BRAIN NOCICEPTIVE IMAGING IN RATS USING ^{18}F-FLUORODEOXYGLUCOSE SMALL-ANIMAL POSITRON EMISSION TOMOGRAPHY

Y.-Y. I. SHIH,[a] Y.-C. CHIANG,[a,b1] J.-C. CHEN,[c,d]
C.-H. HUANG,[a,b] Y.-Y. CHEN,[e] R.-S. LIU,[f] C. CHANG[a*]
AND F.-S. JAW[b*]

[a]*Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, Republic of China*

[b]*Institute of Biomedical Engineering, National Taiwan University, Taipei, Taiwan, Republic of China*

[c]*Department of Biomedical Imaging and Radiological Sciences, National Yang-Ming University, Taipei, Taiwan, Republic of China*

[d]*Department of Education and Research, Taipei City Hospital, Taipei, Taiwan, Republic of China*

[e]*Department of Electrical and Control Engineering, National Chiao-Tung University, Hsinchu, Taiwan, Republic of China*

[f]*School of Medicine, National Yang-Ming University, Taipei, Taiwan, Republic of China*

Abstract—Preclinical exploration of pain processing in the brain as well as evaluating pain-relief drugs in small animals embodies the potential biophysical effects in humans. However, it is difficult to measure nociception-related cerebral metabolic changes *in vivo*, especially in unanesthetized animals. The present study used ^{18}F-fluorodeoxyglucose small-animal positron emission tomography to produce cerebral metabolic maps associated with formalin-induced nociception. Anesthesia was not applied during the uptake period so as to reduce possible confounding effects on pain processing in the brain. The formalin stimulation at the hind paw of rats resulted in significant metabolic increases in the bilateral cingulate cortex, motor cortex, primary somatosensory cortex, secondary somatosensory cortex, insular cortex, visual cortex, caudate putamen, hippocampus, periaqueductal gray, amygdala, thalamus, and hypothalamus. Among the measured areas, clear lateralization was only evident in the primary somatosensory cortex and hypothalamus. In addition, pretreatment with lidocaine (4 mg/kg, i.v.) and morphine (10 mg/kg, i.v.) significantly suppressed formalin-induced cerebral metabolic increases in these areas. The present protocol allowed identification of the brain areas involved in pain processing, and should be useful in further evalu-ations of the effects of new drugs and preclinical therapies for pain. © 2008 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: PET, pain, morphine, lidocaine, rat.

Novel pain-relief drugs and therapeutic techniques have been developed in recent years to alleviate human suffering from pain. However, the mechanisms and circuits underlying pain processing are extremely complex, involving not only sensory responses to noxious stimuli, but also cognitive and emotional factors (McMahon and Koltzenburg, 2005). This makes it difficult to conclusively identify the brain areas that specifically process nociceptive stimuli. Imaging approaches such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) allow functional mapping of the intact brain and measurement of the responses in multiple areas simultaneously, thus bringing the study of pain into a deep level (Phelps et al., 1979; Ogawa et al., 1990a,b; Phelps, 2000).

Preclinical verification for evaluating the efficacy of pain-alleviation strategies is usually essential. Of the numerous pain-testing models in animals, formalin test is one of the most commonly used techniques for generating nociception since it evokes inflammatory pain responses without influencing other sensory modalities; furthermore, the associated behavioral responses have been well investigated (Tjolsen et al., 1992). Our recent animal fMRI studies have shown that formalin stimulation of the rat hind paw significantly activates the cingulate cortex (Cg), motor cortex (M), primary somatosensory cortex (S1), secondary somatosensory cortex (S2), insular cortex (IC), visual cortex (VC), caudate putamen (CPu), hippocampus (HIP), periaqueductal gray (PAG), thalamus (Th), and hypothalamus (HT) (Shih et al., in press, 2008b). However, it is usually essential to apply anesthesia during fMRI experiments in order to sedate the animal and reduce motion artifacts. This tackles a very difficult technical obstacle when imaging the representation of pain in anesthetized animals. Small-animal positron emission tomography (microPET) might be more suitable for imaging brain activation in the conscious animals (Schiffer et al., 2007; Ohashi et al., 2008). An animal can react to stimuli outside the scanner without stresses during the uptake of radionuclide, and then be lightly anesthetized for imaging the accumulated responses, thus minimizing possible confounding variables that could influence central nociceptive processing.

The present study aimed to elucidate formalin-induced nociceptive responses by using ^{18}F-fluorodeoxyglucose (^{18}F-FDG) microPET with the aid of a maximum a posteriori (MAP) reconstruction algorithm to further improve the spatial resolution (Qi et al., 1998). In addition, MRI and a rat brain atlas were used to anatomically align the rat brain (Paxinos and Watson, 1998; Shih et al., 2007). The new information provided by the current study is that nociception-induced metabolic maps can be imaged in the conscious rat brain, in contrast to previous studies only revealing indirect hemodynamic responses during anesthesia (Morrow et al., 1998; Tuor et al., 2000; Shah et al., 2005; Shih et al., in press, 2008b). The effects of pain-relief drugs such as morphine and lidocaine were also examined. This pain-measuring protocol can be used to evaluate the effectiveness of new pain-relief drugs and therapeutic techniques at the preclinical stage.

EXPERIMENTAL PROCEDURES

Subjects

Nineteen adult male Wistar rats (8–10 weeks old; weighing approximately 250–300 g; National Laboratory Animal Center, Taipai, Taiwan, Republic of China) were used in the present study. The animals were housed in a well-controlled environment with a 12-h light/dark cycle and constant humidity and temperature. Rats were housed in plastic cages at three animals per cage with free access to food and water. All experimental procedures were approved by the Institutional Animal Care and Use Committee, National Taiwan University, College of Medicine. All experiments conformed to international guidelines on the ethical use of animals. All efforts were made to minimize the number of animals used and their suffering.

Imaging experiments

Seven rats were used to produce the formalin-induced nociceptive maps. The ^{18}F-FDG was used as a radiotracer to reveal brain glucose metabolism. Each rat was initially lightly anesthetized using ether, and 0.5 ml of ^{18}F-FDG with an activity of 1.0–1.2 mCi was administered i.v. via the tail lateral vein, after which the rat was returned to its cage in a quiet environment for 45 min uptake in the conscious state. Following the uptake, the rat was lightly anesthetized using 1.5% isoflurane and fixed in a custom-built stereotaxic head holder by two ear bars and an incisor fixer so as to minimize motion artifacts (Shih et al., 2007). The body temperature was maintained using a warming lamp whose light field was restricted to avoid additional visual stimulation. MicroPET imaging

(R4, Concorde Microsystems/Siemens, Knoxville, TN, USA) was performed for 30 min, with the images reconstructed using the MAP algorithm (Qi et al., 1998). After 1 week, 50 μl of 5% formalin was injected into the left hind paw prior to ^{18}F-FDG injection. The imaging procedures were identical to those described above.

Another two groups containing six rats each were used to evaluate the effects of lidocaine and morphine. The drugs were given prior to the formalin stimulation, followed by an identical imaging protocol. Rats were i.v. injected with 4 mg/kg lidocaine in one group and 10 mg/kg morphine in the other group.

MRI anatomical images were captured using a 4.7-T Biospec 47/40 spectrometer to define the brain margin. A 72-mm volume coil was used as the RF transmitter, and a 2-cm quadrature surface coil placed on the head was used as the receiver. A T_2-weighted scout image was taken in the mid-sagittal plane to localize the anatomical position by identifying the anterior commissure (bregma -0.8 mm). T_2-weighted template images were then acquired using RARE sequence with a repetition time of 4000 ms, echo time of 80 ms, field of view of 2.56 cm, slice thickness of 1.2 mm, number of excitation of 2, and an acquisition matrix of 256×128 (zero-filled to 256×256).

Data analysis

Images were analyzed using PMOD (PMOD Technologies, Adliswil, Switzerland) and a custom-built ISPMER system (Shih et al., 2007). MicroPET images were initially coregistered among the subjects using a mutual-information algorithm and then averaged to generate the incidence images. A pixel value in incidence images represents the averaged percentage injected dose per gram (%ID/g) of an experimental group, where a higher pixel value indicates a greater number of rat responses consistent with the given task.

The statistical analysis was based on the %ID/g values sampled from different brain structures of each rat. Repeated-measures ANOVAs were used to examine whether formalin stimulation induced metabolic changes in the corresponding brain regions in both hemispheres, with the significance level set at $P<0.05$. Factorial ANOVAs were used to assess differences in ^{18}F-FDG uptake among the groups with formalin stimulation alone, formalin stimulation with lidocaine pretreatment, and formalin stimulation with morphine pretreatment, with $P<0.05$ again considered to be significant. Fisher's post hoc tests were used to assess differences between groups.

RESULTS

Formalin-induced nociceptive maps

The present study used ^{18}F-FDG microPET to elucidate the nociception-induced glucose metabolic changes in the brains of conscious rats. In order to further improve the
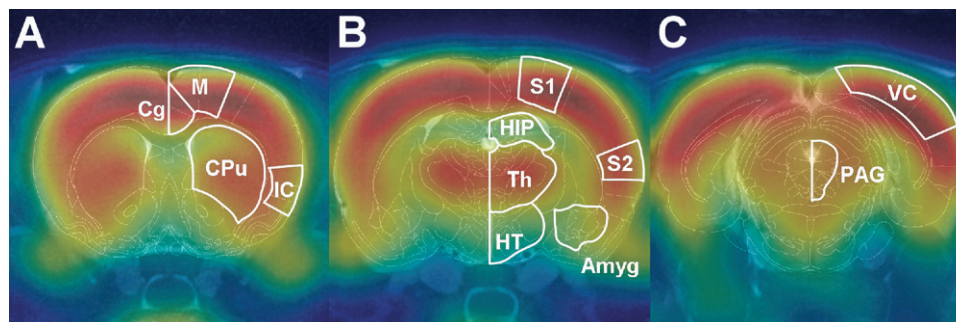
Fig. 1. Fusion of the rat brain atlas, ^{18}F-FDG microPET images, and T_2-weighted MRI images. These images provide anatomical alignment and spatial references for selecting regions of interest. The three images are at bregma $+0.7$ mm (A), bregma -1.8 mm (B), and bregma -7.8 mm (C).
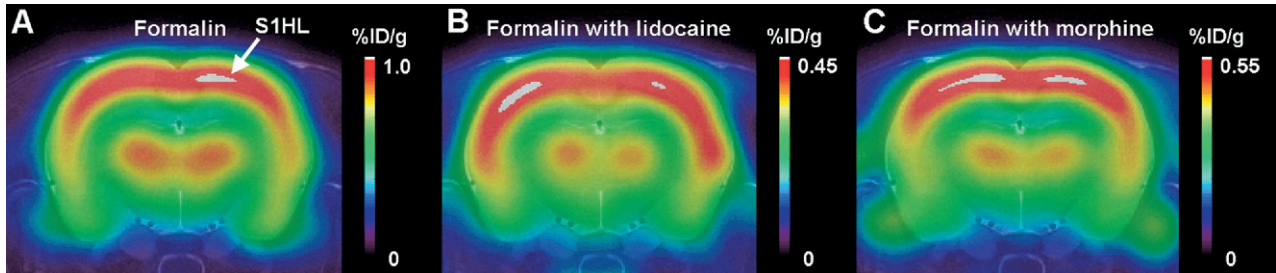
Fig. 2. Incidence ^{18}F-FDG microPET maps overlaid on the MRI images showing the cerebral metabolic changes in three groups of rats, quantified as the averaged %ID/g. (A) Averaged response of seven rats subjected to left hind-paw formalin stimulation alone. (B) Averaged response of six rats subjected to left hind-paw formalin stimulation with lidocaine pretreatment. (C) Averaged response of six rats subjected to left hind-paw formalin stimulation with morphine pretreatment. Clear lateralization is only evident in (A), in which the ^{18}F-FDG uptake is highest in the contralateral S1 and the responsive region match with that of the hind-limb region defined by the rat brain atlas. The image position was 1.8 mm posterior to the bregma.

accuracy with which anatomical locations were determined, microPET and MRI images were coregistered and fused with a digital atlas of the rat brain (Paxinos and Watson, 1998). This method allowed regions of interest to be selected based on clear spatial references (Fig. 1). Averaged formalin-induced metabolic maps overlaid on the MRI images are shown in Fig. 2A. The averaged ^{18}F-FDG uptake in the hind-limb area of the S1 (S1HL) was higher on the contralateral side than on the ipsilateral side. Statistical comparisons of the ^{18}F-FDG uptake in the control and formalin groups are shown in Fig. 3. Repeated-measures ANOVAs with Fisher's post hoc tests indicated the presence of significant activations in the bilateral Cg, M, S1, S2, IC, VC, CPu, HIP, PAG, amygdala (Amyg), Th, and HT, whereas no changes were evident in muscle tissue (Mu). In addition, clear lateralization was only observed in S1 and HT (Fig. 3).

Antinociceptive effects of lidocaine and morphine

The effects of antinociceptive drugs are shown in Fig. 2B, C, and Fig. 4. These reduced the brain ^{18}F-FDG uptake, with no clear lateralized differences evident in the sensory cortices. Factorial ANOVAs with Fisher's post hoc tests have shown that ^{18}F-FDG uptake in the bilateral Cg, M, S1, S2, IC, VC, CPu, HIP, PAG, Th, Amyg, and HT was lower for pretreatment with lidocaine and morphine than for

formalin stimulation alone. Among the measured areas, ^{18}F-FDG uptake in the CPu was higher for morphine treatment than for lidocaine treatment.

DISCUSSION

The present study clearly revealed formalin-induced nociceptive maps, with strong activations evident in the Cg, M, S1, S2, IC, VC, CPu, HIP, PAG, Amyg, Th, and HT. Although functional imaging techniques have been used previously to examine pain-related responses in rodent brains, such as a blood-flow-based autoradiographic method (Morrow et al., 1998) and fMRI (Tuor et al., 2000; Shah et al., 2005; Shih et al., in press, 2008b), the method employed in the current study provides new information and methodological improvements for preclinical pain research. First, our findings complement the previous pain studies in animals by revealing the relative metabolic changes instead of the responses related to blood flow. Second, the ^{18}F-FDG microPET technique provides an opportunity for *in vivo*, longitudinal follow-up of the response in the same animal, whereas the blood-flow-based autoradiographic method can only be applied once in each animal. Third, fMRI is usually performed under anesthesia, which would alter the representation of behavior-related brain functions and has been reported to strongly influence
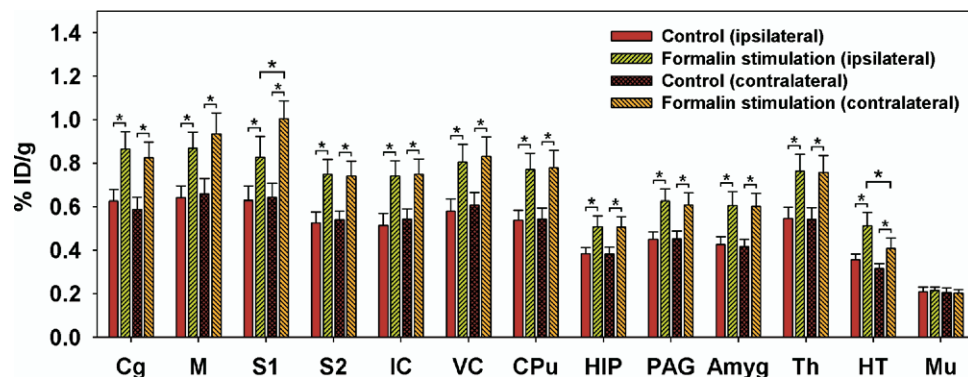
Fig. 3. Formalin-induced metabolic increases and cerebral laterality. Repeated-measures ANOVAs with Fisher's post hoc tests indicated activations in the bilateral Cg, M, S1, S2, IC, VC, CPu, HIP, PAG, Amyg, Th, and HT. Among the measured areas, clear lateralization was evident in S1 and HT, with ^{18}F-FDG uptake being higher in the contralateral S1 than on the ipsilateral side, and in the ipsilateral HT than on the contralateral side. * Denotes $P<0.05$. Error bars represent S.E.M. values.
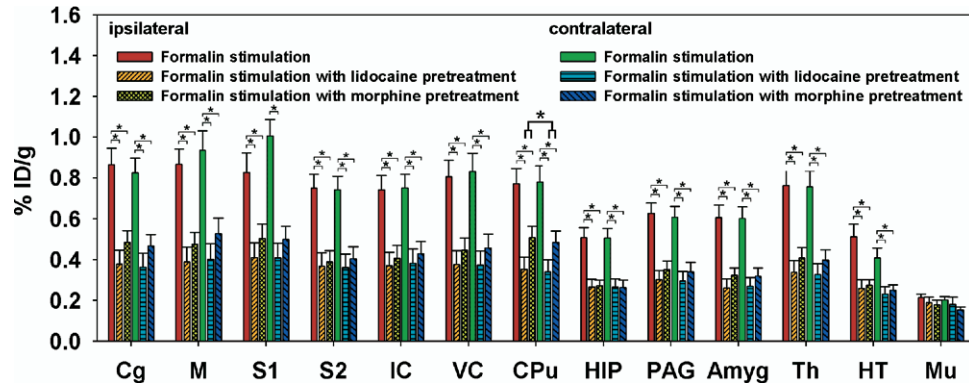
Fig. 4. Effects of lidocaine and morphine pretreatments on formalin-induced metabolic changes. Pretreatment with lidocaine and morphine reduced ^{18}F-FDG uptake by different amounts. Factorial ANOVAs with Fisher's post hoc tests indicated significant decreases in the bilateral Cg, M, S1, S2, IC, VC, CPu, HIP, PAG, Amyg, Th, and HT compared with the groups subjected to formalin stimulation alone. * Denotes $P<0.05$. Error bars represent S.E.M. values.

neuronal activity (Lindauer et al., 1993; Matsumura et al., 2003). Fourth, most fMRI studies in rodents have utilized invasive surgical procedures such as cannulation to control anesthesia, deliver pharmacological compounds, or monitor physiological parameters. Neuronal activation might be affected by the associated surgical pain that would activate the responsive brain region prior to stimulation. In other words, neurovascular coupling around responsive nuclei might already be in a responding state rather than a baseline state due to the surgical wound. These invasive procedures therefore inevitably generate confounding pain reactions. Moreover, if the invasive procedures induce bleeding, the measured hemodynamic responses are very likely to be affected by blood flow changes resulting from the alteration of blood pressure. It is therefore difficult to determine whether the observed activation responses are purely caused by the nociceptive stimulus. The current study avoided these invasive procedures, with the resulting activation responses appearing to be more distinguishable than in previous fMRI studies (Tuor et al., 2000; Shah et al., 2005; Shih et al., in press, 2008b). Lastly, fMRI is often criticized for the difficulty of detecting subcortical responses due to the position of the receiver coil, which usually results in stronger signals from cortical regions than from subcortical regions of the brain. Furthermore, the brain areas influenced by strong susceptibility artifacts such as the Amyg cannot be uniformly measured by fMRI. These disadvantages can be overcome by using microPET, which also shows better homogeneity and fewer artifacts near the air-tissue interface.

Among the measured areas, bilateral activations of the IC and VC as well as S1, S2, Th, and Cg in the spinothalamic tract are consistent with fMRI data (Tuor et al., 2000; Malisza et al., 2003; Shah et al., 2005; Shih et al., in press, 2008b). Although fMRI can consistently detect these activations, clear lateralization is difficult to be observed. Our results indicate that ^{18}F-FDG microPET shows better delineation of the sensory-motor laterality of formalin-induced nociception than fMRI, since the ^{18}F-FDG uptake was significantly higher in the contralateral S1 than on the ipsilateral side (Figs. 2 and 3). A possible explanation of

this feature is the lack of anesthesia, and the use of a glucose analog rather than hemodynamic compensation, which is less sensitive to the blood-pressure-induced nonspecific activation in fMRI (Tuor et al., 2002). It has also been suggested that the CPu and M are activated by painful stimuli (Chudler et al., 1993; Lorenz et al., 2003; Malisza et al., 2003), with bilateral activation in these areas possibly associated with the representation of mirror pain behavior in animals, where licking of the contralateral untreated paw was observed following unilateral formalin stimulation (Aloisi et al., 1993).

In the subcortical regions, the PAG and HT were also activated, both of which are known to be intimately involved in pain modulation. The PAG is considered a major integration site for nociception signals and a center of analgesic action for opiates that influences the activity of the spinal cord dorsal horn through projections from the ventromedial medulla (Mason, 2005). Previous studies have shown that a nociception signal in the HT can simultaneously excite the central autonomic nervous system, and that the HT might play a complex role in antinociceptive reactions (Pinto-Ribeiro et al., 2008). Our findings suggest that the ^{18}F-FDG uptake was higher in the ipsilateral HT than on the contralateral side; however, the underlying mechanism remains obscure. Increased glucose metabolism in the Amyg during activation has been observed in a rat model of neuropathic pain (Mao et al., 1993). The findings of several studies indicate that the Amyg is associated with unconscious memorization of nociceptive stimuli and involved in emotional processes and automatic functions (Bingel et al., 2002; Alkire and Nathan, 2005; Neugebauer, 2007). As far as the activity in HIP is concerned, an immunohistochemical study has shown that a unilateral injection of formalin induced bilateral c-fos expression in the HIP, suggesting that the HIP is linked by numerous reciprocal neural connections and further implicating that this area is involved in the perception of chronic pain (Aloisi et al., 1997).

In addition to formalin-induced nociception, the present study also examined the antinociceptive effects of lidocaine and morphine. Fig. 4 shows that pretreatment with

lidocaine and morphine resulted in different reductions in ^{18}F-FDG uptake in the measured brain areas, indicating that formalin-induced metabolic changes can be inhibited by these two drugs. Moreover, the inhibition by lidocaine might also result from the blocking effect of voltage-gated sodium channels, thus reducing glucose metabolism globally (Abdi et al., 1998; Gold and Thut, 2001; Rykacze-wska-Czerwinska, 2006), whereas morphine has been widely used for pain alleviation (Yaksh et al., 1988; Frank-lin et al., 1990; Lamas et al., 1994; Tuor et al., 2000; Shah et al., 2005) and also reportedly induces additional anesthesia, thus attenuating glucose utilization in widespread brain regions (Cohen et al., 1991; Chudler and Dong, 1995). Although the mechanisms underlying widespread inhibition in the brain are not fully understood, it cannot be ruled out that the down-regulation of global metabolic states is initiated by the brain processing of antinociceptive signals aimed at reducing sensory-motor reactions, and also emotional responses. Furthermore, the differences in the effects of lidocaine and morphine at the used dosages were largest in the CPu, which suggests that this region exhibits higher neuronal activities under morphine regulation.

CONCLUSION

The present study established an ^{18}F-FDG microPET protocol that allowed both serial and longitudinal measurements of nociception-induced metabolic changes in the brains of conscious rats. The formalin-induced nociception maps revealed several brain regions that are possibly involved in various aspects of pain processing. The whole-brain formalin-induced nociceptive responses following lidocaine and morphine pretreatment were also examined. This microPET imaging protocol should be useful in further evaluations of the effects of new pain-relief drugs and in developing preclinical therapeutic strategies for pain.

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