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

Timing matters: the impact of repeated restraint stress on NGF induced sensitization of spinal dorsal neurons in an animal model of myofascial low back pain

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

Non-specific low back pain (nsLBP) is considered as the global leading cause of years lived with disability affecting more than half a billion people. Adverse childhood experiences (ACEs), are one of the major risk factors for the development of both mental and physical disorders in later life. In humans, stress and ACEs are known risk factors for the chronicity of LBP and the development of chronic widespread pain. However, very little is known about the neuronal mechanisms that contribute to the development of this enhanced chronicity and sensitization in cases of nsLBP. The present studies aimed at investigating the impact of repeated restraint stress in an animal model of nerve growth factor (NGF) - induced myofascial low back pain.

To answer the importance of timing of the stressors, in two separate experimental approaches, the animals were stressed repeatedly in a narrow plastic restrainer on 12 consecutive days for 1 hour every day. In one experiment, the animals were stressed in adulthood and in the other experiment at early adolescence. Behavioral tests to assess for mechanically-induced pain-like behavior was assessed on the low back for local pain and at the distal hind paw for spreading of pain. The tests were performed before and after stress and in conjunction with saline/NGF injections.

In deeply anesthetized rats that experienced stress in adulthood, recordings were made with glass microelectrodes from the dorsal horn neurons (DHNs) in the spinal L2 segment. To induce hyper excitability, NGF was injected into the multifidus (MF) muscle at the vertebral level L5 directly before the *in-vivo* recordings started. As control, animals were handled but not repeatedly restrained and also received NGF injection before the recordings.

Restraint stress in adulthood slightly lowered the low back pressure pain threshold (PPT) (Cohen d = 0.8) and a subsequent NGF injection led to an increase in the proportion of DHNs with input from deep tissues (fascia and/or muscle) of the low back (14% vs. 39%; p = 0.041). This increased proportion was also observed for neurons with receptive fields outside the low back (7% vs. 26%; p = 0.081). Furthermore, the proportion of neurons with resting activity significantly increased (28% vs. 55%; p = 0.039) and this was especially in neurons having deep input (0% vs. 26%; p = 0.004). The proportion of neurons with convergent input (input from two types of tissues) showed an increased trend (7% vs. 23%; p = 0.147) but no changes were observed for neurons only with skin input (65% vs. 61%, p = 0.793) which are the majority in the dorsal horn.

In rats that experienced restraint stress in adolescence followed by two intramuscular injections of saline or NGF or both in adulthood, animals were transcardially perfused and lumbar spinal cord sections were extracted. Immunohistochemistry was performed on spinal L2 segments and were stained for ionized calcium-binding (Iba-1), a protein specifically expressed in microglia. Morphological analysis was performed on the Iba-1 postive cells.

Adolescent restraint stress significantly lowered the local low back PPT (d = 2.4) and remote paw withdrawal threshold (d = 2.0) (PWT) immediately after the stress. While the lowered local PPT was maintained throughout adulthood (d = 1.2) the distal PWT (d = 0.9) was stabilized but showed large effect. A subsequent NGF injection in adulthood in previously stressed animals slightly lowered the PPT (d = 0.9) but not PWT. These animals also showed a significant increase in the proportion and number of microglial cells in the phagocytic state compared to the stress + 2 saline group (36% vs. 18%; p = 0.013) and control + 2 NGF injections (36% vs. 16%; p = 0.031) group. The proportion and number of cells in resting state in those animals was significantly lower compared to the control + 2 NGF group (11% vs. 35%; p = 0.024).

The electrophysiological findings suggest that stress in adulthood followed by mild-nociceptive input causes manifest sensitization of the spinal neurons. The behavioral findings from animals stressed in adolescence show long-term central sensitization and a mild-nociceptive input in adulthood exacerbates this behavior and alters the morphology of microglial cells to phagocytic state. These findings indicate that stress in adulthood induces a state of latent sensitization and is manifested when presented with a mild-nociceptive input. While, stress in adolescence induces a long-term central sensitization and an additional insult in later life worsens this effect and that activated spinal microglial cells are involved in the development of manifest sensitization.