

Published in final edited form as:

Neurosci Lett. 2009 November 20; 465(3): 267–271. doi:10.1016/j.neulet.2009.07.065.

Cocaine-induced metabolic activation in cortico-limbic circuitry is increased after exposure to the histone deacetylase inhibitor, sodium butyrate

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Abstract

Drug-induced inhibition of histone deacetylase (HDAC) results in the modification of many behavioral changes resulting from exposure to cocaine and other stimulant drugs-of-abuse, but a comprehensive map of the neuronal circuitries involved is lacking. The present study used blood-oxygen-level-dependent functional magnetic resonance imaging (BOLD fMRI) in awake rats to determine the effects of the HDAC inhibitor, sodium butyrate (SBt) on brain metabolic activation patterns during the initial stage of repeated cocaine administration. Three groups of rats received cocaine during BOLD fMRI, (i) acutely for the first time, or pretreated for two days with either (ii) saline or (iii) SBt 30 min prior to cocaine. Acute but not repeated exposure to cocaine resulted in widespread BOLD activation in fore- and midbrain. Pretreatment with SBt restored BOLD signals in the forebrain after repeated cocaine exposure, including a pronounced activation in the anterior thalamus, the hippocampus/amygdala and various portions of limbic and sensory cortex. Mesocorticolimbic areas showed a similar trend, but did not reach statistical significance. These findings suggest that HDACi modulation after repeated stimulant exposure involves cortico-limbic circuitry regulating emotion, motivation and memory.

Keywords

cocaine; stimulant; histones; lysine; deacetylase; epigenetic; sodium butyrate; fMRI; craving

Introduction

It is thought that [1] stimulant-induced changes in gene expression, particular in the core constituents of the mesolimbic circuitry—ventral tegmental area, ventral striatum/nucleus accumbens and prefrontal cortex—play a key role for the development and maintenance of the addicted state and chromatin remodeling [2], including the chemical modification of histone

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tail residues, is involved in cocaine-related behavioral and molecular adaptations [17]. To this end, the acetylation of histone lysine residues, which is associated with a loosening of chromatin structures and transcriptional activation, could become an attractive target for pharmacological interventions aimed at cocaine abuse. In particular, drugs that act as broad inhibitors of class I/II histone deacetylase (HDAC), profoundly affect the behavior of animals exposed to cocaine and other stimulants. For example, when cocaine, amphetamine or dopamine D₁-receptor agonist is co-administered with drugs acting as class I/II HDACi, there is robust enhancement of the stimulant's effects on locomotor sensitization and reward behavior [10,18].

Presently, little is known about the neuronal circuits mediating the enhancements in stimulant-related behaviors after HDACi treatment. Using blood-oxygen-level-dependent (BOLD) fMRI in fully awake rats [5,6], the goal of the present study was to obtain a comprehensive map of cocaine-sensitive neuronal circuits that are modified by sodium butyrate (SBt), a broad inhibitor of histone (protein) deacetylases previously shown to enhance the behavioral reinforcements of cocaine [10,18]. We report, for the first time, that SBt dramatically enhances the BOLD response to cocaine upon re-exposure in multiple brain regions. Unexpectedly, HDACi-mediated changes were not significant within in the mesocorticolimbic system, but pronounced in other cortico-limbic circuitry including the hippocampus/amygdala, the anterior thalamus and multiple portions of the cerebral cortex. These findings suggest that cortico-limbic circuitry regulating emotion and memory is responsive to HDACi treatments in the context of cocaine exposure.

Methods

Adult Long Evans male rats were treated with cocaine hydrochloride and sodium butyrate acetate (SBt) dissolved in 0.9% sterile saline solution. Cocaine was prepared fresh at a dose of 15 mg kg⁻¹ and SBt at 200 mg kg⁻¹ and both were injected i.p. at a volume of 0.1 cc 100 g⁻¹. The SBt and cocaine administration protocol used in the present study was partly adopted from a previous study [13]. For imaging experiments, cocaine was injected intracerebroventricularly (ICV) during functional scan acquisition at a concentration of 20 µg in 10 µL artificial cerebrospinal fluid as previously described [5,6]. A schematic of the treatment groups, dose regimen and imaging protocol is shown in Fig 1. The activation maps shown in Fig 2 are from the dose regimens shown in red in Fig 1.

All imaging experiments were done in fully awake, unanesthetized male rats (see [5]). Animals were acclimated to the restrainer and imaging protocol prior to an imaging session as previously described [7]. Magnetic resonance imaging was conducted in a Bruker Biospec 4.7-T/40-cm horizontal magnet (Oxford Instrument, Oxford, U.K.) using multislice, fast spin echo (RARE) pulse sequences for both anatomical and functional data acquisitions (for details see Supplementary data, file 1). Random-effects analyses using a fully segmented, 3D rat MRI atlas was used for statistical comparisons between treatment groups [7]. Once each animal was fully registered and segmented in the atlas, the statistical responses for each were averaged on a voxel-by-voxel bases comparing control (32 scan repetitions) to experimental (32 scan repetitions) time periods (see Fig 1) For each voxel (4,800 in number) in the brain a significance test and false discovery test were applied (independent of other voxels) to deem them as activated. Once these voxels for each animal were identified they were assigned to different regions of interest (ROI's) based on atlas information. The mean volume of activation (number of voxels occupying a given ROI) between treatment groups were statistically analyzed using a non-parametric Neuman-Keuls test for multiple comparisons.

Results

BOLD activation maps, co-registered on 2-D, coronal sections for each of the four experimental conditions are shown in Fig. 2. These activation maps from the four conditions (day 1 Saline/Cocaine; day 2 Saline/Cocaine; day 2 SBt/Cocaine; day 2 SBt/Saline) are a composite of six subjects each, fully registered into a 3D rat MRI atlas and segmented for volumes of interest (VOI). Visual inspection shows overall much more robust activation in fore- and midbrain of animals treated with a single dose of cocaine, or two once daily doses of SBt/Cocaine (Fig. 2). In contrast, metabolic activation patterns after two daily doses of single drug cocaine or SBt/saline were overall less pronounced (Fig. 2). The increased signals in the SBt/Cocaine treated animals include much of the cortical mantle, the medial temporal lobe including amygdala/entorhinal cortex/hippocampus and the anterior and medial dorsal nuclei of the thalamus (Fig. 2).

The only brain areas showing significant differences in BOLD signal between the three cocaine treatments, i.e. Saline/Cocaine and 1 and 2 day SBt/Cocaine are listed in Table 1 and include the somatosensory cortex, CA3 and CA1 fields of the hippocampus, cortical nuclei of the amygdala, the anterior thalamic nuclei and the septum. These significant different sites were identified from 93 brain areas, including the substantia nigra, ventral tegmental area, nucleus accumbens, dorsal striatum, prefrontal areas such as the prelimbic, infralimbic and orbital cortices (not all listed in table). Metabolic activation in the amygdala and somatosensory cortex was also higher after 2 days of SBt/Cocaine when compared to a single dose of the stimulant (Table 1). Shown in Fig 3 are 3D renderings of BOLD activation in the reward circuitry and the Papez circuit. These 3D volumes of activation from the three experimental cocaine groups are a composite of six subjects each and provide a visual representation comparing the difference in the number of activated voxels across experimental conditions. There were no significant differences in any of the brain areas comprising the mesocorticolimbic dopaminergic system. In contrast, areas comprising the Papez circuit, e.g. amygdala, hippocampus, and anterior thalamus, were significantly different and show an ostensibly greater volume of activation in animals treated with 2 day SBt/cocaine.

Discussion

The present study shows unexpected patterns of brain activation in awake rats exposed to SBt, using a two day treatment paradigm previously reported to increase cocaine sensitization [13]. An acute ICV dose of cocaine resulted in widespread BOLD activation in the fore- and midbrain, but cocaine-induced activation was significantly reduced after repeated exposure to the stimulant, corroborating an earlier work [5] and consistent with related changes in glucose metabolism [9]. Interestingly, SBt co-treatment restored the pronounced and widespread BOLD activation to successive cocaine treatments. Taken together, these findings suggest that the brain's initial response to repeated cocaine exposure triggers an adaptive, desensitization-like mechanism which can be overturned by pre-treating animals with SBt prior to receiving cocaine. Does this mechanism involve chromatin modification? To this point, a recent report identified HDAC1, a potential target of SBt [2], as an essential factor for the desensitization of the immediate early gene, *c-fos*, upon repeated exposure to the stimulant and drug-of-abuse, amphetamine [17].

Many of the areas responsive to the HDACi also play significant roles in learning, memory (CA3 and dentate gyrus of the hippocampus, anterior nucleus of the thalamus) and social recognition (central nucleus of the amygdala, septum). These same areas form an integrated neural circuit. The anterior thalamic nuclei of the rat receive synaptic projections through the subcommissural fornix from the pre- and parasubicular cortex but not the hippocampal subfields (CA1-CA4) [20]. This projection also extends in the rat to the mammillary nuclei of the

hypothalamus [20]. The subfields of the hippocampus proper communicate directly with the septal nuclei [20]. A case can therefore be made that during the initial exposure to cocaine, chromatin alterations occur in specific brain circuitry to enhance associative learning and memory. However, this remains to be tested experimentally. With regards to the somatosensory cortex it is unclear what the role of cocaine-associated chromatin modifications with this region may be, however it has been shown to have a significant degree of synaptic plasticity in response to cocaine administration in the rat [3].

The neural circuitry underlying this putative epigenetic mechanism contributing to cocaine sensitivity was not the mesocorticolimbic dopaminergic system, i.e., “reward system” as would be expected. Instead, cortico-limbic circuitry regulating emotion and memory appear to play a greater role for the HDACi-related alterations of reward behaviors. The anterior thalamus is the cornerstone of “Papez circuit,” connecting emotional expression from the hypothalamus with emotional experience from the limbic system [16]. Information from the anterior thalamic nuclei is conveyed to anterior cingulate and retrosplenial cortices where it is passed to forebrain cortex and hippocampus, respectively (see Fig 3).

Of note, there is evidence that HDACi-mediated enhancement of a stimulant’s sensitizing effects is context-specific and involves associative learning [10], and furthermore, drug-induced histone acetylation improves performance in other hippocampal learning and memory paradigms [12,14]. In addition, electrical stimulation of the ventral subiculum in the 4–8 Hz (theta) range reinstates self-administration of cocaine [21], while lidocaine-induced silencing of subicular/hippocampal neurotransmission reduced cocaine seeking behaviors [19].

It is remarkable that human subjects craving cocaine during functional neuroimaging activate the same limbic and cortical areas involved in emotion, motivation and memory that were identified in the present preclinical study, including the amygdala, and various portions of the frontal and parietal cortex and the thalamus [4,8,11,15]. Therefore, it is tempting to speculate that drug-induced changes in histone acetylation may provide clues for novel treatment strategies that are not only aimed at the reinforcing effects of cocaine and other stimulants, but also address some of the other key problems when dealing with addiction, including craving and relapse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Ms. Tara L. Stolberg for the superb technical assistance throughout the course of the study. This work was supported by NIH National Institute on Drug Abuse grants to Craig F. Ferris (DA13517), Marcelo Febo (DA019946) and Schahram Akbarian (DA017660). Its contents are solely the responsibility of the authors and do not represent the official views of the NIDA.

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Dose Regimen

Four experimental groups with 6 animals in each group (G)

- G-1 Day 1 - saline i.p. – 30 min later cocaine i.c.v (20 μ g)
 G-2 Day 1 - saline i.p. – 30 min later cocaine i.p. (15 mg/kg)
 Day 2 - saline i.p. – 30 min later cocaine i.c.v (20 μ g)
 G-3 Day 1 - sodium butyrate i.p. (200 mg/kg) – 30 min later cocaine i.p. (15 mg/kg)
 Day 2 - sodium butyrate i.p. (200 mg/kg) – 30 min later cocaine i.c.v. (20 μ g)
 G-4 Day 1 – sodium butyrate i.p. (200 mg/kg) – 30 min later saline i.p.
 Day 2 – sodium butyrate i.p. (200 mg/kg) – 30 min later saline i.p.

(adapted from Kumar et al., Neuron 2005)

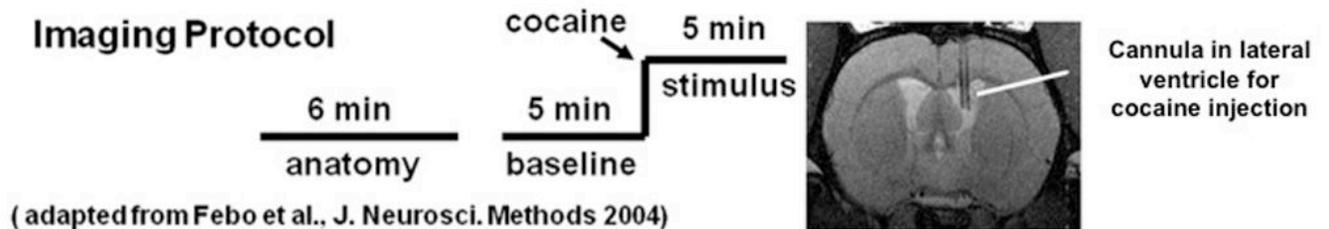


Figure 1.

Schematic diagram outlining drug treatment and imaging protocols. Imaging data acquired during treatment protocols shown in red are presented as activation maps in Fig 2.

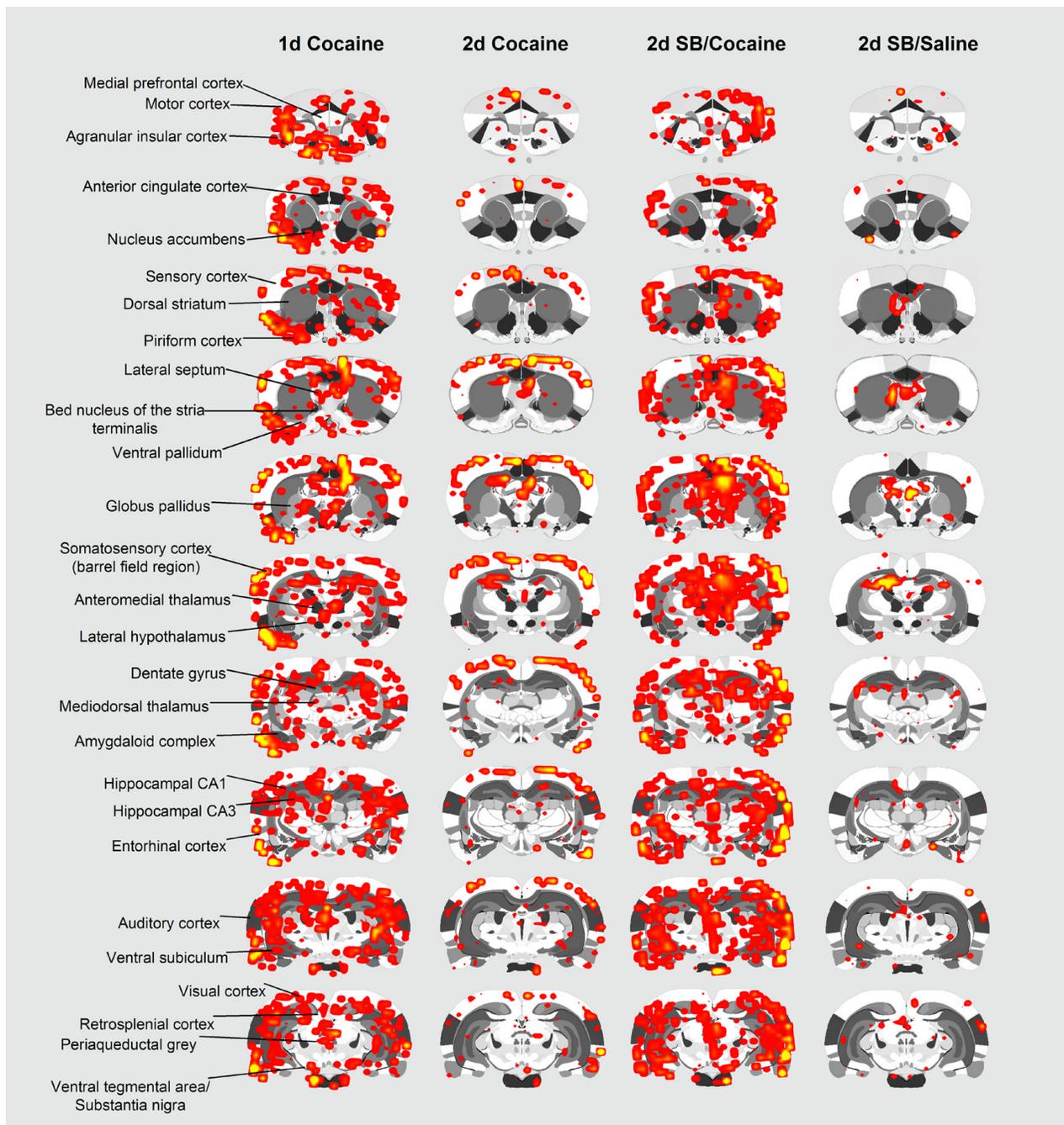


Figure 2.

BOLD signal changes in response to cocaine saline in rats pretreated with and without SBt. Shown are 2D rat atlas maps segmented into various regions of interest as indicated. Colored areas correspond to brain regions that showed statistically significant increases in BOLD signal intensity with cocaine administration ($p < 0.05$, corrected for multiple t -tests). $N = 6$ animals/group. Percent increase in BOLD ranged from 2% (red) to 11% (yellow). Notice pronounced metabolic activation after single dose of cocaine (day 1) and two daily doses of cocaine plus sodium butyrate (Cocaine 2day with SBt) but not two daily doses of (single drug) cocaine (Cocaine 2 day no SBt) or 2day SBt plus saline.

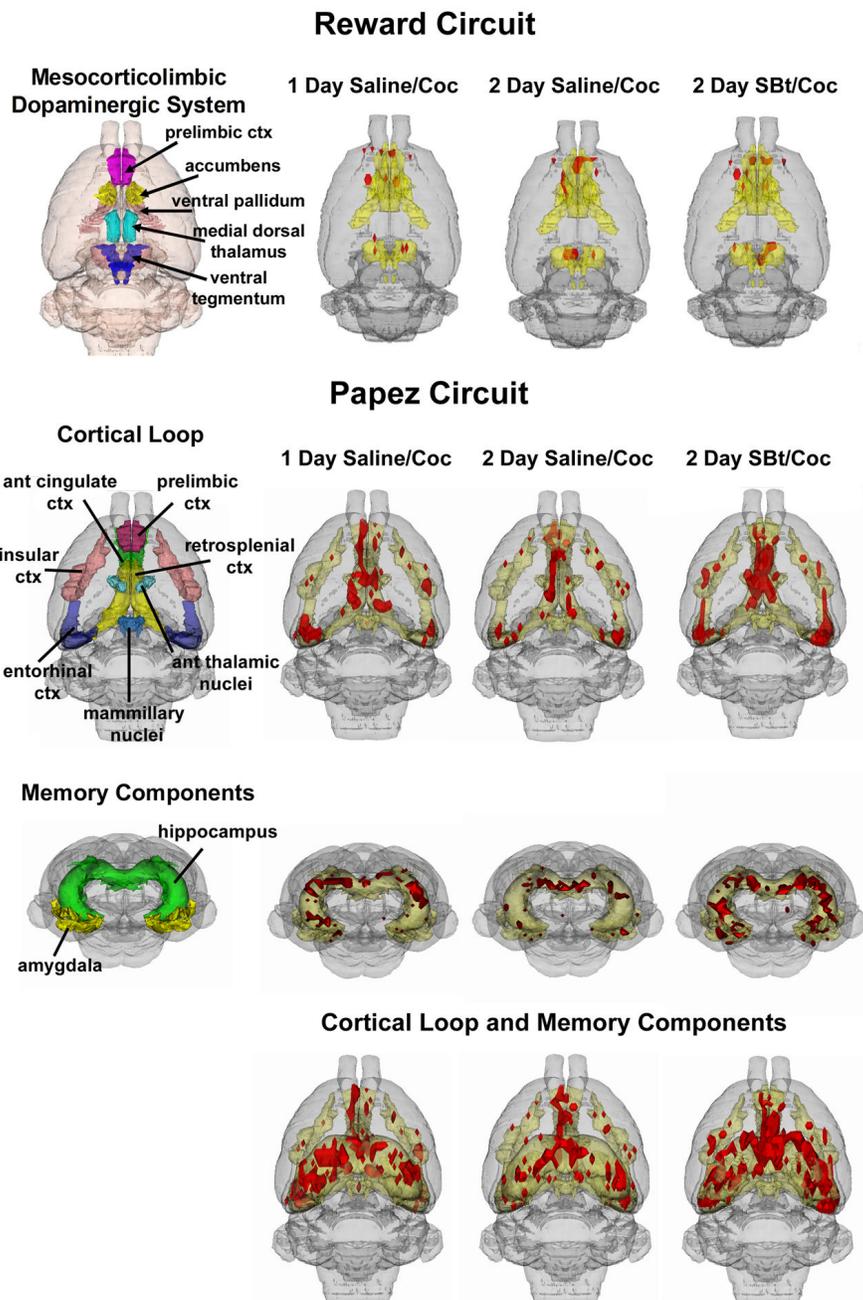


Figure 3. Three-dimensional atlas maps showing cocaine-induced voxel activations for the (top) mesocorticolimbic system and (bottom) cortico-thalamic and amygdala/hippocampal territories of the Papez circuit. The pictures show translucent shells of the brain viewed from a caudal/dorsal perspective. The red depicts the localization of activated voxels interpolated into a 3D volume of activation for the three cocaine treatment groups. The volumes of activation for each experimental condition are composed of six rats each. The volumetric data shown in the top panel denoted Reward Circuit and lower panel denoted Papez Circuit are localized to the different brain areas identified in the far left images. The geometric volumes constituting

the respective reward and Papez circuits identified in the pictures on the left have been melded into a single volume shown in yellow.

Table 1

Activated voxels in regions of interest of rats treated with chromatin sodium butyrate or vehicle prior to ICV cocaine.

Volume of Interest	1 Day Saline/Coc	2 Day Saline/Coc	2 Day SBT/Coc
somatosensory cortex	90 (44,115) *	98 (61,119) *	149 (105,222)
CA3 hippocampus	17 (10,34)	9 (1,17) *	20 (8,22)
CA1 hippocampus	17 (8,33)	8 (2,24) *	20 (15,64)
cortical n. amygdala	4 (3,15) *	7 (1,16) *	15.5 (3,29)
Septum	8 (2,14)	6 (0,10) *	14.5 (7,23)
anterior n. thalamus	0 (0,0)	1 (0,1) *	4.5 (0,3)

Data for voxel activations presented as median and minimum-maximum values in parenthesis. Asterisks denote significance for between groups using Neuman-Keuls multiple comparison test

* $p < 0.05$ compared to 2 Day SBT/Coc.