Distinct BOLD fMRI Responses of Capsaicin-induced Thermal Sensation Reveal Pain-related Brain Activation in Non-Human Primate

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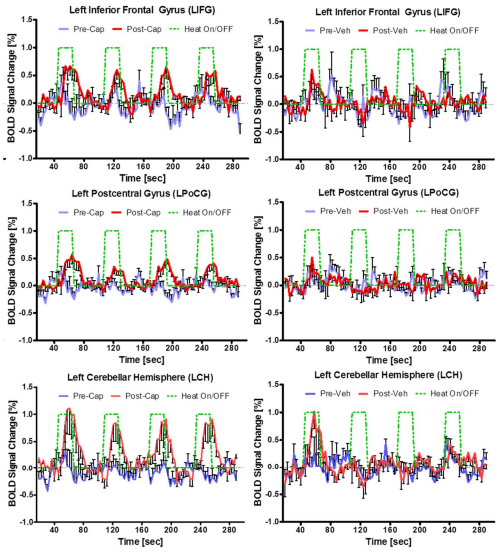
Target Audience: Researchers involved in pain imaging research and pre-clinical animal model development for drug discovery.

Purpose: Capsaicin applied either topically or intra-dermally has been used in experimental pain studies, where both routes of capsaicin administration can induce primary and secondary hyperalgesia to thermal stimuli as well as brief episodes of on-going pain. Thus, capsaicin-induced allodynia and hyperalgesia in rodent, non-human primate (NHP) and human subjects have been explored to evaluate pharmacodynamic antinociceptive effects of novel therapeutics, allowing better understanding of drug actions and perhaps mechanisms of underlying chronic pain. However, assessment of pain responses is often confounded by many factors related to psychological, and cognitive aspects of illness, and thus quantitative measurements, such as of pain imaging[1, 2], permitting objective evaluation of drug's analgesic actions have been developed[3]. Herein we established a capsaicin pain fMRI NHP model using heat stimuli[3-5], which could afford a translational biomarker to bridge the gap between preclinical research and clinical investigation. We hypothesize potentiation in heat-induced cortical activation in response to the capsaicin application. The activation pattern should highlight brain regions associated with the 'pain matrix' observed in human pain fMRI experiments[1, 2]. Additionally, we examined the time-course of BOLD signals obtained from capsaicin- and vehicle-treatment groups to delineate the various aspects of pain versus thermal sensations and their corresponding neural circuitry.

Table 1 Activated brain vol. of group activation Cap (n=8) or Veh (n=5) (pre *vs* post, paired t-test, z>2.3).

Brain Region	Vehicle Act. Vol. [mm^3]		Capsaicin Act. Vol. [mm3]	
	1st Cycle (Right/Left)	2nd-4th Cycle (Right/Left)	1st Cycle (Right/Left)	2nd-4th Cycle (Right/Left)
Cingulate cortex (CC)	43.27 / 47.38	0 / 0	53.34 / 44.42	130.83 / 175.61
Inferior frontal gyrus (IFG)	0 / 0	0 / 0	0 / 17.75	104.61 / 82.67
Superior frontal gyrus (SFG)	6.89 / 0	0 / 0	55.62 / 103.41	291.81 / 233.59
Middle frontal gyrus (MFG)	0 / 0	0 / 0	0 / 24.31	78.06 / 64.17
Precentral gyrus (PrCG)	0 / 0	0 / 0	7.19 / 121.34	285.45 / 531.02
Postcentral gyrus (PoCG)	0 / 0	0 / 0	5.23 / 58.59	102.08 / 208.89
Thalamus (TH)	0 / 0	0 / 0	0 / 0	13.34 / 0
Hippocampus (HC)	0 / 0	0 / 0	0 / 0	5.41 / 0
Insula (INS)	0 / 0	0 / 0	0 / 0	0 / 4.22
Superior temporal gyrus (STG)	0 / 0	0 / 0	0 / 36.03	216.61 / 168.3
Middle temporal gyrus (MTG)	0 / 0	0 / 0	0 / 0	46.3 / 86
Inferior parietal lobulus (IPL)	0 / 32.39	0 / 0	40.88 / 33.72	279.08 / 268.77
Superior parietal lobulus (SPL)	79.97 / 138.17	0 / 0	168.09 / 63.83	515.56 / 317.45
Cerebellar hemisphere (CH)	0 / 0	0 / 0	32.64 / 13.02	292.53 / 262.22
Cerebellar Vermis (CV)	20.72	0 / 0	92.33	237.83
Superior Occipital Gyrus (SOG)	14.67 / 62.7	0 / 0	93.78 / 13.48	388.11 / 358.81
Lateral Occipital Gyrus (LOG)	15.34 / 109.09	0 / 0	50.89 / 66.44	457.73 / 448.66

Methods: ***Animals and Capsaicin Application:*** Female cynomolgus macaques (6-7 yrs, 3-4 kg, n=8) were studied at Maccine's facility (Maccine Pte Ltd, Singapore) in accordance with IACUC guidelines. Animals were sedated with ketamine (10 mg/kg IM) and anesthetized with isoflurane (~1%) during imaging, while vital signs were monitored. Capsaicin (98% pure; Sigma-Aldrich, St. Louis, MO) solution was dissolved 1mg/ml 70% ethanol and 0.1ml solution was topically administered to each animal (1.5×1.5 cm^2 patch, for 25~30 min)[5] at the right forearm. ***Heat BOLD fMRI:*** The thermal stimulation was delivered on capsaicin-treated skin using an MRI-compatible thermal stimulator (Pathway, Ramat Yishai, Israel) that interfaced to a 1.6×1.6 cm^2 heat probe (ATS Thermode). The thermode maintained a steady baseline ambient temperature of 35 ^{o}C at rest, and then heat stimuli were applied at 42 ^{o}C (ON/20 s) with an inter-stimulus interval of (OFF/40 s) for 4 cycles. BOLD fMRI data was collected by a gradient-echo EPI pulse sequence (TR/TE = 3 s /30 ms, in-plane pixel size = 1×1 mm^2, slice thickness = 2 mm, and 24 slices with 0.2 mm slice gap). The heat fMRI was collected at baseline and immediately after capsaicin patch removal. High resolution brain anatomical images (TR/TE = 2 s/5.7 ms, in-plane pixel size= 0.33×0.33 mm^2) of individual animals were also acquired to co-register with a standard monkey brain atlas for further group-level and ROI analyses. ***Data Analysis:*** All data analyses were conducted using FSL software and Matlab programs. A GLM was used for the subject-level analysis, where unbiased univariate linear regression analyses with nuisance regressors accounting motion parameters and mean time-course signals extracted from ventricles and white matter were performed. Group comparisons were done using a fixed-effects paired *t*-test to determine the group mean of the differential effect of capsaicin and vehicle on heat fMRI BOLD signals.



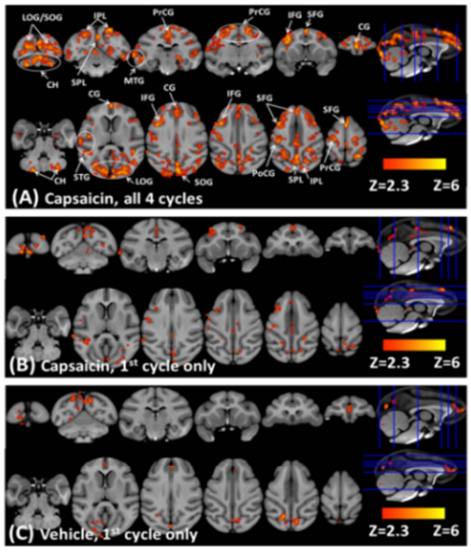


FIG 1 (left) Regional time-course heat/42 ^{o}C fMRI BOLD signals (mean ±SEM) acquired at pre- and post-capsaicin (n=8) or vehicle (n=5) application. **FIG 2** (right) Group comparisons of brain activation patterns showing the effect of capsaicin hypersensitization (n=8) or vehicle (n=5) on heat/42 ^{o}C BOLD fMRI signals (paired *t*-test, pre- vs post-capsaicin, p<0.02): (A) all 4 cycles time-course data analyzed, (B and C) only the first cycle data analyzed; cingulate gyrus (CG), superior and inferior frontal gyrus (SFG and IFG), precentral and postcentral gyrus (PrCG and PoCG), superior and middle temporal gyrus (STG and MTG), lateral and superior occipital gyrus (LOG and SOG), inferior and superior parietal lobules (IPL and SPL), and cerebellar hemisphere (CH).

Results and Discussion: Figure 1 illustrates time-course data obtained from ROIs, showing capsaicin application potentiated BOLD signals under 42 ^{o}C heat challenge. Notably, for certain brain regions the vehicle treatment also produced sizable increases in heat fMRI signals; however, these unexpected changes appear to occur only during the first cycle of heat stimulation. To further investigate this observation, group brain activation patterns were analyzed separately, using the entire time-course data involving all 4 cycles or the data collected during the first cycle only. Figure 2A and Table 1 illustrate group comparisons of brain activation patterns and activated volumes, showing regions with significant increases in BOLD fMRI signals between pre- and post-capsaicin application (paired *t*-test, p<0.02), including frontal, cingulate, precentral and postcentral gyrus, and cerebellum, while these areas are known to be associated with the brain pain matrix in human[1, 2]. The potentiation in the pain matrix ensuing capsaicin administration allows the objective evaluation of experimental pain in this NHP model. From the analyzed first peak data, enhanced BOLD responses were found bilaterally in cingulate cortex and occipital gyrus after vehicle treatment (Figure 2C), while additional, and mostly contralateral, brain regions were highlighted in the capsaicin data (Figure 2B). Brain activation observed from the vehicle first peak data may relate to an early phase response reflecting the autonomic evaluation of afferent aversive stimuli and being representative of thermal sensation rather than pain responses[4].

Conclusion: Our results might provide insights into differentiating brain regions involved with pain response or thermal sensation.

References: [1] Tracey I (2008) Imaging pain. Br J Anaesth 101: 32-39. [2] Borsook D, et al. (2006) Nat Rev Drug Discov 5: 411-424. [3] Upadhyay J et al. (2011) Neuropsychopharmcology 36: 2659-2673. [4] Moulton EA, et al. (2012) J Neurosci 32: 6024-6031. [5] Mohr C, et al. (2008). Pain 139: 416-430.