Stress and Memory in Health and Disease: Impact on Alzheimer's disease and Memory Mechanisms
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Exposure to stressful experiences, either early or later in life, can have a strong impact on learning and memory in adult and ageing individuals. Early life experiences in particular have been implicated in determining the vulnerability and resilience for cognitive decline, for instance when the brain is already vulnerable, such as seen in Alzheimer's disease (AD). The first aim of this thesis was to study the effects of experiences early in life (albeit positive or negative) on aging- or AD-related cognitive decline, and to better understand the underlying mechanisms. I particularly focused on the role of the hypothalamus-pituitary-adrenal (HPA)-axis, and on the expression and functionality of glutamate receptors in this process. The second aim of this thesis was to investigate why stressful memories are retained so well. Recent studies suggest that only a subset of neurons is required for any memory trace ('engram cells'). Using novel genetic and molecular approaches, these engram cells were visualised, characterised and manipulated, to unveil effects of glucocorticoids on memory formation.

In **Chapter 1**, I reviewed the effects of early life experiences on later behaviour and functional plasticity of the brain. I discussed the evidence that early life experiences long-lastingly alter HPA axis (re-)activity, thereby shaping behaviour and brain function during adult life and aging. Finally, I reviewed data supporting the hypothesis that early life experiences, either positive or negative, can alter the vulnerability to develop AD, and I investigated elements of that hypothesis in chapters 2, 3, 4, 5 and 6.

In **Chapter 2,** I showed that manipulating the amount of maternal care that pups receive can have profound effects on the lifespan and pathological markers in mice with transgenic overexpression of APP and tau (biAT mice), the main neuropathological features of AD. Increased levels of maternal care ("early handling") was induced by separating the dam and her pups for fifteen minutes per day during the first week after birth (postnatal day 2-9). Upon reuniting the dam and her pups, the dam intensifies licking and grooming behaviour towards the pups, which results in an 'enriched environment' for the pups. Remarkably, early handling resulted in a longer lifespan and lower β-amyloid (Aβ) levels later in life. On the other hand, reduced levels of maternal care ("early life stress"), induced by placing the litter under impoverished housing conditions, shortened the lifespan and increased Aβ levels later in life (i.e., all at an early stage of the disease).

Using a different genetic mouse model for amyloid-β-associated neuropathology (APPswe/PS1dE9 mice), I validated in **Chapter 3** in more detail the early handling mouse model that resulted in enhanced levels of maternal care. Early handling reduced hippocampal plaque pathology, while plaque load in the amygdala remained unaffected. Importantly, when adult APPswe/ PS1dE9 mice were tested in spatial, hippocampus-dependent cognitive tasks, early handling prevented the APPswe/PS1dE9-induced cognitive impairments. This was not the case in amygdala-dependent memory tests.

In **Chapter 4,** I investigated the role of the HPA axis in the effects of early life adversity on AD-related changes. Early life stress increased Aβ neuropathology from adult age (6 months) onwards, ultimately resulting in cognitive impairments at older age (12 months), in particular in the domain of cognitive flexibility. HPA-axis responsiveness was increased in these mice, which negatively correlated with cognitive performance. Interestingly, a brief, 3-day treatment with mifepristone (which targets glucocorticoid receptors), at an age at which cognitive impairments were already present, was found to reverse the early life stress-induced impairments in cognitive flexibility and Aβ neuropathology. This highlights the important role of glucocorticoid hormones in the development of AD neuropathology and symptomatology.

While declarative memory is often impaired in AD, emotionality is frequently enhanced in AD patients. In **Chapter 5,** I report that APPswe/ PS1dE9 mice exposed to early life stress show an enhanced responsiveness to fearful cues, but also to non-fearful cues. I next investigated whether this was associated with alterations in hippocampal synaptic plasticity, i.e. the cellular substrate of learning and memory, in one year old mice. I found that APPswe/ PS1dE9 mice exposed to early life stress also showed an atypical, enhanced form of synaptic plasticity, that coincided with a reduced

sensitivity to a GluN2B receptor antagonist. These findings may point to a central role for the NMDA receptor in mediating effects of early life stress on adult plasticity.

To further explore the underlying mechanisms of altered synaptic plasticity and cognitive deficits, in **Chapter 6**, I treated APPswe/PS1dE9 mice with the glutamate modulator riluzole throughout their life. While riluzole prevented the impairments in synaptic plasticity from an early age onwards, it even improved cognitive performance in older APPswe/PS1dE9 mice that were exposed to early life stress. The effects of early life stress, aging and AD, and the rescue by riluzole, were accompanied by changes in the expression of the excitatory amino acid transporter 2, which is important for the synaptic reuptake of glutamate. These findings point towards an important role for glutamatergic signalling in early life stress-induced cognitive impairments in AD.

In order to better understand how early life stress determines learning and memory processes in aging and AD, I addressed in **Chapter 7** how early life stress affects short-term and long-term synaptic plasticity in wild type mice. I found that early life stress impaired both these forms of synaptic plasticity. In addition, early life stress reduced the expression of the NMDA receptor subunit 2B in the hippocampus. Blocking the NMDA receptor subunit 2B had no effect on either memory performance, or on synaptic plasticity in mice exposed to early life stress, while this subunit was critically important for these processes in control mice, suggesting that the effects of early life stress may be mediated, in part, by the NMDA receptor subunit 2B.

In the final part of this thesis, I investigated how acute exposure to stress hormones affects learning and memory. In **Chapter 8,** I showed that the administration of corticosterone immediately after auditory fear conditioning enhanced memory consolidation. Using different auditory fear conditioning paradigms, application of corticosterone was found to have opposite effects on conditioned fear memories in male and female mice. Whereas corticosterone increased memory in male mice, the hormone reduced conditioned fear memory in female mice. Interestingly, corticosterone increased extinction learning in both male and female mice. Together, this indicates that fear memory retention is differently affected by corticosterone in male and female mice.

These findings are expanded on in **Chapter 9** where I investigated the effects of a brief corticosterone treatment on memory memory specificity in male mice. Corticosterone decreases the accuracy of the memory of the event, since these mice displayed generalised fear when they were placed in a safe environment. Using transgenic reporter mice that allow the investigation of cells expressing the immediate-early-gene Arc, it was possible to molecularly and electrophysiologically characterise subpopulations of neurons in the hippocampal dentate gyrus in relation to a memory trace. Corticosterone administration to trained animals increased the number of Arc-positive neurons in the dentate gyrus, and these cells are also activated following the retrieval of the memory. Arc-positive neurons are more active than Arc-negative neurons, yet corticosterone specifically changes the activity in Arc-negative neurons. When we subsequently inhibited these dentate neurons specifically using DREADD technology, this prevented the generalisation of fear induced by corticosterone, while memory for the tone was left unaffected. This illustrates that the training-induced recruitment of a subset of dentate gyrus neurons underlies the generalisation of fear by corticosterone.

In **Chapter 10**, I summarise the main outcomes of this thesis, and discussed them in a broader perspective. First, I discussed how early life experiences can program brain (or neuronal) structure and function in a lasting manner, and how this may impact cognition and neuropathology in an AD background. I speculate that besides a direct modulation of AD neuropathology by the HPA axis, early life experiences may also shape the brain or cognitive ‘reserve’ early on, thereby rendering some individuals more resilient or vulnerable to AD-associated impairments than others. Secondly, I discussed how glucocorticoid hormones influence memory strength and memory specificity, and in particular address the role of memory engram cells. Finally, I formulated some remaining outstanding questions that may help move the field of (early life) stress and memory formation ahead.