# Analgesic action sites of pregabalin by fMRI of spinal cord and brain in anesthetized rats, and its qualification against behavioral assay in awake rats

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

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

**[INTRODUCTION]** Pregabalin (Lyrica) is used for the treatment of epilepsy and pain (i.e., neuropathic pain and postsurgical pain). Both its anatomical targets and its mechanism of action are poorly understood [1]. fMRI can map and quantify the strength of pain-related neural activations in spinal cord and brain induced by noxious stimuli. By comparing the fMRI activations before and after delivery of an analgesic, the anatomical location-dependent effects can be measured, providing information about the anatomical targets of the analgesic. In this study, the suppression effects of pregabalin on noxious electrical stimulation (NES) induced activities were investigated by blood volume (BV) fMRI in anesthetized rats. To investigate the potential confounding effects of anesthesia in interpretation of pain fMRI in anesthetized rats, a behavioral assay (vocalization) was carried out in awake rats with the identical pain induction paradigm and treatment protocol as the fMRI study. The fMRI and behavioral results suggest that 1) pregabalin has efficacy on the NES-induced pain in naïve rats; and 2) the analgesic action sites of pregabalin are not in the primary somatosensory pathway (spinal cord, dorsal column nuclei, thalamic relay, primary somatosensory cortex - S1), but in other activated regions, and (3) the temporal profile of treatment effects detected by fMRI in anesthetized rats is similar to that of the behavioral assay in awake rats.

**[METHODS]** fMRI study was carried out on a 7 T Bruker Biospec in rats (n=10) anesthetized with medetomidine (dormitor) and low isoflurane [2]. The NES (2 ms, 5 mA, 40 Hz) was delivered to bilateral paws as a pain source. After administration of USPIO, BV fMRI data were acquired either in a sagittal slice of spinal cord covering the bilateral dorsal horn, or in sixteen consecutive coronal slices covering the brain from forelimb region to cerebellum, using a single-shot GE EPI [2]. For the behavioral study, a separate set of rats were initially anaesthetized with isoflurane (3% in air) and then secured in a rat restrainer. The study started ~1 hour after cessation of isoflurane to allow the rats to recover. The vocalization thresholds of NES of the left hindpaw and right hindpaw were measured independently in each rat. A train of 20 electrical pulses (2 ms, 40 Hz) which lasts 0.5 sec was delivered starting with a low current. If no vocalization was heard, the electrical current was increased at a step-size of 0.1 mA and delivered 1.5 sec later. The process was repeated till the vocalization was heard, and the pain threshold current recorded. The measurement of the other hindpaw immediately followed, and the pair of measurements repeated every 15 min. Both fMRI and behavioral data were acquired before and after bolus injection of pregabalin (100 or 10 mg/kg) or saline. Student's t-test was used to compare data acquired before and at various times after pregabalin administration to evaluate pregabalin's effect on NES-induced neural activation.

**[RESULTS]** As shown in the top row of Fig. 1, before pregabalin injection, NES of paws induces well-localized activations in spinal cord, S1, cuneate nuclei, caudate putamen, and widespread activations in some other cortical and subcortical regions (collectively referred to as 'Disperse' activations). After pregabalin injection (100 mg/kg), activations in all other regions are totally suppressed except those in spinal cord, S1, thalamus relay, and cuneate nuclei, which comprise the primary somatosensory pathway. Since a dose of 100 mg/kg can cause sedation, low dose pregabalin (10 mg/kg, which has no sedative effect by behavioral studies [3, 4]) was studied by fMRI in brain. The temporal profile of fMRI amplitudes in caudate putamen and the 'disperse' activation regions are shown in Fig. 2A. A clear suppression effect at this low dose pregabalin is observed on the fMRI signals in these regions, reaching statistically significant difference ($p<0.05$) from baseline at ~30 min post injection. To verify that the observed suppression of the fMRI signal by pregabalin is due to its analgesic action, a behavioral study to measure the pregabalin (10 mg/kg) effect on the NES threshold to induce vocalization was performed in awake rats. Fig. 2B shows the temporal profiles of the vocalization thresholds measured by this behavioral study. After pregabalin injection, the vocalization threshold slowly increases, and reaches the maximum ~ 2 hours after pregabalin injection, which is consistent with the temporal response from other behavioral studies [5]. The increase in vocalization threshold reached statistical significance ($p<0.05$) 30 min after pregabalin injection. Comparing Fig. 2A and 2B, within 60 min after pregabalin injections, the fMRI signals monotonically decrease while the magnitudes of vocalization threshold monotonically increase, indicating the treatment effect by fMRI in anesthetized rats is associated with behavioral effects in awake rats. Saline injection in the separate group of rats shows no change in vocalization threshold.

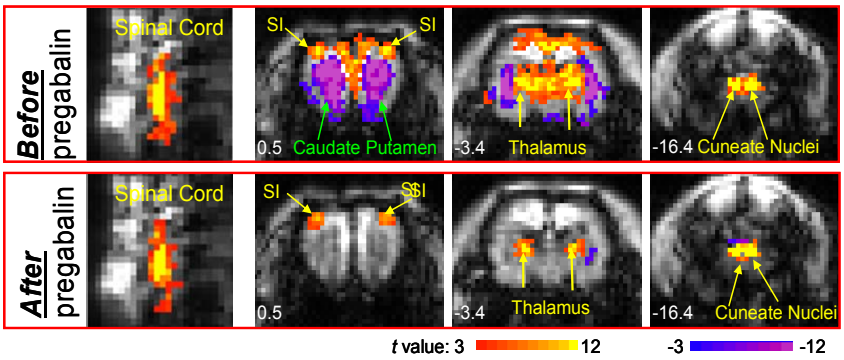


**Fig. 1 Effects of pregabalin (100 mg/kg) on the NES-induced fMRI activations in brain and spinal cord.** A saggittal slice of the spinal cord covering left and right dorsal horns and 3 brain slices covering primary somatosensory cortex (S1), thalamus, and cuneate nuclei (CN) from one animal are shown before (top row) and after (bottom row) pregabalin injection. The numbers in the left-bottom corner of each brain slice represent anterior-posterior coordinates with respect to bregma.

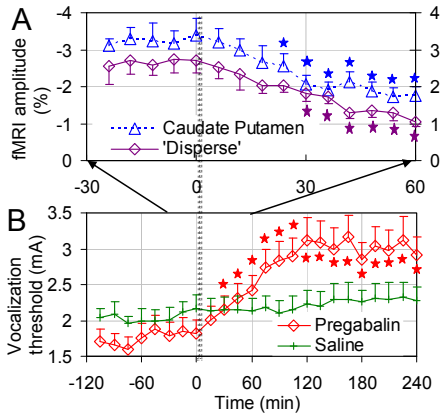


**Fig. 2 Effects of pregabalin (10 mg/kg) on the fMRI and the vocalization thresholds.** Temporal profiles (Mean ± SEM, n=5) of fMRI amplitudes in the caudate putamen and the 'disperse' activation regions (**A**) and of the vocalization threshold (**B**). The gray vertical bar indicates the time of injection. * indicates statistical significant difference ($p<0.05$) from pre-treatment.

**[Discussion]** Our fMRI and behavioral results suggest that 1) pregabalin has efficacy on NES-induced pain in naïve rats; 2) the analgesic action sites of pregabalin are not in the primary somatosensory pathway, but in other activated regions., and (3) the temporal profile of treatment effect by fMRI in anesthetized rats is associated with behavioral effects in awake rats. This information on the anatomical targets should help in understanding the mechanism of action of pregabalin. It has been suggested that pregabalin targets the calcium channel α2δ-1 subunit (Cavα2δ-1) for both its analgesic action [6, 7] and anti-epilepsy action [8]. However, our results show that pregabalin has no effect on the neural activities in the primary somatosensory pathways where Cavα2δ-1 also exists (see Fig. 3 in [9]), suggesting that pregabalin may not act on Cavα2δ-1. Whatever the molecular targets of pregabalin might be, they must not act on transmitters of neural activities induced by NES in the primary somatosensory pathway of naïve rats.

[**Reference**] 1. Taylor, Pain, 2009. 145: 259-261. 2. Zhao, Pain, 2009. 145: 110-9. 3. Yokoyama, J Pain, 2007. 8: 422-9. 4. Vartanian, Epilepsy Res, 2006. 68: 189-205. 5. Wallin, Eur J Pain, 2002. 6: 261-72. 6. Taylor, Pain, 2009. 142: 13-6. 7. Field, PNAS, 2006. 103: 17537-42. 8. Taylor, Epilepsy Res, 2007. 73: 137-50. 9. Taylor, Neuroscience, 2008. 155: 510-21.