Published in final edited form as:

Anesth Analg. 2009 January ; 108(1): 334–344. doi:10.1213/ane.0b013e31818e0d34.

The Effect of Intravenous Lidocaine on Brain Activation During Non-Noxious and Acute Noxious Stimulation of the Forepaw: A Functional Magnetic Resonance Imaging Study in the Rat

Zhongchi Luo, MS^{*}, Mei Yu, BS[†], S. David Smith, PhD[‡], Mary Kritzer, PhD[§], Congwu Du, PhD^{†,‡}, Yu Ma, PhD[†], Nora D. Volkow, MD^{||}, Peter S. Glass, MD[†], and Helene Benveniste, MD, PhD^{†,‡}

^{*}*Biomedical Engineering, State University of New York at Stony Brook, Stony Brook, New York*

[†]*Anesthesiology, State University of New York at Stony Brook, Stony Brook, New York* [‡]*Department of Medicine, Brookhaven National Laboratory, Upton, New York* [§]*Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, New York* ^{||}*National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland*

Abstract

BACKGROUND—Lidocaine can alleviate acute as well as chronic neuropathic pain at very low plasma concentrations in humans and laboratory animals. The mechanism(s) underlying lidocaine's analgesic effect when administered systemically is poorly understood but clearly not related to interruption of peripheral nerve conduction. Other targets for lidocaine's analgesic action(s) have been suggested, including sodium channels and other receptor sites in the central rather than peripheral nervous system. To our knowledge, the effect of lidocaine on the brain's functional response to pain has never been investigated. Here, we therefore characterized the effect of systemic lidocaine on the brain's response to innocuous and acute noxious stimulation in the rat using functional magnetic resonance imaging (fMRI).

METHODS—Alpha-chloralose anesthetized rats underwent fMRI to quantify brain activation patterns in response to innocuous and noxious forepaw stimulation before and after IV administration of lidocaine.

RESULTS—Innocuous forepaw stimulation elicited brain activation only in the contralateral primary somatosensory (S1) cortex. Acute noxious forepaw stimulation induced activation in additional brain areas associated with pain perception, including the secondary somatosensory cortex (S2), thalamus, insula and limbic regions. Lidocaine administered at IV doses of either 1 mg/kg, 4 mg/kg or 10 mg/kg did not abolish or diminish brain activation in response to innocuous or noxious stimulation. In fact, IV doses of 4 mg/kg and 10 mg/kg lidocaine enhanced S1 and S2 responses to acute nociceptive stimulation, increasing the activated cortical volume by 50%–60%.

CONCLUSION—The analgesic action of systemic lidocaine in acute pain is not reflected in a straightforward interruption of pain-induced fMRI brain activation as has been observed with opioids. The enhancement of cortical fMRI responses to acute pain by lidocaine observed here has also been reported for cocaine. We recently showed that both lidocaine and cocaine increased intra-cellular

Address correspondence and reprint requests to Helene Benveniste, MD, PhD, Department of Medicine, Brookhaven National Laboratory, Upton, NY 11973. Address e-mail address to E-mail: benveniste@bnl.gov.

Peter S. Glass is editor of Ambulatory Anesthesiology for the Journal. This manuscript was handled by Steven L. Shafer, Editor-in-Chief and Dr. Glass was not involved in any way with the editorial process or decision.

Section Editor: Tony L. Yaksh/Quinn H. Hogan

calcium concentrations in cortex, suggesting that this pharmacological effect could account for the enhanced sensitivity to somatosensory stimulation. As our model only measured physiological acute pain, it will be important to also test the response of these same pathways to lidocaine in a model of neuropathic pain to further investigate lidocaine's analgesic mechanism of action.

Lidocaine (LIDO), when administered systemically at doses that result in low plasma concentrations in the range of 1–10 $\mu\text{g/mL}$, can alleviate acute pain^{1–5} and chronic neuropathic pain in humans and experimental animal models.^{6–9} Interestingly, when effective, pain relief after a single IV LIDO treatment can last for days or months,¹⁰ which is far beyond LIDO's known pharmacokinetic longevity.^{11,12} The plasma concentration of LIDO that has proven therapeutic in alleviating pain is significantly below what is required to block electrically evoked peripheral nerve conduction in nociceptive C-fibers and nonnoxious A β - and A δ fibers, which require near-lethal LIDO concentrations >250 $\mu\text{g/mL}$.¹³

Several studies have explored the mechanisms that may underlie the unusually long-lasting analgesic effects of IV LIDO. For example, in animal models of chronic pain, it was shown that systemic low-dose LIDO was effective in blocking tonic neural discharges in injured peripheral A δ - and C-fiber nociceptors in dorsal root ganglia, which might explain its mechanism of action.^{13–15} Other suggested targets for LIDO's analgesic action include sodium channels and other receptor sites not in the periphery, but in the brain.^{16–18} Information on LIDO's effects in the brain or the contributions of these to the intriguing analgesic effects that have been observed in both acute and chronic pain is limited. Although LIDO's effect on neurotransmitter release,^{19–22} seizure induction,^{23–26} and neurotoxicity^{27–32} in the central nervous system has been extensively investigated, brain responses during exposure to painful stimuli with systemic LIDO have, to our knowledge, not been characterized.

Noninvasive imaging techniques, such as functional magnetic resonance imaging (fMRI), have been used to characterize changes in brain function in small animal models in response to a wide variety of drugs and/or somatosensory stimuli,^{33–39} including acute nociception.^{40,41} For example, in the rat, acute stimulation via the sciatic nerve of nociceptive A δ and C-fibers resulted in bilateral activation of known brain pain pathways which were attenuated by systemic morphine.⁴⁰ As a first step in characterizing the effects of LIDO on the pain response in the brain, we therefore used fMRI and blood-oxygen-level-dependent (BOLD) contrast to assess the activation pattern induced by innocuous somatosensory and acute noxious electrical stimulation in the rat brain with and without LIDO. Based on the knowledge that systemic LIDO does not block somatosensory or nociceptive peripheral nerve conduction, we hypothesized that LIDO would not interfere with brain activation as measured by fMRI in response to either innocuous somatosensory or acute nociceptive stimulation.

METHODS

All surgical and experimental procedures were approved by the institutional animal care and use committee. Female Sprague-Dawley rats (210–290 g) were used for the studies.

Animal Preparation for MRI

All rats were initially anesthetized with 3% isoflurane and an intraperitoneal (i.p.) dose of methohexital sodium (50 mg/kg) and were also pretreated with glycopyrolate (0.04 mg/kg i.p.). After oral endotracheal intubation, mechanical ventilation was initiated (Harvard Apparatus, Inspira ASV). Anesthesia was maintained with 1.5%–2% isoflurane delivered in a 1:1 air/O₂ gas mixture. The femoral vein and a tail vein were catheterized for fluid and drug administration and the femoral artery for arterial blood pressure monitoring and collection of blood gas measurements during the study.

MRI and Forepaw Stimulation Protocol

All imaging was performed on a superconducting 9.4T/210 horizontal bore magnet (Magnex) controlled by an ADVANCE console (Bruker) using a 30-mm diameter surface radiofrequency coil secured above the head of the rat. All vital signs were continuously monitored during the experiment (SAM PC monitor, SA Instruments, Inc). Needle electrodes were inserted under the skin of each forepaw, between digits 2 and 3 and between 4 and 5. After positioning and insertion of needle electrodes, the anesthesia was switched from isoflurane to α -chloralose (Sigma); α -chloralose was administered first as an IV bolus of 40 mg/kg over a 10–15 min time period followed by a continuous infusion of 25 mg $\cdot$ kg⁻¹ $\cdot$ h⁻¹. Two to three trials of forepaw stimulations were administered to assure that the anesthetic plane was adequate. If the forepaw stimulation did not induce changes in mean arterial blood pressure (MABP), heart rate or the respiratory waveforms, the anesthetic depth was considered adequate. Muscle paralysis with vecuronium (0.01 mg/kg) was then achieved.

For fMRI a single-shot echo-planar sequence was used with the following parameters: TR = 1500; TE = 30 ms; effective bandwidth = 227272 Hz, field of view = 2.56 $\times$ 2.56 cm²; 64 $\times$ 64 matrix with a resulting in-plane resolution of 400 μ m; 8 axial 1.4 mm-thick slices spaced 0.15 mm apart. Each scan was acquired in 3 s. To more accurately identify the anatomical location of the functional activation maps, higher resolution T2-weighted spin-echo images were acquired with identical spatial geometry using a RARE pulse-sequence.

The pulse train forepaw stimulations were generated using a commercial current stimulator (Isolated Pulse Stimulator 2100, A-M Systems). Negative short rectangular current pulses of 0.3 ms duration were applied at a frequency of 3 Hz to either right or left forepaw. Preliminary data from the literature indicate that a 2 mA forepaw electrical stimulus seems to be innocuous (i.e., brain fMRI activation is limited to the somatosensory cortex and does not include other pain-related brain regions), whereas stronger stimulation currents are not. We therefore divided the animals into three groups based on the stimulation current used: Group 1 ($n = 4$) 2 mA; Group 2 ($n = 2$) 4 mA; and Group 3 ($n = 3$) 8 mA. The 2 mA paradigm consisted of 23 scans acquired during rest, 10 scans acquired during stimulation (total stimulation time 30 s), and followed by a poststimulation rest period of 30 scans (total acquisition time 63 $\times$ 3 s = 3.15 min). To minimize painful exposures to the higher stimulation currents, we shortened the stimulation time for the 4 and 8 mA trials. The 4 mA paradigm consisted of 13 scans acquired during rest, 3 scans during stimulation (total stimulation time 9 s), and followed by a poststimulation rest period of 20 scans (total acquisition time = 36 $\times$ 3 s = 1.8 min). The 8 mA paradigm consisted of 13 prestimulation scans, 1 scan during stimulation (total stimulation time 3 s) and 22 scans acquired after stimulation (total acquisition time: 36 $\times$ 3 s = 1.8 min). For each of the stimulation current paradigms, the BOLD signal amplitude as well as the activation map distribution acquired during control conditions were established before LIDO administration. The baseline was obtained by repeating the stimulation paradigm (i.e., 2 mA, 4 mA or 8 mA) 4 times at an interval of 6–10 min (Fig. 1). After the baseline acquisitions, the animals were allowed to rest for approximately 20 min before administration of the first of the 3 escalating doses of LIDO (see below). The entire fMRI experiment required approximately 3.5 h.

IV LIDO Administration

In clinical studies using LIDO for treatment of neuropathic pain or acute nonneuropathic pain, LIDO was administered IV to achieve plasma levels between 1 to 5 μ g/mL.⁶ Thus, previous animal studies with systemic LIDO have used IV computerized infusions to achieve similar LIDO plasma concentration ranges to mimic the clinical studies and to keep the LIDO in a nontoxic range.^{8,9} We did not use a continuous infusion of LIDO. However, we performed experiments to determine the plasma LIDO concentration over time in rats anesthetized and

prepared as described for the imaging experiments. The rats were similarly exposed to the escalating doses of LIDO as shown in Figure 1. Blood samples were collected at set time intervals via the femoral artery and placed in heparinized microcentrifuge tubes. The blood volume removed from the animals was replaced with an equal volume of blood containing heparin to avoid hemodynamic instability secondary to blood loss. The collected blood was centrifuged at 3000 rpm for 5–10 min, and the plasma was extracted and stored at -80°C until analysis. The blood sampling times were 5 and 35 min after the 1 mg/kg LIDO dose and 5, 15, 25, and 35 min after the 10 mg/kg LIDO dose (no samples were collected for the 4 mg/kg/dose).

LIDO Analysis

LIDO and five major unconjugated metabolites were quantified using a specifically developed and validated liquid chromatography procedure. Reference standards for LIDO and xylidide (XYL) were purchased from Sigma Chemical Co. (St. Louis, MO) and Aldrich Chemical Co. (Milwaukee, WI), respectively. Metabolites monoethylglycinexylidide (MEGX), glycinexylidide, 4-hydroxyxylidide (4-OH-XYL), 3-hydroxymonoethylglycinexylidide, 3-hydroxylidocaine (3-OH-LIDO), and internal standard pipercoloxylidide (PPX) were generously supplied by AstraZeneca Pharmaceutical Co., (Södertälje, Sweden). The extraction procedure involved a 0.2 mL sample of plasma (in 0.01 M HCl) and the addition of 25 μL (250 ng) PPX, 0.5 mL of 0.5 M NaOH, and 5.0 mL of ethyl acetate: methyl-tert-butyl ether (1:1). The mixture was shaken for 10 min at a slow speed on a tilt-platform rocker and then centrifuged at 2000 rpm for 15 min. The top organic layer was transferred to a conical centrifuge tube containing 150 μL of 0.1 M HCl. The contents were mixed for 10 min and then centrifuged for 10 min. The organic phase was aspirated to waste and the remaining acid layer evaporated to dryness using a vacuum centrifuge with moderate heat (approximately 45°C). The dried residue was reconstituted with 125 μL of mobile phase and transferred to the autosampler inserts for chromatography.

Separation of the extracted LIDO, its metabolites and internal standard was performed using an octadecylsilyl reversed phase column (Phenomenex Luna C-18, 3 μ , 150×4.6 mm, Torrance, CA) with a mobile phase consisting of 0.05 M monobasic potassium phosphate: acetonitrile (84:16) with the addition of 1 mL/L phosphoric acid (85%), 1.2 mL/L triethylamine, and 7 mL/L heptane sulfonate (20%). The eluted compounds were detected by ultraviolet absorbance at 210 nm. Using a flow rate of 1.5 mL/min with a column thermostated at 32°C , the retention times of the eluted compounds were: 4-OH-XYL, 3.2 min; 3-hydroxymonoethylglycinexylidide, 4.7 min; 3-OH-LIDO, 6.5 min; glycinexylidide, 13.7 min; XYL, 15.6 min; MEGX, 19.0 min; PPX, 26.0 min; LIDO, 28.5 min. Every set of samples was preceded with a 7-point calibration curve, which included the expected concentration range of samples, a blank, and three sets of quality controls. The limit of quantification for LIDO and the metabolites (except for 4-OH-XYL) was about 12.5 ng/mL.

Figure 2 shows the time course of the plasma LIDO concentration after 1 mg/kg and 10 mg/kg (other metabolites not shown). As can be seen, the plasma LIDO concentration is at the “therapeutic” target range of $>1 \mu\text{g/mL}$ at the 5-, 15-, and 25-min time points after the 10 mg/kg LIDO dose. For the 1 mg/kg dose, the plasma concentration is $<1 \mu\text{g/mL}$ at both the 5-min and 35 min time point.

In all imaging experiments, the rats were exposed to 1 mg/kg, 4 mg/kg, and 10 mg/kg over the course of the study as illustrated in Figure 1. Each dose of LIDO was administered IV over 3–5 min, which translated into approximately 0.5–0.1 mg/min. After each of the IV LIDO dose challenges, forepaw stimulation trials were conducted at 5-min, 15-min, 25-min, and 35-min (Fig. 1). One hour was allowed between each of the escalating doses of LIDO to assure that the LIDO plasma concentration was negligible before the next dose was administered.

MRI Data Analysis

The fMRI images were analyzed using Stimulate V6.01 (CMRR, University of MN). After a background masking, the intensity time course of each pixel during the scan was cross-correlated with a boxcar template according to the known stimulation profile. We used a correlation coefficient (r) ≥ 0.3 which corresponds to a P value of 0.01 to calculate the activation maps and detect the areas with a statistically significant BOLD signal. A 4-neighbor 2D clustering was performed to remove scattered false activation. For each rat, a sector-shape region of interest was delineated covering the entire forepaw area of the somatosensory cortex (which spanned 2–3 of the acquired slices and included both primary and secondary somatosensory cortex). Both the number of pixels (nominally, each pixel accounts for a volume of $0.4 \times 0.4 \times 1.5 \times 0.24 \text{ mm}^3/\text{pixel}$) and the average time course of the statistically significantly active pixels (pixels with $r \geq 0.3$) within the region of interest were extracted for each trial, and used to represent the spatial and temporal profile of the BOLD response, respectively. Each time course was then normalized to its mean value during the prestimulation baseline and the peak value was extracted to assess the magnitude for each stimulation trial. All calculated and color-coded functional activity maps were overlaid on the echo-planar images and the anatomical location of activated pixels identified by comparing with the corresponding high resolution T2-weighted MRI. Intra-group differences in the baseline BOLD signal amplitude and magnitude of BOLD activation (number of pixels with statistically significant BOLD signal increases) were analyzed using an F-test. Statistical differences between the average baseline BOLD signal amplitude (average of the four individual stimulations before injection of LIDO) and amplitudes obtained after the LIDO administrations were analyzed using a paired t -test. Statistical differences between the cortical volume activated during baseline conditions (average of the 4 trials) was compared with cortical volumes activated 5-min (where the plasma LIDO concentration was highest) after each of the escalating doses of LIDO using a paired t -test.

RESULTS

Physiological Values

All rats had normal blood gas values during the fMRI experiments. Stimulation with 2 mA (30 s), 4 mA (9 s) or 8 mA (3 s) did not produce significant increases in MABP or heart rate. There was no significant effect of IV LIDO on the MABP or the heart rate regardless of dose administered (data not shown).

Baseline Brain BOLD Responses and Activity Maps with 2 mA and 8 mA Stimuli

Figure 3 shows typical activation maps of the BOLD signal response in the rat brain elicited by each of the 4 baseline 30 s-2 mA forepaw stimulation trials. As expected, right (or left) forepaw stimulation produced BOLD signal increases in the contralateral primary somatosensory cortex (S1) area corresponding to the forepaw territory. In the 4 Group 1 rats exposed to 2 mA for 30 s, the S1 area was the only significant brain region eliciting a BOLD response; although this suggests the 30-s-2-mA stimulus was an innocuous stimulus, because with an $r \geq 0.3$ threshold for data analysis, it is possible that subthreshold activation of other areas went undetected. The number of pixels (pixels with a statistically significant BOLD signal) in S1 activated by the 2 mA stimulus was highly consistent from trial to trial in individual animals (Fig. 3), although modest variation in the total number of pixels activated was observed across subjects. Analysis of all Group 1 rats demonstrated that the average amplitudes of the 4 individual 2 mA-induced BOLD responses acquired during control conditions were $4.1\% \pm 1.7\%$, $3.9\% \pm 2.5\%$, $4.5\% \pm 0.3\%$ and $4.5\% \pm 1.7\%$, with statistical analyses revealing no significant trial to trial differences (F-test, $P > 0.05$) thus indicating that a stable stimulation baseline was present before IV LIDO challenges.

Forepaw stimulation with 4 mA elicited the expected contralateral S1 activation. However, areas with significant BOLD signal increases were also observed in the ipsilateral (in relation to the paw stimulated) thalamus, bilaterally in the ventral tegmental area (VTA) and hypothalamus, albeit somewhat inconsistently from stimulation trial to trial. Because of this inconsistency, data derived from 4 mA stimulation were not assessed further.

Forepaw stimulation with 8 mA for 3 s consistently elicited BOLD signal increases in the contralateral S1 and S2. However, in all of the animals, several other brain areas associated with activation of nociceptive C-fibers, such as the thalamus, insula, hypothalamus and VTA, ⁴² were also activated although not always coincidently. Nonetheless, because the stimulation trials always included 2–3 of the regions listed above in addition to S1/S2 activation, it was concluded that this stimulation current was noxious. Table 1 lists the regions of the brain that were observed to be activated in response to the 8 mA forepaw stimulation during control conditions in 3 different animals. Figure 4A shows an example in one of the rats and demonstrates activation in the following areas contralateral to the forepaw stimulated: S1, secondary somatosensory cortex (S2), thalamus, posterior hypothalamus and the VTA. Figure 4B shows another example of brain activation in response to the noxious stimulation with additional activation of the insula. Under control conditions, the BOLD signal amplitude and total activated pixel volume in S1/S2 elicited by forepaw stimulation with 8 mA was $5.8\% \pm 0.4\%$ and $7.4 \pm 2.6 \text{ mm}^3$, respectively.

In a few control animals, we also investigated the stability of the forepaw stimulation brain activation signal over a subsequent 3-h period during which no IV LIDO was administered. Figure 5 shows the result of a control experiment using a 8 mA, 3 s forepaw stimulus according to the experimental stimulation design but without the LIDO challenges. As can be seen, the amplitude of the BOLD signal response measured in primary somatosensory cortex (average of all activated pixels) for each stimulation trial was relatively stable over the 2–3 h study time. The corresponding cortical volume activated by the 8 mA, 3 s forepaw stimulus in S1 for each trial was also relatively consistent over this timeframe.

Effect of IV LIDO

Innocuous 2 mA, 30 s Stimulus—The effect of the increasing doses of IV LIDO on the BOLD signal elicited by the 2 mA, 30 s stimulus is shown in Table 2. First, the BOLD signal amplitude elicited by 2 mA in S1 did not change significantly with either dose of LIDO when compared with the baseline amplitude. Second, the average volume of pixels in S1 activated at baseline conditions was $5.9 \pm 3.2 \text{ mm}^3$, and there were no statistically significant changes in the total volume of activated pixels in S1 observed with either dose of LIDO. However, the S1 response varied considerably from animal to animal. For example, at 5-min after the 10 mg/kg IV LIDO challenge, the volume of activated pixels elicited by the 2 mA, 30 s stimulus in S1 increased from approximately 6 mm^3 to approximately $20 \pm 18 \text{ mm}^3$. The large variation in the number of activated pixels is a consequence of 3 animals that demonstrated clear increases in the volume of activated pixels in S1 after the 10 mg/kg dose, but one animal which did not. Collectively, the data from the group approached, but did not reach, statistical significance ($P = 0.056$).

Noxious 8 mA, 3 s Stimulus—Figure 6 shows the brain activation pattern in response to the noxious 8 mA forepaw stimuli at baseline and 5-min after 4 mg/kg and 10 mg/kg IV LIDO. First, Figure 6 demonstrates that brain regions involved in nociception are still activated, including S1 and S2 in spite of the IV challenges with 4 mg/kg and 10 mg/kg LIDO. In addition, Figure 6 shows that the fMRI BOLD response in S1/S2 is enhanced (increased total number of pixels) immediately after administration of LIDO. Figure 7 shows brain activation patterns in response to the nociceptive stimulus 5-min after 10 mg/kg LIDO in 2 different rats. In the

first rat (left forepaw stimulation), brain activation is elicited contralateral in the right in S1, VTA, thalamus and posterior hypothalamus (Fig. 7). In the second rat (right forepaw stimulation), brain activation was elicited in the left S1/S2 and thalamus (Fig. 7). Table 3 shows that the average amplitude of the BOLD signal in somatosensory cortex immediately after 1 mg/kg LIDO was $7.1\% \pm 1.0\%$, which was not statistically different from the baseline BOLD signal amplitude (Table 3). Further, the pixel volume activated in S1 in response to the 8 mA forepaw stimulus 5-min after 1 mg/kg LIDO was also similar to baseline conditions. However, the amplitude of the BOLD signal after 10 mg/kg LIDO was 6.9% and significantly higher than at baseline (5.8 ± 0.4 vs 6.9 ± 0.4 , $P = 0.0025$). In addition, analysis of the 4 mg/kg and 10 mg/kg LIDO brain activation maps, demonstrated that the total volume of activated S1/S2 cortex was significantly larger than that observed during control conditions. For example, 5-min after the 4 mg/kg LIDO challenge the activated pixel S1/S2 volume increased from 7.4 to 15.7 mm^3 ($P = 0.008$), and for the 10 mg/kg LIDO challenge it increased from 7.4 to 19.2 mm^3 ($P = 0.03$).

DISCUSSION

The major finding of this study was that LIDO, when administered IV, did not abolish or diminish the brain's response as measured by fMRI BOLD to innocuous or acute noxious electrical stimulation of the forepaw in normal rats. We also showed for the first time that LIDO enhanced the somatosensory cortical fMRI BOLD response to acute noxious stimulation as evidenced by an increase in the number of activated pixels.

Previous experimental animal studies have demonstrated that nerve conduction in peripheral nerves is not blocked by therapeutic, low dose LIDO.^{9,43,44} Other targets for LIDO's analgesic action have therefore been suggested, including sodium channels and other receptor sites, not in the peripheral nervous system, but in the central nervous system.^{16–18} Here we used fMRI to visualize forebrain activation during brief acute pain and demonstrated that the brain circuits and pathways activated in response to peripherally evoked acute noxious stimulation were not disrupted by systemic LIDO in contrast to what has been observed previously for opioid analgesics, such as morphine.^{45–49} These results raise several questions. First, does the animal model of acute pain used here to test the analgesic action of LIDO mimic the clinical condition? Second, what causes the LIDO-induced somatosensory fMRI BOLD enhancement observed during acute nociception? And third, is the LIDO-induced cortical fMRI BOLD enhancement of importance for its analgesic efficacy?

Animal Model of Acute Pain: Clinical Relevance

To elicit acute pain in the rat, we applied an 8 mA electrical current to the forepaw for 3 s which elicited activation in brain areas known to be involved in acute nociception. Our data agree with previous documentation of neuroanatomical pathways involved in acute pain observed in humans^{42,45,46,48,49} and animals.^{40,50–52} For example, neuroimaging studies in humans have demonstrated hemodynamic brain responses to acute pain in somatosensory cortex (S1 and S2) and insula contralateral to stimulation, anterior cingulate cortex and thalamus primarily contralateral to stimulation but often bilateral (for review⁴²).

Preclinical studies with fMRI require that animals be anesthetized and thus a possible confound in these studies is the potential interaction between the anesthetic, the stimulus and/or the drugs tested. However, we used α -chloralose as an anesthetic which interferes the least with brain activation during nociceptive stimulation of A- δ and c-fiber.^{40,50–52} For example, electrical stimulation of A- δ and c-fibers via the sciatic nerve elicited activation in somatosensory cortex, cingulate cortex, thalamus and hypothalamus, which could be partially inhibited by morphine.⁴⁰ The slightly different distribution of brain activation in previous animal studies and those reported here is probably attributable to differences in the magnitude and length of the noxious

stimulus applied (e.g., in previous studies the stimulus was applied for several minutes, whereas it only lasted for 3 s in our study). Thus, based on the brain activation patterns that we observe with the noxious 8 mA forepaw stimulus, it is reasonable to conclude that the animal model used is relevant as an acute pain model from a clinical point of view given the fact that some of the same brain regions observed here are activated in humans exposed to acute pain.⁴²

It is important, however, to emphasize that we tested the effect of systemic LIDO on brain responses to acute, brief pain exposures which activate only the normal physiological pain pathways. One could argue, therefore, that a more clinically relevant experimental model is one that is representative of pathological pain that occurs secondary to both peripheral and central hypersensitization. In studies in which LIDO has shown analgesic efficacy, pathological pain is an important component and thus the potential mechanism of LIDO may be in altering components of pathological pain pathways in the brain, which clearly warrants further investigation. However, from our results it seems unlikely that LIDO's mechanism of action in providing analgesia is by affecting physiological pain pathways.

LIDO-Induced fMRI Enhancement of Cortical Activation with Acute Nociception

The LIDO-induced enhancement of the fMRI BOLD activation in somatosensory cortex to acute noxious stimulation is in agreement with data previously reported for cocaine⁵³ which, like LIDO, has local anesthetic actions. Cocaine, when administered IV (0.75 mg/kg), was shown to augment the electrophysiological response of thalamic neurons to vibrissae stimulation.^{54,55} Furthermore, Devonshire et al.⁵³ demonstrated that the neuronal response as measured by summed field potential to intense somatosensory (whisker) stimulation after cocaine was enhanced in barrel cortex. In parallel with the electrophysiological recordings, they also measured the hemodynamic responses during somatosensory stimulation using a combination of optical and BOLD fMRI techniques.⁵⁶ The data demonstrated that, because cocaine in itself increased the baseline cerebral blood flow and BOLD signals over time in the somatosensory cortex, the ensuing responses recorded were not accurately reflecting the enhanced neuronal response.⁵⁶ We observed that LIDO enhanced the fMRI BOLD activation in somatosensory cortex in response to the acute noxious stimulation and, because LIDO unlike cocaine does not seem to affect baseline cerebral hemodynamics to the same degree,⁵⁷ we suggest therefore that the fMRI enhancement observed truly represents enhanced neuronal activation.

Implications for the Mechanisms of Action of LIDO in Treatment of Pain

Both LIDO and cocaine block sodium channels, which is a mechanism that is believed to contribute to the analgesic effects of LIDO in neuropathic pain.⁸ However, because sodium channels are responsible for the initiation and propagation of action potentials,⁵⁸ their blockade by LIDO would have been expected to decrease and not increase cortical activation with somatosensory stimulation. However, we recently showed that both cocaine and LIDO (1 mg/kg) increased the intracellular calcium concentration $[Ca^{2+}]_i$ in somatosensory cortex.⁵⁹ Since fluctuations in $[Ca^{2+}]_i$ levels affect neuronal excitability,⁶⁰ we propose that LIDO's ability to increase $[Ca^{2+}]_i$ in somatosensory neurons could also underlie the enhanced cortical activation to noxious and nonnoxious stimulation. Moreover, the increase in $[Ca^{2+}]_i$ in somatosensory cortex by LIDO may also be relevant for its analgesic effects since studies have proposed a decrease in neuronal calcium currents in somatosensory neurons as the mechanism underlying neuropathic pain.^{61–63}

CONCLUSION

We studied the effects of LIDO on acute painful stimulation in the brain and demonstrated an increase in the somatosensory fMRI BOLD signal to brief noxious stimuli after LIDO

administration. Our results suggest that LIDO's analgesic efficacy is unlikely to be through an action on normal physiological pain pathways. To further determine the potential mechanism of LIDO's analgesic action, future studies are required in animal models of neuropathic pain to assess if under these conditions LIDO alters signaling in the pain pathways in the brain. We are also planning future fMRI studies to characterize the effect of selective calcium channel inhibitors on the brain's response to acute and chronic pain.

Acknowledgements

Supported by Department of Energy Office of Science and Biological Research (Contract No. DE-AC02-98CHI-886), and New York State Office of Science, Technology, and Academic Research.

References

1. Bonica, J. The management of pain. Philadelphia: Lea & Febiger; 1953.
2. Cassuto J, Wallin G, Hogstrom S, Faxen A, Rimback G. Inhibition of postoperative pain by continuous low-dose intravenous infusion of lidocaine. *Anesth Analg* 1985;64:971-4. [PubMed: 3898920]
3. Groudine SB, Fisher HA, Kaufman RP Jr, Patel MK, Wilkins LJ, Mehta SA, Lumb PD. Intravenous lidocaine speeds the return of bowel function, decreases postoperative pain, and shortens hospital stay in patients undergoing radical retropubic prostatectomy. *Anesth Analg* 1998;86:235-9. [PubMed: 9459225]
4. Matharu MS, Cohen AS, Goadsby PJ. SUNCT syndrome responsive to intravenous lidocaine. *Cephalalgia* 2004;24:985-92. [PubMed: 15482363]
5. Jonsson A, Cassuto J, Hanson B. Inhibition of burn pain by intravenous lignocaine infusion. *Lancet* 1991;338:151-2. [PubMed: 1677068]
6. Petersen KL, Rowbotham MC. Will ion-channel blockers be useful for management of nonneuropathic pain? *J Pain* 2000;1:26-34. [PubMed: 14622840]
7. Strichartz GR, Zhou Z, Sinnott C, Khodorova A. Therapeutic concentrations of local anaesthetics unveil the potential role of sodium channels in neuropathic pain. *Novartis Found Symp* 2002;241:189-201. [PubMed: 11771646]discussion 202-5, 226-32
8. Amir R, Argoff CE, Bennett GJ, Cummins TR, Durieux ME, Gerner P, Gold MS, Porreca F, Strichartz GR. The role of sodium channels in chronic inflammatory and neuropathic pain. *J Pain* 2006;7:S1-S29. [PubMed: 16632328]
9. Araujo MC, Sinnott CJ, Strichartz GR. Multiple phases of relief from experimental mechanical allodynia by systemic lidocaine: responses to early and late infusions. *Pain* 2003;103:21-9. [PubMed: 12749955]
10. Chaplan SR, Bach FW, Shafer SL, Yaksh TL. Prolonged alleviation of tactile allodynia by intravenous lidocaine in neuropathic rats. *Anesthesiology* 1995;83:775-85. [PubMed: 7574057]
11. Bennett PN, Aarons LJ, Bending MR, Steiner JA, Rowland M. Pharmacokinetics of lidocaine and its deethylated metabolite: dose and time dependency studies in man. *J Pharmacokinet Biopharm* 1982;10:265-81. [PubMed: 7175699]
12. Thomson AH, Elliott HL, Kelman AW, Meredith PA, Whiting B. The pharmacokinetics and pharmacodynamics of lignocaine and MEGX in healthy subjects. *J Pharmacokinet Biopharm* 1987;15:101-15. [PubMed: 3612496]
13. Tanelian DL, MacIver MB. Analgesic concentrations of lidocaine suppress tonic A-delta and C fiber discharges produced by acute injury. *Anesthesiology* 1991;74:934-6. [PubMed: 2021210]
14. Devor M. Neuropathic pain and injured nerve: peripheral mechanisms. *Br Med Bull* 1991;47:619-30. [PubMed: 1794075]
15. Devor M, Wall PD, Catalan N. Systemic lidocaine silences ectopic neuroma and DRG discharge without blocking nerve conduction. *Pain* 1992;48:261-8. [PubMed: 1589245]
16. Rykaczewska-Czerwinska M. Antinociceptive effect of lidocaine in rats. *Pharmacol Rep* 2006;58:961-5. [PubMed: 17220556]
17. Waxman SG, Hains BC. Fire and phantoms after spinal cord injury: Na⁺ channels and central pain. *Trends Neurosci* 2006;29:207-15. [PubMed: 16494954]

18. Zhao P, Waxman SG, Hains BC. Sodium channel expression in the ventral posterolateral nucleus of the thalamus after peripheral nerve injury. *Mol Pain* 2006;2:27. [PubMed: 16916452]
19. Hernandez L, Guzman NA, Hoebel BG. Bidirectional microdialysis *in vivo* shows differential dopaminergic potency of cocaine, procaine and lidocaine in the nucleus accumbens using capillary electrophoresis for calibration of drug outward diffusion. *Psychopharmacology (Berl)* 1991;105:264–8. [PubMed: 1796132]
20. Fujitani T, Adachi N, Miyazaki H, Liu K, Nakamura Y, Kataoka K, Arai T. Lidocaine protects hippocampal neurons against ischemic damage by preventing increase of extracellular excitatory amino acids: a microdialysis study in Mongolian gerbils. *Neurosci Lett* 1994;179:91–4. [PubMed: 7845632]
21. Chen NH, Reith ME. Effects of locally applied cocaine, lidocaine, and various uptake blockers on monoamine transmission in the ventral tegmental area of freely moving rats: a microdialysis study on monoamine interrelationships. *J Neurochem* 1994;63:1701–13. [PubMed: 7931325]
22. Woodward JJ, Compton DM, Balster RL, Martin BR. In vitro and in vivo effects of cocaine and selected local anesthetics on the dopamine transporter. *Eur J Pharmacol* 1995;277:7–13. [PubMed: 7635175]
23. Ikeda M, Dohi T, Tsujimoto A. Protection from local anesthetic-induced convulsions by gamma-aminobutyric acid. *Anesthesiology* 1982;56:365–8. [PubMed: 7073000]
24. Niederlehner JR, DiFazio CA, Foster J, Westfall TC. Cerebral monoamines and lidocaine toxicity in rats. *Anesthesiology* 1982;56:184–7. [PubMed: 7059026]
25. Kragh J, Bolwig TG, Woldbye DP, Jorgensen OS. Electroconvulsive shock and lidocaine-induced seizures in the rat activate astrocytes as measured by glial fibrillary acidic protein. *Biol Psychiatry* 1993;33:794–800. [PubMed: 8373917]
26. Hirsch E, Danover L, Simler S, Pereira de Vasconcelos A, Maton B, Nehlig A, Marescaux C, Vergnes M. The amygdala is critical for seizure propagation from brainstem to forebrain. *Neuroscience* 1997;77:975–84. [PubMed: 9130779]
27. Beardsley D, Holman S, Gantt R, Robinson RA, Lindsey J, Bazaral M, Stewart SF, Stevens RA. Transient neurologic deficit after spinal anesthesia: local anesthetic maldistribution with pencil point needles? *Anesth Analg* 1995;81:314–20. [PubMed: 7618722]
28. Sakura S, Kirihaara Y, Muguruma T, Kishimoto T, Saito Y. The comparative neurotoxicity of intrathecal lidocaine and bupivacaine in rats. *Anesth Analg* 2005;101:541–7. [PubMed: 16037173] table of contents
29. Yamashita A, Matsumoto M, Matsumoto S, Itoh M, Kawai K, Sakabe T. A comparison of the neurotoxic effects on the spinal cord of tetracaine, lidocaine, bupivacaine, and ropivacaine administered intrathecally in rabbits. *Anesth Analg* 2003;97:512–9. [PubMed: 12873946]table of contents
30. Radwan IA, Saito S, Goto F. The neurotoxicity of local anesthetics on growing neurons: a comparative study of lidocaine, bupivacaine, mepivacaine, and ropivacaine. *Anesth Analg* 2002;94:319–24. [PubMed: 11812691]table of contents
31. Kanai Y, Katsuki H, Takasaki M. Graded, irreversible changes in crayfish giant axon as manifestations of lidocaine neurotoxicity in vitro. *Anesth Analg* 1998;86:569–73. [PubMed: 9495417]
32. Sakura S, Bollen AW, Ciriales R, Drasner K. Local anesthetic neurotoxicity does not result from blockade of voltage-gated sodium channels. *Anesth Analg* 1995;81:338–46. [PubMed: 7618726]
33. Bock C, Krep H, Brinker G, Hoehn-Berlage M. Brainmapping of alpha-chloralose anesthetized rats with T2*-weighted imaging: distinction between the representation of the forepaw and hindpaw in the somatosensory cortex. *NMR Biomed* 1998;11:115–9. [PubMed: 9699494]
34. Keilholz SD, Silva AC, Raman M, Merkle H, Koretsky AP. Functional MRI of the rodent somatosensory pathway using multislice echo planar imaging. *Magn Reson Med* 2004;52:89–99. [PubMed: 15236371]
35. Spenger C, Josephson A, Klason T, Hoehn M, Schwindt W, Ingvar M, Olson L. Functional MRI at 4.7 tesla of the rat brain during electric stimulation of forepaw, hindpaw, or tail in single- and multislice experiments. *Exp Neurol* 2000;166:246–53. [PubMed: 11085890]

36. Austin VC, Blamire AM, Allers KA, Sharp T, Styles P, Matthews PM, Sibson NR. Confounding effects of anesthesia on functional activation in rodent brain: a study of halothane and alpha-chloralose anesthesia. *Neuroimage* 2005;24:92–100. [PubMed: 15588600]
37. Kida I, Hyder F, Behar KL. Inhibition of voltage-dependent sodium channels suppresses the functional magnetic resonance imaging response to forepaw somatosensory activation in the rodent. *J Cereb Blood Flow Metab* 2001;21:585–91. [PubMed: 11333369]
38. Kida I, Smith AJ, Blumenfeld H, Behar KL, Hyder F. Lamotrigine suppresses neurophysiological responses to somatosensory stimulation in the rodent. *Neuroimage* 2006;29:216–24. [PubMed: 16112588]
39. de Zwart JA, Silva AC, van Gelderen P, Kellman P, Fukunaga M, Chu R, Koretsky AP, Frank JA, Duyn JH. Temporal dynamics of the BOLD fMRI impulse response. *Neuroimage* 2005;24:667–77. [PubMed: 15652302]
40. Chang C, Shyu BC. A fMRI study of brain activations during non-noxious and noxious electrical stimulation of the sciatic nerve of rats. *Brain Res* 2001;897:71–81. [PubMed: 11282360]
41. Tracey I, Becerra L, Chang I, Breiter H, Jenkins L, Borsook D, Gonzalez RG. Noxious hot and cold stimulation produce common patterns of brain activation in humans: a functional magnetic resonance imaging study. *Neurosci Lett* 2000;288:159–62. [PubMed: 10876085]
42. Peyron R, Laurent B, Garcia-Larrea L. Functional imaging of brain responses to pain. A review and meta-analysis (2000). *Neurophysiol Clin* 2000;30:263–88. [PubMed: 11126640]
43. Huang JH, Thalhammer JG, Raymond SA, Strichartz GR. Susceptibility to lidocaine of impulses in different somatosensory afferent fibers of rat sciatic nerve. *J Pharmacol Exp Ther* 1997;282:802–11. [PubMed: 9262344]
44. Mao J, Chen LL. Systemic lidocaine for neuropathic pain relief. *Pain* 2000;87:7–17. [PubMed: 10863041]
45. Balasubramanian S, Morley-Forster P, Bureau Y. Opioids and brain imaging. *J Opioid Manag* 2006;2:147–53. [PubMed: 17319448]
46. Borras MC, Becerra L, Ploghaus A, Gostic JM, DaSilva A, Gonzalez RG, Borsook D. fMRI measurement of CNS responses to naloxone infusion and subsequent mild noxious thermal stimuli in healthy volunteers. *J Neurophysiol* 2004;91:2723–33. [PubMed: 15136603]
47. Hagelberg N, Kajander JK, Nagren K, Hinkka S, Hietala J, Scheinin H. Mu-receptor agonism with alfentanil increases striatal dopamine D2 receptor binding in man. *Synapse* 2002;45:25–30. [PubMed: 12112410]
48. Wise RG, Rogers R, Painter D, Bantick S, Ploghaus A, Williams P, Rapeport G, Tracey I. Combining fMRI with a pharmacokinetic model to determine which brain areas activated by painful stimulation are specifically modulated by remifentanyl. *Neuroimage* 2002;16:999–1014. [PubMed: 12202088]
49. Wise RG, Williams P, Tracey I. Using fMRI to quantify the time dependence of remifentanyl analgesia in the human brain. *Neuropsychopharmacology* 2004;29:626–35. [PubMed: 14679387]
50. Baron R, Baron Y, Disbrow E, Roberts TP. Brain processing of capsaicin-induced secondary hyperalgesia: a functional MRI study. *Neurology* 1999;53:548–57. [PubMed: 10449119]
51. Shah YB, Haynes L, Prior MJ, Marsden CA, Morris PG, Chapman V. Functional magnetic resonance imaging studies of opioid receptor-mediated modulation of noxious-evoked BOLD contrast in rats. *Psychopharmacology (Berl)* 2005;180:761–73. [PubMed: 15778889]
52. Lowe AS, Beech JS, Williams SC. Small animal, whole brain fMRI: innocuous and nociceptive forepaw stimulation. *Neuroimage* 2007;35:719–28. [PubMed: 17300960]
53. Devonshire IM, Berwick J, Jones M, Martindale J, Johnston D, Overton PG, Mayhew JE. Haemodynamic responses to sensory stimulation are enhanced following acute cocaine administration. *Neuroimage* 2004;22:1744–53. [PubMed: 15275930]
54. Rutter JJ, Baumann MH, Waterhouse BD. Systemically administered cocaine alters stimulus-evoked responses of thalamic somatosensory neurons to perithreshold vibrissae stimulation. *Brain Res* 1998;798:7–17. [PubMed: 9666059]
55. Rutter JJ, Devilbiss DM, Waterhouse BD. Effects of systemically administered cocaine on sensory responses to peri-threshold vibrissae stimulation: individual cells, ensemble activity, and animal behaviour. *Eur J Neurosci* 2005;22:3205–16. [PubMed: 16367787]

56. Berwick J, Devonshire IM, Martindale AJ, Johnston D, Zheng Y, Kennerley AJ, Overton PG, Mayhew JE. Cocaine administration produces a protracted decoupling of neural and haemodynamic responses to intense sensory stimuli. *Neuroscience* 2005;132:361–74. [PubMed: 15802189]
57. Lam AM, Donlon E, Engl CC. The effect of lidocaine on cerebral blood flow and metabolism during normocapnia and hypocapnia in humans. *Am Soc Anesthesiologist* 1993;79:A202.
58. Goldin, AL. Diversity of mammalian voltage-gated sodium channels. In: Ruby, B.; Seeburg, P., editors. *Molecular and functional diversity of ion channels and receptors*. New York: New York Academy of Sciences; 1999. p. 38-50.
59. Du C, Yu M, Volkow ND, Koretsky AP, Fowler JS, Benveniste H. Cocaine increases the intracellular calcium concentration in brain independently of its cerebrovascular effects. *J Neurosci* 2006;26:11522–31. [PubMed: 17093073]
60. Verkhratsky A. Calcium ions and integration in neural circuits. *Acta Physiol (Oxf)* 2006;187:357–69. [PubMed: 16776661]
61. Fuchs A, Rigaud M, Hogan QH. Painful nerve injury shortens the intracellular Ca^{2+} signal in axotomized sensory neurons of rats. *Anesthesiology* 2007;107:106–16. [PubMed: 17585222]
62. Hogan QH. Role of decreased sensory neuron membrane calcium currents in the genesis of neuropathic pain. *Croat Med J* 2007;48:9–21. [PubMed: 17309135]
63. McCallum JB, Kwok WM, Sapunar D, Fuchs A, Hogan QH. Painful peripheral nerve injury decreases calcium current in axotomized sensory neurons. *Anesthesiology* 2006;105:160–8. [PubMed: 16810008]

Figure 1.

Outline of experimental stimulation paradigm. The rat was allowed to rest 6–10 min between each forepaw stimulation trial. For the 2 mA experiment the stimulation time was 30 s. For the 4 mA and 8 mA currents the stimulation time was 9 s and 3 s, respectively. Lidocaine was injected IV after establishment of a functional magnetic resonance imaging (fMRI) baseline obtained over the first 1 h (4 set of stimulations). The first lidocaine dose was 1 mg/kg, the second dose 4 mg/kg, and the third 10 mg/kg. One hour was allowed between each of the escalating doses of lidocaine.

Figure 2.

The time course of the average plasma lidocaine concentration after 1 mg/kg and 10 mg/kg in 3 rats. As can be seen, the plasma lidocaine concentration is at the “therapeutic” target range of $>1 \mu\text{g/mL}$ at the 5-, 15-, and 25-min time points after the 10 m/kg lidocaine dose. For the 1 mg/kg dose the plasma concentration is $<1 \mu\text{g/mL}$ at both the 5-min and 35 min time point.

Figure 3.

Blood-oxygen-level-dependent (BOLD) activation maps of the 4 responses to 2 mA forepaw stimulation for 30 s obtained during control conditions (no lidocaine) derived from EPI magnetic resonance imaging (MRI) images in a Group 1 rat. The activated pixels were calculated using a cross-correlation of $r > 0.3$ (STIMULATE). The color bar to the right is a 10-step transition from red to yellow which corresponds to a positive BOLD signal increase from 1% to 10%. In this particular animal the total volume of pixels with statistically significant BOLD signal increase over the 4 individual baseline stimulation trials was 3.6 mm³, 1.9 mm³, 3.6 mm³, and 2.4 mm³. The corresponding BOLD signal amplitudes of the 1st, 2nd, 3rd, and 4th stimulation was 2.3%, 2.1%, 4.8% and 2.4%, respectively.

Figure 4.

(A) Brain activation map during forepaw stimulation with 8 mA for 3 s obtained under control conditions before the lidocaine challenges. In this particular animal, brain regions with statistically significant blood-oxygen-level-dependent (BOLD) signal increases were observed contralateral to the forepaw stimulated in the following regions: primary somatosensory cortex (S1), secondary somatosensory cortex (S2), hippocampus, posterior hypothalamus and ventral tegmental area (VTA). Thalamic activity was also observed ipsilateral to the forepaw stimulated. (B) Example with additional activation of the insula region in another rat. Th = thalamus; PH = posterior hypothalamus; Hip = hippocampus. Activated brain regions were identified by comparing locations on the raw EPI images with their anatomical counterparts and labeled rat brain atlas templates. BOLD signal changes are also observed outside of the brain corresponding to the surface of the brain and/or muscles and are related to large venous structures. In both A and B the color bar to the right is a 10-step transition from red to yellow which corresponds to a positive BOLD signal increase from 1% to 10%.

Figure 5.

The result of a control experiment using the repetitive 8 mA, 3 s forepaw stimulation paradigm as outlined in Figure 1 but without the IV lidocaine challenges. As can be seen the amplitude of the blood-oxygen-level-dependent (BOLD) signal response measured in primary somatosensory cortex (average of all activated pixels) for each stimulation trial was relatively stable over the 2–3 h study time. The corresponding cortical volume activated by the 8 mA, 3 s forepaw stimulus in S1 for each trial was also relatively consistent over time although some variation is seen.

Figure 6. Brain activation patterns in response to the noxious 8 mA forepaw stimuli at baseline and 5-min after 4 mg/kg and 10 mg/kg IV lidocaine. As can be seen brain regions involved in nociception are still activated including S1 and S2 in spite of the lidocaine. In addition Figure 4 shows that the functional magnetic resonance imaging (fMRI) blood-oxygen-level-dependent (BOLD) response in S1/S2 is enhanced (increased total number of pixels) after administration of lidocaine. The color bar to the right is a 10-step transition from red to yellow which corresponds to a positive BOLD signal increase from 1% to 10%.

Figure 7.

Brain activation patterns in response to the nociceptive stimulus 5-min after 10 mg/kg lidocaine in 2 different rats. In the first rat, (left forepaw stimulation) brain activation is elicited contralateral in the right in S1, ventral tegmental area (VTA), thalamus, and posterior hypothalamus. In the second rat (right forepaw stimulation) brain activation was elicited in the left S1/S2 and thalamus. The color bar to the right is a 10-step transition from red to yellow which corresponds to a positive blood-oxygen-level-dependent (BOLD) signal increase from 1% to 10%.

Table 1
Brain Regions Activated by the Noxious 8 mA Forepaw Stimulus

Animal No.	Brain regions activated contralateral to the forepaw stimulated	Brain regions activated ipsilateral to the forepaw stimulated
1	S1/S2	
	Thalamus	Caudate putamen
	Entorhinal cortex	Temporal cortex
	Posterior cingulate cortex	Thalamus
	VTA	VTA
2	S2/S2	
	Entorhinal cortex	Posterior cingulate cortex
	Insula	
	Thalamus	
3	Temporal cortex	
	S1/S2	
	Insula	
	VTA	Entorhinal cortex
	Posterior hypothalamus	VTA
	Entorhinal cortex	
	Temporal cortex	
	Posterior cingulate cortex	

VTA = ventral tegmental area.

Table 2
Amplitude of BOLD Signal Changes and Total Volume of Activated Pixels of Group 1 Rats

BOLD signal	Condition	5-min	15-min	25-min	35-min
Amplitude%	Average baseline			4.3 ± 0.3	
	1 mg/kg lidocaine	5.6 ± 1.2	4.6 ± 1.9	6.2 ± 0.8	5.5 ± 1.4
	4 mg/kg lidocaine	5.3 ± 1.1	5.3 ± 0.5	4.8 ± 1.4	5.5 ± 1.1
	10 mg/kg lidocaine	6.4 ± 1.6	5.5 ± 1.1	5.4 ± 1.3	5.5 ± 0.4
No. of activated pixels (mm ³)	Average baseline			6 ± 3	
	1 mg/kg lidocaine	13 ± 7	15 ± 6	14 ± 6	14 ± 7
	4 mg/kg lidocaine	18 ± 16	20 ± 15	17 ± 15	14 ± 11
	10 mg/kg lidocaine	20 ± 18	25 ± 15	17 ± 11	21 ± 11

Data are mean ± SD and are derived from Group 1 animals.
BOLD = blood-oxygen-level-dependent.

Table 3

Number of Pixels with Statistically Significant Blood-Oxygen-Level-Dependent (BOLD) Signal Increases and the Average BOLD Signal Amplitude (1st Stimulus) in Somatosensory Cortex (S1/S2) Induced by Forepaw Stimulation with 8 mA for 3 s Acquired During Baseline Conditions and 5-min After IV Lidocaine

8 mA, 3s stimulus	Condition	Somatosensory cortex (S1/S2)
BOLD signal amplitude (%)	Baseline	5.8 ± 0.4
	Lidocaine 1 mg/kg (5-min)	7.1 ± 1.0
	Lidocaine 4 mg/kg (5-min)	6.4 ± 0.8
	Lidocaine 10 mg/kg (5-min)	6.9 ± 0.4 ^a
Volume activated mm ³	Baseline	7.4 ± 2.6
	Lidocaine 1 mg/kg (5-min)	11.0 ± 9.4
	Lidocaine 4 mg/kg (5-min)	15.7 ± 3.9 ^b
	Lidocaine 10 mg/kg (5-min)	19.2 ± 1.5 ^c

BOLD = blood-oxygen-level-dependent.

^aThe average BOLD signal amplitude elicited by 8 mA in S1/S2 approximately 5-min following 10 mg/kg was statistically significant from baseline (5.8 ± 0.4 vs 6.9 ± 0.4, $P = 0.0025$).

^bThe S1/S2 volume activated by 8 mA in S1/S2 approximately 5-min following 4 mg/kg was statistically significantly larger than that activated before lidocaine (7.4 ± 2.6 mm³ vs 15.7 ± 3.9 mm³, $P = 0.008$).

^cThe S1/S2 volume activated by 8 mA in S1/S2 approximately 5-min following 10 mg/kg was statistically significantly larger than that activated before lidocaine (7.4 ± 2.6 mm³ vs 19.2 ± 1.5 mm³, $P = 0.03$).