Localization and characterization of the enkephalin and dynorphin expression interneuron popultions in the mouse spinal cord and validation of DREADD expression induced by virus injection.

Harmen Kornelis Koning - Popular science summary

Pain and itch are two basal modalities of the nervous system, everybody is familiar with the feeling of pain and the satisfaction of scratching an itch. Both pain and itch are indicators of adverse stimuli coming from the environment or from within the body, it is obvious that pain and itch are highly functional in learning and interacting with the world around us. It is when pain or itch persists after its functional period or occurs without a reason that they become maladaptive. Examples are allodynia, a syndrome in which normally non-painful stimuli like stroking the skin are perceived as highly painful or chronic itch, which is a common side effect of surgery. Currently available pain and itch relieving medicines are quite unspecific and are paired with many unwanted side effects. To be able to provide better pain and itch relieving medicine we need to better understand their neural pathways and the cells involved in their modulation.

The pathways of pain and itch are quite similar, an itchy or painful stimulus is “recorded” by a primary sensory neuron in the periphery, this primary cell sends the signal from the periphery to the spinal cord, here the signal is transduced to a secondary neuron which takes the signal from the spinal cord up to the brain where the signal is processed. The location where the primary and secondary neurons connect to each other is called the dorsal horn of the spinal cord, at this location small, local cells called interneurons adjust the signal. This modulation is the interest of this study.

This study focused on two closely related interneuron populations which both express a specific subtype of opioid neurotransmitter; enkephalin or dynorphin. It is known that these two populations play a role in the modulation of pain and itch but exactly how is still unclear. We used designer receptors exclusively activated by designer drugs (DREADDs) to study this populations. DREADDs are neurotransmitter receptors that have been designed to only be activated by a exogeneous ligand called CNO. Using the Cre-Lox genetic tool in combination with a virus to introduce the DREADD and a red fluorescent marker we could express the DREADDs in either the enkephalin or dynorphin population. Two types of DREADDs were used, one that silenced the cells after application of CNO, and one that forced the cells to continuously send signals after CNO application.

This thesis covers the validation of the virus injection driven DREADD expression and aimed to characterize the neurotransmitter phenotype of the enkephalin and dynorphin expressing populations, both parts were executed using fluorescence immunohistochemistry. The populations were rather similar. Using an antibody marking recently activated cells confirmed that the DREADDs were injected in the correct region of the spinal cord and covered an area of about 2 millimetres long. After co-localization of the populations of interest with several antibodies that mark inhibitory neurons we can conclude that about half of the populations is of an inhibitory nature. And localization compared to a topographic marker teaches us that circa 20% of the populations resides in the most dorsal layer of the dorsal horn, 15% resides in the second layer and the rest of the population is positioned in the deeper layers of the spinal cords dorsal horn.