Published in final edited form as:
Pharmacol Biochem Behav. 2012 March ; 101(1): 174–180. doi:10.1016/j.pbb.2011.12.016.

CM156, a Sigma Receptor Ligand, Reverses Cocaine-Induced Place Conditioning and Transcriptional Responses in the Brain

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Abstract

Repeated exposure to cocaine induces neuroadaptations which contribute to the rewarding properties of cocaine. Using cocaine-induced conditioned place preference (CPP) as an animal model of reward, earlier studies have shown that sigma (σ) receptor ligands can attenuate the acquisition, expression and reactivation of CPP. However, the underlying molecular mechanisms that are associated with these changes are not yet understood. In the present study, CM156, a novel antagonist with high selectivity and affinity for σ receptors was used to attenuate the expression of cocaine-induced CPP in mice. Immediately following the behavioral evaluations, mouse brain tissues were collected and alterations in gene expression in half brain samples were profiled by cDNA microarray analysis. Microarray data was analyzed by three distinct normalization methods and four genes were consistently found to be upregulated by cocaine when compared to saline controls. Each of these gene changes were found by more than one normalization method to be reversed by at least one dose of CM156. Quantitative real time PCR confirmed that a single administration of CM156 was able to reverse the cocaine-induced increases in three of these four genes: metastasis associated lung adenocarcinoma transcript 1 (malat1), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein (ywhaz), and transthyretin (ttr). These genes are involved in processes related to neuroplasticity and RNA editing. The data presented herein provides evidence that pharmacological intervention with a putative σ receptor antagonist reverses alterations in gene expression that are associated with cocaine-induced reward.

Keywords

CM156; Gene Expression; Neuroadaptation; Place Conditioning; Plasticity; Sigma Receptor

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1. Introduction

Rewarding effects are important contributors to cocaine abuse and addiction (Dackis and O'Brien, 2001). To study the rewarding effects of drugs, conditioned place preference (CPP) and self administration are the most accepted animal models (Bardo and Bevins, 2000; Izhak and Martin, 2002; Sanchis-Segura and Spanagel, 2006). Of the two, CPP has the advantage of being simpler, faster and less invasive to implement. CPP is based on classical conditioning principles where a drug such as cocaine (unconditioned stimulus) is paired with a particular environment such as a distinctive section of a testing chamber (unconditioned stimulus) over a series of exposures. When subjects are subsequently allowed access to the conditioned environment in the absence of cocaine, they spend more time in that environment (vs. a neutral environment). Due to the tendency of animals in a drug-free state to spend more time in the environment in which they previously experienced a rewarding drug such as cocaine, CPP has also been postulated to model drug seeking behaviors (Bardo and Bevins, 2000; Tzschentke, 1998).

Cocaine-induced CPP is associated with numerous neuroadaptations, including alterations in gene and protein expression (Kalivas and O'Brien, 2008; McClung and Nestler, 2008; Robinson and Kolb, 2004). A recent study reported that 214 transcripts were differentially regulated in the hippocampus and 39 genes were differentially expressed in the frontal cortex after cocaine paired training using a CPP model (Krasnova et al., 2008). The genes that were identified are involved in many functions, including RNA processing, protein synthesis, and cytoskeletal organization (Krasnova et al., 2008). While it is well documented that persistent changes occur in the brain in response to repeated cocaine exposures, traditional medication development efforts have focused on manipulating early drug targets, rather than normalizing resulting neuroadaptations.

Sigma (σ) receptors have emerged as viable medication development targets that mitigate both the acute and subchronic effects of cocaine (Matsumoto, 2009). σ Receptors are structurally unique proteins that modulate many cellular activities, including neuroplasticity (Hayashi and Su, 2005; Takebayashi et al., 2002, 2004). There are at least two subtypes of σ receptors, designated σ-1 and σ-2, which are distinguished by their drug selectivity patterns, anatomical distribution, and molecular biological profiles (Hayashi and Su, 2005; Maurice et al., 2002). Cocaine has micromolar affinity for both subtypes of σ receptors (Matsumoto et al., 2002; Maurice et al., 2002). Pharmacological antagonists and antisense oligonucleotides for σ receptors attenuate many cocaine-induced effects in vivo including locomotor activity, convulsions, lethality and behavioral sensitization (Liu and Matsumoto, 2008; Matsumoto, 2009; Matsumoto et al., 2002; McCracken et al., 1999; Maurice et al., 2002).

The involvement of σ receptors in cocaine-induced CPP is also well established. σ Receptor antagonists and antisense oligonucleotides significantly attenuate the acquisition and expression of cocaine-induced CPP (Romieu et al., 2000, 2002). Neuroactive steroids also modulate cocaine-induced reward through σ receptors (Romieu et al., 2003). Further, σ receptor activation is a key step for the reactivation of cocaine-induced CPP by drug priming (Romieu et al., 2004).

In this paper, the feasibility of targeting σ receptors to mitigate the rewarding effects of cocaine is further investigated using a CPP model. Following the acquisition of cocaine-induced CPP, mice were treated with CM156 (3-(4-(4-cyclohexylpiperazin-1-yl)butyl)benzo[*d*]thiazole-2(3*H*)-thione), a σ receptor antagonist, on the post-conditioning day to determine whether it could attenuate the conditioned response. CM156 is a novel compound with high affinity for both subtypes of σ receptors; it is selective for σ receptors, exhibiting > 1,000-fold preference compared to nearly 80 other binding sites (Xu et al.,

2010). CM156 attenuates a number of cocaine-induced behaviors: convulsions, locomotor activity, the expression of behavioral sensitization and the expression of cocaine-induced CPP (Xu et al., 2010). The mechanisms however, by which σ ligands are able to attenuate many cocaine-induced behaviors have yet to be elucidated. The ability of CM156 to reverse cocaine-induced behaviors such as the expression of behavioral sensitization and CPP makes it an ideal tool for the study of the molecular mechanisms by which σ receptor ligands reverse cocaine-induced behavioral changes. The current study utilized cocaine-induced CPP coupled with microarray analysis to identify genetic changes in the brain that may be responsible for these behavioral adaptations and which of these genetic changes are reversed by treatment with the σ receptor ligand CM156.

2. Materials and methods

2.1. Experimental subjects

Male, C57/BL mice (21–26 g, Jackson Laboratories, Bar Harbor, ME) were used for all studies. The mice were housed in groups of four and maintained in a 12:12 light:dark cycle with food and water available *ad libitum*. The procedures were conducted as approved by the Institutional Animal Care and Use Committees at the University of Mississippi and West Virginia University.

2.2. Drugs and chemicals

CM156 was obtained from Dr. Christopher R. McCurdy (University of Mississippi, University, MS), following synthesis as previously described (Mesangeau et al., 2008). Cocaine hydrochloride was obtained from Sigma Chemical Company (St. Louis, MO).

2.3. Behavioral pharmacology

The conditioning chambers were made of Plexiglas (45 × 24 × 20.5 cm) painted gray on one half, and black with white vertical stripes on the other half. The flooring consisted of smooth Plexiglas in the gray half of the chamber and textured plastic matting in the striped side of the chamber. The movements of the mice were recorded using an automated video tracking system (SMART, San Diego Instruments, San Diego, CA). Pre-conditioning, conditioning, and post-conditioning sessions were conducted.

During the pre-conditioning session, mice were allowed access to the entire chamber for 30 min. The time spent on each half of the chamber was measured to confirm that they did not have a preference for a particular side. The criterion for an unbiased response and to proceed to the conditioning phase was less than 2/3 of the time spent on a particular side of the chamber.

During the conditioning sessions, the mice received cocaine (20 mg/kg, i.p.) or saline (vehicle control) and were subsequently confined to one half of the chamber for 30 min; on alternate sessions the mice received saline and were confined to the other half of the chamber. The cocaine dose was selected based on earlier dose response characterizations that showed robust place conditioning with 20 mg/kg, i.p. cocaine (Xu et al., 2010). The mice received a total of eight pairings during the conditioning phase (four cocaine or vehicle control, and four saline sessions). The side of the chamber in which the animals received cocaine was assigned randomly and counterbalanced.

During the post-conditioning session, mice were allowed access to the entire chamber for 30 min and the time spent in each half was recorded. For the expression of place conditioning studies, saline or CM156 (0–20 mg/kg, $N = 4$/group) was administered 15 min before the post-conditioning session. The conditioned score was calculated by subtracting the pre-

conditioning time from the post-conditioning time on the side of the chamber in which the mice were trained to cocaine during the conditioning phase.

The data were evaluated using analysis of variance (ANOVA), followed by post-hoc testing with Tukey's multiple comparisons tests. The statistical comparisons were used to confirm that the group means were representative of previous results in our lab involving larger sample sizes (Xu et al., 2010). This was done to ensure that no outlier samples were included in the microarray analyses.

2.4. Microarray

Immediately after completion of the behavioral measurements in the post-conditioning session, animals were sacrificed and half brain samples were frozen in liquid nitrogen for later analysis. Half brain samples were analyzed instead of specific brain regions for several reasons. First, the compounds were administered systemically, thereby affecting the entire brain to produce its effects. Second, although various brain regions have been shown to influence different aspects of the response to cocaine, there is no data showing that pharmacological interventions can reverse cocaine-induced changes in gene expression in the brain, making a priori selection of a specific brain region to focus on a risk. Therefore, as a first step, half brain was used to evaluate whether reversal of cocaine-induced changes in gene expression were achievable, with the analyses biased toward the identification of the largest, most robust alterations.

Total RNA was extracted from the half brain samples, concentrations were quantified by spectral absorption, and the purity of the samples checked to confirm that the 260/280 ratio was in the range of 1.8–2.0. The integrity of 18s and 28s rRNA was confirmed by electrophoresis. Samples of cDNA were prepared by reverse transcription. The microarray hybridization was conducted using GeneChip Mouse Genome 430 2.0 arrays (Affymetrix, Santa Clara, CA) and the data were generated using Affymetrix GeneChip Operating Software (Affymetrix, Santa Clara, CA).

Data generated from the microarrays were normalized by three distinct methods: PLIER (probe logarithmic intensity error), MAS 5.0. (Affymetrix microarray analysis suite), and RMA (robust multichip average) with and without GC correction. Normalized data were then analyzed using Partek Genomics Suite (St. Louis, MO). ANOVA and contrast analyses were performed using the following criteria to determine statistical significance: 1) overall analysis of variance, $P \leq 0.05$, 2) t-test saline vs. cocaine group, $P \leq 0.05$ and 3) t-test CM156 vs. cocaine group, $P \leq 0.05$.

Four genes from these analyses were selected for further study. These four genes were all found to be altered by cocaine administration when compared to saline in each of the three normalization methods. In addition, the cocaine-induced upregulation of these four genes were all found to be reversed by at least one dose of CM156 in two or more normalization methods.

In addition, hierarchical clustering was performed on the data by standardizing $\log_2$ signal values by row mean centering and scaling to root means. Unsupervised hierarchical clustering of genes and samples were achieved with UPGMA (Unweighted Pair-Group Method using Arithmetic Averages) using Pearson correlation distance as the similarity metric.

Principal component analysis was also performed using the four significant microarray genes that were altered by cocaine and reversed by CM156. Principal component analysis can reduce a complex dataset to a lower dimension to reveal hidden, simplified structures in

the data, with the principal components representing the variability in gene expression levels observed in the dataset.

2.5. Quantitative real time PCR

The same samples of total RNA that were used for the microarray studies were also employed for the quantitative real time PCR confirmations. The concentrations and quality of RNA in each sample were re-checked as above. First strand cDNA synthesis was performed using a high-capacity cDNA reverse transcription kit according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). The reaction included MultiScribe TM Reverse Transcriptase and random primers, with thermal cycler conditions as follows: step 1 at 25°C for 10 min, step 2 at 37°C for 120 min, step 3 at 85° C for 5 s, and step 4 at 4° C for 10 min.

For the quantitative real time PCR amplification, TaqMan® Universal PCR Master Mix and the following primers were obtained from Applied Biosystems (Foster City, CA): transthyretin (Ttr; Mm0044327_m1), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (Ywhaz; Mm01158417_g1), metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA) (Malat1; Mm01227912_s1), and splicing factor, arginine/serine-rich 18 (Sfrs18; Mm01219232_m1 and two Sfrs18-ANY from Custom TaqMan® Gene Expression Assays Service). The primers were tested against 18s rRNA (Hs03003631_g1) as the endogenous control. The reaction mixture was prepared according to the manufacturer's instructions, with the following thermal cycling conditions: initial holding at 50°C for 2 min which is required for optimal AmpErase® UNG activity, followed by a first denaturing step at 95°C for 10 min, then 40 cycles at 95°C for 15 s, and at 60°C for 1 min.

The data from the quantitative real time PCR measurements were calculated using the ΔΔCt method. The threshold value was set at 0.2 and the threshold cycle (Ct value) of each gene was normalized to 18s rRNA. The calculated fold change in the expression of each gene was analyzed using ANOVA (GraphPad Prism, San Diego, CA) to determine statistical significance; post-hoc analyses were performed using Tukey's multiple comparisons tests.

3. Results

3.1. CM156 attenuates the expression of cocaine-induced conditioned place preference

ANOVA confirmed a significant difference between the experimental groups ($F[4,15] = 23.19$; $P < 0.0001$). Post-hoc comparisons using Tukey's multiple comparison tests confirmed that cocaine produced significant CPP in the animals (Fig. 1; $q = 13.48$; $P < 0.001$). For those animals receiving CM156 on the post-conditioning day, Tukey's post-hoc tests revealed that each dose produced a significant decrease in the expression of cocaine-induced CPP: 1 mg/kg CM156 ($q = 5.43$; $P < 0.05$), 10 mg/kg CM156 ($q = 6.40$; $P < 0.01$), 20 mg/kg CM156 ($q = 7.27$; $P < 0.001$) (Fig. 1).

3.2. Cocaine-induced changes in gene expression are reversed by CM156

ANOVA analysis performed on normalized microarray data identified 222 genes by the PLIER method, 182 genes by the MAS method, and 14 genes by the RMA method (Fig. 2). The following four genes were common to the datasets of all three normalization methods: transthyretin (ttr), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (ywhaz), metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA) (malat1), and splicing factor, arginine/serine-rich 18 (sfrs18).

Contrast analysis for malat1, ywhaz, ttr and sfrs18 prior to ANOVA analysis revealed significant differences between saline controls and cocaine-induced CPP animals in these four genes using all three normalization methods ($P < 0.05$, Sal/Sal vs. Coc/Sal). Contrast analysis also revealed that the cocaine-induced upregulation of these four genes was significantly reversed by at least one dose of CM156 using at least one normalization method ($P < 0.05$, Coc/Sal vs. Coc/CM156 dose).

The reversal of cocaine-induced upregulation of the four significant genes (malat1, ywhaz, ttr and sfrs18) by CM156 is summarized in Table 1. CM156 1 mg/kg treatment reversed the cocaine-induced increase in the expression of only malat1 (MAS 5.0, $P < 0.05$). CM156 10 mg/kg treatment resulted in the reversal of malat1 (GC-RMA, $P < 0.005$; MAS 5.0, $P < 0.05$), ywhaz (PLIER, $P < 0.05$), sfrs18 (GC-RMA, $P < 0.001$; MAS 5.0, $P < 0.001$) and ttr (MAS 5.0, $P < 0.001$; PLIER, $P < 0.01$) through the use of at least one normalization method. CM156 20 mg/kg treatment was found to reverse the upregulation of malat1 (GC-RMA, $P < 0.01$), ywhaz (GC-RMA, $P < 0.01$; MAS 5.0, $P < 0.01$; PLIER, $P < 0.05$) and ttr (MAS 5.0, $P < 0.0005$; PLIER, $P < 0.05$).

Principal component analysis of the data from the significant genes is illustrated in Fig. 3. This analysis takes into account genetic changes in each animal from the respective groups. The four animals from each respective group tend to appear in distinct clusters corresponding to their treatment group (i.e. all the cocaine animals cluster together, all of the saline animals form another distinct cluster, etc.). This is indicative of similar patterns of genetic changes occurring in each treatment group. As seen in Fig. 3, increasing doses of CM156 (light to dark blue dots) on the post-conditioning day tends to shift the animals from having a cocaine-like to a more saline-like genetic profile.

3.3. Quantitative real time PCR confirmation of select genes

Changes in three of the four significant genes identified in the microarray analyses were confirmed by quantitative real time PCR (Fig. 4): malat1, ywhaz, and ttr. For malat1, ANOVA revealed a significant difference between the groups ($F[4,35] = 12.51$; $P < 0.0001$), with Tukey's post-hoc multiple comparison tests confirming significant differences between the following key groups: saline vs. cocaine ($q = 8.93$; $P < 0.001$), cocaine vs. 10 mg/kg CM156 ($q = 7.78$; $P < 0.001$), cocaine vs. 20 mg/kg CM156 ($q = 5.17$; $P < 0.01$). For ywhaz, ANOVA confirmed a significant difference between the experimental groups ($F[4,35] = 12.48$; $P < 0.0001$), with post-hoc analysis showing significant differences between the following groups: saline vs. cocaine ($q = 7.99$; $P < 0.001$), cocaine vs. 10 mg/kg CM156 ($q = 6.44$; $P < 0.001$), cocaine vs. 20 mg/kg CM156 ($q = 4.95$; $P < 0.05$). For ttr, ANOVA demonstrated an overall significant difference between the groups ($F[4,35] = 14.50$; $P < 0.0001$), with post-hoc comparisons showing differences between the following key pairs: saline vs. cocaine ($q = 9.09$; $P < 0.001$), cocaine vs. 10 mg/kg CM156 ($q = 4.28$; $P < 0.05$), cocaine vs. 20 mg/kg CM156 ($q = 6.62$; $P < 0.001$). Utilizing three distinct primer sets, the changes in sfrs18 were unable to be confirmed using quantitative real time PCR: primer set 1 ($F[4,35] = 0.79$; n.s.; shown in Fig. 4), primer set 2 ($F[4,15] = 0.49$; n.s.), primer set 3 ($F[4,15] = 1.05$; n.s.).

4. Discussion

In this study, CM156, a novel compound with high affinity and selectivity for both subtypes of σ receptors was used to block the expression of cocaine-induced CPP. Microarray analysis of half brain samples revealed that a single administration of CM156 resulted in the reversal of four genes: malat1, ywhaz, ttr and sfrs18. Quantitative real time PCR confirmed that three of these four genes, malat1, ywhaz and ttr, were reversed with CM156 treatment. All of these genes are related to either cellular plasticity or RNA processing, suggesting that

CM156 has the potential to convey persistent normalizing effects on maladaptive changes in nervous system function conferred through repeated cocaine exposures.

Half brain samples were used for analysis in this study to enable the identification of large changes in the brain that occurred in response to systemically administered drugs (cocaine and CM156). Since knowledge about the brain regions that may be involved in the reversal process and restoration of normal brain function following cocaine-induced CPP are currently incomplete, the entire brain was profiled to detect the most significant changes which could then form the basis for future studies.

Malat1 is non-coding RNA that is highly expressed in many types of cancer and also in normal cells (Guffanti et al., 2009; Ji et al., 2003). Although not much is known about the role of malat1 in the brain, there is increasing information on the function of non-coding RNA. Traditionally, it was assumed that most genetic information encodes proteins. However, recent evidence suggests that the majority of the mammalian genome is transcribed into non-coding RNAs that can regulate gene expression through various mechanisms including RNA splicing, editing, translation and turnover, gene silencing, imprinting, and DNA demethylation (Costa, 2005; Mattick and Makunin, 2006; Mehler and Mattick, 2006; Szymanski et al., 2003). They perform critical functions during development, cell differentiation, nervous system function, and neurological diseases (Costa, 2005; Mehler and Mattick, 2006). Thus, malat1 may play a role in the modulation of gene expression in response to cocaine and environmental cues.

The ywhaz gene encodes tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide. Ywhaz is an adaptor molecule that binds to phosphoproteins involved in signaling, protein folding, ubiquitin mediated proteolysis, and energy metabolism; it has also been implicated in plasticity in the central nervous system (Gouraud et al., 2007; Puri et al, 2008). Therefore, similar to the ttr gene discussed below, ywhaz may serve as a target through which cocaine induces neuroadaptions. Moreover, it appears to be highly and quickly responsive to pharmacologic intervention with CM156, reverting toward normal levels after only a single exposure.

The gene ttr encodes transthyretin (Ttr) protein which is synthesized by epithelial cells in the choroid plexus of the central nervous system. It is involved in the transport of thyroid hormones and retinoic acid from the blood into the brain and cerebrospinal fluid (Richardson, 2007). The increased expression of ttr suggests increased capacity of Ttr protein to transport thyroid hormones and retinoic acid into the brain, where they have critical roles in neural plasticity (Bernal and Nunez, 1995; McCaffery et al., 2006; Mey and McCaffery, 2004; Smith et al., 2002). The receptors of thyroid hormones and retinoic acid are nuclear receptors that function as ligand-inducible transcription factors (Umesono et al., 1988). Thyroid hormones control the expression of genes which regulate different forms of synaptic plasticity and memory formation (Rivas and Naranjo, 2007), whereas thyroid dysfunction in adults can cause deficits in brain function and cognition (Smith et al., 2002). Similarly, retinoic acid influences neural plasticity in the adult brain through processes such as long term potentiation, long term depression, neurite outgrowth, and neurogenesis (McCaffery et al., 2006).

In an attempt to confirm changes in sfrs18, three pairs of primers were tested and failed to confirm any changes in the gene in response to either cocaine treatment or by the administration of CM156. One potential reason for this is the presence of multiple variants of the sfrs18 gene. Nevertheless, the overall pattern of results suggests that sfrs18 gene expression is not a significant factor in cocaine-induced CPP or the ability of σ receptor ligands to attenuate these behaviors.

Many investigations have shown that repeated exposures to cocaine in CPP result in behavioral changes which are mediated by neuroadaptations in the brain reward system (Hyman et al., 2006). This is a complex process, with neuroadaptations occurring at different levels such as alterations in gene expression, synaptogenesis, synaptic plasticity, and structural reorganization (Hyman et al., 2006; Kalivas and O'Brien, 2008; McClung and Nestler, 2008; Robinson and Kolb, 2004). The regulation of malat1, ywhaz and ttr are consistent with such studies in that these genes have functional consequences on central nervous system targets which can drive neural plasticity. The ability of CM156 after a single administration to reverse these genetic alterations may be a potential mechanism by which CM156 attenuates the expression of cocaine-induced CPP.

A potential limitation of this study is that the changes in gene expression were not confirmed at the protein level. Unfortunately, commercially available antibodies suitable for Western blots are not available for the significant genes that were identified. The only commercially available antibody is for Ttr; however this antibody provided no signal in brain tissue, although robust labeling could be detected in blood serum and plasma (data not shown). Although no splice variants of ttr are known, the protein can be post-translationally modified in several ways through a single cysteine (Kingsbury et al., 2007), possibly affecting antibody reactivity specifically in brain samples. Alternately, because changes in genes such as malat1 were noted, it is possible that the most significant alterations do not occur at the protein level, and treatments that instead target RNA may offer improved therapeutic outcomes. The ability of CM156 to reverse both the behavioral effects of cocaine and genetic alterations involved in RNA editing suggest that RNA editing may play a role in the subchronic behavioral effects of cocaine. Future studies may provide evidence that specifically targeting RNA or processes related to the post-transcriptional editing of RNA may be one way of reversing neuroplastic changes that have occurred as a result of cocaine usage.

A very conservative analysis approach was used in this study to identify significant changes, and clearly other genes are also influenced by cocaine and σ ligands. However, usage of a conservative approach has allowed the identification of plasticity-related processes which have previously gone unidentified as being important targets for the putative σ receptor antagonist CM156 in an animal model of reward and drug seeking. Specifically, data presented in this study is the first to show that treatment with a σ receptor ligand, CM156, is able to reverse genetic alterations that have occurred in a behavioral reward model with cocaine. It also reinforces the hypothesis that pharmacological intervention after repeated cocaine exposures can normalize brain function and behavior. Additional studies to further characterize these processes are warranted and suggest a novel therapeutic strategy for the treatment of cocaine abuse.

Acknowledgments

We appreciate the technical expertise of Dr. Jamaluddin Shaikh (University of Mississippi, Oxford, MS) during the behavioral studies and Dr. Meenal Elliott (West Virginia University, Morgantown, WV) for performing Western blots. These studies were supported by grants from the National Institute on Drug Abuse: DA023205 for the synthesis of CM156, and DA011979 for other aspects of the study. Some of the microarray data analysis was performed using Partek Genomics Suite that was purchased by INBRE grant P20RR016477. The funding sources were not involved in the conduct of the research; preparation of the article; study design; collection, analysis and interpretation of the data; writing of the report; or decision to submit the paper for publication.

Abbreviations

ANOVA analysis of variance

CM156	3-(4-(4-cyclohexylpiperazin-1-yl)butyl)benzo[d]thiazole-2(3H)-thione
CPP	conditioned place preference
Ct	threshold cycle
malat1	metastasis associated lung adenocarcinoma transcript 1
MAS 5.0	Affymetrix microarray analysis suite
PLIER	probe logarithmic intensity error
GC-RMA	GC corrected robust multichip average
RMA	robust multichip average
sfrs18	splicing factor, arginine/serine-rich 18
ttr	transthyretin
UPGMA	Unweighted Pair-Group Method using Arithmetic Averages
ywhaz	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein

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Highlights

- CM156 prevents the expression of cocaine-induced conditioned place preference.
- CM156 reverses cocaine-induced changes in the expression of select genes.
- Cocaine-induced genes that can be reversed by CM156 are involved in processes related to plasticity and RNA editing.

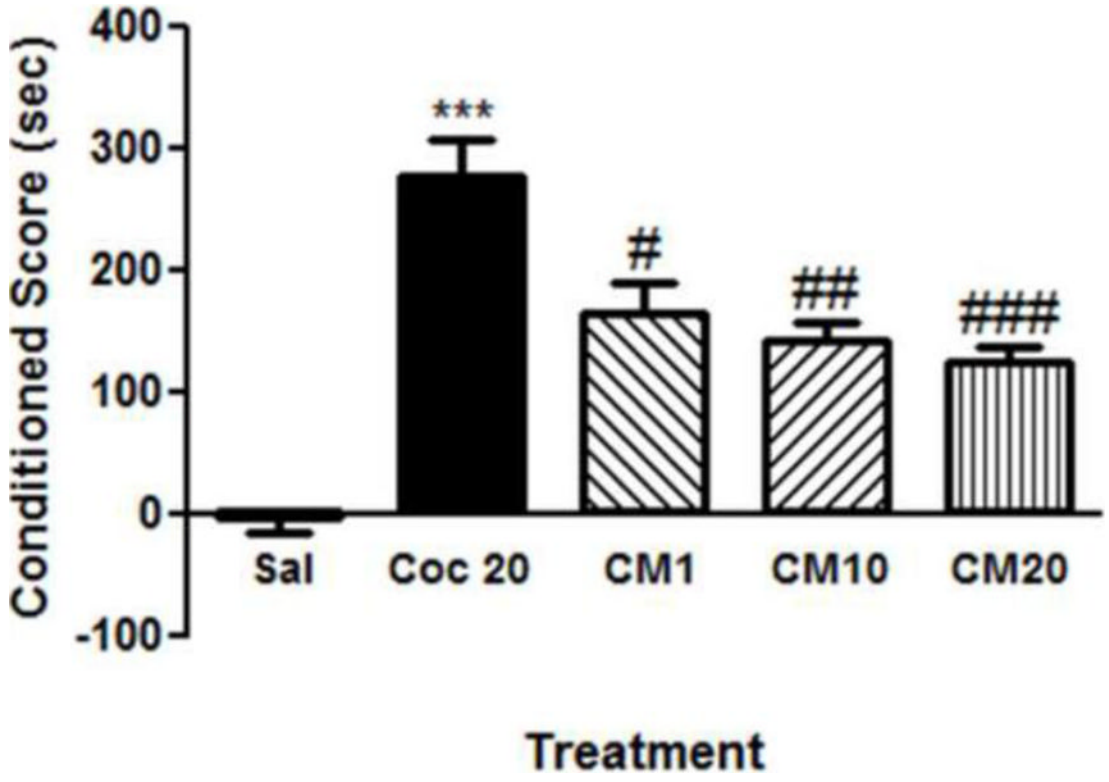

Fig. 1.

Attenuation of the expression of cocaine-induced conditioned place preference by CM156. Male, C57/BL mice were injected (i.p.) with saline (Sal) or cocaine (Coc) during the conditioning sessions. On the post-conditioning day, the mice received CM156 (0–20 mg/kg, i.p.) 15 min prior to the behavioral testing session. Cocaine produced robust CPP (*** $P < 0.001$, saline vs. cocaine). All of the tested doses of CM156 significantly attenuated the expression of cocaine-induced CPP (# $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ CM156 (1–20 mg/kg) vs. cocaine group). CM1 = 1 mg/kg CM156, CM10 = 10 mg/kg CM156, CM20 = 20 mg/kg CM156.

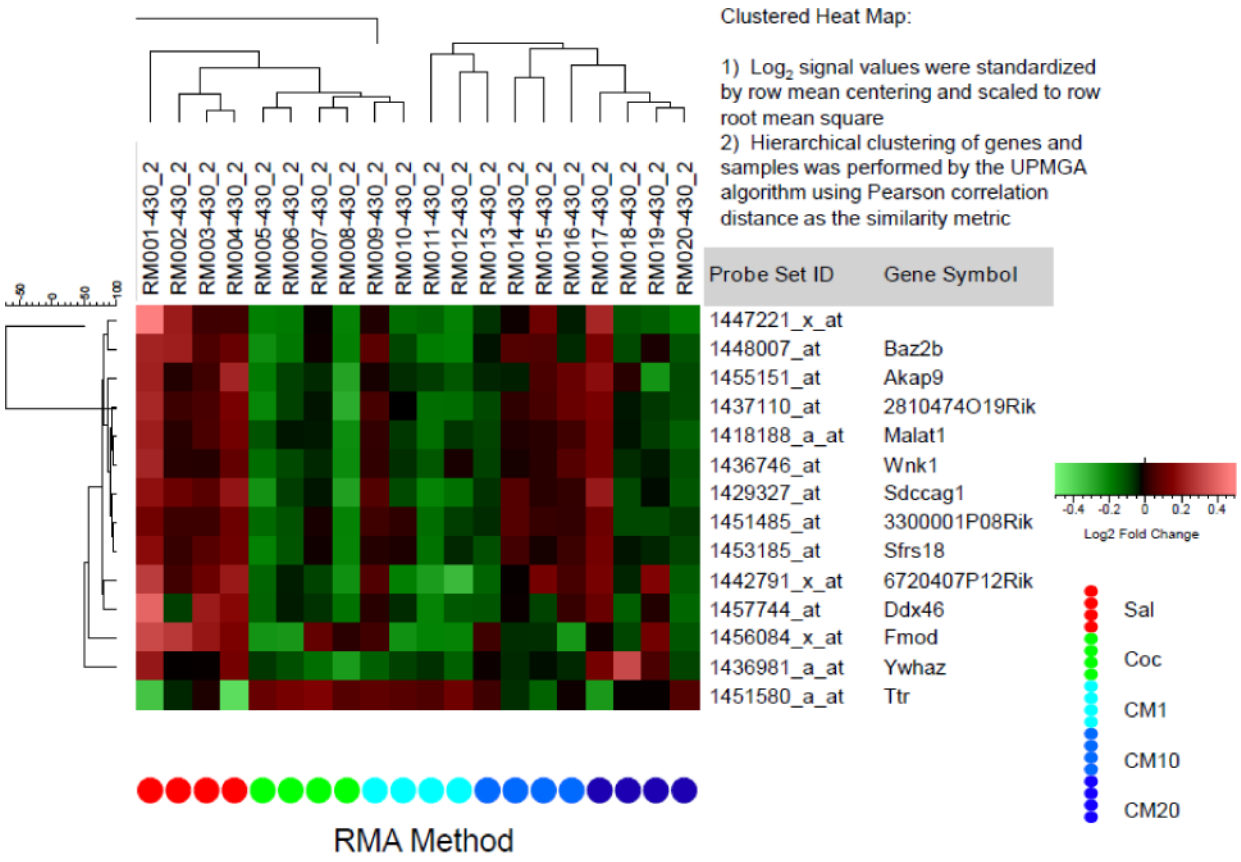

Fig. 2.

Heat map of microarray data using the RMA normalization method. Red dots = saline, green dots = cocaine, light blue dots = 1 mg/kg CM156, medium blue dots = 10 mg/kg CM156, dark blue dots = 20 mg/kg CM156.

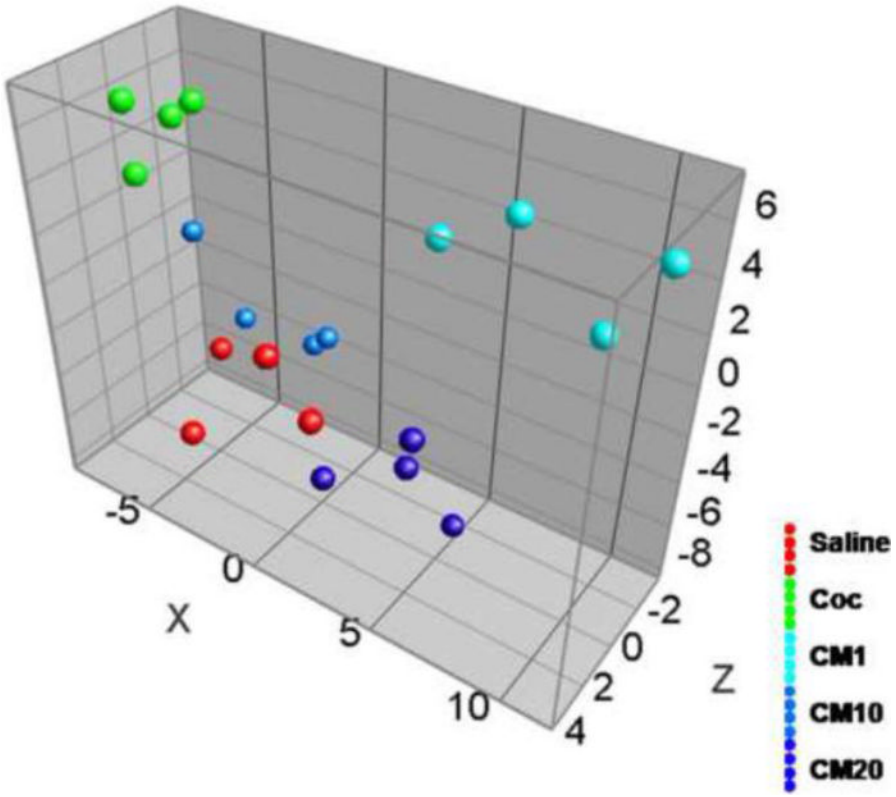

Fig. 3.

Principal component analysis of significant microarray genes. The principal components represent the variability in gene expression levels observed within the dataset, with the top three principal components (X, Y, Z) used to generate the 3-dimensional graph shown. Red dots = saline, green dots = cocaine (20 mg/kg), light blue dots = CM1 (1 mg/kg CM156), medium blue dots = CM10 (10 mg/kg CM156), dark blue dots = CM20 (20 mg/kg CM156). Each dot represents an animal from each respective treatment group. The analysis uses data from the PLIER microarray normalization method. Saline and cocaine treatments are clustered in distinct groupings. With increasing doses of CM156 on the post-conditioning day, the animals display a profile more similar to saline treated animals as opposed to cocaine animals. This is reflective of CM156 treatment reversing genetic changes that occurred as a result of cocaine treatments.

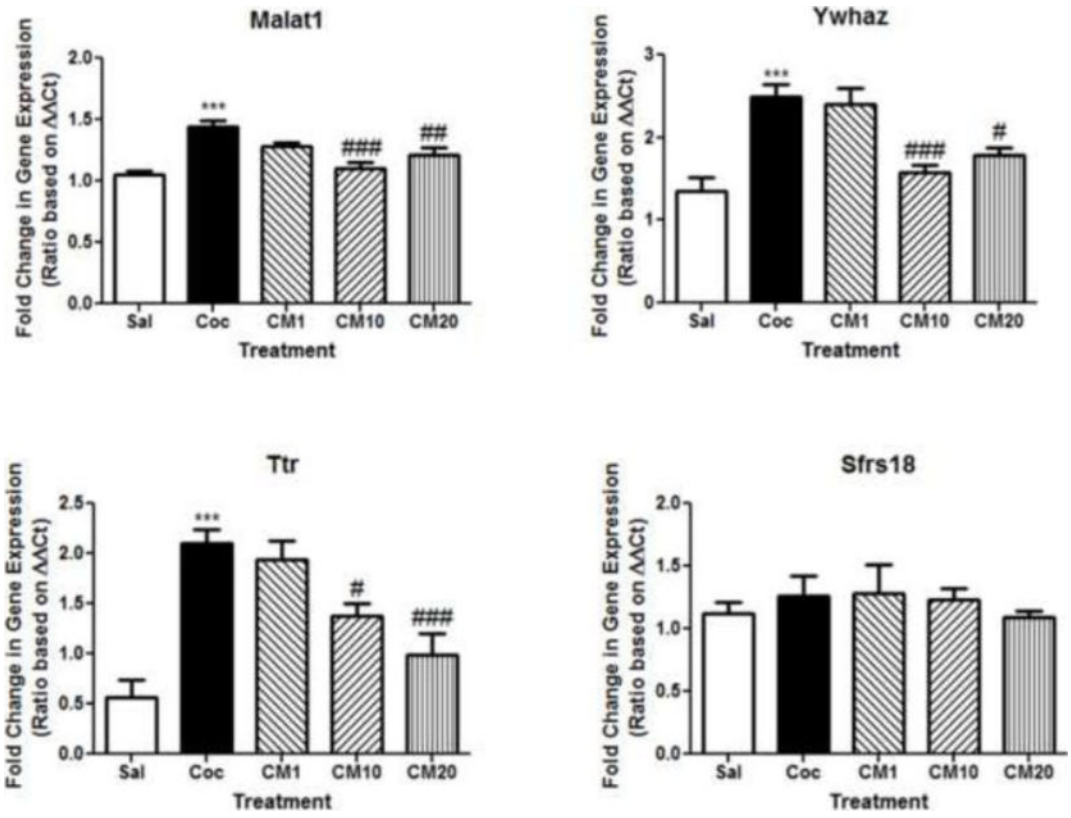

Fig. 4.

Quantitative real time PCR confirmed changes in three of four genes: malat1, ywhaz, ttr. *** $P < 0.005$; saline (Sal) vs. cocaine (Coc) in post-hoc comparisons. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ CM (1–20 mg/kg) vs. cocaine (Coc) in post-hoc comparisons. CM1 = 1 mg/kg CM156, CM10 = 10 mg/kg CM156, CM20 = 20 mg/kg CM156.

Table 1

Statistics summary of genes from microarray selected for quantitative real time PCR confirmation

Gene Name	Overall ANOVA	Sal/Sal vs. Coc/Sal	Coc/Sal vs. Coc/CM(1)	Coc/Sal vs. Coc/CM(10)	Coc/Sal vs. Coc/CM(20)
malat1					
GC-RMA	*P = 0.00568*	*P = 0.00239*	n.s.	*P = 0.00263*	*P = 0.00905*
MAS 5.0	*P = 0.01572*	*P = 0.00418*	*P = 0.02396*	*P = 0.03130*	n.s.
PLIER	*P = 0.03390*	*P = 0.01395*	n.s.	n.s.	n.s.
ywhaz					
GC-RMA	*P = 0.01983*	*P = 0.01396*	n.s.	n.s.	*P = 0.00524*
MAS 5.0	*P = 0.02396*	*P = 0.02786*	n.s.	n.s.	*P = 0.00563*
PLIER	*P = 0.01497*	*P = 0.01698*	n.s.	*P = 0.03318*	*P = 0.04229*
sfrs18					
GC-RMA	*P = 0.00220*	*P = 0.00945*	n.s.	*P = 0.00063*	n.s.
MAS 5.0	*P = 0.01020*	*P = 0.03345*	n.s.	*P = 0.00063*	n.s.
PLIER	*P = 0.03821*	*P = 0.00773*	n.s	n.s.	n.s.
ttr					
GC-RMA	*P = 0.01193*	*P = 0.00164*	n.s.	n.s.	n.s.
MAS 5.0	*P = 0.00009*	*P = 0.00002*	n.s.	*P = 0.00022*	*P = 0.00024*
PLIER	*P = 0.01054*	*P = 0.02453*	n.s.	*P = 0.00789*	*P = 0.04708*

[a]Sal = saline; Coc = cocaine (20 mg/kg); CM(1) = CM156 (1 mg/kg); CM(10) = CM156 (10 mg/kg); CM(20) = CM156 (20 mg/kg); n.s. = not significant ($P > 0.05$)