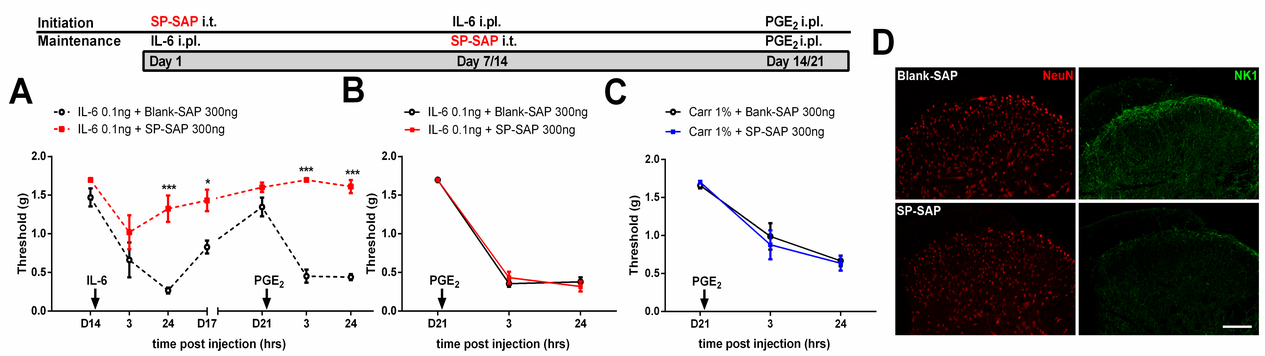
Spinal dopaminergic projections control the transition to chronic pain via a D1/D5-mediated mechanism

Ji-Young Kim[1], Dipti V Tillu[2], Tammie L Quinn[3], Galo L Mejia[1,2], Adia Shy[1], Marina NK Asiedu[1,2], Elaine Murad[1], Robert E Sorge[3], Patrick W Mantyh[1], Gregory Dussor[1,2], Theodore J Price[1,2]

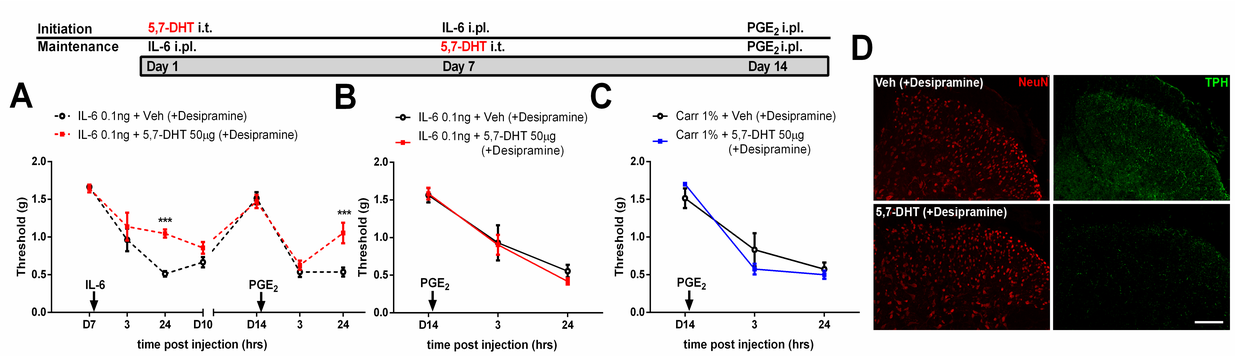
[1]University of Arizona, Department of Pharmacology [2]University of Texas at Dallas, School of Behavioral and Brain Sciences [3]University of Alabama at Birmingham, Department of Psychology

Results

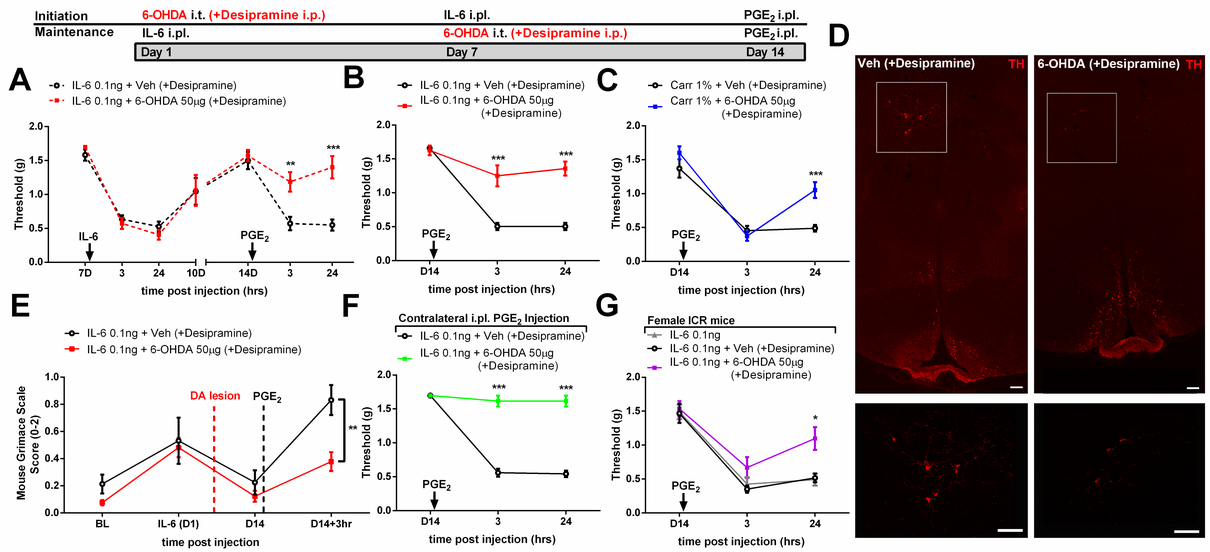
1. NK1-postive projection neurons are required for initiation of hyperalgesic priming but not for its maintenance.



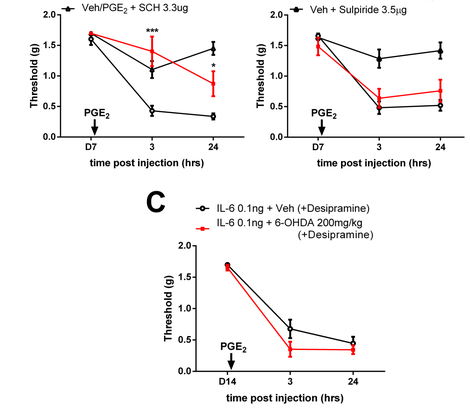
2. 5-HT neurons differentially influence the initiation and maintenance of hyperalgesic priming.



3. Dopaminergic spinal projections from A11 are required for hyperalgesic priming.



4. Hyperalgesic priming is regulated by D1/D5 dopamine receptors



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 E_2 (PGE_2) precipitates 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 neurokinin 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 PGE_2 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 PGE_2 response reflecting differential mechanisms driving plasticity in a primed 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 PGE_2 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.

Materials and Methods

Behavioral testing

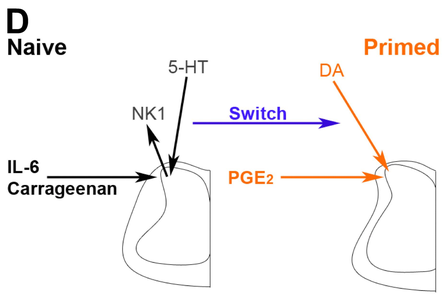
Mechanical withdrawal threshold testing was conducted using the up down method of Dixon with modification. Animals were placed in acrylic boxes with wire mesh floors and habituated for a minimum of 1 hour prior to the measurement of mechanical withdrawal thresholds of their left hindpaw using calibrated von Frey filaments (Stoelting). In order to establish hyperalgesic priming, we injected 0.1 ng of human IL-6 (R&D systems) in 25 µL sterile 0.9% saline or 1% carrageenan (w/v, Sigma Aldrich) in 30 µL sterile water into the left hindpaw and measured their mechanical withdrawal thresholds at 3, 24 and 72 hr post injection. On day 7, animals were baselined and subsequently injected in the left hindpaw with 100 ng of PGE_2 (Cayman Chemical) in 25 µL sterile 0.9% saline. Afterward, mechanical withdrawal thresholds were measured at 3 and 24 hr. For all intrathecal (i.t.) injections, drugs were administered in 5 µl sterile 0.9% saline or water to animals anesthetized with isoflurane for no longer than 3 minutes. For all intraperitoneal (i.p.) injections drugs were dissolved in sterile 0.9% saline and administered in 200 µL volume.

Immunohistochemistry

Tissue sections were washed with 100 mM PBS (pH 7.4) and permeabilized and blocked with 0.3 or 1% triton-X100 in 100 mM PBS (pH 7.4) containing 3% goat or donkey serum (depending on antibodies). Sections were incubated in permeabilization / blocking solution containing primary antibodies and incubated overnight at 4°C. After the primary antibody incubation, slides were washed with PBS three times and then incubated for 1 hr with secondary antibody at room temperature. Slides were washed with PBS three times once the secondary incubation was completed and then mounted in ProLong Gold mounting media (P36930, Life Technologies). Tissues from all groups were processed together under identical conditions with the same reagents.

Conclusions

1. NK1-positive spinal cord neurons are involved in initiation but nor maintenance of hyperalgesic priming
2. Neither serotonergic nor noadreneric 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.



Acknowledgements

This work was funded by NIH R01 **NS065926** to TJP