Use of functional Magnetic Resonance Imaging (fMRI) to map the neural circuitry of the brain involved in pain perception and provide an imaging biomarker of analgesic efficacy independent of behavioral assays in conscious rats

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Abstract

The objective of this study was to evaluate the utility of functional Magnetic Resonance Imaging (fMRI) to map the neural circuitry of the brain involved in pain perception. In addition, the identification of imaging biomarkers independent of behavioral assays in conscious rats was evaluated as an indication of efficacy of an analgesic. Functional MRI in awake rats was used to assess changes in brain activity to formalin challenge with and without drug treatment. BOLD (Blood Oxygen Level Dependent) fMRI imaging was employed to study the areas of brain activation and deactivation following injection of formalin in the hind foot pad of awake rats. Following acclimation, animals were placed in the restrainer and positioned in the magnet for imaging. Vehicle or TRPA1 antagonist (HC-030031) was injected ip approximately 30 minutes prior to formalin challenge. Scans were subsequently collected before and after administration of formalin injection continuously for about 1 hour post-formalin. Animals challenged with formalin showed changes in brain activity in several brain areas identified from the literature as part of the distributed neural network involved in the perception and processing of pain. The change in BOLD signal measured over the 1 hour imaging session following formalin injection showed a biphasic response in some regions. Prior treatment with a positive control significantly affected activity in these areas and much of the cortical mantle. In particular, activity in the prelimbic cortex, showed one of the greatest significant differences and might be used as an imaging biomarker for future studies evaluating novel analgesics.

Materials & Methods

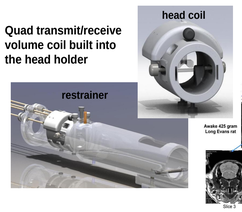
Acclimation for Imaging

Adult, male Sprague Dawley rats were acclimated to the holding device used for imaging (AIR, Holden, MA) for a minimum of 4 days before imaging in accordance with the Testing Facility SOP. On each day of acclimation, animals were anesthetized with 2-3% isoflurane and secured into the imaging system. When fully conscious, the imaging system was placed into a black opaque tube “mock scanner” with a tape-recording of an MRI pulse sequence for 60 minutes to simulate the bore of the magnet and imaging protocol. A significant decline in respiration, heart rate, motor movements, and plasma corticosterone has been measured when the first and last acclimation periods are compared (King et al., *J Neurosci Methods.* 2005;148[2]:154-160). The reduction in autonomic and somatic measures of arousal and stress improve the signal resolution and quality of the magnetic resonance (MR) images. The imaging system designed and employed isolated all of the body movements from the head restrainer and minimized motion artifact

Imaging Technology

Bruker BioSpec 7T / 20cm USR

Quad transmit/receive volume coil built into the head holder



Optimal signal-to-noise and field homogeneity

Studies were performed with a quad transmit/receive head coil and rat restrainer developed by Animal Imaging Research, Holden, MA

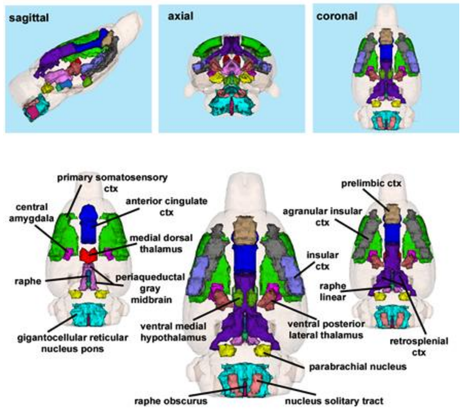
Experimental Design and Analysis

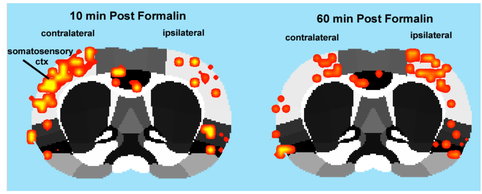
Group	No. of Animals	Test Compound	Dose (mg/kg)	Conc (mg/mL)	Dose Vol (mL/kg)	ROA	Image Acquisition
1	10	Vehicle	NA	NA	10	IP	Animals had vehicle or HC administered about 30 minutes prior to induction of pain. Baseline scans pre-formalin were acquired followed by formalin administration. Functional scans were collected for about 1 hr- post-challenge with formalin.
2	10	HC-030031	150	15	10		

Images were aligned and registered to a 3D rat brain atlas (Ekam Solutions, LLC), which is segmented and labeled with 152 discrete anatomical regions. The alignment process was facilitated by an interactive graphic user interface. The registration process involved translation, rotation and scaling independently and in all three dimensions. Matrices that transformed each subject’s anatomy were used to embed each slice within the atlas. All pixel locations of anatomy that were transformed were tagged with major and minor regions in the atlas. This combination created a fully segmented representation of each subject within the atlas. The inverse transformation matrix [Ti]-1for each subject (i) was also calculated.

Main Results

Pain Neural Circuit





The figure above illustrates activation (yellow/red) maps of the average significant change (n=9) in positive BOLD following formalin challenge. Note the lateralization of the BOLD signal to the contralateral somatosensory cortex as would be predicted by formalin-induced pain in the affected, ipsilateral hindpaw. Interestingly, the signal overtime is more highly represented in the ipsilateral somatosensory cortex.

Using a 3D segmented atlas (Ekam Solutions, LLC) and computational analyses of over 150 different brain areas it is possible to observe activation patterns across distributed, integrated neural circuits. The distributed neural circuit for acute experimental pain is illustrated above. The pain neural circuit is comprised of seventeen areas based on tract tracing studies in rats showing connectivity to and from the parabrachial nucleus of the brainstem (Raboisson et al., 1996) and critical nodes identified from meta-analysis from human pain imaging studies (Apkarian et al., 2005).

TABLE 1

Volume of Activation - Positive BOLD - 5 min Post Formalin

Region of Interest	Formalin Control			Formalin/CB			
	med	max	min	med	max	min	P value
motor secondary ctx	87	164	53	35	70	0	0.002
parietal ctx	9.5	24	2	0	10	0	0.003
prelimbic ctx	19	47	0	2	14	0	0.007
ventral medial thalamus	1.5	7	0	0	1	0	0.011
reticular thalamus	1.5	4	0	0	0	0	0.011
pontine gray	13	26	3	5	11	0	0.011
simple lobule cerebellum	73.5	123	13	19.5	45	0	0.016
substantia nigra compacta	2	4	0	0	1	0	0.018
orbital ctx	11	43	0	3	11	0	0.019
motor ctx primary	52.5	146	17	13	54	2	0.021
visual ctx	53.5	184	20	13.5	58	1	0.021
nucleus posterior commissure	0.5	4	0	0	0	0	0.027
motor nucleus trigeminal nerve	0.5	4	0	0	0	0	0.027
culmen cerebellum	75.5	79	12	33.5	55	1	0.031
medial dorsal thalamus	12	19	4	5.5	11	0	0.035
retrosplenial ctx	46	155	9	19	55	0	0.04
anterior lobe pituitary	17.5	30	1	6	17	0	0.046
paraflocculus cerebellum	22.5	59	6	11	23	2	0.046
superior colliculus	66.5	99	20	30.5	59	6	0.046
somatosensory ctx primary	78	397	23	25	75	2	0.046
declive cerebellum	24	51	3	4.5	30	0	0.058
gigantocellular reticular n. pons	52	96	20	18	76	5	0.059
anterior pretectal nucleus	3	10	0	0.5	6	0	0.059
premammillary hypothalamus	0	2	0	0	0	0	0.064
raphe linear	0	2	0	0	0	0	0.064
ansiform cerebellum	46	103	10	16.5	56	0	0.066
submedial thalamus	1	4	0	0	1	0	0.08
raphe	2	3	0	0	1	0	0.08
lateral posterior thalamus	4.5	13	0	1	3	0	0.086
central nucleus thalamus	3	8	0	0	3	0	0.088
CA1 hippocampus dorsal	6.5	49	1	3.5	22	0	0.09
lateral septal nucleus	17.5	33	11	12.5	22	0	0.091
reuniens thalamus	4.5	7	0	1.5	5	0	0.097

TABLE 2

Volume of Activation - Negative BOLD - 5 Min Post Formalin

Region of Interest	Formalin Control			Formalin/CB			
	med	max	min	med	max	min	P value
uvula cerebellum	0	0	0	6.5	18	0	0.001
dorsal lateral striatum	5.5	17	0	16.5	48	6	0.009
paramedian cerebellum	3	16	0	40	81	1	0.01
ansiform cerebellum	20.5	84	1	109	125	15	0.013
declive cerebellum	6	40	0	47	79	5	0.013
nodulus cerebellum	2.5	16	0	16.5	22	0	0.015
somaotsensory ctx secondary	4	34	0	18	49	9	0.018
inferior olivary complex	0	2	0	3	9	0	0.018
paraventricular nuclus	0	2	0	2	3	0	0.033
anterior pretectal nucleus	0	3	0	2	12	0	0.043
ventral medial striatum	1.5	10	0	12	18	1	0.049
accumbens core	1.5	5	0	5	14	1	0.05
gustatory ctx	7	23	0	20	44	4	0.051
lateral geniculate	0	4	0	2	7	0	0.057
principal sensory n. trigeminal	12	41	0	29.5	45	3	0.058
premammillary hypothalamus	0	1	0	0	0	0	0.063
posterior thalamus	0	5	0	1.5	8	0	0.064
ventral lateral striatum	2	23	0	16.5	53	6	0.065
subiculum hippocampus	17.5	81	0	44.5	100	8	0.074
visual ctx	25	107	0	67.5	156	24	0.074
agranular insular ctx	12	42	1	33	60	2	0.082
insular ctx	9	19	0	18	30	3	0.082
interposed nucleus cerebellum	0	2	0	1.5	3	0	0.084
orbital ctx	1.5	30	0	15	46	0	0.091
reticular thalamus	0	2	0	1.5	2	0	0.092
superior vestibular nucleus	7.5	22	0	23	29	0	0.092
CA1 hippocampus ventral	15.5	29	1	31	69	5	0.092
simple lobule cerebellum	24	73	3	62	112	10	0.093
somatosensory ctx primary	68	131	2	115	225	17	0.093

Positive and negative voxels in a given brain region (representing the volume of activation) were analyzed at early-acute (5 min) and late-chronic (60 min) phases of brain activity following formalin injection into the right hind paw as compared to formalin following pretreatment with TrpA1 antagonist (CB). The brain areas are listed in their rank order of significance after a multiple comparisons non-parametric Kruskal-Wallis Test. The lists of brain areas from Table 1 and Table 2 above show a significant difference between treatment groups for positive and negative BOLD in the early-acute phase.

Results

Figure 2

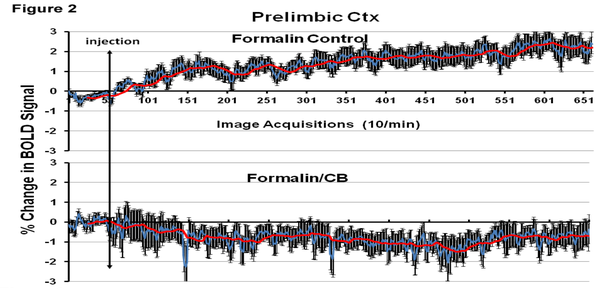
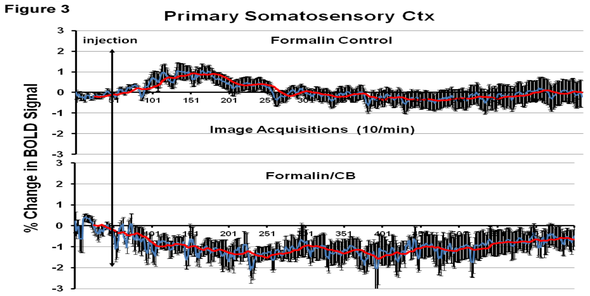


Figure 3



Activation curves for the prelimbic cortex (top) and primary somatosensory cortex (bottom) comparing the response to formalin challenge over time for formalin control (n=9) and formalin following pretreatment with 150 mg/kg HC-030031 (n=9). These times course data represent the mean (±SE) signal intensity for all voxels, both positive and negative for each of 650 acquisitions (50 pre-formalin and 600 post-formalin). A trend line represented in red is shown as the average of every 2.5 minutes. A vertical line in each of the graphs indicates the time in which formalin was injected into the footpad of the animal remotely while being imaged.

Conclusions

The data from formalin-induced pain show a “fingerprint” or a change in brain activation overtime that suggests a biphasic effect with an early activation occurring within the first 10 min of formalin injection and one later at about 60 min post-formalin challenge. This temporal change in brain activation is blocked by pretreatment with test article at a dose of 150 mg/kg. Imaging the pain response with fMRI is possible in awake animals and provides for evaluation of analgesic activity using imaging biomarkers. Integration of this technology as part of a discovery tool independent of behavioral assays in conscious rats may provide important and translational data valuable in the development of novel therapeutics.

References

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Raboisson et al., 1996 *J Comp Neurol* 1996 Apr 15; 367[4]: 503-517
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