Proceedings of the Master's Programme Cognitive Neuroscience of the Radboud University

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From the Editors-in-Chief

Dear reader,

We feel honoured to present the second issue of the 13th volume of the Proceedings of the Master's Programme Cognitive Neuroscience, consisting of a selection of outstanding research done by the alumni of the research master programme Cognitive Neuroscience in Nijmegen.

First, we would like to congratulate our authors. Their theses met the standards of high quality research in order to publish in this journal. As there are less February graduates, we decided to make this issue smaller to guarantee high quality of the journal.

This issues shows the diversity of innovative methods used in cognitive neuroscience, ranging from functional connectivity in resting state fMRI to oscillatory synchronization in EEG to mouse models, often combined with behavioural measures. In addition, this issue reflects the diversity of populations being studied in cognitive neuroscience. For example, it covers studies using clinical populations with autism spectrum disorder or obsessive-compulsive disorder but also touches upon animal research. We are proud that the journal is such a good representation of what cognitive neuroscience research is like.

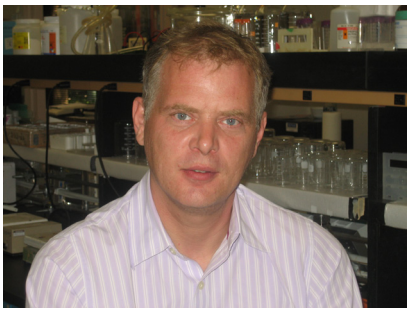
Lastly, we would like to thank our journal team. This issue reflects their dedication and hard work. We are very happy to have the opportunity to run this journal, providing a place for excellent graduates to publish their theses and for current students to learn about publication processes.

We hope you enjoy reading this new issue full of exciting and fascinating research!

Nijmegen, May 2018

Your Editors-in-Chief
Lisanne and Antonia

Innovative and personalised brain science



At the time when I was a PhD student, a Congressional Resolution signed by President Bush Sr. designated the 1990's as the "Decade of the Brain". This initiative was globally embraced and brought with it a considerable amount of enthusiasm and optimism regarding advances in knowledge about the brain – how it works, what goes wrong when it is injured or diseased – and the prospects for novel treatments. A renewed interest in the localisation of neural phenomena to distinct anatomical regions of the brain led to a rapid uptick in more direct study of the brain and its psychological implications. The Decade of the Brain brought us many new discoveries, for example neuro-epigenetics, and amazing new technologies such as neuroimaging. It also raised public awareness of the brain and brain disorders. However, today in 2018, almost 20 years after the ending of the Decade of the Brain, we still do not have a clear understanding of most brain disorders and do not have effective prevention or treatment programs.

Perhaps the most important insight that the Decade of the Brain has brought us is that brain disorders are usually complex syndromes, with innumerable subtypes and variations. These different subtypes might relate to specific abnormalities in different brain processes. For example, neurodevelopmental disorders such as autism spectrum disorder and obsessive compulsive disorders are highly heterogeneous disorders with a wide spectrum of possible symptom manifestations. On the other hand, there is also high overlap of symptoms across these disorders. Similarly, large individual differences in symptoms can emerge in posttraumatic stress disorder following traumatic stress, but a majority of people show remarkable resilience and do not develop any lasting symptoms after trauma. There is therefore a need for joint efforts between clinical and preclinical research to study the neurobiological mechanisms underlying such individual symptoms, to study resilience factors and to define subtypes on the basis of biological markers, since each subtype may call for specific targeted psychotherapy and pharmacotherapy.

Precision medicine is already being used in some clinical disciplines such as cancer, where molecular diagnosis is leading to better treatment. However, in psychiatry, biologically-based precision medicine is just beginning to be adopted. The papers in this issue of the CNS journal speak to this topic and provide some excellent examples of innovative and personalised investigations. The novel ideas presented in these papers may provide a breakthrough and lead the way to a better understanding of the brain processes underlying these individual behavioural or cognitive symptoms and the identification of patients who are most likely to respond to targeted treatments. We have enough reason to be optimistic, but lessons from the past three decades have also taught us that progress is slow and that no single approach or technique will give us all the answers. We have to show patience and set realistic goals and expectations. The brain "continues to intrigue scientist and layman alike. Over the years, our understanding of the brain has increased dramatically. However, we still have much more to learn" (Bush Sr., 1990).

Benno Roozendaal
Professor of Behavioural Neuroscience

Low Frequency Effects of Targeted Memory Reactivations on Subsequent Recall Processes

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There is an unmistakable link between the sleeping state and memory. The leading theory to explain this effect is the systems consolidation theory (Rasch & Born, 2013): a memory is consolidated through repeated, unconscious reactivations of prior learned memories. This project furthers the research into this theory by studying both the behavioural and oscillatory effects of these reactivations through targeted memory reactivations. Participants learned word-image pairs, of which half were then reactivated during a period of sleep by playing back the words in the pair. The long-term effects of this manipulation were then studied during subsequent cued recall. We clearly observed that behavioural performance increased as a result of cueing, as well as alpha band desynchronisation related to successful recall and stronger lateralised activity. Our results suggest that memory replay helps preserve the memory trace and, thus, makes the memory more accessible during recall.

Keywords: sleep, memory, consolidation, systems consolidation theory, alpha desynchronisation, recall

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Everyone knows about the restorative effects of a good night of rest, and many of us have observed the negative cognitive effects of not sleeping enough; sleep is essential. While it is generally a critical part of our lives, it is less known that sleep is also paramount in the formation of new memories. An example of this is the necessity of a night of rest for studying to actually take effect, the memories being more easily accessible the following day. In this project we will explore the biological underpinnings of this memory strengthening effect. Firstly, I will address our knowledge to date regarding this link.

Memory

Memory is generally thought to be comprised of three main processes: encoding, consolidation, and recall. Encoding is the process during which stimuli are perceived and eventually represented in the brain. This process depends on executive control and requires attention to the stimulus to function (Chun & Turk-Browne, 2007; Craik, Govoni, Naveh-Benjamin, & Anderson, 1996; Fernandes & Moscovitch, 2000). The representations created by this process are cortically distributed (Ahmad & Hawkins, 2015), yet depend on hippocampal activation to be accessed; the hippocampus in this case acting as an index of those representations (Moscovitch, Nadel, Winocur, Gilboa, & Rosenbaum, 2006).

Consolidation is the process through which encoded memories are stored and either further integrated into our current knowledge, or discarded. We currently do not know all the factors that influence if a certain memory is discarded or integrated, but reactivations (replay of the neural activity observed during learning) when awake could play a role, by increasing the likelihood of the reactivated memories to be replayed during sleep (Carr, Jadhav, & Frank, 2011; Diekelmann & Born, 2010; Diekelmann, Büchel, Born, & Rasch, 2011; Jafarpour, Horner, Fuentemilla, Penny, & Duzel, 2013; O'Neill, Pleydell-Bouverie, Dupret, & Csicsvari, 2010). Consolidation begins immediately after encoding and can take various amounts of time, but most evidence points to the fact that sleep plays a pivotal role in this regard (Buzsáki, 1998; Fenn, Nusbaum, & Margoliash, 2003; Lee & Wilson, 2002; Vertes, 2004). Consolidation is thought to be produced mainly by spurious memory reactivations that occur during the sleeping state (Born & Wilhelm, 2012; Dudai, Karni, & Born, 2015; Lewis & Durrant, 2011; Stickgold, 2005). This hypothesis has been previously tested by provoking memory reactivations through playing back a part of a learned stimulus (one word of a pair) and measuring the effect this has on the recall of the entire memory. The beneficial effect of memory cueing during sleep, as well as replay of previously encoded memories, has been observed both in humans and rodents (Peigneux et al., 2004; Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009; Schreiner & Rasch, 2015; Wilson & McNaughton, 1994).

The third process involved in the memory system is retrieval, through which a stored memory is reactivated and made consciously available. The ease of retrieval is dependent on the integration of the memory and, therefore, dependent on how much the memory has been consolidated. Another phenomenon that has been observed in relation to retrieval that may hold some functional significance is the encoding-retrieval match, activity present during encoding of a piece of information closely resembles activity present during recall (Reijmers, Perkins, Matsuo, & Mayford, 2007).

This study focuses on the consolidation and retrieval aspects of memory, as our manipulation has effects on the consolidation processes and the outcome measures can only be related to recall (as the study of consolidation on its own is not easily achieved). By targeting our manipulation either in sleep or waking states, and measuring retrieval processes multiple times, we may also gain a more complex understanding of when different consolidation processes take place, and what effect these have on the memory trace.

Sleep and memory – Systems consolidation theory

Sleep is essential for many processes that happen in the brain, as well as the rest of the body. The restorative effects of sleep are well known to everyone, and the cognitive toll that comes with sleep deprivation has not only been felt by most, but also tested and measured in laboratory settings (Pilcher & Huffcutt, 1996). Sleep deprivation has been proven to cause a marked decline in executive functioning, and can lead to serious cognitive symptoms such as confusion, hallucinations and delirium (Samkoff & Jacques, 1991). From a physical health perspective, Samkoff and Jacques also noted that sleep deprivation can lead to irregular heart rhythms, irregularities in body temperature, and has a negative impact on the immune system and can even lead to death if the deprivation is extended.

Aside from the restorative biological and cognitive roles of sleep, it also seems to play an important part in memory.

One study on the link between sleep and memory selectively deprived people of a single stage of their sleep and examined what effects the loss of that stage had on different types of memory (Rauchs, Desgranges, Foret, & Eustache, 2005; Rotenberg, 1992). Through these approaches, it was discovered that Rapid Eye Movement (REM) sleep was more important for procedural memories (how to do things), as well as memories with high emotional content, than for episodic (personal events) and declarative memories (common knowledge, facts, not tied to personal self). Rauchs et al. (2005) also noted in their review that non-REM, and more specifically, in most cases slow-wave sleep (SWS) has been found to be important for the consolidation of associative and episodic memories.

The current leading theory to explain how consolidation happens during sleep is the systems consolidation theory (Born & Wilhelm, 2012; Frankland & Bontempi, 2005; Pavlides & Winson, 1989). This theory states that memories are consolidated through multiple unconscious reactivations of prior learned memories. The first evidence of replay was found in rodents (Pavlides, 1989): Place cells that were active during waking periods were more likely to become active during subsequent sleep. Replay of more complex memories was observed, using single unit recordings of place cells, cells that specifically record an animal's position in a certain environment, during learning of a path and subsequent sleep (Wilson & McNaughton, 1994). Wilson and McNaughton (1994) observed that the place ensembles, being more complex representations of the followed path, that were active in the hippocampus during the exploration of a certain maze, reactivate during sleep.

The underlying neural activity behind this replay seems to be a case of first synchronisation, then communication between the hippocampus and neocortical areas (Girardeau, Benchenane, Wiener, Buzsáki, & Zugaro, 2009; Girardeau & Zugaro, 2011). Sharp wave ripples (SWR) in the hippocampus seem to be the events during which replay occurs most often, and blocking these events has been shown to cause the consolidation effect to disappear completely. These ripples travel over the brain, acting as carriers of the memory information. Slow wave sleep represents a state where large populations of neurons are either active or inactive at the same time. This is then a prime example of a period when neocortical areas and subcortical areas are synchronised. During these synchronised states, the memory information can be transmitted in the form of thalamo-cortical spindles which spread across the neocortex. Nested in these thalamo-cortical spindles, SWR form in the hippocampus, in which the actual memory trace is encoded. By repeating and spreading the activation related to a memory trace during synchronised periods of SWS, the memory is transformed from a temporary form stored in the hippocampus, to an integrated form stored in the neocortex (Rasch & Born, 2013).

The research in this field relies on being able to provoke memory reactivations in a reliable way, in order to study their functional significance. It has been observed that these reactivations can be provoked by presenting one of a pair of associated stimuli (Oudiette, Antony, Creery, & Paller, 2013; Oudiette & Paller, 2013; Rasch & Born, 2007; Rasch, Büchel, Gais, & Born, 2007; Rudoy, Voss, Westerberg, & Paller, 2009; Schönauer, Geisler, & Gais, 2014). Participants would learn pairs of stimuli (sounds paired with targets which could be other sounds or images), and then to unobtrusively stimulate the replay of the full association, those sounds are presented during non-REM. By presenting these cues during sleep, it has been found that the associated memories become stronger (have a higher chance to be recalled) than the memories for which cueing was not performed.

Importantly, this cueing effect appears to be hippocampus-dependent; this can be one of the reasons why the hippocampus is essential for the formation of long-term memories. An experiment conducted by Fuentemilla and colleagues (2013) has shown a strong negative correlation between the amount of hippocampal damage and the strength of the cueing effect in epilepsy patients. They showed that the effect is hippocampus-dependent, as patients with bilaterally removed hippocampi did not benefit from cueing at all (Fuentemilla et al., 2013).

Oscillatory recall effects

As previously mentioned, recall is the process through which memories become active and consciously available, and has been associated with a marked desynchronisation in the alpha and beta frequency ranges, reflecting reactivation of the memory trace (Dujardin, Bourriez, & Guieu, 1994; Klimesch, 1999). The role of alpha oscillations has been proposed to be one of targeted inhibition of task-irrelevant information, as increases in alpha power have been observed in relation to the number

of distractors involved in visual processing tasks (Bonnefond & Jensen, 2012). The alpha power increase also seemed to be lateralised: if task irrelevant information was presented in the right visual field, alpha power increased in the contralateral occipital cortex, and vice-versa. In the case of memory retrieval, a review from Hanslmayr, Staudigl and Fellner (2012) proposed the information through desynchronisation hypothesis, which explains how desynchronisation in the alpha and beta ranges could reflect the richness of information carried by those oscillations, and therefore, aid in the recall of memories. An example of this effect can be seen in the study of Waldhauser et al. (2012), in which participants learned two separate memories, stored either in the left or right side of the visual cortex, associated with one cue. One of the memories was the target, the other a distractor. During recall, they observed increased alpha and beta activity in the hemisphere with the distractor memory, and a marked alpha desynchronisation in the side where the target was stored. This indicates that there was increased inhibition in the areas related to distractor information, while the target areas were more accessible (through the alpha desynchronisation) for recall.

Another phenomenon that has been observed in relation to recall is epochry: the reactivation of activity present during encoding. Another study by Waldhauser and colleagues (2016), demonstrated that episodic memory relies on reactivation of sensory information. By presenting stimuli in a lateralised manner during encoding, followed by retrieval using centrally presented retrieval cues, they were able to find that successful retrieval was associated with alpha and beta decreases in the visual cortex contralateral to the visual field at encoding. In another experiment Waldhauser et al., 2016), they tried disrupting this activation using transcranial magnetic stimulation (TMS), and found significant memory performance decreases only for memories stored in the disrupted visual field.

In this study, we focus on tracking the evolution of memories, more specifically related to memory reactivations during sleep, as well as the oscillatory effects of cueing during sleep on subsequent recall processes. In this regard, we asked participants to learn a list of associated word-picture pairs, use targeted memory cueing (presenting the words at low volume) as a way to provoke memory reactivations during sleep, and study the oscillatory and behavioural effects of this manipulation during recall. We expected to first reproduce the memory effect of desynchronisation in the alpha and beta ranges as well as find differences between cued and uncued words in this regard. As alpha desynchronisation has been associated with better memory, we propose that cued words would be remembered better and would have a greater associated alpha power decrease. As we present the pictures in a lateralised manner during encoding, we also hypothesise there to be significant lateralisation effects and we expect these effects to vary between cued and uncued words. More specifically we expect to find stronger lateralisation in cued words, as these would have a better-preserved memory trace.

Method

As this project is a part of a larger experiment meant to measure the effects of wakeful reactivations in comparison to sleep reactivations, here I will present the entire experimental protocol for reproducibility. This project strictly focuses on the final recall phase of the experiment, as well as the sleep cueing condition of the study.

Participants

Data from 15 participants, aged between 19 and 35 (M = 24, SD = 4.26; 8 female, 7 male), were collected. The participants were all Dutch native speakers in order to minimize any possible language effects associated with the task. None of the participants reported taking any medication at the time of the experiment nor any chronic medication and none had a history of neurological or psychiatric disorders. Participants who had any metal implants (including dental wires) were also excluded from the experiment to reduce noise in the Magnetoencephalogram (MEG) signal. Participants were also informed of the sleeping conditions and were required to consider themselves "good sleepers", in order to ensure that they would be able to sleep in the lab. Also in this regard, on experimental dates, subjects were instructed to restrict their sleep to six hours. This study was approved by the ethics board of the Radboud University, and all participants gave informed consent to the experiment.

General procedure and task design

The experiment was performed in the MEG laboratory of the Donders Institute, Radboud University, Nijmegen. Participants came in for a total of four sessions during the experiment: one adaptation evening, two experimental evenings, and

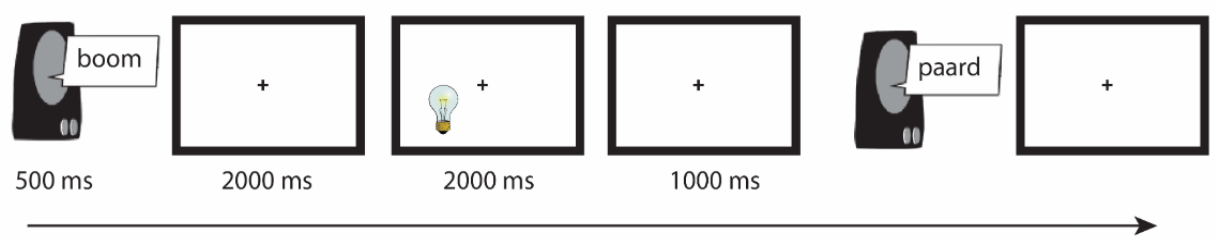

Fig. 1. Learning form of the task, adapted from Schreiner & Rasch (2015).

one Magnetic Resonance Imaging (MRI) session, with the two experimental conditions being separated by one week, creating a repeated measures design, where we compared the performance and neural underpinnings between conditions. During the first adaptation evening, participants were exposed to the MEG environment and allowed to sleep for a total of 60 minutes. The role of this initial meeting was to get participants habituated to the novel environment and to ensure that they would be able to sleep in the MEG. The second and third nights were the experimental evenings, during which a memory task was learned and tested repeatedly after a period of active wakefulness (60 minutes) and one of sleep (120 minutes). The memory task had three forms: learning, cued recall with feedback, and cued recall without feedback. In the learning form, participants were instructed to learn as many word picture associations as they could, with the words being presented in an auditory manner (500 ms), followed (after a fixation cross of 2000 ms) by a lateralised presentation of the picture stimulus (2000 ms, and finally another fixation for 1000 ms; see Figure 1). A total of 140 word-picture pairs were presented, with 70 pictures appearing in the right visual field and the other 70 in the left. The entire task was presented and solved in Dutch.

In the feedback form of the task, after the word was presented, a question mark appeared, during which participants had to try to name the object previously presented in the picture associated with the word (see Figure 2). After their response was recorded, participants were presented with the associated picture again, in order to strengthen the memory. The recall version of the task was the same as the feedback version, except for the fact that participants no longer received feedback (the correct image) after their response, and its role was to measure the strength of the memory at different steps during the experimental design.

In order to have a more robust measure of memory, and the role of waking and sleeping reactivations, multiple measures of recall were needed. During experimental dates, participants first completed the initial learning and feedback learning versions of the task, after which a first recall was measured. After this, a period of relaxed wakefulness ensued (of 60 minutes) during which participants had to complete a number sorting task (odd-even), in order to ensure vigilance. Recall was then measured again, before allowing participants to sleep for 120 minutes. Finally, recall was measured one last time. During one of the experimental nights, cueing was applied during the waking phase, while in the other, cueing was applied during the sleeping phase (see Figure 3 for full experimental design). Different word lists were used for the experimental evenings, to avoid learning effects. The order of the word lists, as well as the order of the experimental evenings were counterbalanced across participants.

Of the 140 words on the list, half of the remembered pairs and half of the forgotten pairs were randomly chosen as cue targets. Whether a pair was marked as remembered or not remembered, was decided by the performance on the recall, immediately prior to the cueing phase. As such, half of the remembered words associated with left or right lateralised pictures were cued or not cued

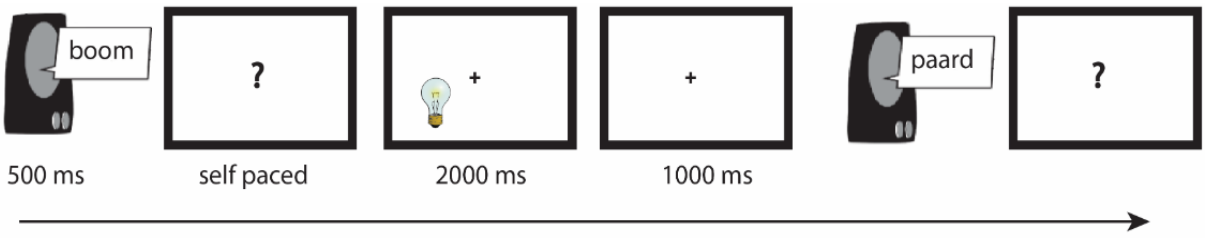

Fig. 2. Cued recall form of the task, adapted from Schreiner & Rasch (2015).

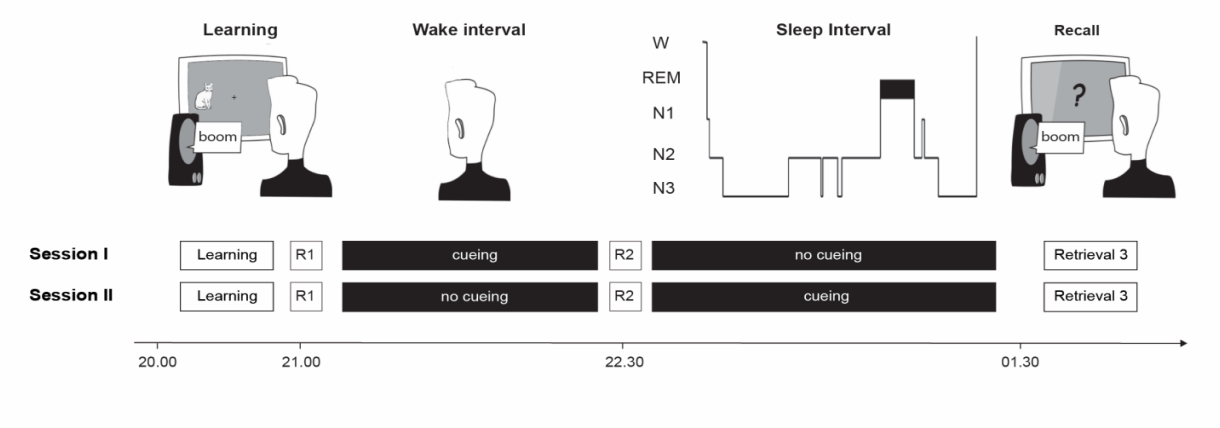

Fig. 3. Experimental design, figure adapted from Schreiner & Rasch (2015).

respectively. Cueing was realised by playing back the previously associated words at a low volume level (approximately 50dB), either during the number sorting task, or during non-REM sleep, depending on the experimental condition. Presentation occurred every 5800 - 6200 ms in a randomized order for a total of 60 minutes, resulting in ~10 exposures to each word. Participants' sleep was constantly monitored to ensure that the cueing did not wake them up.

Recording, software, pre-processing

Electroencephalogram (EEG) data were collected from four electrodes (F3, F4, C3, C4) with a left mastoid reference (A1) placed according to the 10-20 system, at a sampling rate of 600 Hz. The MEG recorded data from 275 axial gradiometers at a sampling rate of 600 Hz (VSM/CTF Systems, Port Coquitlam, British Columbia, Canada). Head position relative to the helmet was monitored with the use of three head-localisation coils (placed in the ears and on the nasion). The data from these coils were analysed using a real-time head localiser (Stolk, Todorovic, Schoffelen, & Oostenveld, 2013). Four electrodes were also placed on the participants' faces for the collection of vertical and horizontal Electrooculogram (EOG).

The stimuli were presented using presentation software and all analyses were conducted in Matlab. The fieldtrip toolbox created for Matlab (Oostenveld, Fries, Maris, & Schoffelen, 2011) was also used in the oscillatory analyses. Data were divided into single trials of 3 seconds after the auditory stimulus presentation. Trials were corrected for cardiac and eye movement artifacts using independent component analysis (Makeig, Bell, Jung, & Sejnowski, 1996) after manual artefact rejection. The trials were sorted into conditions as a function of final recall performance (remembered versus forgotten), cueing condition (cued versus uncued) and initial stimulus presentation (left versus right). Frequency decomposition of the data was achieved via Fourier analysis based on sliding time windows (moving forward in 10 ms increments). The settings were optimised for low frequency ranges (2-29 Hz, 1-Hz steps), the window length was set to five cycles of a given frequency (for example, 500 ms for 10 Hz; 250 ms for 20 Hz), and the windowed data segments were multiplied with a Hanning taper before Fourier analysis. The resulting power maps were normalised by dividing over the averaged −1 s pre-stimulus baseline window and subjected to direct comparison between conditions of interest.

A lateralisation index was computed, adapted from D'arcy et al. (2013), by subtracting the activity observed in the left condition from activity observed in the right condition and dividing by the sum of these. In this way, we can see which activity is specific to processing information presented in one visual field.

Results

Behavioural results

The behavioural data were analysed using IBM SPSS Statistics 20, using the standard settings. We used repeated measures two-way Analysis of Variance (ANOVA) for factorial analysis of effects, and post-hoc t-tests for confirmation and detailing. Repeated measures t-tests were also used to exclude certain possible confound effects (equivalency between pre-cue periods was assured).

Already from the behavioural results we can deduce that cueing words led to an increase in performance, independent of whether cueing was performed during the waking or sleeping state. We

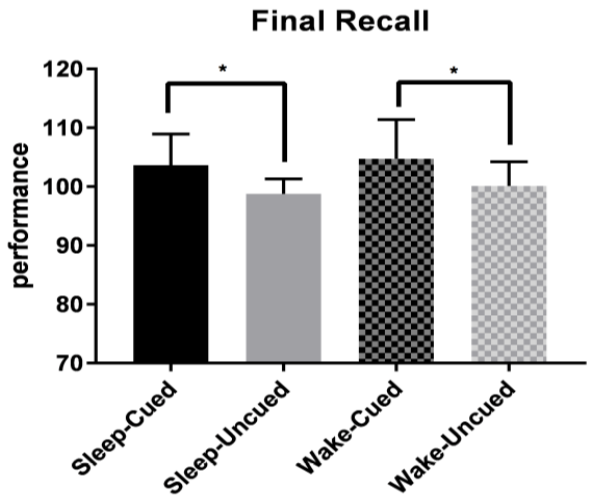

Fig. 4. Hit increases between second recall and final recall expressed in percentage; * denotes significant differences at $p < .05$.

used a repeated measures ANOVA to measure the comparative effects of cue presence (cued versus uncued), cue time (wake versus sleep cueing) and lateralisation (words presented on the left versus right during encoding), as well as any interactions between these effects (see Figure 4). Firstly, cueing has an effect independent of the cue time on the performance during the final recall, with cued words being better remembered than uncued ones (main effect of cue presence: $F(1,14) = 11.89$, $p = .004$). Secondly, this cueing effect is not different between the conditions (cue time effect: $F(1, 14) = .6$, $p = .44$, interaction cue time X cue presence: $F(1, 14) = .13$, $p = .91$). Post-hoc repeated measures t-tests between cued and uncued words revealed this effect in both the sleep ($t(14) = 2.85$, $p = .01$) and wake ($t(14) = 2.51$, $p = .02$) conditions.

When comparing performance in the recalls immediately pre and post cueing using paired samples t-tests, we observed that only sleep cueing has an immediate effect ($t(14) = -2.35$, $p = .03$), while wake cueing does not ($t(14) = -1.9$, $p = .07$). This suggests that wake cueing requires subsequent sleep in order to have an effect on performance. Thus, when comparing the effects of cueing, lateralisation, and time of cueing, the only significant factor is whether the pairs were cued or not. An interesting fact that needs to be pointed out is that performance during the final recall was almost always at least as good as the performance in the previous recall stage (the performance not dropping below 100% of the previous round). This could be due to the fact that even uncued words may be reactivated without the aid of cueing.

Furthermore, we used paired samples t-tests to check whether the pre-cue periods were equivalent between conditions. This is necessary due to the fact that in the sleep cueing condition, the participant goes through two recall sessions before cueing takes place (and one after), while in the wake cueing condition, there is one recall before cueing and two afterwards. This analysis proved, as expected, that before cueing the participants had the same performance in the wake and sleep cueing conditions, meaning that the differences observed during the final recall were due to the cueing manipulation ($t(14) = .5$, $p = .5$).

Finally, to assure that there were no immediate effects of cueing in the wake condition, we ran another paired samples t-test between the pre-cue and post-cue interval in the wake condition. This analysis showed that sleep was necessary for the wake cueing to function by showing that wake cueing on its own did not produce the observed final effects ($t(14) = -1.9$, $p = .07$).

Oscillatory results

Seeing that there is a behavioural effect of cueing, the next step in the analysis was to look for the neural underpinnings of this effect. In other words, what changes does cueing provoke from an oscillatory perspective that correlate with our behavioural results? Before looking into more detailed, fine-grained cueing effects, we first searched for the oscillatory underpinnings of successful retrieval in order to further orient our search. In this regard, we ran a memory contrast, between remembered and forgotten words across the entire epoch and with the frequency range between 3 and 20 Hz, as most memory effects are found within this range. A single negative cluster was observed in the alpha range, between 0.6 and 1.7 s, not only confirming the main memory effect, but also informing the following analyses (see Figure 5). We used the time and frequency ranges found in the first analysis to inform the following analyses, as cueing directly impacts the strength of the memory, and as such we expected the effects to be similar. Also important to note is the localization of this effect, the topography suggesting the source of the activity being localised in the parietal region, which is in line with many recent memory studies (Gluth, Sommer, Rieskamp, & Büchel, 2015; Tanaka et al., 2014).

The next step in searching for the oscillatory underpinnings was to look for differences between the cued and uncued words, as this was the main effect found in the behavioural analysis. Running

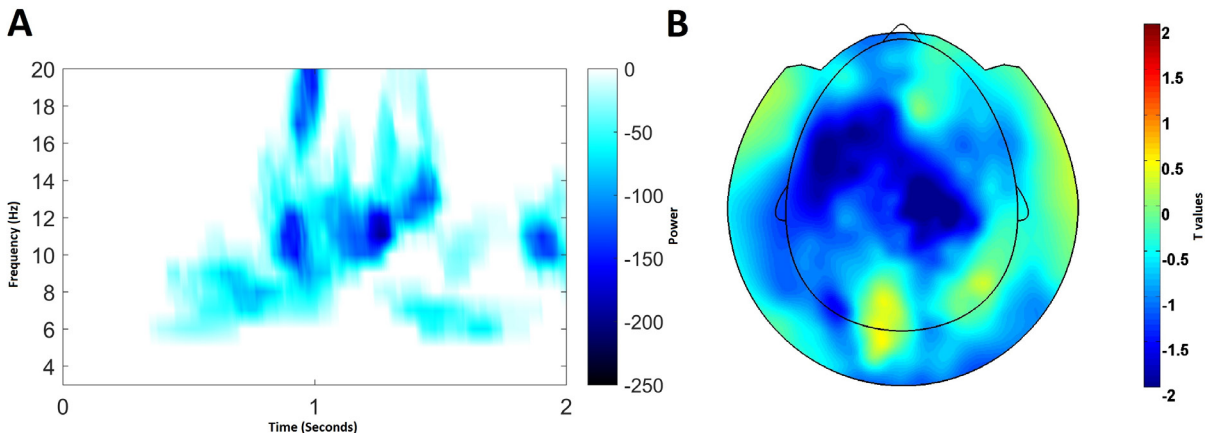

Fig. 5A. Power contrast between remembered and forgotten words during the final recall. **B.** Localisation of effect found in A in the form of t-values, taking into account all sensors.

a contrast between cued and uncued words that were remembered yielded no significant clusters, nor did the same contrast for words that were forgotten (neither for the full time interval and over all frequencies, nor for specifically 0.6-1.6s, 10-14 Hz). These results suggest that there is no combined effect of cueing and memory, yet this clashes with the behavioural results. While not finding an effect in the case of forgotten words can be expected, in this case, the memory is not accessible, and thus, any memory effects would disappear. Not finding a cueing effect in the remembered words contradicts the systems consolidation theory.

Contrasts between remembered and forgotten words between 10-14 Hz from 0.6-1.7 s post stimulus in the cued and uncued conditions, on the other hand, revealed a negative cluster in the uncued conditions (p = .05) and a negative trend (p = .06) in the cued condition (see Figure 6). These results, while being more in line with previous findings revealing the previous memory effect, still do not explain the difference between cued and uncued words, as the effects are in the same direction.

As these effects do not fully explain our behavioural results, we decided to look more closely at the phenomenon of epochry and how it behaves as a function of cueing. As such, we prepared a lateralisation index, by subtracting the activity observed when recalling images presented on the right side of the screen from the activity when recalling

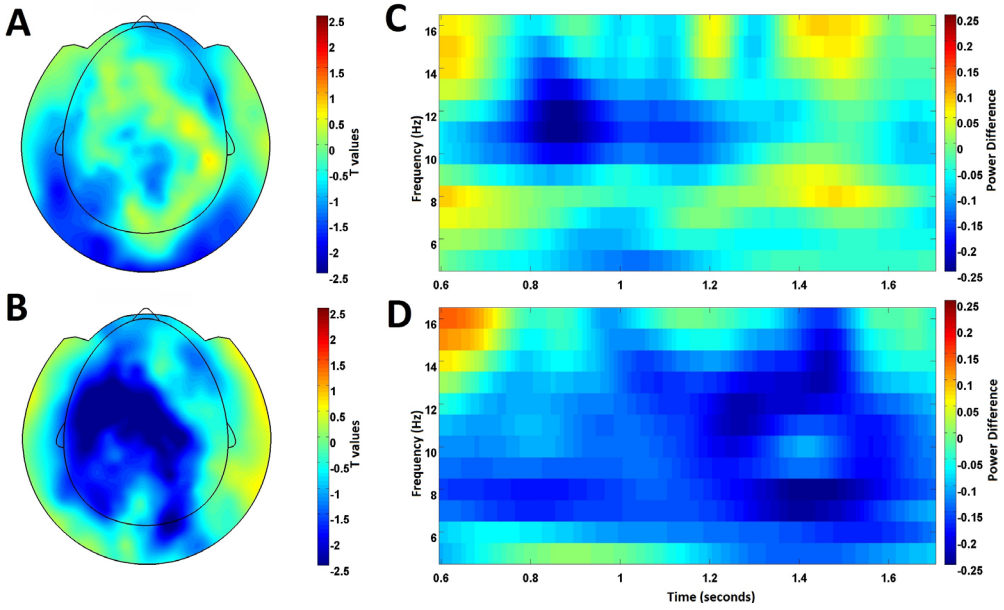

Fig. 6. Contrast between remembered and forgotten words in the cued (**A** and **C**) and uncued (**B** and **D**) conditions. **C** and **D** represent the average power values of the difference between all significant sensors observed in **A** and **B**.

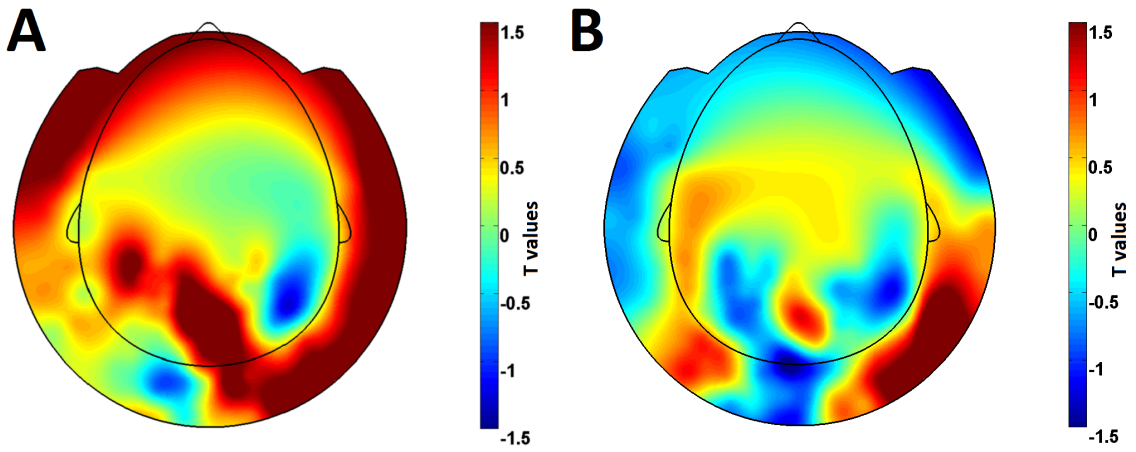

Fig. 7. Topography of the difference between the left and right lateralisation indices in the cued (**A**) and uncued (**B**) conditions, excluding frontal sensors. Time range 0.7-1 seconds, Frequency range 10-14 Hz.

images on the left side of the screen and divided by their sum (left-right/left+right). By contrasting the lateralisation indices for remembered, cued words, and excluding frontal sensors, a significant cluster was observed in the alpha range (p = .04, from 0.7 to 1 s and between 10-14 Hz). Applying this same contrast to uncued words, on the other hand, yielded no significant clusters (see Figure 7).

This result is pivotal, as it shows a clear effect of cueing that is not present in the uncued condition, and as such could underlie our behavioural effects. The fact that there is a difference between words encoded on the left side and words encoded on the right side simply confirms the phenomenon of epochry. The fact that this effect changes as a function of cueing shows that cueing words helps preserve the memory trace, the reactivation of the memories being stronger in the cued condition. The fact that this is a lateralised effect could also explain why differences were not found earlier in the analysis, as it may act as a confound in the other analyses.

Discussion

This study aimed to take a look at the effects of memory reactivations during sleep on subsequent recall, by comparing the neural activity observed when recalling words that were cued during prior sleep with those which were not. In this regard, we asked participants to learn word-picture pairs, with the words being presented in an auditory manner. Then, during a period of subsequent sleep we cued half of the words (by replaying at a low volume), while leaving the rest to be consolidated under normal conditions. Finally, we observed the differences between these words during the final recall period from behavioural and oscillatory points of view.

Our behavioural results provide evidence sustaining the systems consolidation theory, and offer a good starting point to look for the oscillatory mechanisms that sustain this cueing effect. Firstly, and most clear of all, there is a behavioural effect of cueing on memory. This provides evidence for the fact that memory reactivations strengthen memories. This result also fits with previous cueing studies which have found similar effects when cueing pairs of associated stimuli (Rasch, Büchel, Gais, & Born, 2007; Schreiner, Göldi, & Rasch, 2015; Schreiner & Rasch, 2015).

The neural underpinnings of this behavioural effect were more difficult to isolate, yet the effects that we found fall in line with previous literature about systems consolidation theory, as well as epochry. The initial memory contrast provided data that confirms previous work on memory-related effects (Dujardin et al., 1994; Hanslmayr, Staudigl, & Fellner, 2012) and offered us a starting point for the rest of our analysis, narrowing down both the time and frequency windows to memory-related activity.

In accordance with previous findings, we observed desynchronisation in the alpha band related to successful retrieval of memories. This effect has been previously described in multiple other studies and is thought to reflect the memory trace becoming active, a greater desynchronisation in the alpha band allowing for easier access to the memory through lowered inhibition (Hanslmayr et al., 2012). Furthermore, this activity seemed to not differ significantly between cued and uncued conditions, which is a relatively surprising result

taking into account that cued memories were easier to recall than uncued ones, from a behavioural perspective. This apparently indicates that there is no combined effect of cueing and memory, yet this does not fall in line with our behavioural results.

As such we decided to look more closely at how the memory itself changes as a function of cueing. Words presented on the left side of the screen during the learning period cause an increase in the activity present in the contralateral (right) occipital cortex. Because we expect this activity to correlate with the activity present during retrieval (leading to contralateral increases during retrieval), we used this as a marker of the strength of the memory. In this way, we found clear effects in the cued condition that were not found in the uncued condition. In other words, the activity present during retrieval was more lateralised in the cued condition, leading to the conclusion that cueing increases epochry.

This lateralisation effect could also be a possible explanation for the lack of direct differences between cued and uncued conditions when looking at alpha desynchronisation. Because the desynchronisation is lateralised, by averaging over conditions the activity related to left and right words would cancel each other out, and this canceling would be more powerful in the cued condition since all words would present a stronger lateralisation. Meanwhile in the uncued condition, some words would be spontaneously replayed, leading to a less lateralised trace that would not cancel out as strongly through averaging.

In the context of systems consolidation theory, our data suggest that memory reactivations during sleep cause the engram of the memory to be more accessible during recall, preventing the deterioration of the memory trace. The observed lateralised activity in the cueing condition suggests that cueing helps preserve the engram. Meanwhile, the lack of these reactivations results in a weaker memory trace, and as such does not lead to any increases. The fact that cueing during sleep has this effect is in accordance with the systems consolidation theory, which states that reactivations during sleep cause memories to be transformed from a labile short term state to a more stable long term representation (Rasch & Born, 2013). The fact that we cannot be sure that uncued words are not replayed automatically during sleep can explain why we did not observe drops in performance for uncued words, and can also be one of the reasons why the neural effects were more difficult to see.

In order to continue this line of research and build on these results, the next logical step would be to follow this lateralisation effect and its lateralisation across the rest of the recall periods. In this way, we would be able to quantify the memory losses due to the passage of time, as well as see how this degradation is affected by cueing in the short term (immediate effects), as well as in the long term (post-sleep effects). By following the evolution of this effect in the wake cueing condition as well as the sleep cueing one, we would be able to have a clearer, larger picture, which has better ecological validity, as memory reactivations in humans are almost never cued (in a way similar to the one we tested), and happen spuriously throughout the day and during sleep (Atherton, Dupret, & Mellor, 2015). For example, in the wake cueing condition, we would expect that the memory trace becomes weaker up to sleep, after which it would bounce back to the original levels.

Another analysis that could strengthen our argument and could be further applied to the data would be to use a more specific measure of the memory trace such as Representational Similarity Analysis (RSA) in order to capture the reactivations occurring during sleep. RSA could be used to compare the activity observed during encoding with the activity observed immediately post-cue, which, if positive, would be the first direct evidence of human memory reactivations during sleep. The functional relevance of these reactivations could then be studied by comparing these to memory performance. Furthermore, this type of analysis could also yield insights into how wake reactivations work and what their role is, by searching through the sleeping period for the activity of previously cued words.

Using a different experimental protocol, not dependent on cueing, with this kind of analysis, it might also be possible to capture and study natural memory reactivations, by provoking them through different means. For example, by taking advantage of the directed attention effect on memory (Dulas & Duarte, 2013) we can increase the likelihood of certain words to be replayed. We can then search for the representations of those specific words during subsequent waking or sleeping periods and thus have a model for natural memory formation and integration.

Conclusion

This thesis is part of a larger project with the aim of disentangling the effects of wake reactivations of memories from the effects of reactivations occurring during sleep. This thesis aids in reaching

that goal, not only by demonstrating the effects of cueing during sleep, but also by outlining a way to measure the effects of cueing directly on the memory trace. We have found both behavioural and oscillatory effects of cueing during sleep. Starting with demonstrating the alpha desynchronisation related to memory and building up to demonstrating that sleep cueing directly increases the accessibility to the memories that were cued, this project adds to the current knowledge in the field of sleep and memory research. By design, these findings provide clear evidence that cueing during sleep has a positive effect on memory. Furthermore, this project adds to the current understanding of how memory reactivations strengthen memories by demonstrating that the original memory is more active after cueing during sleep. From a methodological perspective, this project adds to the field by outlining a way to search for reactivations of encoded memories. To be fair, the reactivations that were described in this thesis are related to recall, but there is no reason that the same statistical methods could not be used to study reactivations pertaining to the integration of memories. By applying these methods to other parts of the experiment, we could glean knowledge about the differentiated effects of sleep and wake cueing. In conclusion, this project adds both to the field of sleep research as a whole, and to the larger project that it is a part of.

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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

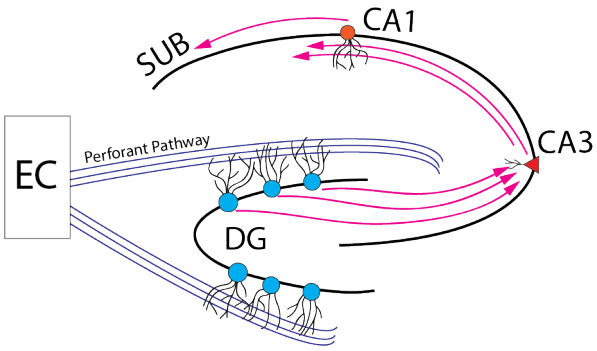

Fig. 1. Schematic representation of the hippocampus. The entorhinal cortex performant projections to, for example, the molecular layer of the dentate gyrus, which in turn project to the CA3 of the hippocampal formation. [EC = Entorhinal cortex, SUB = subicular cortex, DG = dentate gyrus, CA3 = hippocampal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

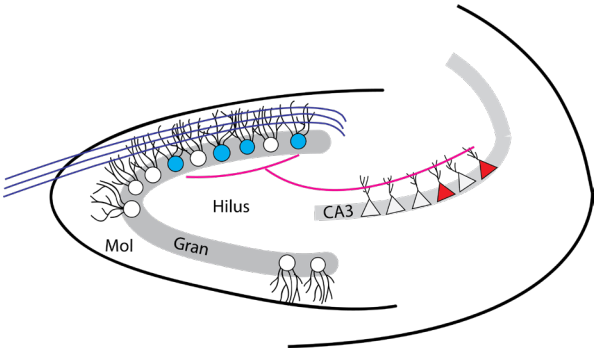

Fig. 2. Schematic representation of pattern separation in the DG. The memory representation enters via the molecular layer towards the neurons in the principal cell layer. From there information travels via the hilus towards the hippocampal formation CA3 region, where fewer nodes are activated and, thus, 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

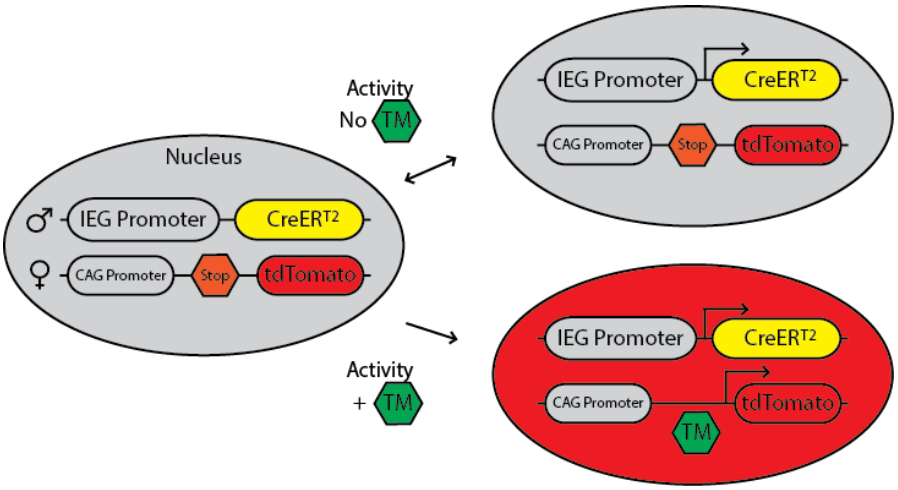

Fig. 3. A heterozygous male knock-in mouse CreER^{T2} from the activity dependent Fos immediate early gene (IEG) was crossed with a mouse with a homozygous knock-in allele of the Rosa26 locus that allowed expression of the effector gene *tdTomato* under the control of Cre-recombinase. In the absence 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).

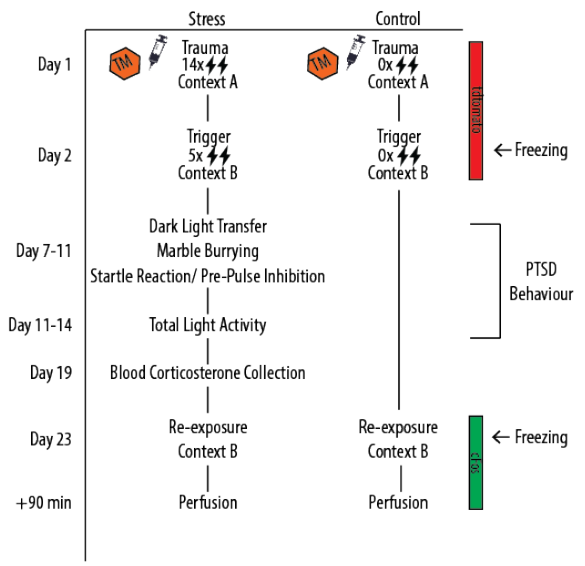

Fig. 4. Timeline of the PTSD induction model and behavioural tests. Injection of tamoxifen (TM) prior to the trauma ensured optimal *tdTomato* labeling and permanent labeling of all cells active during memory encoding. At the last day of our model, mice were perfused 90 minutes after the re-exposure 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).

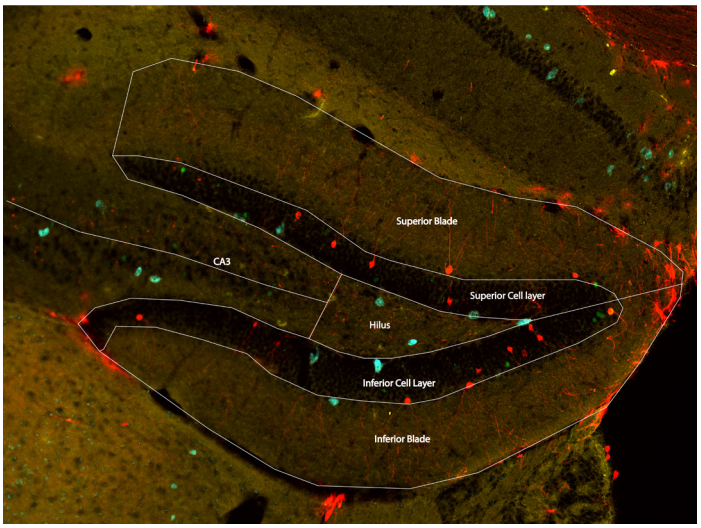

Fig. 6. Region boundaries for cell counting. Regions were divided into the blades (superior and inferior), the granule cell layers (superior and inferior), and the hilus region. The hilus region was considered to be the triangle within the superior and inferior cell layer, which ended at the beginning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

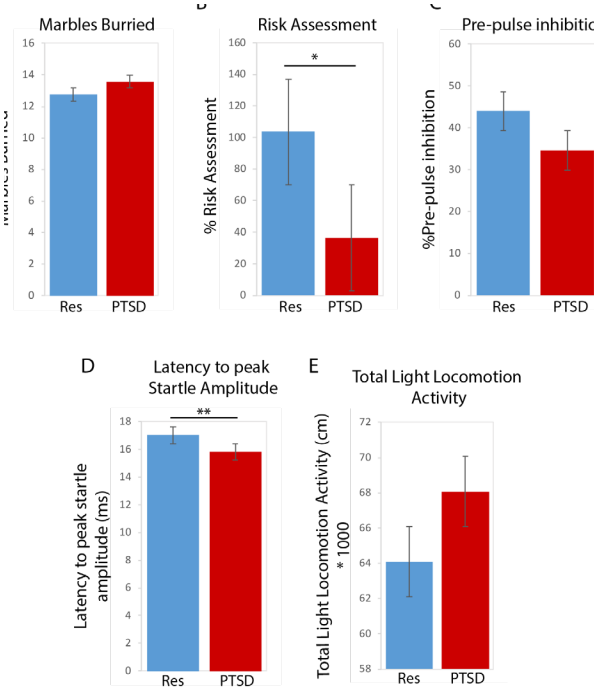

Fig. 7. A. Resilient (n = 8) and PTSD-like mice (n = 9) show no differences in behaviour in amount of marbles buried. **B.** Resilient mice show significantly more risk assessment behaviour than PTSD-like mice. **C.** There is no significant difference between mice in startle response, although **D.** 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. *: $p < .05$; **: $p < .01$.

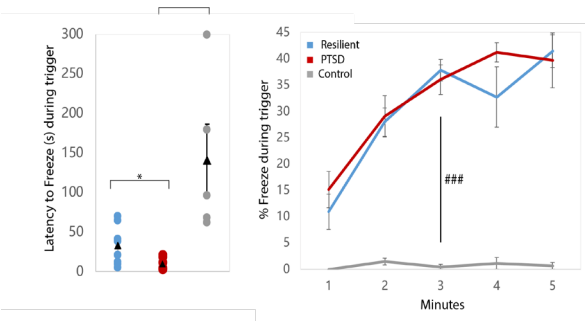

Fig. 8. PTSD-like mice (n = 8) show a significant faster latency to freeze during the trigger session than control mice (n = 5) due to the inescapable administration of foot shocks. Also, PTSD-like mice (n = 9) froze significantly faster than resilient mice (left). Total freezing was also significantly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

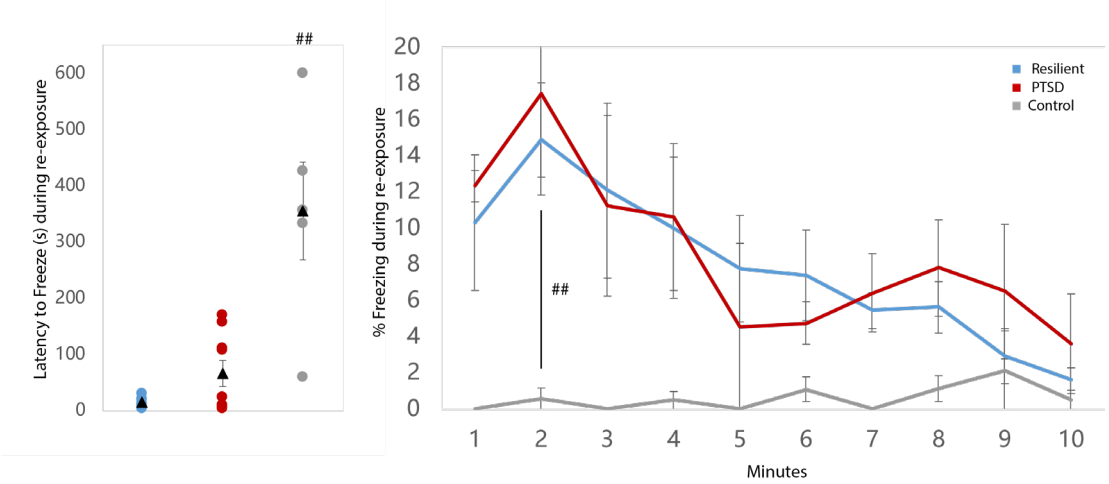

Fig. 9. Trauma-exposed mice (n = 17) froze significantly faster during the re-exposure to the traumatic context in comparison to control mice (n = 5) (left). They also froze significantly longer than control mice. There were no significant differences between susceptible (n = 8) and resilient mice (n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

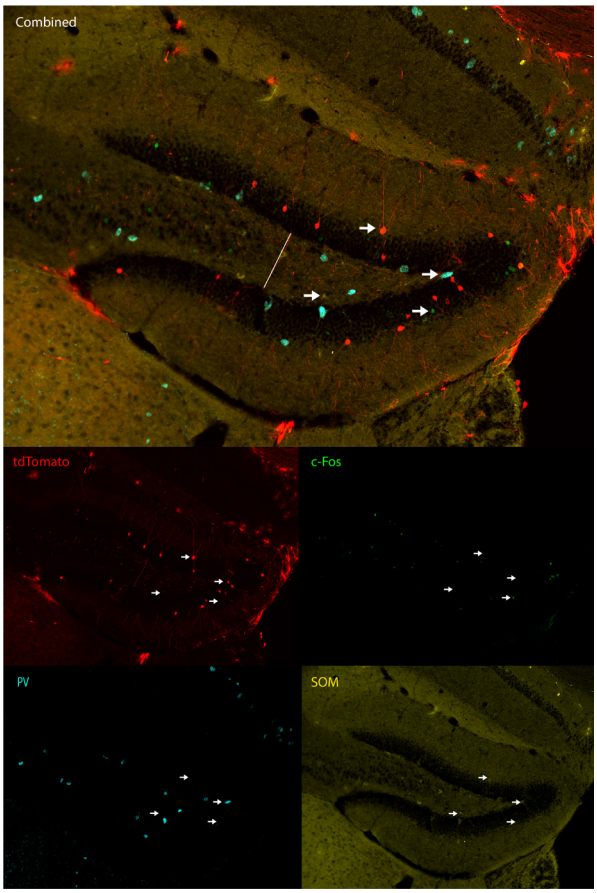

Fig. 10. Image of the DG. Cells were counted along the superior and inferior blades of the DG. Cells were considered to be in the hilus when located between the granule cell layers and the beginning of the CA3; depicted here with a white stripe. Arrows demonstrate a cell example of *tdTomato*, c-Fos,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

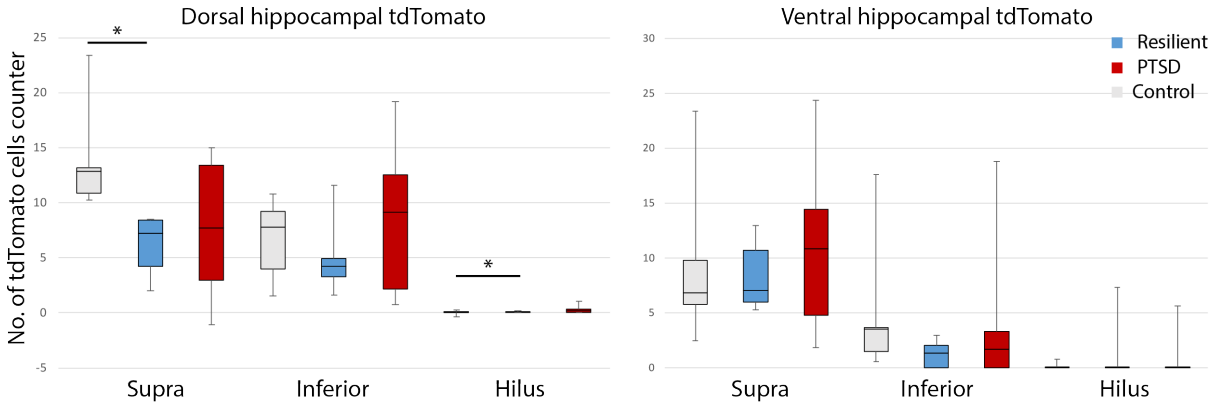

Fig. 11. tdTomato cell count differences in the dorsal and ventral hippocampus. There is a significant difference in the suprapyramidal region and the hilar region of the dorsal hippocampus between resilient and control mice. *: $p < .05$.

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

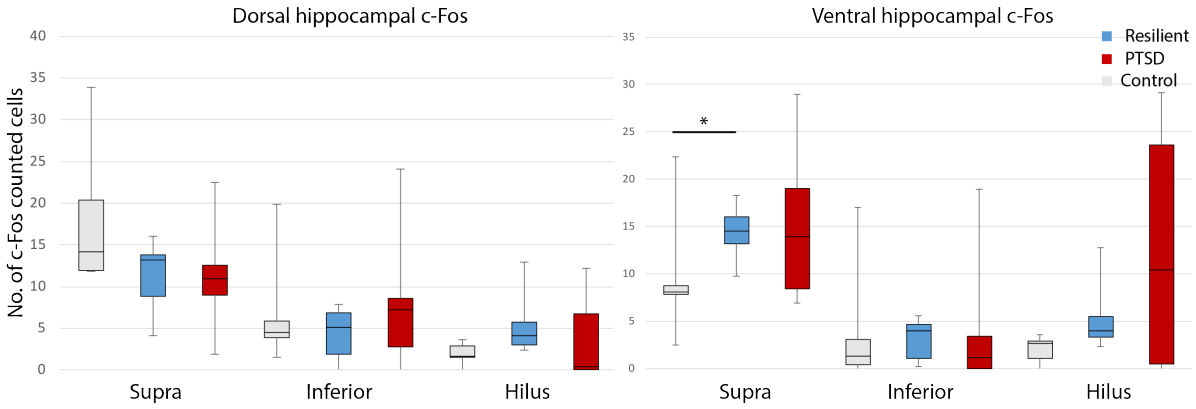

Fig. 12. c-Fos cells labeling in the dorsal and ventral hippocampus. There appeared to be a significant difference in the suprapyramidal region in the ventral hippocampus between resilient and control mice. * = $p < .05$.

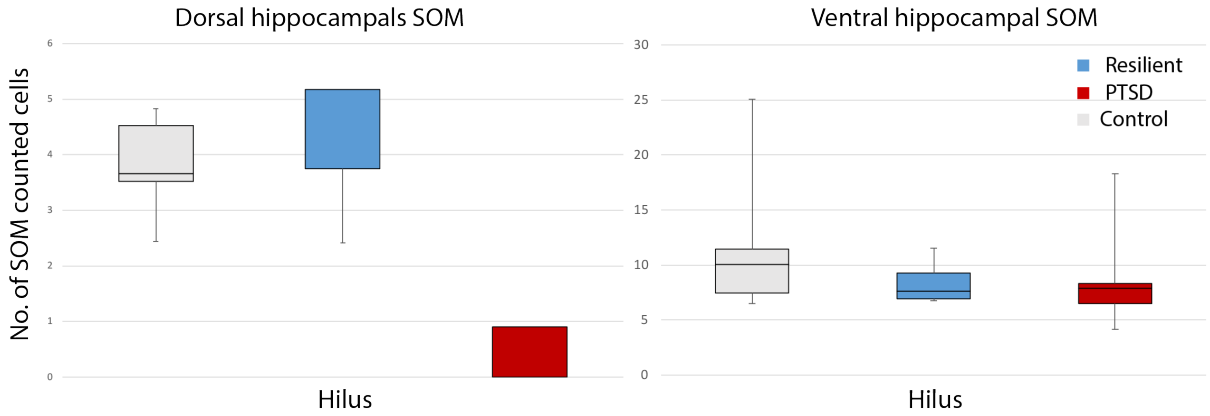

Fig. 13. SOM cell counts in the DG hilus of the dorsal and ventral hippocampus. There appeared to be no differences between the groups of mice.

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

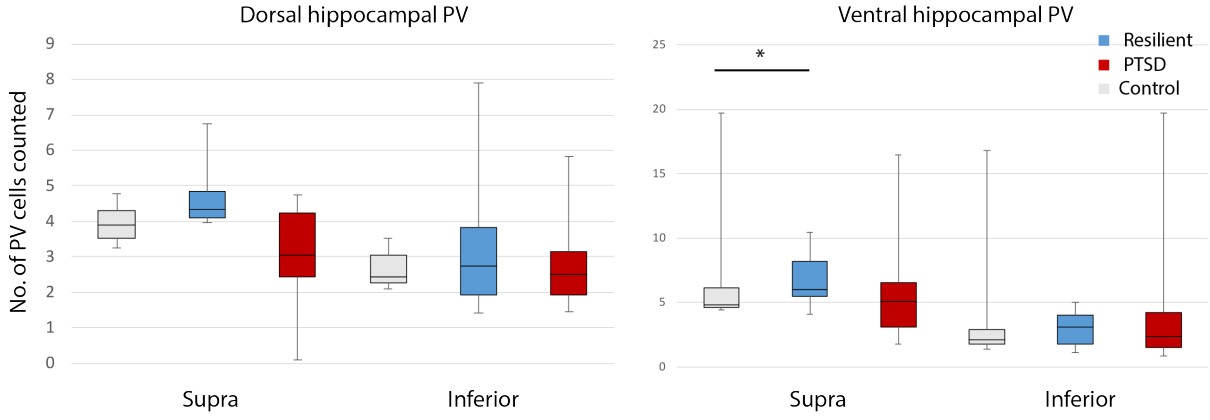

Fig. 14. PV cell counts in the suprapyramidal and infrapyramidal DG blades in the dorsal and ventral hippocampus. There appeared to be a significant difference between resilient mice and control mice in the suprapyramidal region of the ventral hippocampus. *: $p < .05$

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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Altered Frontostriatal Resting State Connectivity in Compulsivity-related Neurodevelopmental Disorders

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Obsessive Compulsive Disorder (OCD) and Autism Spectrum Disorder (ASD) share compulsivity-related symptoms and previous research has indicated similar underlying frontostriatal connections. Nevertheless, previous studies have solely focused on one disorder at a time, hampering direct comparison. Furthermore, inconsistent findings might have occurred due to heterogeneity within diagnostic groups and a direct focus on overlapping symptom dimensions might generate more coherent results. Here, we addressed functional connectivity of frontostriatal circuits in OCD and ASD and additionally in relation to compulsive behaviour across groups. Resting state functional magnetic resonance imaging data as well as the Repetitive Behavior Scale-Revised were obtained from children with ASD (N= 25), OCD (N=21) and controls (N=24). A seed-based functional connectivity analysis was conducted by correlating the time series of striatal seed regions to the frontal lobe. Subsequently, we performed group comparisons and analysed cross-disorder associations between connectivity and compulsive behaviour. We detected no significant differences between groups. Across groups, more severe compulsive behaviour was related to lower functional connectivity between the nucleus accumbens and orbitofrontal cortex as well as lower connectivity between the caudate and supplementary motor area. These results show that compulsivity is related to decreased frontostriatal connectivity across disorders, possibly underlying the inability to inhibit behaviour.

Keywords: compulsivity, repetitive behaviour, striatum, frontal cortex, Autism Spectrum Disorder, Obsessive Compulsive Disorder, resting state, functional connectivity, frontostriatal circuit

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Compulsivity is the irresistible urge to perform a certain behaviour repeatedly (Robbins, Gillan, Smith, de Wit, & Ersche, 2012), such as skin picking or excessively washing one's hands. These routines affect the daily functioning of children with Obsessive Compulsive Disorder (OCD), who suffer from an inability to inhibit intrusive repetitive thoughts (obsessions) and behaviours (compulsions). Likewise, Autism Spectrum Disorder (ASD) is characterised by increased repetitive behaviour as well as deficits in communication and social interaction (American Psychiatric Association, 2013). ASD and OCD often coexist, either within the same individual or clustering within a family (Meier et al., 2015). The increased comorbidity rate between the two disorders and the overlapping symptoms of compulsivity suggest shared underlying neural correlates (Naaijen et al., 2017). Accordingly, the disorders ASD and OCD have been related to impairments in functional connectivity between areas in the frontal cortex and the striatum (Bernstein et al., 2016; Chen et al., 2016; Delmonte, Gallagher, O'Hanlon, McGrath, & Balsters, 2013; Di Martino et al., 2011; Harrison et al., 2009; Jung et al., 2013; Vaghi et al., 2017).

The striatum is divided into three nuclei, which form circuits with different areas of the frontal cortex. The nucleus accumbens (NAcc) of the striatum forms the limbic network with the anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC) and has been related to motivational control and reward processing (Haber & Knutson, 2010; Langen, Durston, Kas, van Engeland, & Staal, 2011). The caudate nucleus of the striatum creates a circuit with the dorsolateral prefrontal cortex (DLPFC), which is involved in cognitive control (Langen et al., 2011; Levy, Friedman, Davachi, & Goldman-Rakic, 1997). The putamen creates a circuit with pre-motor and motor regions. This so-called sensorimotor circuit is involved in motor learning and performance (Alexander & Crutcher., 1990; Langen et al., 2011). Di Martino et al. (2011) replicated these findings on frontostriatal circuits when investigating functional connectivity in resting state functional magnetic resonance imaging (R-fMRI). A benefit of investigating connectivity with R-fMRI is that multiple functionally distinguishable networks can be assessed within a single setting and without giving any demanding task to the participants (Oldehinkel, Francx, Beckmann, Buitleaar, & Mennes, 2013). Therefore, this approach has allowed researchers to study alterations of frontostriatal connectivity in patients with OCD and ASD, who might have difficulties in enduring longer scanning sessions and comprehending demanding tasks.

When compared to healthy controls, participants with OCD showed higher resting state functional connectivity of the NAcc with the OFC and ACC (Harrison et al., 2009; Vaghi et al., 2017). Additionally, symptom severity was related to higher connectivity of the NAcc with the medial (Jung et al., 2013) and anterior (Harrison et al., 2009) OFC and lower connectivity with the lateral OFC (Jung et al., 2013). Jung et al. (2013) reason that the medial OFC processes emotions and positive rewards, whereas the lateral OFC is related to behavioural inhibition and the processing of punishments. Decreased connectivity with the lateral OFC might therefore underlie the inability to inhibit compulsive behaviour. Other studies report decreased connectivity of the putamen from the sensorimotor circuit, with the OFC, medial- and inferior frontal gyrus (MFG; IFG) in OCD (Bernstein et al., 2016; Harrison et al., 2009; Vaghi et al., 2017). Decreased connectivity of the caudate with the DLPFC (Chen et al., 2016) and the inferior frontal gyrus (Vaghi et al., 2017) has also been found in OCD, pointing to an involvement of the cognitive circuit. Increased connectivity of the caudate with the ventrolateral prefrontal cortex was related to cognitive flexibility in OCD, suggesting that abnormalities in the cognitive circuit underlie the inability to switch flexibly between behavioural alternatives (Vaghi et al., 2017). Additionally, Vaghi et al. (2017) found decreased connectivity between the caudate and an area in the precentral gyrus, which is part of the motor systems. This indicates that also connectivity between the cognitive and sensorimotor circuit is altered in OCD.

Similar to research on OCD, a study reported increased functional connectivity of the NAcc with the right ACC and OFC as well as the MFG in ASD (Delmonte et al., 2013). Additionally, increased connectivity of the caudate with the MFG (Delmonte et al., 2013) and premotor areas was found in ASD (Turner, Frost, Linsenbardt, McIlroy, & Müller, 2006). Especially increased connectivity between the right caudate and the right MFG was related to more severe symptoms of restricted repetitive behaviour (Delmonte et al., 2013). Another study on ASD found elevated connectivity between the putamen and the left precentral gyrus, which contains the primary motor cortex (Di Martino et al., 2011). Overall, alterations within and between the three frontostriatal circuits seem to occur in both OCD and ASD. Yet, in order to identify similarities and differences in

frontostriatal dysfunctioning more specifically and directly, studies comparing both disorders in one set-up together with healthy controls are required. Therefore, the first aim of this study is to explore whether there are group specific and overlapping abnormalities within frontostriatal resting state connectivity in children with OCD and ASD as compared to healthy controls.

Furthermore, previous alterations of frontostriatal connectivity in ASD and OCD have not consistently been found and studies report altered connectivity between the striatum and several different areas in the frontal cortex (Bernstein et al., 2016; Delmonte et al., 2013). This might be due to the diversity of symptoms within a disorder such as ASD or OCD (Langen et al., 2011). Robbins et al. (2012) claim that current diagnostic labels create groups that are too heterogeneous, and a more direct analysis of symptom dimensions should be applied when studying compulsivity related disorders. Additionally, by comparing compulsivity across different disorders, commonalities in the biological mechanisms underlying these disorders might be identified, which could lead to new genetic and therapeutic insights (Robbins et al., 2012). Hence, a second aim of the present study is to assess the relation between resting state connectivity within frontostriatal circuits and compulsive behaviour across diagnostic groups.

Another reason for the diverse findings might have been the age groups of the chosen samples. A study on age-related effects in OCD found that differences in connectivity between the dorsal striatum and the ACC were especially represented in the youngest participants around the age of 11 years (Fitzgerald et al., 2011). Likewise, a study on ASD found that differences between participants with ASD and healthy controls in functional connectivity and interhemispheric correlation decrease with increasing age (Anderson, Locke, Kretzmann, Kasari, & the AIR-B Network, 2011). This might be due to the plasticity of the brain and the development of compensatory mechanisms. In order to improve early diagnosis, prognosis and treatment, more studies on connectivity in childhood are necessary (Hull et al., 2017). Therefore, we focussed the study on children between the ages of 8 and 16 years. We expected to find overlapping abnormalities in frontostriatal connectivity in OCD and ASD groups compared to healthy controls, as well as abnormally connected circuits in relation to compulsive behaviour across disorders.

Method

Participants

The current sample originated from the multicentre study 'Compuls' which was performed at four locations across Europe (Naaijen, 2016). Initially, participants for the ASD group met the criteria for a DSM-IV diagnosis (American Psychiatry Association, 2000). Additionally, patients with ASD were excluded if they had a comorbid diagnosis of OCD or attention-deficit/hyperactivity disorder (ADHD). Participants for the OCD group were included if they had a clinical diagnosis and/or a total score on the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS; Scahill et al., 1997) of seven or higher. In this group, participants with any comorbid disorder were included, except for a comorbid diagnosis of ASD. Healthy controls were excluded if they or a first-degree family member had a psychiatric disorder. Additionally, controls were only included if they scored within a normal range on the Child Behavior Checklist and Teacher Report Form (Bordin et al., 2013). All participants were between 8 and 16 years old, of Caucasian descent and did not have any contra-indications for MRI scanning. Only subjects with an IQ above 70 and the ability to communicate fluently in their native language were included in the study. Additionally, they were not allowed to have a major physical illness, past or present head injuries or neurological disorders.

The participants of two scan-sites were excluded from the present analysis, because the recruitment numbers of OCD participants were too low. This resulted in 29 included subjects from King's College London, London, United Kingdom and 104 subjects from Radboud University Medical Center and the Donders Institute for Brain, Cognition and Behaviour, Nijmegen, The Netherlands, with a complete resting state scan. 34 of these participants were diagnosed with ASD, 24 with OCD and 75 children did not have a diagnosis of ASD or OCD respectively. However, one participant from the ASD group was excluded due to comorbid symptoms of OCD and one participant was excluded because he did not have sufficient symptoms for a DSM-IV diagnosis. A participant from the control group was excluded due to too many symptoms of OCD, according to our phenotypic assessment. Additionally, a participant from the OCD group was excluded due to an incidental finding in the structural scan. Another seven subjects were

Table 1.
Demographic information of participants across groups

		Control	ASD	OCD	Test Statistic
N	**Nijmegen**	18	16	12	-
	London	6	9	9	-
% Male		60	58.33	61.91	$\chi^2(2, N = 70) = 0.26, p = .88$
Mean Age in years *(SD)*		11.99 (2.07)	11.64 (2.21)	12.25 (2.16)	$F(2,67) = 0.46, p = .64$
Mean IQ[a] (*SD*)		105.36 (12.27)	104.06 (13.82)	108.32 (17.18)	$F(2,53) = 0.44, p = .65$
RBS-R[b] (*SD*)		1 (1.51)	21.01 (18.07)	26.1 (23.29)	$F(2,65) = 13.97, p < .001$
% left handed		0%	8%	9.52%	-
Medication-use (*N*)		0	2	4	-

Abbreviations: *N* = Number of subjects; *SD* = standard deviation; RBS-R = Repetitive Behavior Scale-Revised; IQ = intelligence quotient

[a]Group mean and SD of estimated IQ based on four subtests of the Wechsler Intelligence Scale for Children-III (Canivez & Watkins, 1998). The estimated IQ was unknown for 13 subjects.
[b]Group mean and SD of total score on RBS-R (Lam & Aman, 2007).

excluded after a quality control procedure as described in the paragraph on pre-processing.

The remaining number of controls in Nijmegen, consisting of 63 subjects, was larger than the OCD group with 12 subjects and ASD group with 11 subjects. Therefore, a smaller healthy control group from Nijmegen was matched to the OCD group from Nijmegen, based on age, IQ and gender, using the full matching procedure of the package MatchIt in R (Ho, Imai, King, & Stuart, 2011; The R Core Team, 2014). The final sample consisted of 25 children with ASD, 21 children with OCD and 24 healthy controls between the ages of 8 and 16 years. Detailed group characteristics are listed in Table 1. Ethical approval for the project was obtained for each site individually. Before the start of participation, the parents filled in a written informed consent and children over the age of 12 filled in an informed assent. From children younger than 12, an oral informed assent was required.

Phenotypic Assessments

The ASD diagnosis and symptom severity was confirmed with a structured interview with the parents using the Autism Diagnostic Interview Revised (ADI-R; Lord, Rutter & Le Couteur, 1994). OCD symptoms were rated with the Children's Yale Brown Obsessive Compulsive Scale (CYBOCS; Scahill et al., 2014), in form of an interview with the parent(s) and child present. In order to assess possible comorbidities, such as conduct disorder, oppositional defiant disorder, anxiety disorder and the presence of tics and Tourette's syndrome, all parents were interviewed with either the structured Diagnostic Interview Schedule for Children (DISC; Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000) in London or the semi-structured Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS; Kaufman et al., 1997) in Nijmegen.

Compulsive behaviour was defined as the total score on the Repetitive Behavior Scale-Revised (RBS-R; Lam & Aman, 2007). This questionnaire was filled in by the parents and could yield a total score between 0 and 129. Up to two missing items were replaced with the participant's mean score of the respective subscale. As none of the subjects were missing more than two answers, no subjects had to be excluded during this step. The full intelligence quotient (IQ) was estimated by administration of four subtests of the Wechsler Intelligence Scale for Children-III (WISC-III; Canivez & Watkins, 1998): block design, vocabulary, similarities, and picture completion. Past and present use of prescribed medication was assessed with a questionnaire for the parents.

Data Acquisition

Prior to the fMRI scan, a practice session was

Table 2.
Scan parameters for the structural and resting state MRI across sites

Scan	Site	TR/TE/ TI (ms)	Flip angle	FOV	Matrix RL/AP/ slices	Voxel-size (mm)	Gap (%)	Parallel Imaging
T1	Nijmegen (Siemens)	2300[a]/ 2.98/ 900	9	256	212/256/ 176	1.0 x 1.0 x 1.2	NA	2
	London (GE)	7.31[a]/ 3.02/ 400	11	270	256/256/ 196	1.1 x 1.1 x 1.2	NA	1.75
R-fMRI[b]	Both	2300/ 12 Nijmegen 13 London/ -	80	240	240/240/ 33	3.8 x 3.8 x 3.8	11	2

[a]The manufacturer of GE defines a TR as the time an excitation pulses, while Siemens defines a TR as the time between inversion recovery pulses
[b]R-fMRI: In London TE2 was 31ms and TE3 was 48ms; in Nijmegen TE2 was 28.41ms and TE3 was 44.82ms

held in a simulator in order to familiarise the participants with the scanning environment. The data acquisition was performed with comparable 3 Tesla MR scanners. In London a General Electric MR750 (GE Medical Systems, Milwaukee, WI, USA) was used with an 8-channel head coil and in Nijmegen a Siemens Prisma scanner (Siemens, Erlangen, Germany) was used with a 32-channel head coil. An anatomical T1-weighted scan and T2-weighted R-fMRI scans were acquired from each participant (see parameters in Table 2). Reference T1-weighted anatomical scans were obtained with an MPRAGE parallel imaging sequence. For the R-fMRI scan a multi-echo sequence was used. During the R-fMRI scan, the light was dimmed and participants were instructed to look at a fixation cross and to avoid falling asleep during the scan.

Preprocessing

A standard preprocessing pipeline was applied on the data using the FMRIB Software Library (FSL version 5.0). The first five volumes of the resting state scan were removed in order to allow for signal equilibration. Head motion correction was applied via realignment to the middle volume with MCFLIRT (Jenkinson, Bannister, Brady, & Smith., 2002). Then, grand mean scaling was performed as well as spatial smoothing with a 6mm FWHM Gaussian kernel. Subsequently, signal components corresponding to secondary head motion artifacts were removed with ICA-AROMA (Pruim, Mennes, Buitelaar, & Beckmann, 2015; Pruim et al., 2015). Nuisance regression was used to remove signal from white matter and cerebrospinal fluid. Additionally, high-pass filtering (100 s) was applied. The images were co-registered to the anatomical image of the respective participant with boundary-based registration in FSL-FLIRT (Greve & Fischl, 2009; Jenkinson et al., 2002; Jenkinson & Smith, 2001). The T1-images of the participants were then registered to the MNI152 standard space with 12-parameter affine transformations and refined using non-linear registration with FSL-FNIRT (Andersson, Jenkinson, & Smith, 2010). By applying the resulting warp fields to the concatenated functional image, this image was also brought into standard space. In total seven participants (ASD: N = 1, OCD: N = 0, Control: N = 6) were excluded based on fMRI quality. This included participants with less than 80% of volumes acquired. Additionally, the root mean square of the frame-wise displacement across functional scans (RMS-FD; Jenkinson, 1999) was computed for each participant as an indicator of head motion and the participants belonging to the 5% with on average the most head motion (highest mean RMS-FD scores) were excluded from the analysis (RMS-FD > 0.80).

Seed Definition

In the present study, a seed-based approach

was applied, using subject specific anatomically defined regions of the striatum: NAcc, caudate and putamen (Langen et al., 2011). Due to the functional differences between the anterior and posterior putamen, it was divided into two seeds (Oldehinkel et al., 2016). Left and right volumes were analysed separately yielding eight seed masks in total (see Fig 1). The masks were created by first segmenting all subcortical structures for each participant with FSL-FIRST (Patenaude, Smith, Kennedy, & Jenkinson, 2011). Each subcortical volume was retrieved separately and warped into MNI152 space. The volumes were then binarised in order to create masks. The putamen was divided into anterior and posterior parts by multiplying its volume with a binarised anterior and posterior mask, based on the coordinates of the anterior commissure, leaving a space of 4 mm (2 voxels) between the anterior and posterior regions to minimise signal overlap.

Participant Level Analysis

From these subcortical seed masks, we extracted the first eigenvariate of the timeseries of the R-fMRI activity in MNI152 standard space. Using these timeseries, General Linear Model (GLM) analyses were performed within FSL (FSL version 5.0). In this approach, voxel-wise correlations of the timeseries of each seed region with the voxels in the whole brain were computed, resulting in connectivity maps for subsequent group analysis.

Group Level Analysis

The second level analysis on group level was performed with FSL Randomise (Winkler, Ridgway, Webster, Smith, & Nichols, 2014). Voxel-wise regression of the eigenvariate timeseries of the individual seed regions with the frontal cortex was computed, correcting for gender, age and scan-site. ASD, OCD, and healthy control groups were compared using a group level analysis on the connectivity maps obtained in the previous step. Permutation testing (5000 permutations) was applied using FSL Randomise (Winkler et al., 2014), including covariates for age, sex, and scan-site. Voxel-wise testing was limited to the voxels within a frontal lobe mask, including the insular cortex. An F-contrast was applied on the full model of each seed in order to test if there were any differences between the groups. In T-contrasts, the functional connectivity of each diagnostic group was compared to the control group and the ASD and OCD groups were compared to each other.

The association of compulsive behaviour with functional connectivity was investigated across groups (ASD, OCD and control groups), using a similar approach as described above. Two participants were excluded from these analyses because their parents had not filled in the RBS-R questionnaire. T-contrasts were used to do voxel-wise tests of either positive or negative associations between compulsive behaviour and connectivity of the respective seed region. In order to assess whether the relation of compulsivity with frontostriatal connectivity differed between the diagnostic groups, the analysis was repeated, including the interaction of compulsivity and diagnosis. For every analysis, threshold-free cluster enhancement (TFCE) was used as implemented in FSL Randomise (Winkler et al., 2014). Significance was defined with a threshold of familywise error (FWE) corrected $p < .05$.

Sensitivity Analysis

In order to check whether IQ or average head motion (RMS-FD) influenced functional connectivity of the significant clusters, nonparametric Spearman correlations were computed between IQ or RMS-FD and the z-statistic extracted from clusters showing significant functional connectivity group differences or associations with compulsive behaviour. The influence of medication was not assessed, as only four subjects in the OCD group and two subjects in the ASD group were using medication regularly (see Table 1).

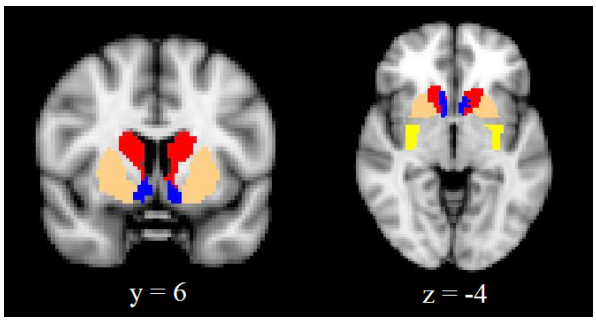

Fig. 1. Seed Regions in the Striatum. An example of the seed regions of a participant, used for connectivity analyses in MNI152 standard space: caudate (red), nucleus accumbens (blue), anterior putamen (orange) and posterior putamen (yellow).

Results

Whole-Brain Functional Connectivity of the Striatal Seed Regions

The whole-brain functional connectivity in the control group roughly corresponded to the frontostriatal circuits described in previous literature (Di Martino et al., 2011). The NAcc was connected with the OFC, ACC and paracingulate gyrus. The network of the caudate included the SMA and the prefrontal cortex as well as the putamen, NAcc and thalamus. The anterior and posterior putamen were both connected with the other striatal seeds, the ACC, the supplementary motor area (SMA) as well as the precentral and postcentral gyrus. Furthermore, they showed connectivity with smaller clusters in the parietal and temporal lobe. Also, the posterior putamen was connected with the thalamus, and smaller clusters in the occipital cortex and the cerebellum. An example of functional connectivity of the right striatal seed regions in controls is displayed in Figure 2.

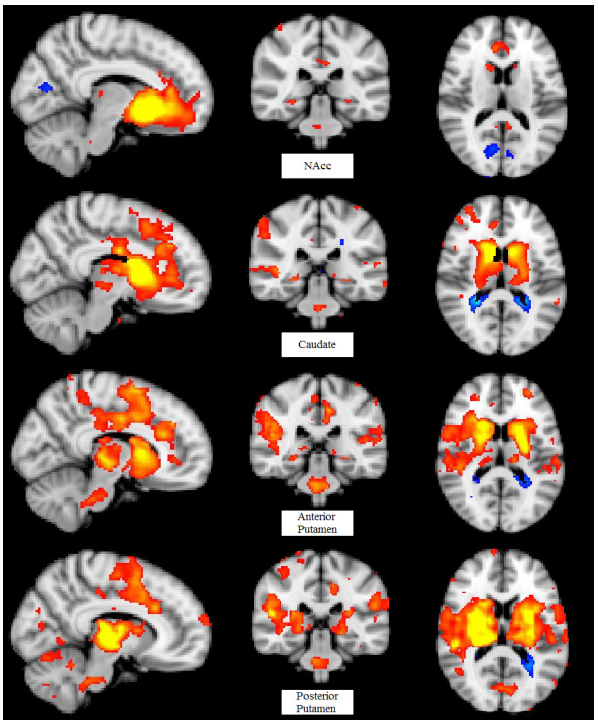

Fig. 2. Global striatal connectivity. Whole-brain functional connectivity of the right striatal seed-regions, nucleus accumbens (NAcc), caudate, anterior and posterior putamen in the control group (N= 24). The figures show T-maps (threshold: $|t| \geq 4$) with MNI 152 coordinates: x= 20, z = 8 and y = 10. Positive connectivity is displayed with a red colour gradient and negative connectivity is displayed with a blue colour gradient.

Group Comparison of Functional Connectivity

When comparing the voxel-wise functional connectivity (within the frontal lobe) of the eight striatal seed regions between groups, none of the F-tests for the overall models yielded significant results. Likewise, the T-contrasts comparing each diagnostic group to the control group and the ASD to the OCD group were not significant.

Association Between Compulsive Behaviour and Functional Connectivity

The analysis on the association between compulsive behaviour (RBS-R total scores) across ASD and OCD groups, and seed-based functional connectivity yielded two significant clusters (results are displayed in Figure 3). Functional connectivity decreased with increasing RBS-R scores between the right NAcc and the left lateral OFC and between the right caudate and the left SMA. The analysis of the interaction effects between compulsivity and diagnosis on the functional connectivity did not reveal any significant clusters. For illustrative purposes, the correlation of the RBS-R with the z-statistic of the significant clusters is displayed group-wise in Figure 4.

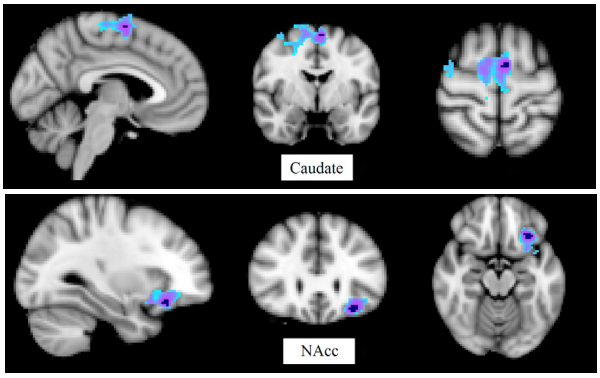

Fig. 3. Compulsivity-related (defined as the total score on the Repetitive Behavior Scale – Revised) hypoconnectivity between striatal seed regions and frontal areas. **A.** Right caudate and left supplementary motor area (p = .045, coordinates: x= -4, y= -4, z= 64). **B.** Right nucleus Accumbens (NAcc) and left OFC (p = .0418; coordinates: x= -30, y= 28, z= -60) The left side in the figures corresponds to the right side of the brain and vice versa. Significant voxels (family-wise error corrected; $p < .05$) are displayed in dark blue. X, y and z are MNI 152 coordinates and correspond to the voxels with the lowest p-values. For illustrative purposes, subthreshold results ($p < .20$) are displayed in a gradient from purple to light blue.

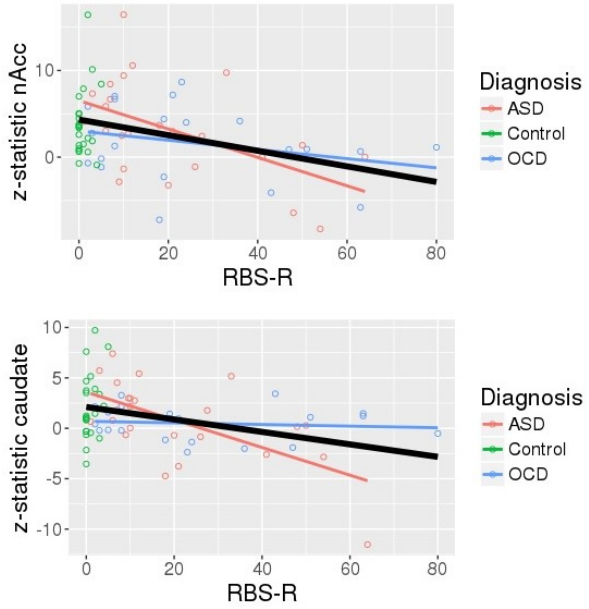

Fig. 4. Correlation of compulsivity with the z-statistic of significant clusters in diagnostic groups. The figures display the correlation of compulsivity (defined as the total score on the Repetitive Behavior Scale – Revised; RBS-R) with the z-statistic of the significant clusters per diagnostic group. The correlation of compulsivity with the z-statistic of the nucleus accumbens (NAcc) and orbitofrontal cortex is displayed on the left and the z-statistic of the caudate and supplementary motor area (SMA) is displayed on the right. The red line shows the correlation in Autism Spectrum Disorder (ASD) and the blue line shows the correlation in Obsessive Compulsive Disorder (OCD). The black line represents the overall correlation across groups.

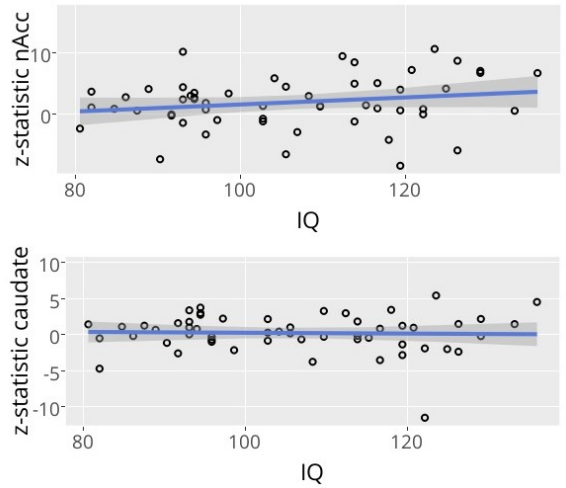

Fig. 5. Correlation of intelligence quotient and head motion with functional connectivity. **A.** Correlation of the estimated intelligence quotient (IQ) based on four subtests of the Wechsler Intelligence Scale for Children-III (Canivez & Watkins, 1998) with the z-statistic of functional connectivity of the nucleus Accumbens (NAcc) with the orbitofrontal cortex. **B.** Correlation of head motion as defined by root mean square of the frame-wise displacement across functional scans (RMS-FD; Jenkinson, 1999) with the z-statistic of functional connectivity of the nucleus accumbens (NAcc) with the orbitofrontal cortex and the caudate with the supplementary motor area.

Sensitivity Analysis

The sensitivity analyses showed no significant correlation of IQ ($\varrho\rho$ = -0.01, p = .92) or RMS-FD ($\varrho\rho$ = -0.14, p = .27) with the connectivity between the right caudate and the left SMA. Likewise, the correlations of IQ ($\varrho\rho$ = 0.21, p = .12) and RMS-FD ($\varrho\rho$ = -0.05, p = .68) with the functional connectivity of the right NAcc and the left OFC were not significant (see Figure 5).

Discussion

The aim of the present study was to investigate overlapping and group-specific abnormalities of resting state frontostriatal connectivity in ASD and OCD. Additionally, we assessed the cross-disorder association between compulsive behaviour and frontostriatal connectivity. We did not detect any abnormalities related to ASD or OCD. More severely compulsive behaviour, on the other hand, was associated with hypoconnectivity between the NAcc and OFC and between the caudate and SMA.

In the present analysis, we could not replicate previous findings on abnormalities related to OCD and ASD in the limbic (Delmonte et al., 2013; Harrison et al., 2009; Jung et al., 2013; Vaghi et al., 2017), cognitive (Chen et al., 2016; Delmonte et al., 2013; Fitzgerald et al., 2011; Vaghi et al., 2017) or sensorimotor (Bernstein et al., 2016; Harrison et al., 2009; Vaghi et al., 2017) frontostriatal circuits. An explanation for the absence of commonalities and differences between groups might be the heterogeneity of symptom representation within the disorders (Robbins et al., 2012). While some patients with ASD, for instance, show increased levels of compulsivity, others might suffer more from symptoms of social deficits. Likewise, children with OCD might differ on the amount of compulsions and obsessions expressed in daily behaviour. When investigating brain circuits underlying the common mechanisms in ASD and OCD, this could have caused a large variance within groups of the present

study. Hence, the hypothesised abnormalities in functional connectivity might be present only in participants with ASD and OCD who show more severe symptoms of compulsivity.

In line with this reasoning, we found that more severely compulsive behaviour was related to decreased connectivity between the NAcc and the lateral OFC. While previous studies on the two disorders mainly reported increased connectivity within the limbic circuit (Delmonte et al., 2013; Harrison et al., 2009; Vaghi et al., 2017), a distinction has to be made between lateral and medial OFC (Jung et al., 2013). Jung and colleagues (2013) found that the NAcc and medial OFC were more connected, whereas the NAcc and the lateral OFC were less connected in relation to symptom severity in OCD. The latter is in line with the present results. Considering the role of the lateral OFC in behavioural inhibition and processing of punishment (Jung et al., 2013), this suggests that compulsivity is related to an inability to inhibit negative repetitive symptoms.

Additionally, the present results reveal a relationship of compulsive behaviour with underconnectivity between the caudate nucleus and the SMA. The SMA is located within the MFG, anterior to the motor cortex (Nachev, Wydell, O'Neill, Husain, & Kennard, 2007) and is part of the sensorimotor circuit of frontostriatal connectivity (Morris et al., 2015). In line with the results of the current study, Vaghi et al. (2017) also found decreased connectivity in OCD between the caudate and an area in the precentral gyrus, which is also part of the sensorimotor circuit. The present results suggest abnormalities of communication between the caudate of the cognitive circuit and the sensorimotor circuit. More specifically, the SMA plays a role in motoric inhibition of behaviour (Bari & Robbins, 2013; Nachev et al., 2007). Together, the present results on underconnectivity of the NAcc with the lateral OFC and the caudate with the SMA indicate that deficits in the ability to inhibit actions might lead to compulsive behaviour.

In accordance with that, studies found a deficit of inhibitory skills in the Go/No-go task in patients with OCD and ASD (van Velzen, Vriend, de Wit, & van den Heuvel, 2014; Christ, Holt, & White, 2007; Kana, Keller, Minshew, & Just, 2011; Uzefovski, Allison, Smith, & Baron-Cohen, 2016). Next to ASD and OCD, also the trait compulsivity in healthy participants has been related to impaired inhibitory skills in a Go/No-go task (Sánchez-Kuhn et al., 2017). In order to explain the emergence of compulsive symptoms in related disorders, however, the direct link of compulsivity, inhibitory skills and functional connectivity should be investigated across disorders in future studies.

Even though the present results show abnormalities in frontostriatal circuits, they differ from the findings of a previous study on ASD (Delmonte et al., 2013). In the present study, mainly hypoconnectivity between the right caudate and the left SMA in the MFG has been related to compulsive behaviour. However, Delmonte et al. (2013) reported hyperconnectivity between the right caudate and the right MFG in relation to repetitive behaviour in ASD. Though these results seem contradictory at first, they are not necessarily inconsistent with one another. Studies on functional connectivity in ASD found that overconnectivity and underconnectivity often coexist. More specifically, some studies found overconnectivity within the right hemisphere of the brain and underconnectivity between the two hemispheres (Hull et al., 2017; Anderson et al., 2011). Accordingly, Delmonte et al. (2013) reported hyperconnectivity within the right hemisphere, whereas the present results revealed hypoconnectivity between the right striatum and the left frontal cortex. Our results suggest that the distinction between intrahemispheric overconnecitivity and interhemispheric underconnectivity could be an interesting avenue for further investigation and that treating left and right seed regions separately is recommended when investigating functional connectivity.

Another reason for the discrepancies between findings might be the chosen age groups. A study on age-related effects of frontostriatal circuits in ASD found that differences diminish with increasing age and that classification is easier in younger subjects (Anderson et al., 2011). Likewise, Fitzgerald et al. (2011) reported age-related effects on frontostriatal connectivity in OCD. This demonstrates the importance of studies on children, since they are diagnosed earlier and the neural plasticity might still compensate for functional connectivity deficits during development in childhood and adolescence (Anderson et al., 2011).

While the present study did focus on younger participants, there are still limitations that should be considered when interpreting the results. The sample size of the current analysis was not large and might therefore limit the power to detect significant effects. Additionally, the scope of the current study did not allow for an analysis of the sub-scales of the RBS-R (Lam & Aman, 2006). In the present analysis, we have exclusively investigated the total score of compulsive behaviour, and

therefore no conclusions can be made on different types of repetitive behaviour, such as self-harming or stereotyped behaviour. Further analyses on the subtypes of compulsive behaviour would be of great value to elucidate more specific relationships between dysfunction in different frontostriatal circuits and behavioural subtypes. Furthermore, the RBS-R used in the present analysis has especially been designed to investigate ASD (Lam & Aman, 2006). Therefore, it might be less sensitive in measuring repetitive behaviour in OCD and alternative assessment tools should be considered in future studies.

Conclusion

In the present study, we found decreased connectivity of the right NAcc with the left OFC and the right caudate with the left SMA in relation to compulsive behaviour across diagnostic groups. This suggests that compulsivity is linked to abnormalities in connectivity of the right striatum with the left frontal cortex. On the other hand, no group differences between OCD and ASD diagnostic groups and healthy controls were detected. This is in line with recent suggestions in the field that diagnostic labels are heterogeneous and that we should strive towards a more direct investigation of cross-disorder phenotypes in order to identify subtypes of compulsivity-related disorders.

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Abstracts

Proceedings of the Master's Programme Cognitive Neuroscience is a platform for CNS students to publish their Master thesis. Given the number of submissions, we select the articles that received the best reviews, under recommendation of our editors, for the printed edition of the journal. The abstracts of the other articles are provided below, and for interested readers a full version is available on our website: www.ru.nl/master/cns/journal.

Biological Motion Feedback as Rewarding Stimuli to the Brain
A Novel fMRI Study on Biological Motion

Laura Bilbao Broch, Emily S. Cross, Elin H. Williams, Jan Buitelaar

Human social motivation is characterised by the pursuit of social reward and the avoidance of social punishment. However, most previous studies have focused on using human faces as social stimuli and little is known about responses of the different populations to other types of social stimuli, such as biologial motion. Biological motion is defined as the visual perception and integration of movement associated with human/animal movement and provides rich information about the identity of an agent as well as the actions and intentions conveyed in the way an agent moves. The aim of the present study was to investigate whether typically developing individuals also assign a high value to positive/negative motion stimuli as feedback as they do with faces and whether the preference for this type of social stimuli is also linked to autistic traits. Thus, we conducted an event-related functional magnetic resonance imaging (fMRI) experiment using a social incentive delay task with dynamic video stimuli of body motion alone (masked faces) as social incentive feedback in order to examine participants motivation for social reward gain and social punishment avoidance. The anticipation phase analysis revealed significant activation of the right thalamus during the avoidance of punishment condition, showing a greater activation when comparing negative biological motion feedback to negative text feedback. Moreover, we found significant activation of brain areas linked to specific processing of biological motion in all the other conditions as well as in the outcome phase. Taken together, these results might provide initial evidence of biological motion feedback possibly being more rewarding to the brain than text feedback.

Investigating the Effect of Sleep on Different Spatial Learning Paradigms and its Underlying Neural Correlates

Anumita Samanta, Lisa Genzel

Sleep has been shown to enhance memory consolidation and improve performance in spatial navigation in virtual environment tasks. The processing of spatial memory can be further disentangled into allocentric (spatial) and egocentric (motor) representations. Not much research has been conducted to disentangle their differential effects on sleep using spatial navigation tasks. A preliminary study done in rats on the water-maze showed sleep enhanced performance in the probe trial under allocentric training condition. We developed a human analogue of the study and conducted an fMRI investigation wherein participants had to locate a treasure box in virtual water maze environment. Groups of participants were trained in either allocentric or egocentric conditions and took a nap or watched a movie between training and retrieval test period. Analogous to findings in the rats, we found that sleep promoted an increased accuracy in marking the location of the treasure box under the allocentric training condition. Performance in the egocentric condition remained independent of sleep effects. Our results were hence able to replicate the behavioural findings in rats showing that the underlying consolidation mechanisms might be conserved in both species.

The Effect of Probing Modality on Neural Signatures and Awareness of Movement Intent

Yvonne F. Visser, Ceci Verbaarschot, Jason Farquhar

Since Libet et al. published their study on the timing of the conscious intention to act in 1983, this timing and neural signatures preceding voluntary movement have been the subject of much discussion. An innovative way to measure the onset of an intention was introduced in 2008, in which awareness of intention was estimated by participants responses to probe stimuli. In this work, the probing modality was manipulated to investigate the effect of 1) stimulus processing speed as well as 2) facilitating/interfering characteristics of the stimulus. The effect of these two stimulus characteristics was investigated for the timing of the awareness of intending to act and the neural signatures Readiness Potential (RP) and Event Related Desynchronization (ERD). Participants were asked to make self-paced voluntary movements while probing them at optimised intervals to target the times at which they are aware of their intention to act. When participants noted a probe while being aware of an intention to move, they were asked to stop (veto) their movement. This allowed us to estimate the time when participants were aware of an intention to act: the intention window. Four different probing modalities were used: auditory, visual, passive tactile and active tactile, chosen for their variance in processing speed and likely interaction with the participants awareness of intention. Probing modality was found to affect the size and onset of the intention window, where its onset can be explained by the different processing speeds of the probe stimuli and through intention facilitation. Trials in which a participant made a movement and trials in which they were not aware of an intention to act were found to differ significantly for both RP and ERD. This could suggest that the neural signatures of a veto lie in between that of a move and an ignored probe: possibly caused by an intention but not an actual move. In conclusion, probing modality influences the intention window, and the neural signatures seem to not only be related to the move itself but also to the intention. The expected difference in timing of neural signatures between different modalities was not found: the effect of modality on the neural signatures remains unclear.

Relation Between Social Imitation of Alcohol use and Social Alcohol cue Reactivity in Young Adults

Pritha Bhandari, Martine Groefsema, Maartje Luijten

For young adults, alcohol consumption primarily occurs in social settings. Social imitation of alcohol use has been found in several studies. Attempts to explain individual differences in social imitation of alcohol use in terms of personality and genetic factors have yielded partial answers. The current study used an incentive sensitisation framework to explain variation in social imitation of alcohol use in drinking young adults. We investigated (1) whether young adults display social imitation of alcohol use in a semi-naturalistic (bar-lab) context, as shown in previous studies (Larsen, Engels, Souren, Granic, & Overbeek, 2010; Quigley & Collins, 1999), (2) whether social alcohol cues elicit activity in the reward-related dopaminergic pathway using a novel passive viewing cue exposure task, with functional magnetic resonance imaging (fMRI), and (3) whether social imitation of alcohol consumption is associated with cue reactivity to social alcohol cues. A total of 157 drinking young male adults took part in the study. Young adults consumed more alcohol in the presence of a heavy drinking confederate than a light drinking one, demonstrating social imitation. Additionally, greater activity in the ventral striatum and ventral medial prefrontal cortex was found for social alcohol cues, suggesting reward-related activity. Finally, we conclude that social imitation of alcohol use is not associated with social alcohol cue reactivity in the reward-related dopaminergic pathway directly.

Reconstructing the Perceptual Organisation of Sound from Neural Responses

Christos-Nikolaos Zacharopoulos, Bernhard Englitz

Background

Sets of stimuli can span different stimulus spaces. Examples include linear, circular or planar. Since the neural system does not know the geometry of this stimulus space, it needs to have a way of estimating it from information contained in the neural population responses. Recently, a set of techniques was proposed that can achieve this estimation, known as representational similarity analysis (RSA).

Methods

We expand the current framework of RSA by establishing a criterion for taking into account the local geometry of the neural response manifold. We refer to this expansion as gRSA (global RSA). To do so, we compute distances between stimuli within the response manifold (Local Distance Matrix, LDM). Once pairwise distances have been identified, we reconstruct the global geometry from the local geometry by recreating the neighborhoods of the manifold (Global Distance Matrix, GDM). The GDM is constructed by stochastic exploration of the LDM. Once a certain value of cross correlation is established, two neighbours are identified based on a local decoder. That way, the path between two stimuli in the response manifold can be thought of as the shortest distance between two responses within that manifold.

Results

We applied gRSA to simulations and real data (neural responses from the auditory cortex of the ferret). We successfully reconstructed the stimulus geometry of the simulated data. The analysis led to a satisfactory reconstruction of the stimulus space geometry for the real responses.

Conclusion

The perseverance of similarity from the external to the internal space (2nd order isomorphism) is only achieved when the local geometry is taken into account. Our results showed that when this local aspect is not taken into account, the 2nd order isomorphism is sometimes violated and the stimulus space reconstruction can fail.

Impaired mPFC-dependent Spatial Working Memory in the APO-SUS rat Model for Schizophrenia

Lydia Pavlidi, Dorien A. Maas, Gerard J.M. Martens

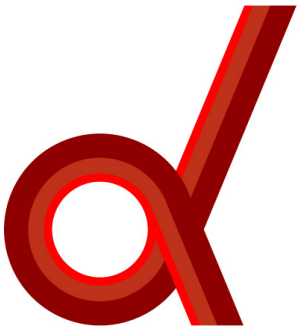
The neurobiology underlying cognitive symptomatology in schizophrenia (SZ) remains poorly understood and untreated. Prefrontal cortex (PFC) dysconnectivity and both a redox imbalance and oligodendrocyte (OL) dysfunction are thought to be involved in SZ. To study the cognitive symptoms, we used the APO-SUS rat model for SZ. At the behavioural level of the symptoms we focused on the medial PFC (mPFC) and working memory (WM). We report a dysfunctional/immature mPFC in the APO-SUS rats as was evident from the deficits observed in the social interaction test and spatial working memory deficits that were repeatedly shown in three different mPFC-dependent tasks: the continuous delayed alternation, repeated reversal learning and spatial win-shifting. At the molecular level and various ages (PND21, PND90, PND365) APO-SUS rats had altered mRNA expression levels of redox-related genes in the cingulate cortex (Cg), the hippocampus (HPC) and barrel cortex (BC), indicating a general redox impairment. Finally, we report that APO-SUS rats do not differ from APO-UNSUS rats in the mRNA expression levels of myelin- or OL-related genes in Cg, HPC and BC, which together with previous studies from our group indicates a mPFC-specific myelin and OL deficit. We conclude that APO-SUS rats have a redox imbalance that is not mPFC specific but rather spread throughout the brain and a spatial working memory deficit that is mPFC dependent.

Predicting Movement Intent in Real-time: From Brain to Subjective Experience

Anne Gerrits, Jason Farquhar, Ceci Verbaarschot

The readiness potential (RP) and the event-related desynchronization (ERD) are neural signals that build up over the motor cortex 1.5-2 seconds prior to movement onset. Bai et al. (2011) were amongst the first to reliably detect movement intent online based on these signals. Interestingly, these brain signals typically build up prior to the moment a person reports to consciously intend to act. However, how these subjective reports relate to these neural preparatory signals remains unclear. To investigate this, we developed a brain-computer interface (BCI), based on the Bai study, that predicts movement intent based on these brain signals and then feeds this prediction back by means of functional electrical stimulation (FES). Three experiments were conducted. In the first experiment we successfully replicated the Bai study offline. We found we could predict movement intent offline based on the ERD (-0.70.17s) and the RP (-0.430.84s) before movement onset. In the second experiment we investigated the effect of FES stimulation on EEG data. We showed that FES stimulation mostly influences the EEG data on and after movement onset and was thus not an issue for our study. In our third experiment we used online classification to investigate if a person is aware of their intention to act when movement preparation is detected in the brain. The online classification did not work as expected due to a high false positive rate. Therefore, we could not answer the main question in this experiment. We believe the online classification was affected by an anticipation buildup over time. By using more time points for classifier training, building in trials that provide a measure of anticipation alone and creating more variance in action timing, we believe it will be possible to predict movement intent in real-time and investigate how the subjective experience of intending to act relate to the RP/ERD.

Institutes associated with the Master's Programme Cognitive Neuroscience



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