Dopaminergic NK1 secondary incubation was completed and then mounted in PBS (pH 7.4) containing 3% goat or donkey serum (depending on antibodies). Sections were incubated in 0.3 or 1% triton X-100 (w/v) or carrageenan injection, IL-6 and carrageenan-induced mechanical hypersensitivity was impaired and subsequent PGE2 response was absent or blunted, respectively. However, when these neurons were lesioned after IL-6- or carrageenan-induced mechanical hypersensitivity had resolved, they had no effect on the PGE2 response reflecting differential mechanisms driving plasticity in an acutely sensitized state. Similarly, ablating noradrenergic neurons after the IL-6 or carrageenan response subsided did not affect maintenance of priming. In stark contrast, animals with spinal dopaminergic lesion prior to IL-6 and carrageenan injection showed intact IL-6- and carrageenan-induced mechanical hypersensitivity but the subsequent PGE2 injection failed to cause mechanical hypersensitivity. Moreover, ablating dopaminergic neurons after the resolution of the IL-6- and carrageenan response also reversed the maintenance of priming. This was reflected both in mechanical hypersensitivity and in spontaneous pain measured with the mouse grimace scale. Pharmacological antagonism of spinal dopamine D1/D5 receptors indicated a key role for these receptors in the maintenance of priming. In stark contrast, animals with spinal dopaminergic lesion prior to IL-6 and carrageenan injection showed intact IL-6- and carrageenan-induced mechanical hypersensitivity but the subsequent PGE2 injection failed to cause mechanical hypersensitivity. Moreover, ablating dopaminergic neurons after the resolution of the IL-6- and carrageenan response also reversed the maintenance of priming. This was reflected both in mechanical hypersensitivity and in spontaneous pain measured with the mouse grimace scale. Pharmacological antagonism of spinal dopamine D1/D5 receptors indicated a key role for these receptors in the maintenance of priming. These findings demonstrate a completely novel role for descending dopaminergic neurons in the maintenance of chronic pain.

Materials and Methods

Mechanical testing

Mechanical withdrawal threshold testing was conducted using the up-down method of Dixon with modification. Animals were placed in acryl boxes with wire mesh floors and habituated for a minimum of 1 hour prior to the measurement of mechanical withdrawal thresholds. For all injections, animals were anesthetized with isoflurane in 0.9% saline and administered in 200 μL sterile water into the left hindpaw and carrageenan injection failed to cause mechanical hypersensitivity. Moreover, ablating dopaminergic neurons after the resolution of the IL-6- and carrageenan response also reversed the maintenance of priming. This was reflected both in mechanical hypersensitivity and in spontaneous pain measured with the mouse grimace scale. Pharmacological antagonism of spinal dopamine D1/D5 receptors indicated a key role for these receptors in the maintenance of chronic pain. The mechanisms that lead to the maintenance of chronic pain states are poorly understood but their elucidation could facilitate the discovery of novel therapeutics. The importance of both peripheral and central sensitization has been confirmed in many preclinical pain models but how and whether these forms of neuronal plasticity maintain a chronic pain state is not known. We investigated the role of spinal dorsal horn neurons and descending circuitry in mediating a transition from acute to chronic pain using hyperalgesic priming model. In this paradigm, interleukin-6 (IL-6) or carrageenan injection into the mouse hind paw elicits a transient acute mechanical hypersensitivity. Following resolution, a subsequent intraplantar injection of a low dose of prostaglandin E2 (PGE2) precipitated prolonged mechanical hypersensitivity exclusively in primed mice -- e.g. those with previous exposure to IL-6 or carrageenan. We found that when spinal dorsal horn neuronskin-1 (NK1) receptor-positive neurons or descending serotonergic neurons were ablated prior to IL-6 or carrageenan injection, IL-6- and carrageenan-induced mechanical hypersensitivity was impaired and subsequent PGE2 response was absent or blunted, respectively. However, when these neurons were lesioned after IL-6- or carrageenan-induced mechanical hypersensitivity had resolved, they had no effect on the PGE2 response reflecting differential mechanisms driving plasticity in an acutely sensitized state. Similarly, ablating noradrenergic neurons after the IL-6 or carrageenan response subsided did not affect maintenance of priming. In stark contrast, animals with spinal dopaminergic lesion prior to IL-6 and carrageenan injection showed intact IL-6- and carrageenan-induced mechanical hypersensitivity but the subsequent PGE2 injection failed to cause mechanical hypersensitivity. Moreover, ablating dopaminergic neurons after the resolution of the IL-6- and carrageenan response also reversed the maintenance of priming. This was reflected both in mechanical hypersensitivity and in spontaneous pain measured with the mouse grimace scale. Pharmacological antagonism of spinal dopamine D1/D5 receptors indicated a key role for these receptors in the maintenance of hyperalgesic priming. These findings demonstrate a completely novel role for descending dopaminergic neurons in the maintenance of chronic pain.

Conclusions

1. NK1-positive spinal cord neurons are involved in initiation but not maintenance of hyperalgesic priming
2. Neither serotonergic nor noradrenergic neurons are required for maintaining hyperalgesic priming. Serotonergic neurons may play a role in establishing priming.
3. Dopaminergic innervation of the spinal cord is required for hyperalgesic priming initiation and maintenance in a D1/D5-dependent fashion.

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Abstract

The mechanisms that lead to the maintenance of chronic pain states are poorly understood but their elucidation could facilitate the discovery of novel therapeutics. The importance of both peripheral and central sensitization has been confirmed in many preclinical pain models but how and whether these forms of neuronal plasticity maintain a chronic pain state is not known. We investigated the role of spinal dorsal horn neurons and descending circuitry in mediating a transition from acute to chronic pain using hyperalgesic priming model. In this paradigm, interleukin-6 (IL-6) or carrageenan injection into the mouse hind paw elicits a transient acute mechanical hypersensitivity. Following resolution, a subsequent intraplantar injection of a low dose of prostaglandin E2 (PGE2) precipitated prolonged mechanical hypersensitivity exclusively in primed mice -- e.g. those with previous exposure to IL-6 or carrageenan. We found that when spinal dorsal horn neuronskin-1 (NK1) receptor-positive neurons or descending serotonergic neurons were ablated prior to IL-6 or carrageenan injection, IL-6- and carrageenan-induced mechanical hypersensitivity was impaired and subsequent PGE2 response was absent or blunted, respectively. However, when these neurons were lesioned after IL-6- or carrageenan-induced mechanical hypersensitivity had resolved, they had no effect on the PGE2 response reflecting differential mechanisms driving plasticity in an acutely sensitized state. Similarly, ablating noradrenergic neurons after the IL-6 or carrageenan response subsided did not affect maintenance of priming. In stark contrast, animals with spinal dopaminergic lesion prior to IL-6 and carrageenan injection showed intact IL-6- and carrageenan-induced mechanical hypersensitivity but the subsequent PGE2 injection failed to cause mechanical hypersensitivity. Moreover, ablating dopaminergic neurons after the resolution of the IL-6- and carrageenan response also reversed the maintenance of priming. This was reflected both in mechanical hypersensitivity and in spontaneous pain measured with the mouse grimace scale. Pharmacological antagonism of spinal dopamine D1/D5 receptors indicated a key role for these receptors in the maintenance of hyperalgesic priming. These findings demonstrate a completely novel role for descending dopaminergic neurons in the maintenance of chronic pain.