



Ruprecht-Karls-Universität Heidelberg
Medizinische Fakultät Mannheim
Dissertations-Kurzfassung

Sex differences in the effects of adolescent stressors in a rat model of chronic primary low back pain

Autor: Deepika Singhal
Institut / Klinik: Mannheim Center for Translational Neuroscience (MCTN).
Neurophysiology
Doktorvater: Prof. Dr. R.-D. Treede

The studies in this thesis explored how adolescent stressors affect males and females differently in a rat model of chronic primary low back pain (cPLBP), focusing on the related behavioural, cellular, and molecular mechanisms. This model probes memory traces in the nociceptive system (“latent sensitisation,” hypothesized to be induced by stress) by mild nociceptive challenges that would not normally lead to behavioural signs of dorsal horn neuron hypersensitivity (manifest sensitisation).

Two distinct stressors were implemented during adolescence: repeated restraint stress (RS) as a model of physical abuse, acute social isolation (SI) as a model of emotional neglect. In adulthood, rats received intramuscular injections of nerve growth factor or saline. Dorsal horn neuron hypersensitivity was assessed behaviourally by pressure pain thresholds (PPT) of multifidus and gastrocnemius muscles and by withdrawal threshold to pinprick of the ipsilateral hindpaw (PWT). Furthermore, alterations of the functional states of spinal dorsal horn microglia due to stress and NGF were examined through immunohistochemistry. Additionally, differentially expressed genes resulting from the interaction between sex and conditions (stress and controls) were identified through snRNA sequencing to evaluate potential molecular pathways associated with adolescent stress predisposed cPLBP in adulthood.

Both early-life stressors were found to trigger widespread muscular and cutaneous hypersensitivity. RS and SI had moderate effects on females, while males experienced a stronger sensitisation with persistence into adulthood. Injections into the low back muscle during adulthood resulted in a moderate additional hypersensitivity in stressed females but not in males. In contrast to the previously examined Sprague-Dawley strain, Wistar Han rats exhibited a greater vulnerability to spinal sensitisation by stress but were less affected by additional nociceptive input. Males were more susceptible to the immediate manifest sensitisation by stress than females. However, females showed signs of a memory trace that increased their vulnerability to subsequent nociceptive challenges (latent sensitisation).

Alterations in the functional state of spinal microglia were assessed using a custom-designed automated phenotyping method. This method classified microglia cell morphology into three main groups: surveillant, primed, and activated. Across all experimental groups, males exhibited a greater proportion of activated microglia, while females showed a higher number of surveillant cells; this was true for both superficial and deep dorsal horn. Priming by stress shifted microglia from surveillant to activated states; this shift was more pronounced in males than females but still significant when pooled across sexes. Comparison of two vs. one NGF injections in non-stressed animals showed that priming by mild nociceptive input shifted microglia towards activated states only in males. Additivity of priming by stress and challenge by NGF was only found as a shift towards primed state in males not in females.

The snRNA-seq analysis demonstrated that the most significantly differentially expressed genes (DEGs) were specific to neuron and oligodendrocyte cell types. A total of 588 genes showed differential expression, with 177 genes being upregulated in stressed males and 411 genes upregulated in stressed females. The gene ontology results showed a predominant activation of DEGs in tissue development and remodelling in males, whereas the DEGs in females were mainly involved in protein regulation and trafficking.

In conclusion, males were more susceptible to manifest sensitisation due to early-life stress, whereas females developed a memory trace (latent sensitisation), causing hyperalgesia upon the second hit in adulthood. The different sensitivity to early-life stress may contribute to the higher prevalence of chronic primary low back pain in females. The different molecular pathways suggest that sex-specific treatments are a valuable strategy for advancing personalised pain therapeutics.