity dependent Fos immediate early gene (IEG) was crossed with a mouse with a homozygous knock-in allele of the Rosa26 locus that allowed expression of the effector gene *tdTomato* under the control of Cre-recombinase. In the absence of tamoxifen (TM), CreER^{T2} is retained in the cytoplasm of the active cells. In the presence of TM, Cre-recombinase re-allocates to the nucleus where recombination occurs and active CreER^{T2} cells permanently express the effector gene *tdTomato*.

expected that PTSD-like mice show higher neuronal levels in the suprapyramidal and hilar DG regions of the ventral hippocampus, and also higher pre-existing PV-expressing interneurons, given its role in emotional regulation. Here, we show that mice that were categorised as resilient, exhibit lower levels of encoding in the dorsal, and higher retrieval and PV expressing cells in the ventral hippocampus compared to control mice.

Methods

Mice

Two founder mouse lines, FosCreER^{T2} and conditional *tdTomato*, were purchased from The Jackson Laboratory and bred as described by Guenthner and colleagues (2013) to generate heterozygote FosCreER^{T2}xtdTomato offspring (Fig. 3). Only male mice were used for this study. Mice were housed IVC on a reverse 12 hour (10.00-22.00h) dark/light cycle in groups of three/four mice per cage. Food and water were provided *ad libitum*. Unless otherwise stated, behavioural testing was performed during the animal's active phase (the dark) between 13.00 - 18.00 h. The experimental protocols were in line with international guidelines and approved by the Central Committee for Animal Experiments, Den Haag, The Netherlands.

General procedure

This protocol was based on the PTSD mouse model as described by Lebow and colleagues (2012). To induce a PTSD-like phenotype, mice were exposed to a traumatic event (severe unpredictable foot shock) followed by a less severe trigger (mild, predictable foot shock) event 24 hours later. After the PTSD-induction and a week of recovery, mice were subjected to a subset of behavioral tests to assess their PTSD-like phenotype. On the final day, mice were re-exposed to the trigger context and perfused 90 minutes later. As we wanted to investigate how PTSD vulnerability links to hippocampal activity, we will compare cell activity in both PTSD vulnerable and susceptible mice. However, given that literature suggests that resiliency is due to adaptation after trauma exposure, we will include a control group that will not undergo a trauma experience in order to compare cell activity to baseline activity. Control mice underwent the same protocol, but did not receive shocks during the traumatic event and trigger, nor were they subjected to the test battery to assess PTSD-like behavior to prevent any stress exposure (Fig. 4).

Tamoxifen

Tamoxifen (TM) was dissolved in an 10% ethanol/corn oil solution at a concentration of 10mg/mL. Animals were weighted on day 0, and intraperitoneal (i.p) injected with tamoxifen on the morning of day 1 (15µl/kg).

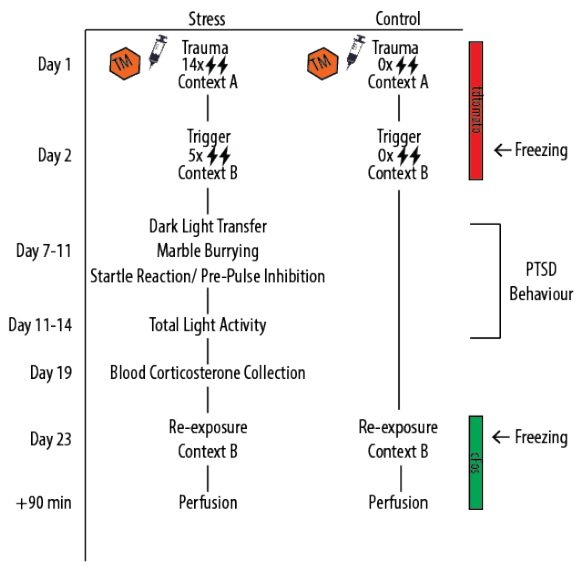

Fig. 4. Timeline of the PTSD induction model and behavioural tests. Injection of tamoxifen (TM) prior to the trauma ensured optimal *tdTomato* labeling and permanent labeling of all cells active during memory encoding. At the last day of our model, mice were perfused 90 minutes after the re-exposure to the (traumatic) context to assess neuronal activity related to memory storage. Control mice (n=5) differed from trauma-exposed mice (n=40) by not receiving any foot shocks during the trauma-induction, nor were the control animals tested in the behavioural tests on day 7-19.

PTSD protocol

Seven hours after the TM injection, the trauma induction started in which mice received 14 individual 1mA shocks in context A. Each shock lasted for a duration of 1s, and shocks were spread over 85min in variable intervals. For the trauma induction, mice were moved to the dark experimental room with two or three mice in dark carton boxes and placed individually in context A boxes which were connected to a fear-conditioning apparatus (Bussey-Saksida, ABET II TOUCH). Context A consisted of a dark, triangular shaped Plexiglas box with a steel grid and metal tray. The boxes were sprayed with 1% acetic acid, and mice were subject to 70 dB background noise and had no illumination during the trauma induction. On the second day, 28 hours after injection, mice were subjected to the trigger phase in context B in which they received 5 shocks of 0.7 mA. Each shock lasted for the duration of 1s and shocks were presented over fixed intervals. For this trigger session, two or three mice were moved to the 70 lux illuminated experimental room in see-through cages. The mice were placed individually in context B boxes. These boxes contained curved white walls and a steel grid with underneath a white tray. Context B was cleaned with 70% ethanol and during the session the house light in the box was turned on.

Dark-light transfer test

This test was based on the dark-light transfer test of Lebow and colleagues (2012). Briefly, the mouse was placed in the dark compartment of the dark-light apparatus and movement of the mouse was recorded and scored automatically with Ethovision XT. The time spent in the risk assessment area, a small area by the opening of the door of the light compartment (6x3), was measured to calculate the percentage risk assessment; the amount of time spent in the risk assessment zone as a percentage of total time spent in the lit arena outside of that zone.

Marble burying

On day 10, mice were individually moved to the experimental room in a covered cage. There the mouse was placed in a 10 lux illuminated black open box (30cmx27cm). The box had a layer of corn cops (5 cm) and 20 marbles were centrally arranged (4x5) on top of that layer. Each mouse was placed in the corner of the box to initiate the task. Mice were videotaped for 25 minutes. Videos were scored by assessing the amount of unburied marbles after 25 minutes.

Prepulse inhibition test

This test was also based on the Acoustic Startle Response test of Lebow and colleagues. (2012). Briefly, at day 12, mice were moved to the experimental room in their home cage. There they were individually placed in small, see-through Plexiglas constrainers mounted on a vibration sensitive-platform inside a ventilated cabinet that contained two high-frequency loudspeakers (SR-LAB, San Diego Instruments). Movements of the mice were measured with a sensor inside of the platform. First, the pre-pulse inhibition test (PPI) started with an acclimatisation period of 5 minutes in which a background noise of 70 dB was presented and which lasted throughout the session of 30 minutes. Thirty-two startle responses of 120 dB, 40 ms in duration and with a random varying inter-trial interval (ITI) were presented

with another 36 startle responses preceded by a 40 ms pre-pulse of randomly 75 dB, 80 dB or 85 dB. Sessions were scored by assessing the latency to peak startle amplitude and the pre-pulse inhibition; the percentage of startle inhibition response to the different pre-pulse stimuli [1 - (mean pre-pulse startle response/ mean startle response without pre-pulse) x 100].

Homecage Locomotion

Immediately after the pre-pulse inhibition test, mice were individually housed (45cmx45cm) (Noldus, Phenotyper) for 72 hours while their locomotion was recorded by an infrared-based automated system (EthoVision XT). The first 24 hours were considered habituation time. For the measurements we assessed total locomotion during the two light phases implementing 10 minutes intervals.

Inclusion criterion for PTSD-like behavior vs resilient-like behaviour

In order to categorise mice as either PTSD-like or resilient, one compound measure was generated by adding five different scores for the four behavioural tests; risk assessment, latency to peak startle, total PPI disruption, total light activity (non-active phase) and marbles buried (Fig. 5). The top 25% of mice showing the most extreme behavior in each test received the maximum score, while the other 75% of the mice received a score of zero. For the risk-assessment test, mice that showed the lowest percentage risk assessment were considered most extreme in their behavior. For the latency to peak startle and total PPI disruption, fastest startle responses and lowest PPI levels were considered most extreme behavior. For the total-light activity, highest activity rates during the light-phase were considered as extreme behavior. And for the marble burying, mice who buried the fastest and most marbles after 25 minutes were considered most extreme in their behaviour. Mice with a total score of five or higher (necessitating extreme behaviour in multiple tests) were included into the PTSD-like group. Mice with a total score of zero were included in the resilient group.

Behavioral Measure		Score
% Risk assessment behavior		3
Latency to Peak Startle Amplitude		3
% Pre-Pulse Inhibition		2
Total Light Activity (Light-Phase)		1
Total Marbles Burried		1
PTSD-like	Top 25%	≥5
Resilient-like	Bottom 25%	<1

Fig. 5. The behavioural tests with their maximum score as assessed by Lebow et al. (2012). Mice that performed within the top 25% of the test received the maximum score for that test. The sum of the scores that each mouse received was calculated, and mice with a score of 5 or higher were categorised as PTSD-like. Mice that received a total score of 0 were categorised as resilient.

Corticosterone collection and measurement

Blood samples were collected by tail bleed under basal conditions and 25, 75 and 120 minutes after stress initiation. Mice were placed in a restrainer for 25 minutes. All blood sample collections started at 1Öö:00 p.m. Blood samples were centrifuged immediately and plasma was extracted and stored at -80°C. Samples remain to be analysed.

Re-exposure

On the final day of the experiment, mice were again placed in context B for the duration of 10 minutes, following the exact same procedures as during the trigger session to induce memory retrieval. No shocks were administered during this context re-exposure session. Afterwards, mice were placed two or three in a cage and anaesthetised and perfused 90 minutes after re-exposure.

Freezing behavior

Mice were videotaped during the trigger induction (day 2) and the re-exposure to the trigger context (day 23) to assess stress coping behaviour and fear memory. Freezing behaviour was manually scored by an observer blinded to the experimental condition (Noldus, The observer XT12). Consistent with previous literature, mice were considered as freezing when they were immobile for more than two seconds (Shoji, Takao, Hattori, & Miyakawa, 2014; Patel et al., 2014).

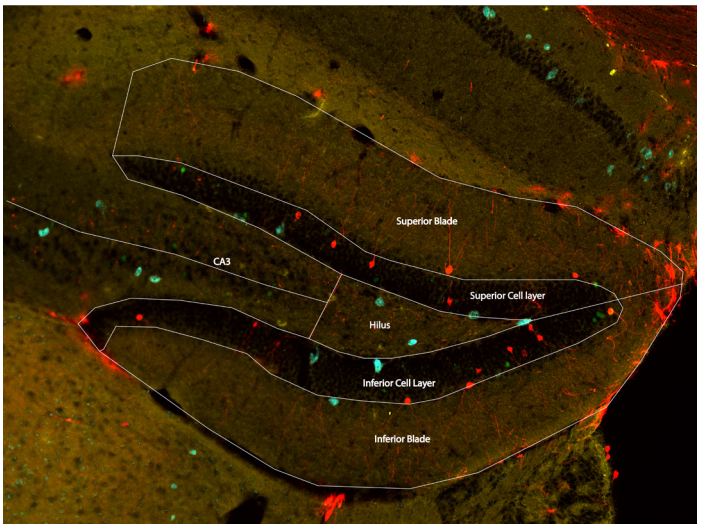

Fig. 6. Region boundaries for cell counting. Regions were divided into the blades (superior and inferior), the granule cell layers (superior and inferior), and the hilus region. The hilus region was considered to be the triangle within the superior and inferior cell layer, which ended at the beginning of the CA3.

Brain tissue collection

90 minutes after the re-exposure session, mice were anaesthetised with inhalation isoflurane and overdosed by i.p. injection with pentobarbital. Then, they were perfused with PBS and 4% paraformaldehyde (PFA), followed by 24 hours of post-fixation in 4% PFA. Next, brains were divided into two hemispheres. Left hemispheres were stored in PBS (1x) at 4°C, and right hemispheres were stored in 1x PBS and 30% sucrose at 4°C until slicing.

Immunohistological analysis

Right hemispheres of PTSD-like (n = 9), resilient (n = 6), and control (n = 5) animals were sliced at 30µm thickness using a freezing sliding microtome and stored in 1x PBS. Floating sections were used for immunohistochemistry. For immunohistochemistry of the dorsal hippocampus, we used 4-6 sections with the anterior posterior coordinates between -1.46 mm and -1.94 mm according to Bregma. For the ventral hippocampus, we used 4-6 sections with the anterior posterior coordinates between -2.92 mm and -3.52 mm according to Bregma. Sections were washed three times in 1x PBS and blocked in PBS-BT (1x PBS with 0.3% Triton X-100 and 1% bovine serum albumin (BSA)) for 30 minutes at room temperature (RT). Incubation of the primary antibody was performed overnight (guinea pig anti-c-fos, 1:750, 226004, Synaptic Systems; rat anti-somatostatin, 1:200, MAB354, Merck Chemicals; rabbit anti-parvalbumin, 1:1000, ab11427, ITK) in PBS-BT for 18 hours at RT. Then sections were washed three times in 1x PBS, and incubated with the secondary antibody (Alexa 647-conjugated donkey anti-guinea pig, 1:200, AP193SA6, Merck Chemicals; Alexa 488-conjugated donkey anti-rat, 1:200, A-21208, Thermo Fisher; Alexa 350-conjugated goat anti-rabbit, 1:200, A-11046, Thermo Fisher) for three hours at RT. Lastly, slices were washed three times in 1x PBS, mounted on gelatin-coated slides using FluorSave™ reagent (345789, Merck Chemicals) and cover slipped. Cell counting was performed on at least 8-12 sections per animal, with a minimum of 4 sections per hippocampal axis.

Image acquisition and cell counting

For cell counting, images were captured through a light microscope (Axio Imager 2, Zeiss) using a 10x objective lens and a LED module (Colibri 2, Zeiss). Cells were manually counted per region in Fiji (Schindelin et al., 2012) by an experimenter blinded to the experimental group (Fig. 6). Moreover, hippocampal region size/length was assessed and corrected for to obtain standardised measures of cell density.

Analysis

To check for normally distributed data, we performed Shapiro-Wilk tests for normality. For normally distributed data we used a univariate and independent t-tests for data analysis. For non-parametric data, we used the independent-samples Kruskal-Wallis test. Differences were considered significant if $p < 0.05$. Tables show medians ± standard error.

Results

Behavioral measures

PTSD-like vs resilient mice. PTSD-like categorised mice showed significantly less time engaged in risk assessment behavior than resilient-like categorised mice ($H(1) = 6.35$, $p = .012$). Moreover, they showed significantly shorter latency to peak startle ($H(1) = 9.20$, $p < .001$). However, no significant difference in PPI response were observed between groups ($t(15) = 1.544$, $p = .14$), nor was there a significant difference in activity rates during the light-phase than resilient-like mice ($t(15) = -.387$, $p = .704$. Finally, there was no significant difference in marbles buried between susceptible and resilient mice ($H(1) = 1.048$, $p = .306$) (Fig. 7).

Freezing behavior

Memory encoding. Univariate tests showed a significant main effect of group in therms of freezing latency ($F(2,16) = 9.446$, $p = .002$), as well for total freezing time ($F(2,17) = 50.727$, $p < .001$) during the trigger. Whereas, as expected, control mice showed longer latencies to start freezing, susceptible, but not resilient mice, froze significantly faster (susceptible; $t(4.04) = 2.91$, $p = .043$, resilient; $t(4.3) = 2.373$, $p = .072$) (Fig. 8). Interestingly, PTSD-susceptible mice also showed a significantly shorter latency to start freezing than the resilient mice ($t(8.91) = 2.37$, $p = .042$). As the first shock is only delivered after 60 seconds, shorter latencies to freeze can be interpreted as increased novelty-induced anxiety (or potentially fear generalisation) in these animals. Regarding total time spent freezing, trauma-exposed susceptible and resilient mice froze significantly longer during the trigger session than did control mice (which were not exposed to shocks) (susceptible; $t(6.09) = 12.19$, $p < .001$, resilient; $t(7.14) = 12.5$, $p < .001$). In this measure there was, however, no significant difference in total amount of freezing between susceptible and resilient mice during the trigger induction ($t(13) = 0.882$, $p = .394$), indicating similar responses to shock exposure (and thus stress coping).

Context re-exposure. Univariate testing revealed a significant effect of group on freezing latency during the re-exposure to the trigger context ($F(2,18) = 17.738$, $p < .01$), as well as total freezing time ($F(2,17) = 3.528$, $p = .05$). During the re-exposure to the trigger context, both susceptible and resilient mice froze significantly faster than the control mice (susceptible; $t(12) = 4.10$, $p < .001$, resilient; $t(4.01) = 3.88$, $p = .018$), indicating the existence

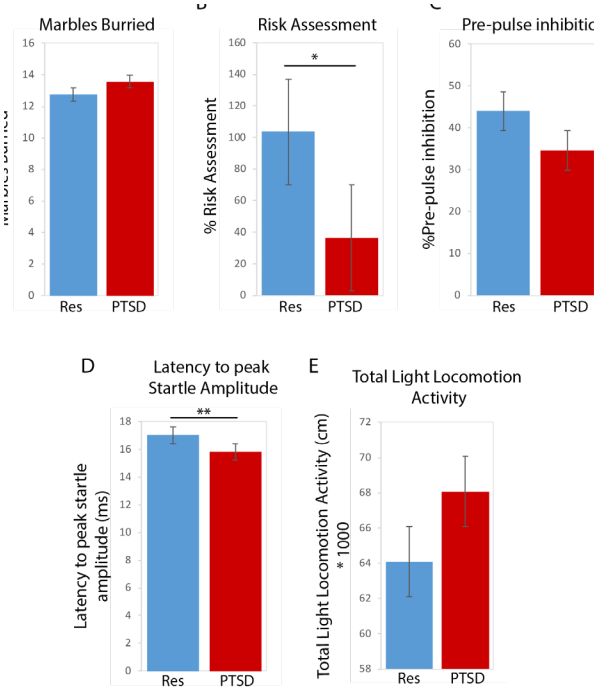

Fig. 7. A. Resilient (n = 8) and PTSD-like mice (n = 9) show no differences in behaviour in amount of marbles buried. **B.** Resilient mice show significantly more risk assessment behaviour than PTSD-like mice. **C.** There is no significant difference between mice in startle response, although **D.** PTSD-like mice do show a significantly faster startle reaction. **E.** Lastly, there was no significant difference between mice in their locomotion activity during the light-phase. *: $p < .05$; **: $p < .01$.

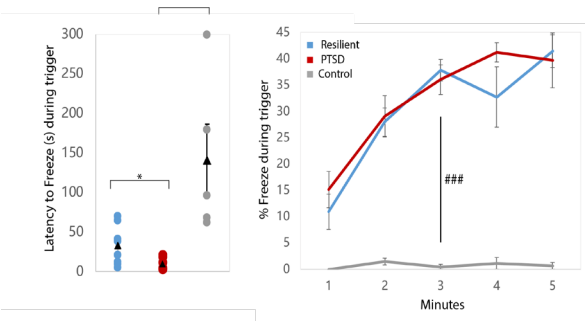

Fig. 8. PTSD-like mice (n = 8) show a significant faster latency to freeze during the trigger session than control mice (n = 5) due to the inescapable administration of foot shocks. Also, PTSD-like mice (n = 9) froze significantly faster than resilient mice (left). Total freezing was also significantly increased in trauma-exposed mice in comparison to control (right). *: $p = < .05$, o: $p < .05$, ###: $p < .001$.

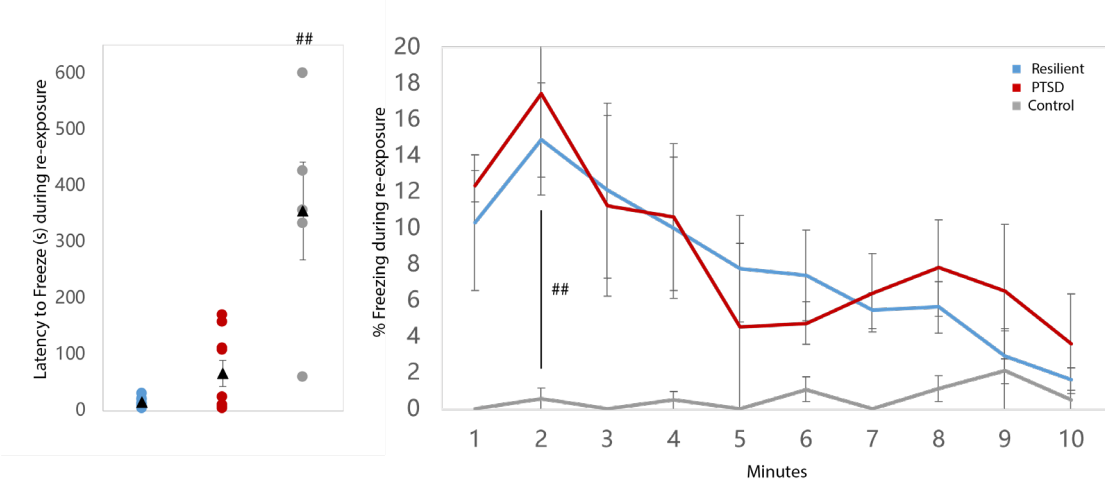

Fig. 9. Trauma-exposed mice (n = 17) froze significantly faster during the re-exposure to the traumatic context in comparison to control mice (n = 5) (left). They also froze significantly longer than control mice. There were no significant differences between susceptible (n = 8) and resilient mice (n = 9). ##: $p < .01$ (right).

of a fear memory for the aversive experience (Fig. 9). Moreover, there was a trend-level significant difference in the latency to freeze between susceptible and resilient mice ($t(14) = 1.9$, $p = .078$), with the resilient animals tending to show shorter latencies.

As expected, susceptible and resilient mice spent significantly more time freezing during the re-exposure to the context than control mice (susceptible; $t(7.10) = 2.87$, $p = .024$, resilient; $t(6.541) = 5.220$, $p = .002$). However, there was no significant difference in total freezing time between PTSD-susceptible and resilient mice ($t(9.175) = .674$, $p = .517$).

Cell counting

Memory encoding. Neuronal activity during the memory encoding of the trauma was permanently labeled by expression of the effector gene *tdTomato*. Since both the axis of the hippocampus, as well as the regions within the DG acquire different in- and output projections and contribute to different behavioural functions, we also tested whether groups structurally differed in cell activity over different regions separately. We found no significant differences in *tdTomato* activity in any regions of the DG between trauma-exposed and control mice. However, we did find significantly higher levels of activity in the superior region, as well as in the hilar region, of the DG in the dorsal hippocampus in resilient mice in comparison to control mice (superior; $H(1) = 7.500$, $p = .006$, hilus; $H(1) = 4.437$, $p = .035$). Further, we found no such differences between control and PTSD-like mice (superior; $H(1) = 0.751$, $p = .386$, hilus; $H(1) = 1.046$, $p = .306$), nor between resilient

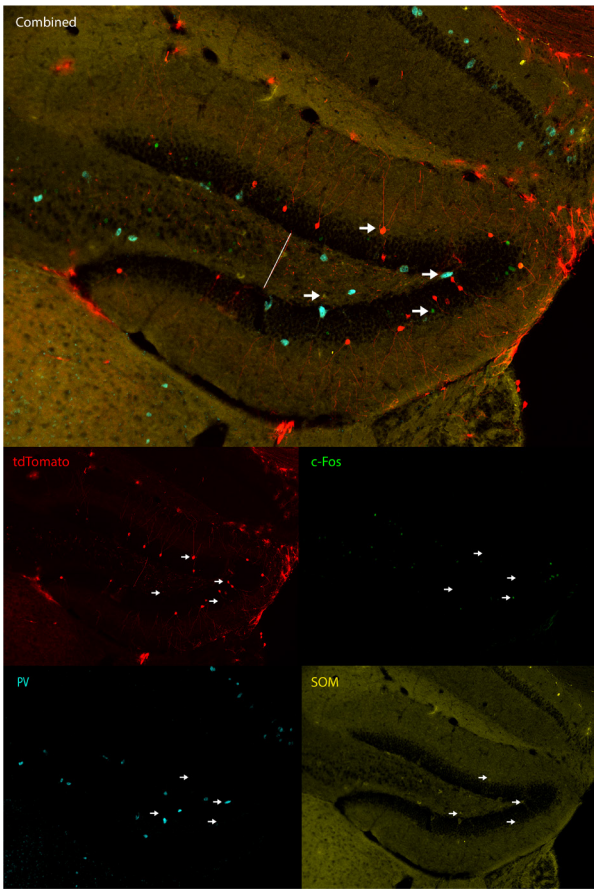

Fig. 10. Image of the DG. Cells were counted along the superior and inferior blades of the DG. Cells were considered to be in the hilus when located between the granule cell layers and the beginning of the CA3; depicted here with a white stripe. Arrows demonstrate a cell example of *tdTomato*, c-Fos, PV or SOM.

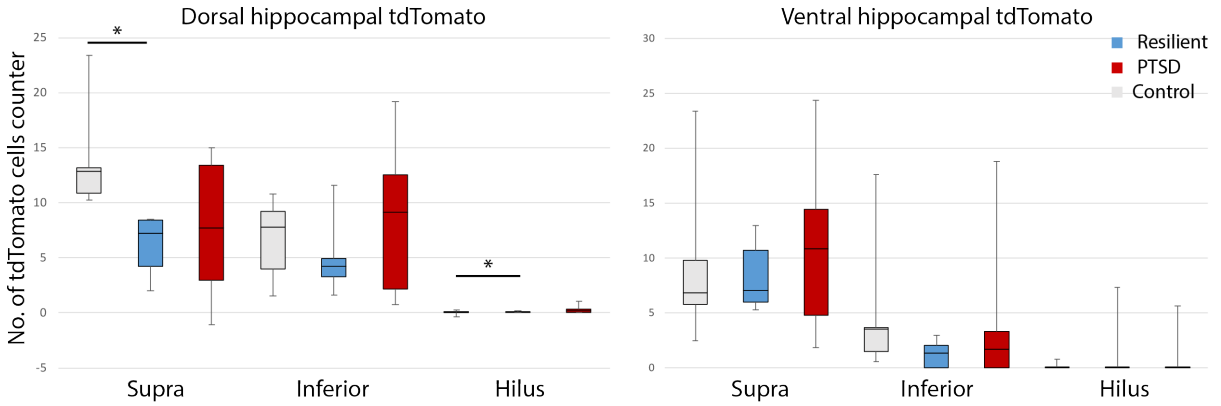

Fig. 11. tdTomato cell count differences in the dorsal and ventral hippocampus. There is a significant difference in the suprapyramidal region and the hilar region of the dorsal hippocampus between resilient and control mice. *: $p < .05$.

and PTSD-like mice (superior; $H(1) = 1.389$, $p = .239$, hilus; $H(1) = .500$, $p = .480$) (Fig. 11).

Memory retrieval. Neuronal activity of memory retrieval was measured by labeling c-Fos cells active during re-exposure to the traumatic context. Here, we found no significant differences between trauma-exposed and control mice in c-Fos labeling during retrieval in any regions. However, we did find significantly higher levels of activity during memory retrieval in the superior region of the DG in the ventral hippocampus in resilient mice in comparison to control mice ($t(9) = 2.794$, $p = .021$). We did not find this significant difference between resilient and PTSD-like mice ($t(10.567) = 1.007$, $p = .336$), nor between PTSD-like and control mice ($t(10.700) = 1.001$, $p = .339$) (Fig. 12).

Memory reactivation. Because we are interested in pattern separation in the DG, it would be interesting to investigate overlap between cells that were active during memory encoding and cells that were active during memory retrieval. However, due to the low number of overlapping cells between memory encoding and retrieving and low power, it was not statistically reliable to test on this data and therefore we did not include this data in our analyses.

Activity of DG interneurons. Given the influence of SOM and PV cells on emotional and spatial memory, we also investigated their presence during memory encoding and retrieval. As our data only showed moderate SOM levels in the hilus region of the DG, we only performed statistical tests there. We found no significant differences in number of SOM-expressing cells in the hilus between trauma-exposed and control mice. We also found no significant differences in SOM-expressing cells in the hilus between phenotypeß-grouped mice (Fig. 13).

Considering our low cell count of PV in the

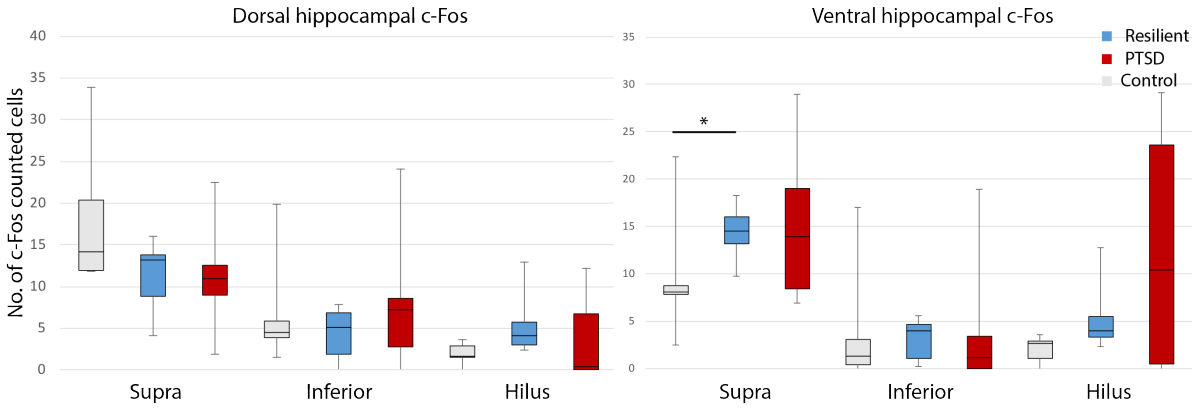

Fig. 12. c-Fos cells labeling in the dorsal and ventral hippocampus. There appeared to be a significant difference in the suprapyramidal region in the ventral hippocampus between resilient and control mice. * = $p < .05$.

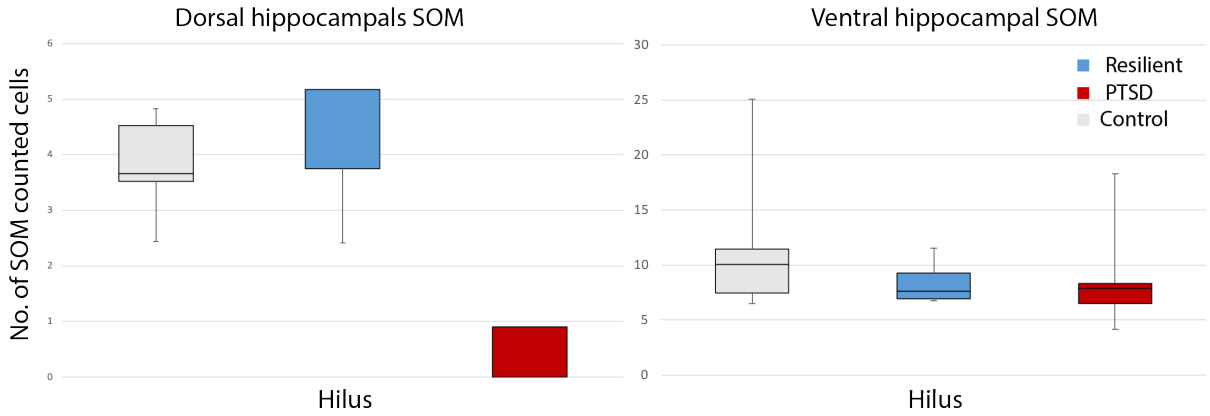

Fig. 13. SOM cell counts in the DG hilus of the dorsal and ventral hippocampus. There appeared to be no differences between the groups of mice.

hilus region, we limited our statistical analyses to the superior and inferior blades of the DG. We found no significant differences of PV-expressing cells between trauma-exposed and control mice. However, we did find significantly more PV-expressing cells in the superior blade of the DG in the ventral hippocampus in resilient mice in comparison to control mice ($H(1) = 4.033, p = .045$). This effect was not reproduced between resilient and PTSD-like mice ($H(1) = 1.067$, $p = .302$), nor between PTSD-like and control mice ($H(1) = .021$, $p = .884$) (Fig. 14).

Next, to investigate the co-localisation of SOM and PV-expressing cells on active cells during memory encoding and retrieving, we also tested the overlap between the DG interneurons with *tdTomato* and c-Fos. Here, we found no significant differences in SOM-expressing cell overlap with either *tdTomato* nor with c-Fos between trauma-exposed and control mice. We also found no significant differences in SOM-expressing cell overlap between phenotype grouped mice. Regarding PV, we also found no significant differences in PV-expressing cell overlap with either *tdTomato* nor with c-Fos between trauma-exposed and control mice. Again, we found no significant differences for PV-expressing cell overlap with *tdTomato* or c-Fos between phenotype grouped mice.

Discussion

In this study we aimed to investigate DG neuronal activity during the encoding and retrieval of a traumatic memory in order to link this to the development of PTSD-like symptomatology. Therefore, we labeled active neurons during trauma encoding using a transgenic mouse model and active neurons during trauma retrieval using immunohistochemistry. Categorisation of mice into the PTSD-like or resilient phenotype by use of different behavioural tests allowed us to investigate the neuronal activity associated with inter-individual

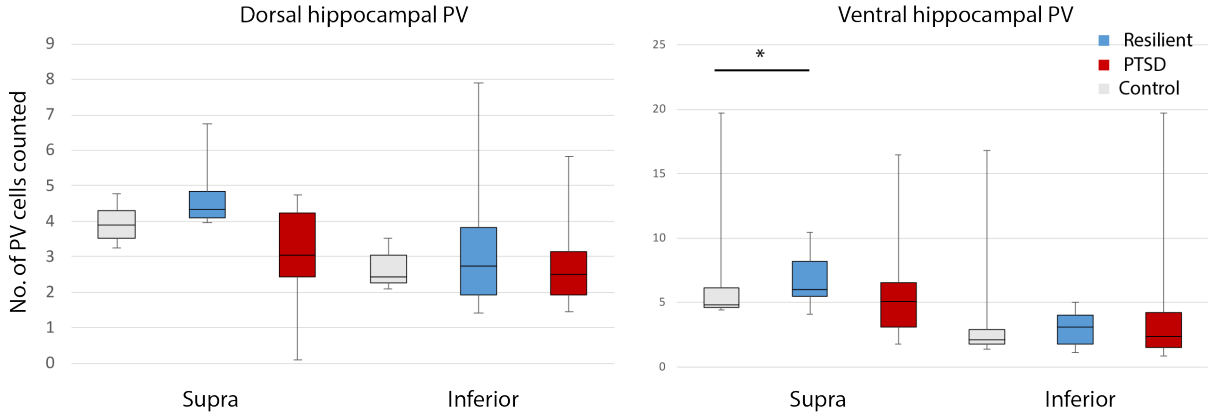

Fig. 14. PV cell counts in the suprapyramidal and infrapyramidal DG blades in the dorsal and ventral hippocampus. There appeared to be a significant difference between resilient mice and control mice in the suprapyramidal region of the ventral hippocampus. *: $p < .05$

differences in susceptibility for PTSD. Our study shows that trauma-exposed mice show faster latency to freeze during the trigger and re-exposure, as well as a higher total amount of freezing during both the trauma-induction and the re-exposure session. However, despite behavioural differences, our study shows no significant differences in neuronal levels between trauma-exposed and control mice.

Further, our study shows that PTSD-like mice freeze significantly faster than resilient and control mice during the trigger session. Also, both PTSD-like and resilient mice freeze significantly longer during the trigger and re-exposure to the traumatic context. However, there were no differences between PTSD-like and resilient mice in total amount of freezing for either the trigger or re-exposure session. Considering memory encoding and retrieval, we show that resilient mice have lower encoding levels in the superior, and lower encoding levels in the hilar region of the dorsal hippocampus in comparison to control mice. We also show that resilient mice exhibit higher retrieval levels in the superior DG region of the ventral hippocampus than control mice. Lastly, we show that resilient mice have higher levels of PV in the superior DG region of the ventral hippocampus, in comparison to control mice. Unfortunately, our data do not show any significant differences in neuronal levels between resilient and PTSD-like mice.

In line with expectations, PTSD-like categorised mice showed less time engaged in risk assessment behaviour than resilient mice. This is consistent with PTSD-patient data where risky behaviour is expressed by drug abuse, violence and suicide (Tarrier & Gregg, 2014; Kotler, Iancu, Efroni, & Amir, 2001; Kofoed, Friedman, & Peck, 1993), but also in previous translational studies (Lebow, Neufeld-Cohen, Kuperman, Tsoory, & Chen, 2012; Quartermain, Stone, & Charbonneau, 1996). Further, PTSD-like categorised mice showed a shorter latency to peak startle after exposure to a loud sound, demonstrating hypervigilance. However, PTSD-like mice failed to show significantly impaired prepulse inhibition (Siegelaar et al., 2006; Pole et al., 2009), a significant difference in locomotor activity during the light-phase (Butler et al., 1990; Inman, Silver, & Doghramji, 1990), nor increased marble burying. Given that the scores that contributed the most for PTSD-categorisation were given for the risk assessment and the latency to startle response, it is not surprising that after tallying the scores, the tests with the least contribution came out as least significant between PTSD-like and resilient mice.

As expected, trauma-exposed mice showed a higher total amount of freezing during the trigger session, which is a natural fear response to inescapable stressors. Also, trauma-exposed mice showed faster freezing responses and more overall freezing during the re-exposure sessions indicating their retrieval for the fear memory of the aversive experience. Interestingly, PTSD-like mice showed faster freezing responses than the resilient mice during the trigger session, suggesting a greater degree of generalisation of their fear to different contexts. This behavioural difference was however not reflected in the neuronal data. This could indicate that a different region than the DG might predispose PTSD-like animals. Lastly, there were no behavioural differences in freezing between PTSD-like mice and resilient-like mice during context re-exposure, indicating that both groups display a similarly strong memory for the traumatic event when re-exposed to the exact same context.

Addressing the neuronal levels, we did not find any significant differences between trauma-exposed mice and control mice in either memory encoding nor retrieval induced activation of DG interneurons. This suggests that the encoding and retrieval of a traumatic memory did not involve different DG interneuron activity from that induced by a neutral memory.

Interestingly, during memory encoding neuronal activity in the superior and hilar dorsal DG are lower in the resilient mice than in control mice. This is in line with previous literature, stating that IEG cell activity in the hilus and the granule cell layer is negatively affected by stress (Gould, Tanapat, McEwen, Flügge, & Fuchs, 1998; Moretto, Duffy, & Scharfman, 2017; Gould & Cameron, 1996). This decrease in DG cell activity in response to acute stress seems to be adaptive, as DG function is adjusted towards environmental demands (Sherry, Jacobs, & Gaulin, 1992). When the environment is enriched, neuronal processes are active, however, when the environment is not suited for positive behaviours, neuronal activity in the granule and mossy cells is decreased (Ohl & Fuchs, 1999). From our data, it can be interpreted that there is a link between cell activity in the suprapyramidal and hilar DG and less maladaptive behaviour. However, more research needs to be done to further investigate this matter.

During retrieval of memory, the superior DG region of the ventral hippocampus has higher levels of activation in the resilient mice in comparison to the controls. Given the role of the ventral hippocampus in emotional memory, this is not surprising. The re-exposure context for the control

mice did not elicit any fear memory, in comparison to the trauma-exposed resilient mice, and therefore specific emotional memory retrieval was not as activated as it was in the resilient mice. However, this difference was not seen between the PTSD-like and control mice. Literature states that different levels of stress are associated with different levels of c-Fos activation of the amygdala and ventral hippocampus (Kogan & Richter-Levin, 2008). Considering the strong bidirectional connectivity between the amygdala and the ventral hippocampus, it is not unlikely that changes in the amygdala due to PTSD-susceptibility alter the stress experience differently than in resiliency. Unfortunately, we did not account for the amygdala in this study, so future studies should investigate the role of the amygdala between PTSD-like and resilient mice.

Interestingly, besides higher levels of cell activity during memory retrieval in the superior DG region of the ventral hippocampus in the resilient versus control mice, we also found higher levels of PV in resilient mice in comparison to control mice. However, we did not find this difference between PTSD-like and control mice. Literature has shown that increased PV activity in the DG can have an anxiolytic effect on fear memory (Zou et al., 2016). This is in concordance with our behavioural data that shows that overall, even after trauma-exposure, resilient mice show less anxiety behaviour, while the PTSD-like animals show high anxiety behaviour.

Further, we could not make conclusions about memory reactivation, given the minimal overlap of the cells active during trauma memory encoding and trauma memory retrieval. This was partially due to the low number of animals in our PTSD-like and resilient group. Another reason for our minimal cell overlap could be explained by our transgenic mouse model. Previous research that used a similar design as ours, used the transgenic ArcCreER^{T2} mice instead of the FosCreER^{T2} mouse line (Denny et al., 2014). Arc, which is a member of the IEG family, is a plasticity protein involved in activation regulation. When comparing our data to the ArcCreER^{T2}-line, the FosCreER^{T2} mouse line seems to have a higher threshold for activation (Guenthner, Miyamichi, Yang, Heller, & Luo, 2013). This is characterised by its lower levels of background activation in comparison to the ArcCreER^{T2}-line, however, in this case it might have also resulted in less overlap between the memory encoding and retrieval cells. Within our lab, we are producing a similar experiment with the ArcCreER^{T2} mouse line, which will contribute to our investigation about memory reactivation.

Lastly, another reason for our low levels of memory encoding and retrieving overlap is due to our main focus on the DG. Literature often specifically addresses the role of the DG in pattern separation and encoding. When discussing pattern completion (Rolls, Treves, & Rolls, 1998), which is the memory retrieval of an event based on a degraded version of that event, evidence guides us more towards the role of the CA3 and CA1 in the hippocampal formation. Due to time-constraints, this study did not include cell count analyses of the entire hippocampal formation. Therefore, we will refrain from making statements about overgeneralisation until we have completed our cell analyses of the entire hippocampal formation. Only then can we fully map the role of the hippocampus and possible overgeneralisation in PTSD patients.

Conclusion

Although PTSD-like mice did not exhibit neuronal differences in comparison to resilient mice, we presented data that suggest a link between a resilient phenotype and neuronal activity in the DG during trauma exposure. Here, we show that resilient mice show decreased neuronal activity levels during memory encoding of trauma in the suprapyramidal and hilar DG region, which could explain their well-adapted behaviour after trauma-exposure. Neuronal levels in the DG suprapyramidal region in the ventral hippocampus of the resilient mice were lower during encoding of stress in comparison to control mice, paired with higher PV-expressing interneurons, whiccomh suggests higher emotional memory in response to re-exposure of the traumatic context, as well as a higher anxiolytic effect on fear memory and behavior. In the future, we will expand more towards the hippocampal formation in order to interpret our data regarding overgeneralisation. Then, we will also include the intermediate categorised mice so that we have more statistical power and correlate our PTSD-categorisation with our behavioural data. Finally, it would be interesting to investigate the role of the resilient phenotype more in depth, which could eventually lead to more clinically effective therapy.

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