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PHARMACOLOGICAL FUNCTIONAL MRI ASSESSMENT OF THE EFFECT OF IBUPROFEN-ARGININE IN PAINFUL CONDITIONS

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Pharmacological functional magnetic resonance imaging (phMRI) is a valuable tool for the investigation of pharmacological effects of a drug on pain processing. We hypothesized that the ibuprofen-arginine combination, in line with its characteristic analgesic properties, may influence the phMRI response at the central level, as compared to placebo. Ten healthy subjects underwent a double-blind, placebo-controlled, randomized, cross-over phFMRI study with somatosensory painful stimulation of the right median nerve. We measured the blood oxygen level dependent (BOLD) signal variations induced in conditions of pain after oral administration of either ibuprofen-arginine or placebo formulations. Independent component analysis (ICA) was used for the analysis of the fMRI data, without assuming a specific hemodynamic response function (HRF), which may be altered by drug administration. Median nerve electrical painful stimulation mainly activated the primary contralateral and the secondary somatosensory cortices, the insula, the supplementary motor area, and the middle frontal gyrus. Placebo and ibuprofen-arginine administration induced activation bilaterally in the premotor cortex, and an overall reduction in the other pain-related areas, which was more prominent in the left hemisphere. A task-related increase of BOLD signal between drug and placebo was observed bilaterally in the primary somatosensory area and the middle frontal gyrus without any changes in subjective pain scores. Overall, our findings show that ibuprofen-arginine, in line with the characteristic analgesic properties of ibuprofen, influences the BOLD response in specific pain-related brain areas with respect to placebo, with a vasoactive effect possibly due to arginine.

Pharmacological functional magnetic resonance imaging (phFMRI) is a functional neuroimaging modality that combines the administration of a given dose of a drug with the imaging of brain activity through the blood oxygen level dependent (BOLD) technique. phFMRI permits to investigate *in vivo* and non-invasively the direct effects of the drug on hemodynamic response according to an on/off paradigm, or the drug-induced modulation on specific functional networks activated by physiologically controlled stimuli (1). Neuronal activation, as a consequence of stimulation, locally

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induces a dynamical change in the oxyhemoglobine/deoxyhemoglobine ratio, thus varying the intensity of the measured BOLD magnetic resonance signal. Although neuronal activation and BOLD response are tightly linked, they can be differently influenced by drugs, which can act either as mediators or as modulators of cerebral activity (2).

Several phFMRI studies have investigated the functional modifications of the brain induced by the administration of central analgesic drugs (e.g. morphine and ramifentalin) on pain-related neuro-vascular activation maps, which may reflect specific neuro-anatomical patterns and spatial distribution of opioid receptors (1, 3). In contrast, a limited number of studies have focused on the analgesic effects on the human brain after the administration of non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs possess anti-inflammatory, antipyretic, analgesic properties by selectively or non-selectively inhibiting cyclooxygenase (COX). COX has two isoforms: COX-1, which is constitutively expressed in most tissues, and COX-2, which is constitutively expressed in the brain and is usually induced by inflammatory stimuli (4). Few phFMRI studies have reported the NSAIDs effect on regional cerebral blood volume (CBV) and regional cerebral blood flow (CBF). Using anesthetized rats, Lawrence showed the effect of indomethacin, a non-selective inhibitor of COX, on changes in cerebral oxidative metabolism and CBF during sensorimotor activation (5). Stefanovic also demonstrated that the administration of meloxicam (COX-2) produced a variation of the BOLD response in anesthetized rats subjected to somatosensory stimulation (6). These results have been recently confirmed in humans (7- 9) using an experimental pain model. The complexity of the physiological mechanisms linked to the neuro-vascular coupling from which the BOLD signal originates reflects the multiplicity of biochemical mediators involved in the functional regional hyperemia (10), such as potassium (K^+) and hydrogen (H^+) ions, prostaglandins, epoxyeicosatrienoic acid and nitric oxide (NO). In phFMRI studies based on BOLD imaging technique, it is important to consider that the administration of some substance contained in the drugs can directly act on the BOLD signal by the release of NO, an endogenous messenger involved in the regulation of the basal tone of cerebral vessels (11, 6). This mechanism is supported by the results of a number of studies, which documented an increase of the NO production in concomitance with synaptic activation, and suggested the important role of NO in modulating the hemodynamic response (12).

The combination ibuprofen and arginine is widely utilized for the treatment of chronic, but especially acute pain, because of its analgesic property and fast onset (13). Ibuprofen is a non-steroidal anti-inflammatory drug that has anti-inflammatory, antipyretic and analgesic effects (4). L-arginine is the biosynthetic precursor of NO. It can be synthesized from L-arginine by nitric oxide synthase (NOS), an enzyme that is present in the brain in its neuronal isoform (nNOS) (14). Ibuprofen has a basic analgesic action; the association with L-arginine preserves the properties of the active principle and increases its solubility and absorption rate (15). The pharmacological characteristics of the ibuprofen-arginine combination, along with its potential neuro-vascular effect, are in line with experimental evidence that the administration of this drug can strongly influence the BOLD signal. Nonetheless, it is still unclear whether this effect can be detected globally in the CNS, instead of a direct selectivity for specific pain areas. To address this issue, we conducted a double-blind, placebo-controlled, randomized, cross-over study. Healthy volunteers underwent measured administration of ibuprofen-arginine against somato-sensory painful stimulation. In this study, we evaluated whether the treatment with ibuprofen-arginine could induce BOLD signal variations in a state of pain, and whether the topography of such variations corresponds to specific pain activation patterns. We also investigated the extent and the direction of such modifications in comparison to placebo.

MATERIALS AND METHODS

Volunteers were recruited and carefully screened based on counter-indication to NSAID treatments and previous CNS diseases. We selected 10 healthy male subjects as determined by medical history, physical examination and vital signs (blood pressure and heart rate), age range 18-45 years, and right handedness according to the Edinburgh Inventory (16). All subjects signed a written

informed consent, which was approved by both the Local and National Ethics Committees.

The participants underwent two separate experimental sessions at a one week interval, considered a wash-out period sufficient to avoid any pharmacological carry-over effect. In each session the participant was subjected to somatosensory electrical stimulation of the right median nerve reaching the pain threshold level. The experimental session was composed of two runs, performed just before and 30 minutes after the administration of the drug (or the placebo). After the end of each experimental session, the subject was kept under observation for 2 hours, in order to complete the safety procedures and to check for adverse events.

The drug used in this study consisted of an oral formulation containing ibuprofen and L-arginine (Spidifen®, Zambon Spa, Italy). The drug was supplied in sachets, with 3 g of powder, each containing 400 mg of ibuprofen, 370 mg of L-arginine and excipients for the remaining part. The placebo was supplied in sachets containing 3 g of powder with the same excipients, but with an additional quantity of sucrose instead of ibuprofen and L-arginine. Each subject received a total single dose of 800 mg (2 sachets of 400 mg each), dissolved in 150 ml of non-sparkling mineral water and administered orally. The dose in a single administration was selected in accordance with previous studies on artificially-induced pain (17-18). All volunteers underwent electrical stimulation of the median nerve on the right wrist, with an electrical stimulus consisting of a rectangular pulse of 1.9 Hz frequency and 400 μs duration, delivered via non-magnetic AgCl electrodes (19-20). Pain intensity was individually scored using an 11-point scale, ranging from 0 (no sensation) to 10 (unbearable pain), where a score of 2 corresponded to the motor threshold level (painless thumb muscle twitch), 5 to the pain threshold and 7 to strong pain. All volunteers underwent somatosensory stimulation at the strong pain level (score 7).

Each phFMRI acquisition run followed a block design, with alternating stimulation and rest periods. For each run, 81 volumes were acquired in blocks of 9 volumes (with 5 rest and 4 stimulation blocks) (19). BOLD functional imaging was performed with a Siemens Magnetom Vision scanner at 1.5 T by means of T2*-weighted echo planar imaging (EPI) free induction decay (FID) sequences with the following parameters: TE 60 ms, matrix size 64 × 64, FOV 256 mm, in-plane voxel size 4 × 4 mm, flip angle 90°, slice thickness 4 mm and no gap. A standard head coil was used and the subject's head was fixed by foam pads to reduce involuntary head movements. Functional volumes consisted of 22 axial slices parallel to the AC-PC line including the cortical and sub-cortical areas, acquired with a volume TR of 3000 ms. Subsequently, a high resolution structural volume was acquired via a 3D MPRAGE sequence (sagittal, matrix 256 × 256, FOV 256 mm, slice thickness 1 mm, no gap, in-plane voxel size 1 mm × 1 mm, flip angle 12°, TR = 9.7 ms, and TE = 4 ms) in order to provide an anatomical reference for the functional scans.

Brain Voyager QX 1.9 (Brain Innovation, Maastricht, Netherlands) was used for BOLD data preprocessing and analysis. Functional image time-series were first corrected for the differences in slice acquisition times, detrended, realigned with T1-volumes, corrected for motion, and transformed into the standard Talairach anatomical space. Spatial ICA was used for the assessment of brain activations in the different experimental conditions without relying on a predefined model of hemodynamic response, necessary for classical general linear model (GLM) analysis (21). ICA is a data-driven method which is able to retrieve independent features from their linear mixtures, with no prior knowledge about their activity waveforms or locations (22). Each BOLD dataset was broken down into independent spatial-temporal patterns of brain activity by means of the deflation approach of the FastICA algorithm (23). For each dataset, we selected the IC with the largest average correlation with the predictor obtained convolving the boxcar function with a canonical hemodynamic response function (HRF) (24). Although we used the HRF-convolved predictor in our analysis, we could obtain spatial maps of activation/deactivation related to a particular treatment (analgesic or placebo), without assuming a predefined HRF. The IC maps from data acquired before any treatment in two separate sessions were averaged to obtain a single-subject IC map. Next, for the data before treatment and after either placebo or ibuprofen-arginine administration, group-level activation maps were created by combining results across subjects by using a random effect analysis on the single-subject spatial maps ($p < 0.01$ corrected with false discovery rate, FDR). A peak-finding algorithm was used on the activation maps to detect the peak voxel of each activated area, which was subsequently classified using anatomical information from the Talairach atlas. The cluster-size, expressed in mm^3, was calculated on the basis of the number of voxels adjacent to the peak voxel and above the selected threshold. We used the cluster-size and the peak t-score in each activated area to allow for a quantitative comparison between experimental conditions. Additionally, a drug *vs* placebo contrast was computed by a paired t-test between the activation maps obtained after ibuprofen-arginine and placebo administration. The resulting spatial map was thresholded, in order to delineate brain areas with significant differences between treatments ($p<0.05$ FDR-corrected).

Table I. *List of brain areas for which activity is significantly related to painful somatosensory stimulation ($p < 0.01$).*

Anatomical regions	*Placebo*			*Ibuprofen-arginine*		
	t-score	*voxels*	*coordinates*	*t-score*	*voxels*	*coordinates*
Superior temporal gyrus (R)	4.3	459	(53,-43,6)	4.3	318	(62,-43,12)
Superior temporal gyrus (L)	4.3	8	(-55,46,1)	4.3	240	(-37,-11,-1)
Middle temporal gyrus (R)	4.3	228	(50,-53,-2)	4.3	7	(36,-43,0)
Superior frontal gyrus (R)	4.3	613	(18,-7,69)	4.3	1173	(31,-6,61)
Superior frontal gyrus (L)	4.3	92	(-27,12,66)	4.3	24	(-16,-2,66)
Middle frontal gyrus (R)	4.3	3744	(39,31,24)	4.3	5669	(48,28,30)
Middle frontal gyrus (L)	4.3	86	(-43,35,32)	4.3	324	(-38,37,24)
Inferior frontal gyrus (R)	4.3	5810	(45,7,17)	4.3	414	(50,4,8)
Inferior frontal gyrus (L)	4.3	1547	(-48,3,10)	4.3	9	(-47,6,5)
Superior paretal lobe (R)	4.3	3274	(39,-48,50)			
Inferior paretal lobe (R)	4.3	335	(52,-46,39)	4.3	3969	(56,-36,36)
Inferior paretal lobe (L)				4.3	15	(-50,-37,33)
Anterior insula (R)	4.3	121	(33,15,10)	4.3	361	(32,11,17)
Anterior insula (L)	4.3	143	(-29,7,15)	4.3	79	(-29,13,16)
Medial insula (L)	4.3	124	(-39,-2,4)	4.3	109	(-40,-5,15)
Posterior insula (R)	4.3	120	(38,0,-2)			
Posterior insula (L)	4.3	121	(-42,0,15)	4.3	151	(-39,-21,15)
Anterior cinguate (R)	4.3	188	(2,2,51)	4.3	108	(7,21,33)
Anterior cinguate (L)	4.3	49	(-1,-29,45)			
Posterior cingulate (R)	4.3	233	(2,-39,44)			
Posterior cingulate (L)	4.3	6	(-14,22,20)			
Precentral gyrus (L)				4.3	1586	(-28,-27,62)
Postcentral gyrus (L)	4.3	146	(-51,33,43)		63	(-48,-33,45)
SII (R)				4.3	12	(63,-21,19)
SII (L)				4.3	146	(-51,-16,18)

The results referring to ibuprofen-arginine and placebo administrations are directly compared in the table. For each brain area the number of voxels, and the Talairach coordinates are shown.

Furthermore, we analyzed the activations revealed by ICA specifically in pain-related areas. To this end, we extracted for each subject and each pharmacological condition, the average z-score in selected regions of interest (ROIs), assumed to be related to local response intensity. One-way analysis of variance (ANOVA) was carried out to detect significant differences across conditions ($p<0.05$). Additionally, we performed post-hoc contrasts between conditions by means of a paired t-test ($p<0.001$).

RESULTS

Ten subjects completed both sessions in the study. No subjects showed side effects linked to the procedure or to the drugs. Pain intensity perception was individually scored without any changes, either after placebo or ibuprofen-arginine administration. In the data collected before any treatment, we found activations induced by painful somatosensory

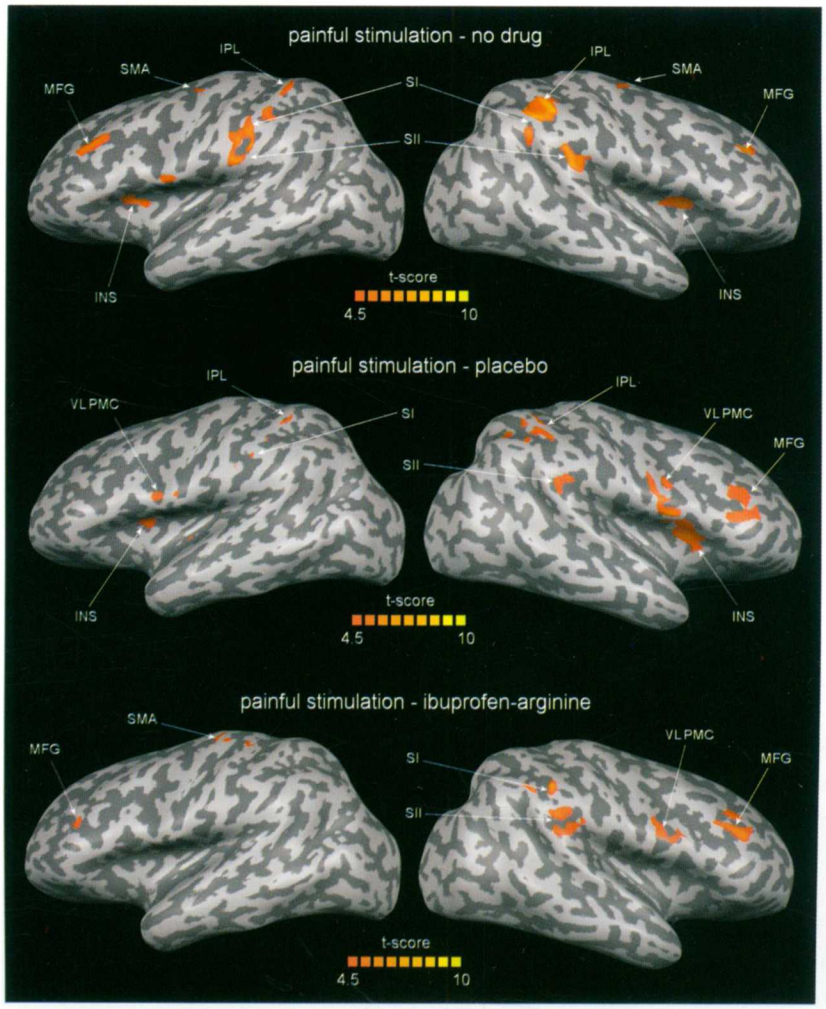

Fig. 1. *Activation maps during pain stimulation with no treatment (**a**), and with placebo (**b**) and ibuprofen-arginine treatments (**c**) ($p < 0.01$, FDR-corrected). IPL: inferior parietal lobule; MFG: middle frontal gyrus; SI: primary somatosensory area; SII: secondary somatosensory area; INS: insula; SMA: supplementary motor area; VLPMC: ventrolateral premotor cortex*

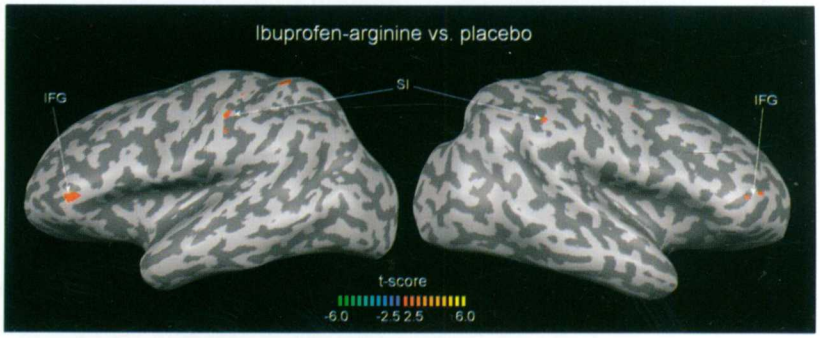

Fig. 2. *Contrast map between pain-related activations with ibuprofen-arginine and placebo. The main areas for which the brain activity with the drug was larger and smaller than that with the placebo ($p < 0.05$, FDR-corrected) are coloured in yellow-orange and green-blue, respectively. IFG: inferior frontal gyrus; SI: primary somatosensory area*

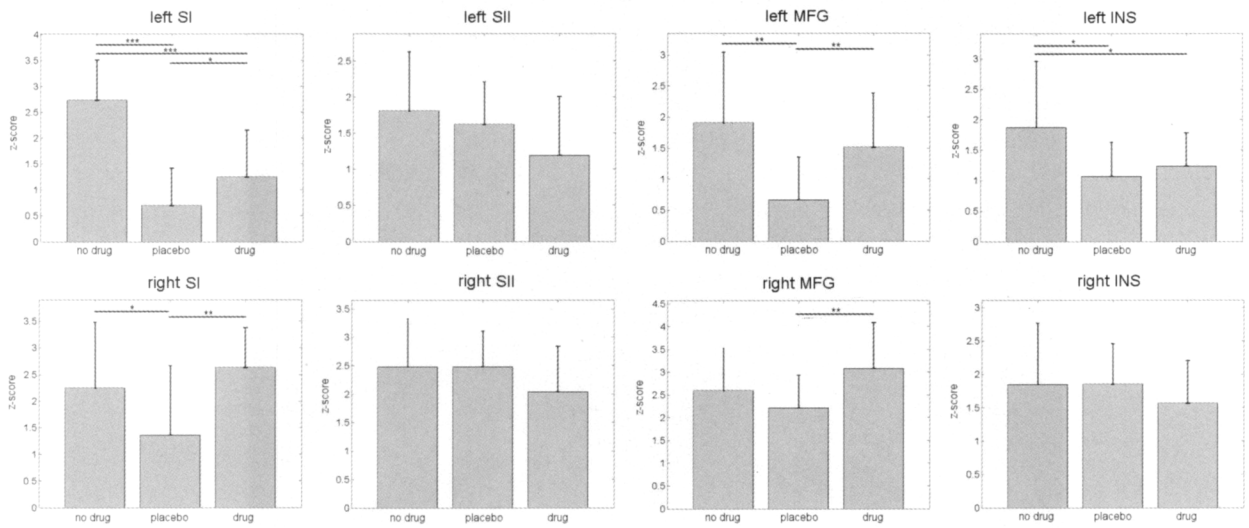

Fig. 3. *Bar plots showing the average and the standard deviation of the activation intensity in the pain-matrix areas with no drug, with placebo and with ibuprofen-arginine, respectively. Statistical differences between conditions are indicated with stars: *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$. SI: primary somatosensory area; SII: secondary somatosensory area; IFG: inferior frontal gyrus; INS: insula*

stimulation mainly in the primary contralateral and the secondary somatosensory cortices, the insula, the supplementary motor area, and the middle frontal gyrus (Fig. 1). Next, we assessed the activations after the administration of ibuprofen-arginine and placebo, respectively (Table I). Placebo and drug maps (Fig. 1) showed additional activations bilaterally in the ventrolateral premotor cortex (VLPMC), and an overall reduction in the other pain-related areas, which was more prominent in the left hemisphere.

When comparing the imaging results obtained in terms of pain-related activations, we observed a set of brain areas that showed a larger activation for the tested drug in respect to the placebo (Fig. 2). These are mainly bilateral, in the primary somatosensory area and the inferior frontal gyrus (Fig. 2). A task-related reduction of BOLD signal between the tested drug and placebo could be observed in the insular cortex (Figs. 1-2).

The statistical analysis on the activations in selected pain-related areas confirmed a general reduction after placebo or drug treatment (Fig. 3). The differences across conditions (i.e., no drug, placebo and ibuprofen-arginine administration), assessed by ANOVA, were significant for the left primary somatosensory area ($F(2,27)=15.01$, $p < 0.001$), the right primary somatosensory area ($F(2,27)=8.10$, $p = 0.002$), and the left middle frontal gyrus ($F(2,27)=16.06$, $p < 0.001$). Conversely, our data did not show significant differences for the secondary somatosensory area and the insula, in which a minor reduction in brain activity was reported with both placebo and drug administrations. We also observed an increase in the activations between ibuprofen-arginine and placebo in the primary somatosensory area and the middle frontal gyrus, in line with the imaging results reported in Fig. 2.

DISCUSSION

phFMRI is an accurate measure of modifications induced in the brain by drug administration (2). In pain investigation, phFMRI in humans provides a measure of the efficacy of specific formulations with analgesic effect, and relies on an objective measure, instead of subjective pain scores. In our study the subjective pain perception, detected by subjective perception scale, was no different for placebo and drug, whereas we could report significantly different activity in specific pain-related areas.

Previous neuroimaging studies applied to pharmacology showed that drugs can produce effects at the neuronal level (25), and hemodynamically (1). Moreover, it has been demonstrated that these

two response types can be uncoupled in particular conditions, for example when a drug interferes with NO availability, inducing rCBV, rCBF and BOLD modifications (26). The modification of the hemodynamic response in case of drug administration is a factor to be considered both in the experimental design of phFMRI studies and in the subsequent data analysis. In our study based on an acute pain model (19, 27), we performed BOLD measurements with a randomized and double-blind administration of ibuprofen-arginine and placebo. The comparison with placebo was chosen for its relevance in phFMRI studies, due to its well-known psychological effects (28). The placebo is a chemically-inert formulation, which can induce therapeutic effects in a non-pharmacological way, utilizing the psychological suggestion of an expected clinical benefit. Although the neurobiology of the placebo effect on central processes is not completely clear, a large fraction of its analgesic effect seems to be related to an increase of the endogenous opioid response to painful stimuli (29).

Due to the problem of neurovascular uncoupling, and the possible alterations in the hemodynamic response induced by the drug administration (2), specific data analysis approaches need to be developed for a reliable detection of task-related activation. Instead of a classical GLM analysis, we used a data-driven approach based on ICA for evaluating the effect of the drug on the measured BOLD response. ICA is a technique able to separate independent spatio-temporal patterns of synchronized neural activity, without prior knowledge about their activity waveforms or locations (21). The use of ICA ensured the comparability of the results of the drug and placebo, as the neurophysiological substrate may be different between the two treatments.

The pain matrix results from the complex interactions of numerous and distinct brain regions which contribute to pain processing. Different aspects of pain processing, as classically reported in neuroimaging studies, can be related to the activated brain areas: an affective-motivational component that is ascribed to parietal and insular regions, a cognitive-evaluation aspect that involves fronto-parietal areas, a motor control component that involves motor, premotor and supplementary motor areas, a sensory-discriminative aspect that involved the primary and secondary somatosensory areas and insula regions (30-31). SI is activated in roughly half of the studies, while insula was consistently activated in almost studies of experimentally-induced pain. Insula acts as a relay station for sensory information while SI encodes spatial information of nociceptive stimuli. Several imaging studies (31) have reported a sensory-motor coactivation during painful electro-stimulation. In our double-blind study, the placebo is supposed to induce emotional and affective cerebral processes related to pain perception, while the drug is supposed to additionally induce a pharmacological effect. With the exception of BOLD signal increase in the VLPMC, we observed a reduction in pain-related activation for both the placebo and the tested drug, with specific differences in the primary somatosensory area and the inferior frontal gyrus, where the drug-related effect could be directly detected.

In the phFMRI literature, few studies have reported the NSAIDs effect on regional cerebral blood volume (CBV) and regional cerebral blood flow (CBF) (2). Although these studies showed a drug-induced reduction of the BOLD signal in pain-related areas, our results showed an increase consistent with the vasoactive effect in the brain of L-arginine. Since NO is mainly involved in the vasodilation effect in the BOLD response (26), the larger NO bio-availability produced by L-arginine administration can induce a greater efficiency of the micro-vascular response in the stimulus-related areas. This effect was confirmed by studies conducted on animals: Morikawa reported that the administration of L-arginine in rats determines an increase of regional CBF in the normal brain, as well as in a marginally perfused brain region distal to middle cerebral artery occlusion (32); Kobari demonstrated that the administration of a NOS inhibitor in cats determines a reduction of CBF and CBV (11), suggesting that NO participates in both the regulation of cerebral micro-vessel basal tone and the CBF autoregulation. Comparing this experimental evidence with the results of the present study, it is possible to hypothesize that ibuprofen-arginine combines the analgesic properties of ibuprofen with a vasoactive effect potentially due to arginine.

It is worth mentioning a number of potential

limitations in our study. First, given that the subjects were informed about the painful stimulation, it is possible that the anticipation of a strong painful stimulus may alter the activation pattern. However, the effect of pain anticipation would be transient, whereas we investigated the task-related brain activity during the entire experiment. As a second point, ICA processing on phfMRI data might partially reduce inter-subject variability in pain-related activation, as random-effect analysis is performed on activation maps in z-scores. Finally, the specificity of our results is limited by the characteristics of the instruments. From this standpoint, the use of a high-field scanner as 3 or more Tesla, instead of 1.5 Tesla, might improve the resolution of the fMRI data, and hence the accuracy of the activation maps.

In conclusion, the present phfMRI study shows that ibuprofen-arginine, in line with its characteristic analgesic properties in the microvascular environment, influences BOLD response in specific pain-related brain areas in respect to placebo with a side vasoactive effect of arginine. More generally, this study demonstrates that phFMRI may represent a valuable objective tool in the study of both analgesic drug mechanism of action and the functional brain networks it might interact with.

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