Spinal cord and brain pain fMRI in rats: anatomical sites of analgesic action of buprenorphine on the noxious electrical stimulation-induced pain

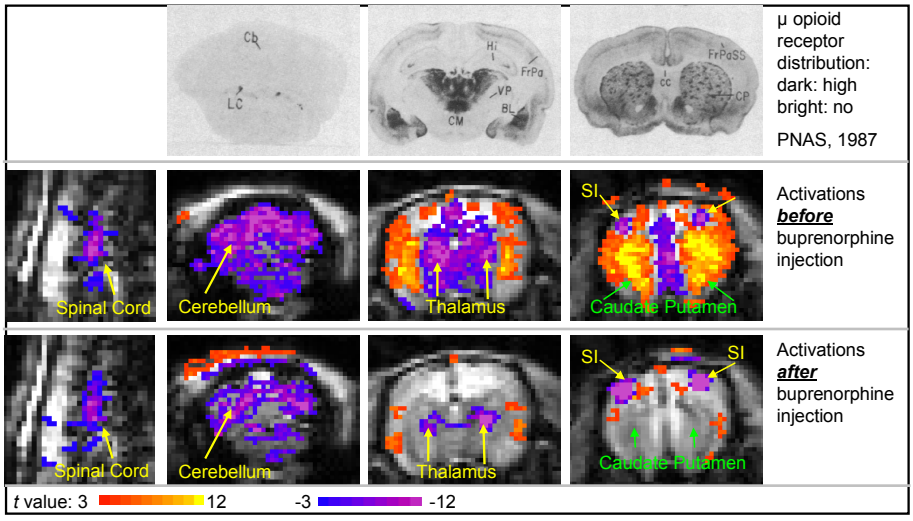
F. Zhao[1], D. Welsh[1], M. Williams[1], A. Coimbra[1], M. O. Urban[2], R. Hargreaves[2], J. Evelhoch[1], and D. S. Williams[1]

[1]Imaging Department, Merck Research Laboratories, West Point, PA, United States, [2]Neuroscience Department, Merck Research Laboratories, West Point, PA, United States

[INTRODUCTION] Functional MRI (fMRI) can non-invasively measure pain-related neural activities in humans and animals, providing a valuable tool to evaluate efficacy, and to study the mechanisms of action of analgesics. As part of our ongoing efforts, fMRI activations induced by noxious electrical stimulation (NES) in spinal cord (*1*) and brain have been developed in rats. To better understand fMRI as a pain biomarker, and to determine its utility in elucidation of mechanisms of action of analgesics, the effect of buprenorphine (BPN), a partial μ-opioid agonist, on pain fMRI signals was investigated. A BPN dose of 1 mg/kg (i.v.) was used because it fully occupies the μ-opioid receptor system (9). Blood volume (BV) fMRI was used to improve sensitivity (*1*).

[METHODS] The animal protocol was approved by the IACUC of Merck Research Laboratories. Sprague-Dawley rats (Taconic Farms Inc) were initially anesthetized with isoflurane in a mixture of O_2 and N_2 gases (3:7) and intubated for mechanical ventilation (MRI-1; Kent Scientific, CT, USA). A continuous subcutaneous infusion of a mixture of 0.15 mg/kg/h medetomidine (Domitor) and 4 mg/kg/h pancuronium bromide was started and isoflurane was stopped 20 minutes later (*2*). All MRI measurements were performed on a 7 T Bruker Biospec system. A 2 cm diameter surface coil positioned either beneath the lumbar spinal cord or on the top of the brain was used as the RF receiver, while an actively-decoupled 72 mm diameter volume coil was used as the RF transmitter. T2*-weighted images were acquired using a single-shot GE EPI with phase-encoding in the dorsal-ventral direction; matrix size = 64 × 64; TE=11 ms. Each fMRI run consisted initial baseline, stimulation on, and recovery periods. To achieve the highest activation, an optimum electrical pulse strain (2 ms, 5 mA, 40 Hz) (*1*) was applied to the bilateral paws simultaneously. For spinal cord fMRI, one single sagittal slice of 2 mm thickness covering the bilateral dorsal horns was chosen. For brain fMRI, sixteen consecutive axial slices covering the brain from forelimb region to cerebellum were chosen. In-house synthesized USPIO was used for BV fMRI (*1*). BPN was injected (i.v.) at a dose of 1 mg/kg (*3*).

[RESULTS] Figure shows pain fMRI activation maps in the spinal cord and the brain at cerebellum, thalamus, and CPu levels induced by the NES of paws before (middle row) and within one hour after (bottom row) BPN injection. Before the BPN injection, Negative blood volume (BV) fMRI responses (blue/purple) indicating an increase in local blood volume are observed in the lumbar enlarge of spinal cord, and several regions including cerebellum, thalamus region, and primary somatosensory cortex (S1), etc. of brain. Robust positive BV fMRI responses (red/yellow), indicating a decrease in local blood volume, are observed in the bilateral CPu. After BPN administration, the activations in caudate putamen and a large part of the thalamic region are totally suppressed, while the activations in the spinal cord and the cerebellum are partly suppressed, and the activations in the thalamic relay of somatosensory pathway and the S1 are not suppressed. The spatial pattern of fMRI signal suppression in response to buprenorphine shows a similarity to the μ opioid receptor distribution maps (*4*) (top row): the pain signals in the regions with high μ receptor density (CPu and thalamus region) are totally suppressed, while the pain signals in the regions (cerebellum, thalamic relay, and S1) with low (no) μ receptor density are less (not) suppressed. These results demonstrate the value of fMRI in providing information on the anatomical sites of the analgesic action of analgesics.



[Discussion] BPN is a well-established opioid analgesic (μ-opioid partial agonist). It has a broad analgesic profile in various rodent models of acute and chronic pain (*3*). NES can directly activate noxious peripheral neural fibers (*5*) bypassing the sensory endings, and has been used in human (*6*) and animal pain studies as an acute pain model. It is suitable to test the central component of analgesic effects of centrally acting analgesics (*6*). Hence it is not surprising that the results from this study show that the pain fMRI signals induced by NES can be suppressed by BPN doses that fully occupy the μ-opioid receptor system (*9*). However, the important message from this study is that fMRI can provide not only the information about the analgesic *efficacy*, but also information about the *anatomical sites of action of the analgesic*. BPN is a partial agonist at μ-opioid receptor and an antagonist at k- and δ-opioid receptors. It has been concluded that its analgesic effect to acute pain originates from μ opioid receptor. Since the pain fMRI signals induced by NES represent acute pain, the anatomical sites of BPN action should relate to μ opioid receptor distribution in CNS. It is known that μ opioid receptor is distributed in spinal cord (*7*), and our data show that BPN does suppress spinal cord pain fMRI signals; these findings are consistent with the previous studies that showed that opioid analgesic morphine reduced the formalin-induced c-fos expression in spinal cord (*8*). However, the suppression efficacy of pain fMRI signals in spinal cord is much lower than the analgesic effect measured by behavioral studies (*3*), suggesting that the analgesic sites of BPN locate not only at spinal level, but also at supraspinal levels. At supraspinal levels, pain fMRI signals in CPu and the thalamic region (where μ opioid receptors distribute with high density) are totally suppressed, indicating the analgesic effect of BPN dominates in these regions. In conclusion, as evidenced in these results, pain fMRI can provide information on the analgesic action sites within the neuraxis. Such information is not easily obtained by other techniques and should help understand the mechanism of action of different types of analgesics.

[Reference] 1. F. Zhao *et al.*, *Neuroimag* 40, 133 (2008). 2. C. P. Pawela *et al.*, *Neuroimag* 46, 1137 (2009). 3. T. Christoph *et al.*, *Eur J Pharmacol* 507, 87 (2005). 4. A. Tempel, R. S. Zukin, *Proc Nat Acad Sci* 84, 4308 (1987). 5. D. Le Bars, et al., *Pain* 6, 283 (1979). 6. W. Koppert *et al.*, *Anesthesiology* 95, 395 (2001). 7. A. G. Hohmann, et al., *Brain Res* 822, 17 (1999). 8. R. W. Presley, et al., *J Neurosci* 10, 323 (1990). 9. D. Rubins, et al., Proc. of World Molecular Imaging Congress, 2008.