Validation of Awake Rat Model for Measures of Pain and Analgesia with fMRI

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Introduction: Functional imaging studies of rodents generally use anesthetics and paralyzers to control stress levels and excessive motion. For pain studies, however, the use of anesthetics introduces confounds that hamper our ability to measure pain responses in the brain and their modulation by analgesics. An awake animal fMRI pain model, with proper control of stress and motion, is necessary. However, for proper interpretation of results obtained with a conscious imaging model, it is critically important to establish its correspondence with assays traditionally used in preclinical drug studies in which animals are unrestrained and drug effects are assessed through behavioral measures (e.g. paw withdrawal latencies). In this work, we further characterize a previously described restraining awake rat imaging model [1,2] with respect to transient effects of animal preparation methods on behavioral and imaging (fMRI) outcome measures. Specifically, we assess the effects of isoflurane, used to facilitate animal handling and preparation for conscious imaging, over time on (i) behavior and (ii) imaging readouts.

Methods: Animals: Male Sprague-Dawley rats (~300g) were used for all our experiments. For behavioral studies, 3 groups (N=8) of animals were used (1 for mechanical and 2 for thermal stimulation studies); in each study one group underwent 15 minutes of 3% isoflurane and the other not. At 15, 45, and 90 minutes post-isoflurane both groups were tested with mechanical (von Frey filaments) or thermal (50 and 52C, hot-plate test) stimuli on the plantar aspect of the hindpaw. For imaging, 2 groups (N=6) of animals were exposed to 15 minutes of isoflurane (3%) to be loaded into the MRI cradle; in group 1 hypercapnia (CO2 challenge) was induced at 30, 60, and 90 minutes to assess global perfusion effects of isoflurane; group 2 was stimulated with a mechanical nylon fiber, similar to a von Frey filament, and heat (47C) stimuli to the dorsum of the hindpaw. Mechanical (thermal) stimuli were applied at 30(45), 60(75), and 90(105) minutes post-isoflurane. (Imaging was carried out in a 4.7T Bruker system, a RARE sequence with TReff/TEeff of 7s/53 ms was used for functional studies; each had Repetitions=90 with 6s-on/10s-off alternations, (5 stimuli/scan), 10% CO2 was applied for 3 minutes during a 10 minute scan).

Results: Table 1 summarizes the results of mechanical and thermal stimulation on standard behavioral experiments. At 15 minutes a significant increase in the von Frey threshold measure is observed, but not at later time points. Heat latencies indicate only residual effects for 50 and 52C 15 minutes post-isoflurane. No effects were seen at other times/temperatures. No significant changes in % BOLD signal due to hypercapnia were observed. The fMRI results to mechanical and thermal stimulation are displayed in Figure 1 in several pain-related brain structures. Although a trend to reduced activation at 30 minutes is observed in some structures no significant differences were observed at this group size.

Mechanical Withdrawal Threshold

	Non-Isolurane		Isoflurane	
Time course	mean (g)	sem	mean (g)	sem
Baseline	318.75	13.52	322.50	7.95
15 min	297.19	11.40	403.13	19.34
45 min	293.44	11.74	295.31	11.78
90 min	287.81	10.43	290.63	11.31

Hot Plate Test (15 min)

		Non-Isolurane		Isoflurane	
	Time course	mean	sem	mean	sem
50°C	Baseline	12.94	1.20	12.23	0.70
	15 min	28.95	3.30	12.09	0.68
52°C	Baseline	9.18	0.77	9.48	0.56
	15 min	19.01	2.39	10.39	0.56

Hot Plate Test (45 min)

	Non-Isolurane		Isoflurane	
Time course	mean	sem	mean	sem
Baseline	18.34	3.58	17.83	2.42
45 min	22.09	3.75	19.16	3.05
Baseline	14.14	2.41	13.20	2.43
45 min	13.14	1.91	11.73	1.14

Table 1: Behavioral measures of residual isoflurane effects on mechanical and thermal stimulation. Threshold of von Frey filament are compared between exposed and unexposed animals to isoflurane. Hot-plate latencies at 15 minutes to 50 and 52 indicate significant differences between exposed and unexposed animals. Conditions for which measurements post-isoflurane were significantly different from measurements taken from animals not treated with isoflurane are highlighted.

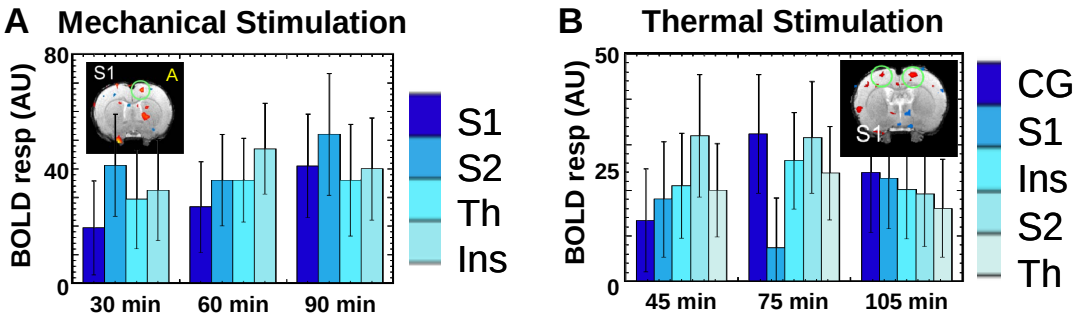

Figure 1 Activation in response to mechanical (A) and thermal (B), stimuli. Activation in various brain structures is displayed across time post-isoflurane withdrawal (in arbitrary units). (CG: cingulate cortex, S1/S2: sensory, Ins: insula, Th: thalamus). Insets show spatial pattern of activation. S1 activation is unilateral for mechanical stimulation and bilateral for thermal.

Discussion & Conclusion: Our results indicate that behavioral effects due to isoflurane are observed at 15 minutes post-isoflurane withdrawal, but not at 45 minutes or later. Imaging data, however, doesn't seem to produce similar results. This is not surprising given that pain responses are elucidated in fully anesthetized animals (Lowe et al. 2007). Nevertheless, activation across structures seems to have similar amplitudes at 45/60 minutes post-isoflurane. It is also important to indicate that innocuous mechanical stimulation induced unilateral activation while noxious heat produced bilateral activation. Similar effects have been observed in healthy human subjects. In conclusion, our studies seem to indicate that an awake restraining animal model with a 30/45-minute waiting period allows for pain studies that can be compared to behavioral experiments in drug development. This model will permit evaluation of drug effects directly in the animal brain.

References: [1] Borsook D, et al (2007) Drug Devel Res 68:23-41. [2] King JA, et al (2005) J Neurosci Methods 148:154-160. [3] Lowe AS, et al. 2007 Apr 1;35(2):719-28.