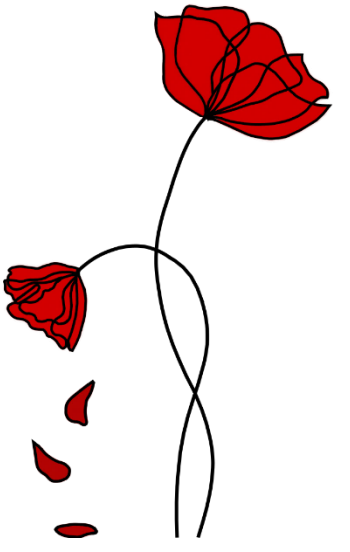
Post-traumatic stress disorder

CHAPTER 9

Summary



The aims of this thesis were to (i) examine how studying resilience factors could be beneficial for deepening our understanding of trauma susceptibility, and (ii) gain insights into epigenetic underpinnings of post-traumatic stress disorder (PTSD) and start exploring whether some of these could potentially serve as diagnostic biomarkers for PTSD. In order to answer these questions, this thesis presented two literature reviews, three experimental studies and one methodological chapter.

First, the available literature on resilience studies was summarized in a review presented in **Chapter 2**. This overview highlights the need to standardize resilience studies, starting with the way resilience is currently being defined. Studying factors that could promote resilience, either through cognitive strategies or pharmacologically by targeting biological pathways of resilience, if and when identified once study designs are optimal, will be beneficial for at-risk individuals. This is further emphasized by the fact that addressing potential mental health issues is currently not always receiving sufficient attention within the military.

In order to get a first understanding of what is known regarding the implications of microRNAs (miRNAs) in PTSD, a second review was presented in **Chapter 3**. By being easily accessible and relatively stable in biofluids and across species, miRNAs hold the exciting potential to serve as biomarkers of disease. In the context of PTSD, most of the work has been done in animals by modeling traumatic stress and studying associated phenotypes, while most human research examined trauma-related miRNAs in military populations. These studies are mostly characterized by small sample sizes and a great variety in the way miRNAs are being analyzed. Although informative as a first exploration of miRNA dysregulations in PTSD, we suggest that future studies use longitudinal designs by incorporating time points prior to and following stress exposure in order to gain insights into the role of circulating miRNAs in the onset and course of PTSD. When possible, combining such studies with animal studies would be of added value in order to track corresponding molecular mechanisms occurring within the brain which could potentially yield novel avenues for treatment strategies.

Next, several experimental studies were performed using blood samples from deployed military members. In **Chapter 4** we performed a first pilot study in order to examine circulating miRNA profiles of deployed military members with or without PTSD. Expression profiles of several miRNAs were found to be dysregulated between individuals with PTSD, trauma-exposed controls and healthy controls, some of which had previously been associated with PTSD. Although preliminary, this study highlights the feasibility and

potential usefulness of clustering miRNAs based on their expression profiles and use this information to distinguish individuals with PTSD from (trauma-exposed) healthy controls.

This study gave rise to several questions, one of which included whether exosomal miRNAs would be better suited for sampling and analysis given the higher stability of encapsulated miRNAs and the potential that these vesicles offer to track their tissue of origin. The protocol needed to isolate and analyze exosomal miRNAs within plasma neuron-derived exosomes (NDEs), i.e. exosomes released by neurons which end up in the blood circulation, was presented in **Chapter 5**. This methodological chapter provides insights into the workflow needed to capture these vesicles and sequence their miRNA content.

In **Chapter 6**, we then applied this knowledge to plasma samples belonging to individuals with PTSD, along with urine samples from the same individuals, and serum samples from an independent cohort of PTSD subjects. This study is the first to assess the miRNA content of NDEs present in limited amounts of several human biofluids using high-throughput sequencing. The findings generated by this pilot study indicate several aspects. First, it is feasible to sequence the miRNA content of NDEs using small amounts of human plasma. Next, two specific miRNAs could potentially, if replicated in larger cohorts, serve as markers for PTSD, especially given the interesting fact that both were expressed in all samples of PTSD subjects, while being absent in all (but one) of the control samples. More research is needed in order to assess whether urine can be used for these analyses, for example by assessing the precise origin of L1CAM+ exosomes present in urine. Finally, our findings suggest that older serum samples might still be useful for NDE miRNA analyses, although the stability of these miRNAs should be assessed further given the potentially considerable degradation of larger RNA fragments.

Finally, in **Chapter 7** we took a different approach of looking at epigenetic mechanisms in PTSD and focused on DNA methylation by performing one of the first studies that used longitudinal measures of DNA methylation. We combined DNA methylation data from three military cohorts from which blood methylation and phenotypic information was collected prior to and following combat exposure. Our findings highlight several differentially methylated positions and regions, some of which had previously been associated with PTSD, thereby enhancing their potential implication in the disorder.