Stress-in-a-dish

Chapter 10

Summary

The aims of this thesis were (1) to develop an *in vitro* model of ELS (i) to investigate the neurobiological effects of chronic cortisol (CORT) in human cortical neurons throughout neuronal development and (ii) study candidate genes associated with PTSD susceptibility, and (2) to highlight the ethical implications that may result from this research. This thesis presents work done on the first aim in Chapter 2 to 6 while aim 2 was covered in Chapter 7 and 8.

This thesis starts with reviewing the available literature on *in vitro* studies investigating glucocorticoids (GCs), which was summarized in a review presented in **Chapter 2**. This overview highlights the different mechanisms and pathways affected by GC exposure in central nervous system cells and the need to standardize GC *in vitro* studies, starting with an extensive definition of stress *in vitro*. Future research ought to focus on how GCs play the role of mediators in different types of stress, cell type-specific vulnerabilities to GCs, GC-mediated mechanisms in stress susceptibility and resilience, the interplay between GCs and other stress-related hormones and explore ways to improve *in vitro* modeling of stress mechanisms.

Next several experimental studies were performed using human embryonic stem cell-derived cortical neurons at different stages of neuronal development. In **Chapter 3**, I investigated the effects of chronic CORT in neural progenitor cells (NPCs), immature young neurons, and maturing neurons. A decrease in proliferation and survival were observed in NPCs, a decrease in differentiation-related markers was observed in young immature neurons, and changes in synaptic plasticity proteins, neuronal activity, and glial marker in maturing neurons. Moreover, genome-wide changes were observed in the distinct stages of neural differentiation including dysregulation in synaptic plasticity, GC signaling, and extracellular matrix organization among other pathways. The findings of this study are supported by previous *in vitro* and *in vivo* studies showing negative effects of GCs on neuronal processes.

To gain better insight in the longitudinal effects of chronic CORT on neuronal development, trajectory modeling of transcriptomic patterns was performed. This yielded patterns of genes driving differentiation, from which subsequently CORT-sensitive genes were identified by analyzing whether their expression changes interacted with chronic CORT. The findings were presented in **Chapter 4** and highlight a set of genes involved in neuronal differentiation and synaptic plasticity as being sensitive to CORT-exposure. Together these findings identify genes and related processes dysregulated in GC-mediated mechanisms, in accordance with pathways previously shown to be implicated in ELS, thereby enhancing their potential implication in SRDs and validating the modeling of stress-related mechanisms *in vitro*.

In **Chapter 5**, I then applied this established chronic CORT ELS model to maturing cortical neurons to investigate candidate genes involved in epigenetic mechanisms underlying susceptibility to post-traumatic stress disorder (PTSD). This study is the first to assess the biological relevance of these genes in relation to stress mechanisms *in vitro*. The findings resulting from this exploratory study highlight the CORT-responsiveness of these genes through the observed changes in DNA methylation and mRNA expression of *DUSP22* and *ZFP57*, respectively. Moreover, this increases the relevance of these genes as potential biomarkers for PTSD. More research is needed to assess the functional relevance of these genes in neuronal processes and in relation to GC-associated mechanisms *in vitro* and *in vivo*.

An overview of the challenges and limitations of GC *in vitro* studies was presented in **Chapter 6**, together with future directions for the field. The existing sources of variability between these studies makes it increasingly challenging to have reproducible and conclusive results on the neurobiological effects of GCs. Although the existing literature exploring the effects of GCs is extensive, future studies should adhere to standardized practices to ensure reproducibility and increased validity of *in vitro* studies investigating neurobiological effects of GCs and related stress hormones. Table 1 summarizes our main findings from Part I of this thesis.

Table 1. A summary of the main findings in Chapter 3, 4, and 5 of this thesis.

	Neural Progenitor Cells (PRO)	Young Immature Neurons (Diffy)	Maturing Cortical Neurons (Diffm)
Chronic CORT-induced phenotype	o Decreased proliferation o Decreased survival o Increased apoptosis o Dysregulation in synaptic plasticity pathway	o No effect on overall differentiation o Decreased Pax6 expression o Dysregulation in GC signaling and extracellular matric organization	o Increased expression of post-synaptic plasticity protein o Age-dependent decreased neuronal activity o Decreased GFAP expression o Dysregulation in transmembrane receptor protein serine/threonine kinase signaling pathway
Chronic CORT effects on neurodevelopment	o Decreased LRRTM2 expression o Increased TSPAN5 expression o Dysregulation in synapse formation processes	o Decreased KCND3 expression o Decreased KCNIP4 expression o Decreased GRIA3 expression o Dysregulation in neuronal excitability and synaptic plasticity processes	
Chronic CORT effects on PTSD susceptibility candidate gene methylation and expression	Not assessed	Not assessed	o Decreased DUSP22 DNA methylation in one CpG o Increased ZFP57 mRNA expression

Part II of this thesis dealt with the ethical implications, starting with **Chapter 7** which highlights the ethical implications of making use of human cerebral organoids (COs) in stress-related research. Ethical considerations pertaining to research ethics frameworks of *in vitro* studies, donor-related issues, and chimera research is discussed, while providing recommendations on how to navigate this uncharted area for future studies. Figure 1 summarizes our main conclusions.

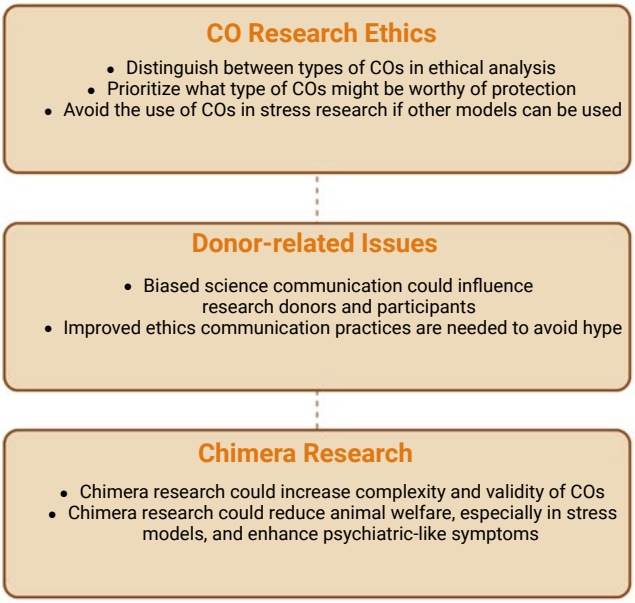

Figure 1. A summary of the main conclusions in Chapter 7.

Finally in **Chapter 8**, I highlight some of the ethical implications associated with the introduction of biomarkers of PTSD susceptibility and resilience in members of the military and law enforcement agencies. The identification of susceptible and resilient individuals brings about unique ethical considerations that require increased attention while the technology is still developing. This may bring about changes in policies that may carry consequences at the ethical, legal, and societal level. Figure 2 summarizes some of the main issues to attend to in the future.

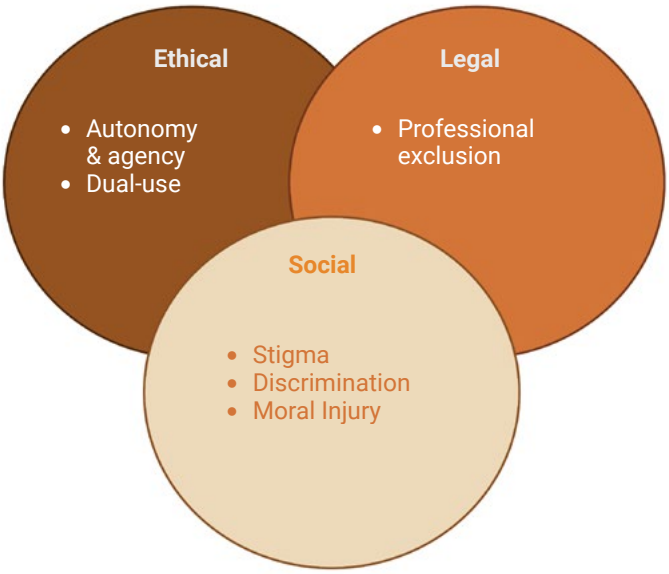

Figure 2. A summary of the main conclusions in Chapter 8.

Thus, the work described in this thesis provides novel insights into biological underpinnings associated with stress-related mechanisms and raises awareness about the ethical considerations that may arise from these pursuits. Nevertheless, it is important to acknowledge that this thesis contributes to a small portion of the larger efforts aimed at discovering the molecular and biological mechanisms underlying differential susceptibility to the effects of stress and SRDs, while also taking the lead in highlighting ethical considerations of stress-related *in vitro* research that require further attention and exploration.