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Non-neuronal BDNF, a key player in development of central sensitization and neuropathic pain

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Pain usually arises as a consequence of activation of primary nociceptive afferences by tissue-damaging stimuli. Sometimes pain comes from the nervous system and can persist for months or years after the initial onset. This type of chronic pain is defined as neuropathic pain. It may have multiple origins such as diabetes, autoimmune disease, infections or nerve injury. Mechanisms underlying neuropathic pain remain elusive. About 25 years ago, it was clearly shown that two sites are actively involved in the early stages of producing pain: primary neurons in the dorsal root ganglia and central neurons into the spinal cord dorsal horn. After injury, the increase in the excitability of the central neurons induces reorganizations of the dorsal horn neurons. All those changes are collectively referred to as 'central sensitization' (Campbell & Meyer, 2006). An early consequence of peripheral injury is the spinal microglial activation which leads to a brain-derived neurotrophic factor (BDNF) release (Tsuda *et al.* 2005). However, mechanisms involved in 'central sensitization' remain poorly understood and the link between pain and BDNF is still unclear. Indeed, the involvement of BDNF in acute pain remains controversial. Some data are consistent with an anti-nociceptive role, whereas others are consistent with a pro-nociceptive effect (Coull *et al.* 2005).

In a recent publication in *The Journal of Physiology,* Lu *et al.* investigated the hypothesis that the 'central sensitization' may involve different effects on inhibitory and excitatory dorsal horn neurons in acute spinal cord slices (Lu *et al.* 2009). Moreover, they investigated the possible role played by BDNF released by activated microglia on this central sensitization on organotypic spinal cord slices. Previous studies have shown central sensitization, microglial activation and specific electrophysiological changes in the neurons of the lamina II after a sciatic nerve chronic constriction injury (CCI) (Balasubramanyan *et al.* 2006). Based on these studies, the authors used CCI as a neuropathic pain model.

The authors were firstly confronted with the complexity of neuronal phenotypes found in the substantia gelatinosa. The first series of experiments were conducted to simplify and re-characterize neurons from substantia gelatinosa using morphological and electrophysiological criteria on acute spinal cord slices. Their first results led the authors to work on two discrete neuronal populations: TIC (tonic islet/central) and RID (radial irregular delay). As 'tonic discharge pattern' and islet morphology have been associated with inhibitory neurotransmitters (GABA and/or glycine), and as 'delay discharge patterns' are rarely GABAergic, the authors defined TIC as inhibitory and RID as excitatory neurons. Of course, one might question the validity of a study based upon observations from about 50% of the total neuronal population. Nevertheless, when the authors checked electrophysiological modifications following CCI, they found that as they expected, sEPSC frequency and amplitude increased in RID neurons. Moreover, they described that sEPSC frequency decreased in TIC neurons.

To assess whether *in vitro* BDNF application can mimic CCI *in vivo*, the authors changed their study model, and used organotypic slice cultures instead of acute slices. This allowed prolonged exposure of neurons to BDNF for about 1 week. It has already been observed that morphological and electrophysiological phenotypes persisted in organotypic slice cultures. Following appropriate controls and results analysis (giant EPSC exclusion), they highlighted that BDNF led to an increase in excitatory drive to RID neurons and an attenuated excitability of TIC neurons, in a similar way to CCI. Lu *et al.* further explored their assumptions about the inhibitory and excitatory neuron phenotypes. This was done by *post hoc* determination of phenotype using histochemical techniques to detect glutamic acid decarboxylase (GAD)-65 and -67, a marker for GABAergic function in recorded neurons. They confirmed the previously described results: GAD^+ neurons showed a decrease in both frequency and amplitude and GAD^- neurons displayed an increase in the amplitude of their sEPSCs. One could ask why the authors did not also characterize TIC and RID neurons as GAD^+ or GAD^-.

BDNF is a well-described molecule released by activated microglia following peripheral nerve injury at the central level. As both CCI and BDNF lead to the same pattern of electrophysiological modifications, the authors asked whether microglial BDNF could mediate the CCI effects. In order to test their hypothesis, they exposed organotypic slice cultures to activated microglia conditioned medium (aMCM). One would expect that the authors firstly quantified BDNF in aMCM, to verify that BDNF concentration was about in the *in vivo* CCI condition range and then performed electrophysiological recordings on these aMCM-exposed neurons, instead of calcium imaging. However, it is worth noting that confocal calcium imaging does lead to elegant results. For the first time, the authors described the effect of high K^+ challenge on dorsal horn neurons cultured with aMCM. They showed that both BDNF and aMCM led to an increase in both the peak amplitude and the area under the curve. One has to appreciate that the authors performed control experiments on cells grown in resting microglia conditioned medium culture. The next question was to know whether the effect of aMCM was due to BDNF. Several strategies were tested (pharmacological Trk receptor inhibition, Trk receptor intracellular transduction pathway inhibition, etc.). They chose an alternative approach to inactivate the neurotrophin using the recombinant protein TrkB-d5 which binds BDNF. Results from these experiments strongly suggested that aMCM excitatory effect is mediated by BDNF in the dorsal horn of the spinal cord. Finally, the authors checked whether the overall excitability of the spinal cord following aMCM exposure was not the consequence of a decrease of the inhibitory network activity. This is because microglial-derived BDNF has been shown to collapse or even reverse the Cl^- gradient (Coull *et al.* 2005). They blocked

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GABAergic neurotransmission with an inhibitor of $GABA_A$ receptors. After such treatment, dorsal horn excitability was still present following BDNF incubation. The conclusion is that BDNF mediated the modifications of excitatory synaptic transmission that lead to central sensitization of the spinal cord. The authors wanted to go further by demonstrating the involvement of depolarization-induced glutamate release in the overall increased excitability. To test, they used two pharmacological agents, a glutamate uptake (DHK) and two glutamate ionotropic receptor inhibitors (CNQX plus AP5). Results from these experiments were consistent with the involvement of ionotropic glutamate receptors in BDNF-induced increases of overall excitability.

This study of Lu *et al.* shows for the first time that both excitatory and inhibitory circuits are differentially modified during 'central sensitization'. Moreover, they paved the way to the explanation of how non-neuronal BDNF is involved in the overall increase in excitability into spinal cord following peripheral nerve injury despite the debate on the role of BDNF in 'central sensitization'. Further work will have to focus on ways to attenuate 'central sensitization' in order to relieve patients who suffer from neuropathic pain.

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