BOLD-signal representation of incisional and inflammatory pain in rat brain after noxious electrical and noxious mechanical stimulation

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TARGET AUDIENCE: Scientists interested in pain studies (Clinical/Preclinical)

PURPOSE: In behavioral pain studies, mechanical stimulation with von Frey filaments is commonly used to explore hyperalgesia.[1] However, fMRI studies frequently use electrical or heat stimuli to evaluate brain responses to pain. The present study aims to investigate the BOLD-response in pain-related brain regions in an incisional and an inflammatory rat model during noxious electrical stimulation (NES) and noxious mechanical stimulation (NMS) of the injured hindpaw under the anesthesia with medetomidine.

MEHODS: A surgical incision[2] (n=12) or induction of inflammation by injection of Complete Freud's Adjuant[2] (CFA; n=12) in the rats hindpaw was performed 24 hours before MR measurement in adult Sprague Dawley rats. A control group (n=12) receiving anesthesia only was used to compensate for the possible anesthesia effect during induction of the injury. Each injury group was divided in two subgroups, one receiving NES and the other NMS. The fMRI data were acquired on a 9.4T Bruker Biospin with a GE EPI sequence (TR/TE:1000/18ms, 12-13 slices, 1.2mm thick, FOV 30*30mm^2, Matrix 80*80, 600 repetitions). The stimulation was applied to the injured hindpaw with a block design paradigm of 10s stimulation and 20s rest (20 cycles). A constant current stimulator was employed for the subcutaneous NES (2ms, 9Hz, 5mA). The NMS was delivered using a von Frey filament (diameter 0.85mm, contact area 0.56 mm^2, equal weight 95g, 1Hz) implemented in a home-made, pneumatically controlled device.[3] fMRI images were realigned and smoothed in SPM8. The time courses were extracted from delineated ROIs on selected anatomical regions using the Marsbar toolbox of SPM8. The percent changes of BOLD signal were calculated and averaged over the stimulation cycles and all the animals in a group.

RESULTS: The BOLD signal changes were assessed in Cinguls (CG), Primary somatosensory cortex (S1), Thalamus (Tha), Retrosplenial cortex (RSC) and Periaqueductal grey (PAG), which are some of the regions associated with pain responses (Fig. 1). Within all studied regions in all groups, the time courses showed a signal increase approximately 2 seconds after start of the applied stimulation. The profile of the response to painful stimulation followed a biphasic pattern with an initial peak followed by a plateau of lower amplitude; this pattern was observed for both types of stimulation (Fig. 1). Only in S1 of the pain models, only the initial peak was observed under NES, while the sham group showed a more typical hemodynamic profile. Comparing the two modes of stimulation differences in the response to NMS and NES were observed. Upon NMS, the response in the inflammation group was higher as compared to both incision and sham group (U-test, $P<0.05$), which both showed similar responses (Fig. 1). Upon NES, significantly higher responses were observed for both pain models in CG, Tha and PAG (U-test, $P<0.05$) as compared with the sham group. Different responses for the two pain models were observed in S1 and RSC (U-test, $P<0.05$, Fig. 1).

DISCUSSION: The feasibility of mechanical stimulation in animal fMRI studies has been shown before in naïve animals.[3,4] The present study reveals for the first time differences between electrically and mechanically induced pain responses for different types of painful injuries in rats. The response of the sham group to both stimulations differed from that in pain models. However, differences between pain models were more pronounced with NMS. NMS has the advantage of specific pain induction through Aδ –fibers and represents a clinical meaningful measure of hyperalgesia. In contrast, NES activates not only the nociceptive fibers, but also non-nociceptive afferents, able to overshadow specific responses to painful stimuli in injured animals. The biphasic pattern of responses after painful stimulation is similar to previously observed patterns in human fMRI studies[5], but has not been reported in animal studies so far. Possible explanations could be the suppressive effect of isoflorane anesthesia used in those studies, or exclusion of the first seconds of the stimulation from the analysis. The higher BOLD response to NMS in the inflammation group corresponds to greater hyperalgesia and the occurrence of allodynia in inflammation compared to incision injury.

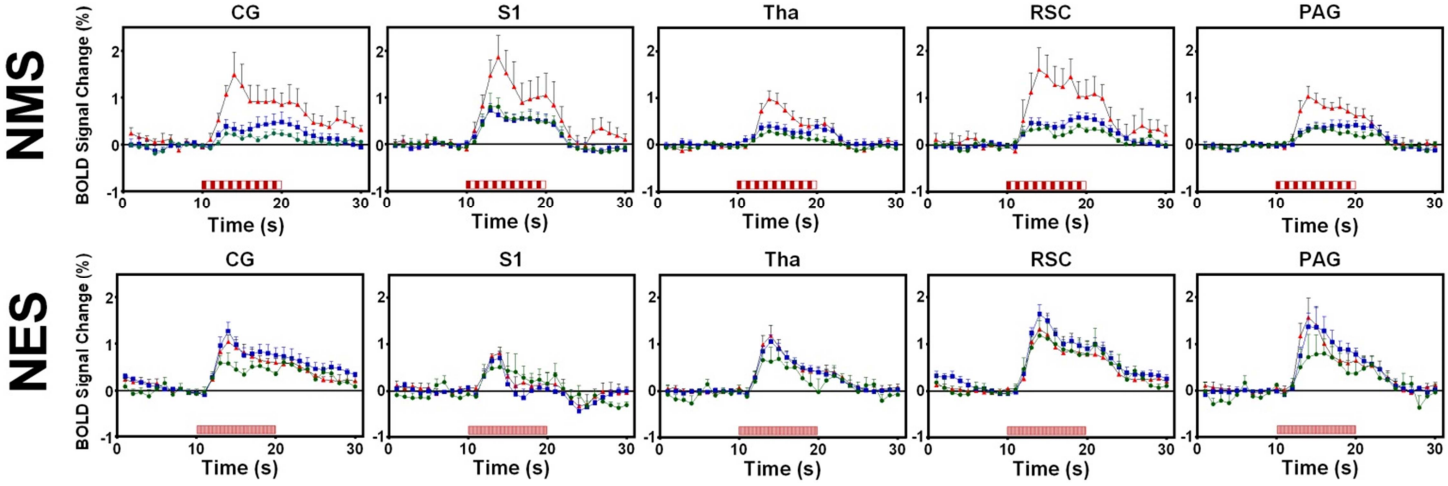

Figure 1: Time courses of BOLD fMRI after stimulation of right hindpaw with NMS (1Hz) and NES (9Hz) in CG, S1, Tha, RSC and PAG in sham (green), incision (blue) and inflammation (red) treated rats. Mean ± SEM n= 6. The red bars under the time courses indicate the 10s stimulation period

References: 1. Reichl, S.,[…]Pogatzki-Zahn, E.M., *Pain* **153,** 129–141 (2012). **2.** Scherer, M. et al., *Anesth Analg*. **110**, 222-227 (2010). **3**.Governo, R.J. et al., *J Neurosci Methods* **163,** 31–37 (2007). **4.** Zhao, F. et al. *Neuroimage* **59,** 1168–1179 (2012) **5**.Moulton, E.A. et al., *J Neurosci* **32,** 6024–6031 (2012).