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Cocaine hydrolase encoded in viral vector blocks the reinstatement of cocaine seeking in rats for 6 months

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Abstract

Background—Cocaine dependence is a pervasive disorder with high rates of relapse. In a previous study, direct administration of a quadruple mutant albumin-fused butyrylcholinesterase (BChE) that efficiently catalyzes hydrolysis of cocaine to benzoic acid and ecgonine methyl ester acutely blocked cocaine seeking in an animal model of relapse. In the present experiments these results were extended to achieve a long duration blockade of cocaine seeking with a gene transfer paradigm using a related BChE-based cocaine hydrolase, termed “CocH”.

Methods—Male and female rats were allowed to self-administer cocaine under a fixed-ratio 1 schedule of reinforcement for approximately 14 days. Following the final self-administration session, rats were injected with CocH vector or a control injection (empty vector or saline), and their cocaine solutions were replaced with saline for 14 days to allow for extinction of lever pressing. Subsequently, they were tested for drug-primed reinstatement by administering i.p. injections of saline (S), cocaine (5, 10, and 15 mg/kg, C), and d-amphetamine (A) according to the following sequence: S, C, S, C, S, C, S, A. Rats then received cocaine-priming injections once weekly for 4 weeks, and subsequently, once monthly for up to 6 months.

Results—Administration of CocH vector produced substantial and sustained CocH activity in plasma that corresponded with diminished cocaine- (but not amphetamine-) induced reinstatement responding for up to 6 months following treatment (compared to high responding controls).

Conclusion—These results demonstrate that viral transfer of CocH may be useful in promoting long-term resistance to relapse to cocaine addiction.

Keywords

Addiction; Cocaine; Cocaine hydrolase; Butyrylcholinesterase; Gene therapy; Relapse; Prevention

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Introduction

Cocaine dependence is a persistent, pervasive, chronically-relapsing disorder for which there is currently no FDA-approved pharmacotherapy. A high rate of relapse with serious adverse social and medical consequences is a hallmark of cocaine abuse (1, 2) and a prime target for intervention. A novel approach to addressing this problem involves interference with cocaine pharmacokinetics through the use of protein therapeutics. Butyrylcholinesterase (BChE) is a naturally occurring enzyme that metabolizes cocaine in human blood plasma (3), and it has been pursued as a potential treatment for cocaine addiction and overdose (4). The catalytic efficiency of native BChE is far too low to be a viable treatment for cocaine addiction, but mutagenesis efforts have greatly increased this property and have led to enzymes that work several hundred times better (5, 6). In previous studies we have shown that the direct administration of a BChE-based cocaine hydrolase abolished cocaine-induced seizures and lethality in a model of overdose (7), temporarily reduced motivation to seek cocaine, as assessed with a progressive-ratio (PR) schedule (8), and acutely blocked cocaine's priming effect in an animal model of relapse (reinstatement) (7).

These results led us to hypothesize that gene transfer of a similar cocaine hydrolase (30), termed "CocH", might produce a long term reduction in the reinstatement of cocaine seeking and prove useful in relapse prevention. We had already established that a single injection of helper-dependent adenoviral vector (hdAD) incorporating CocH cDNA can sustain high plasma levels of cocaine hydrolase activity in rats for several months (9). Our current objective was to use a rat model of reinstatement to explore the effectiveness and duration of this CocH transduction. In particular we set out to determine whether, and how long, an i.v. delivery of hdAD CocH (10^{11} viral particles) would block reinstatement of cocaine seeking when rats trained to self-administer cocaine, and then denied drug access during a 2 week extinction period were later given periodic cocaine-priming injections (10 mg/kg) that occurred throughout a 6 month testing period. Serum levels of CocH were analyzed at multiple points during reinstatement testing to investigate the relationship between effects on behavior and corresponding levels of enzyme expression.

To evaluate the specificity of CocH vector effects and detect any consequences that might have reduced reward-driven behavior in general, we included several control conditions. First were groups that received "empty vector" (no encoded enzyme) or saline solution in place of CocH vector. Next were conditions in which the priming injection of cocaine was replaced with d-amphetamine, a drug that elicits reinstatement responding but is not affected by CocH. Third, monthly extinction sessions were conducted to assess spontaneous recovery of reinstatement responding and were used to test for a potential general suppressant effect of the CocH vector. In this case, rats housed in the home cage for some time after treatment (see Methods), were returned to the self-administration chamber, and that typically elicits an initial burst of responding. A final control condition determined whether CocH vector would alter locomotor activity in an open field. Sex-balanced designs were used throughout, as some studies modeling several phases of the addiction process indicated that female rats and monkeys showed more drug-seeking and drug-taking (10) and responded better to treatment than males (11).

Materials and Methods

Subjects

A total of 36 rats were used in this study. Sixteen female (250 to 300 g) and 24 male (350 to 400 g) 90-day-old Wistar rats (Harlan-Sprague Dawley, Madison, WI) were used in an experimental design that consisted of 6 groups. An additional 7 male rats (3 CocH vector treated and 4 controls) were added to a control condition to examine the effects of CocH

vector on open-field locomotor activity. The experimental protocol (1008A87756) was approved by the University of Minnesota Institutional Care and Use Committee, and the experiments were conducted in accordance with the Principles of Laboratory Animal Care (18) in a laboratory accredited by the American Association for the Accreditation of Laboratory Animal Care.

Self-Administration Apparatus

Following the catheterization surgery rats were housed in octagonal operant conditioning chambers that contained Plexiglas walls alternating with stainless steel walls in which response levers, stimulus lights, a food receptacle, and a water bottle holder were inserted through openings in the steel panels [see (7) and SI Materials and Methods].

Surgical Procedure

Rats were implanted with a polyurethane catheter (MRE-040, Braintree Scientific, Inc., Braintree, MA, USA) in the right jugular vein following methods described by Lynch et al. (2000) (19, SI Materials and Methods).

Self-Administration and Reinstatement Procedures

The experimental procedure is outlined in Table 1. Rats were tested from 9:00 to 11:00 am during each phase of the study (i.e., acquisition of iv cocaine self-administration, maintenance, extinction, and reinstatement). Following the 3-day post-operative recovery period, rats were trained to self-administer cocaine (0.4 mg/kg) under a fixed-ratio 1 (FR 1) schedule until reaching the criteria for stable cocaine intake. This consisted of the following: 1) there were no increasing or decreasing trends in infusions, 2) at least 30 infusions were self-administered, and 3) there was a 2:1 active/inactive lever response ratio for 3 consecutive days. Rats continued to self-administer cocaine for approximately 10 additional days once stability was reached under the maintenance condition. Immediately after the final cocaine self-administration session (at 11:00 am) most rats received a single tail-vein injection of 10^{11} viral particles of helper-dependent vector designed to transduce the mutant cocaine hydrolase, CocH (SI Materials and Methods). Control rats received an equivalent dose of inactive conjugate (empty vector), or saline.

For the next 14 days of daily 2-hr sessions, rats were allowed to extinguish lever pressing. Subsequently, responses on the lever previously associated with cocaine self-administration were counted as 'reinstatement responses'. Responses on the inactive lever were also counted; however, stimulus lights, house lights, and self-administration pumps were deactivated to ensure that responding was the result of the priming injection and represented cocaine seeking, not cue seeking. Rats were then tested on a reinstatement procedure recently found sensitive to pharmacological interventions, such as treatment with ovarian hormones (20), their metabolites such as allopregnanolone (21), and to individual differences such as sex (22), female hormonal status (23), impulsivity (24), proclivity for exercise (25), or sweet preference (26). The reinstatement procedure consisted of 8 days in which i.p. injections of saline (S) or cocaine (5, 10, 15 mg/kg, C) were given at the beginning of experimental sessions. On the final day of the procedure a single i.p. injection of d-amphetamine (2 mg/kg, A) was administered. Thus, the injection order occurred in the following sequence: S, C, S, C, S, C, S, A (see Table 1). Injections (i.p.) of saline and cocaine occurred at 9:00 am throughout this and all subsequent reinstatement testing sessions.

After this 8-day reinstatement condition, saline- and cocaine-priming injections were given once every 7 days for 4 weeks to assess long-term effects of vector treatment on cocaine seeking. Blood sampling continued every 7 days, after each cocaine-primed reinstatement

session (11:00 am) (SI Materials and Methods). Following the 4th cocaine injection during the 4th week, the self-administration harness and tether were detached, and the rats were transferred from their operant conditioning chambers to plastic home cages for 30 days before monthly retesting commenced. On the 30th day, infusion harnesses and tethers were reattached, and the rats were returned to operant conditioning chambers for at least 3 days. Lever presses during the first day back in the operant chamber was considered a measure of spontaneous recovery of reinstatement responding. During the next few days rats were allowed to again extinguish lever pressing before initiating the protracted reinstatement condition. Stimulus lights, house lights, and syringe pumps remained deactivated during this time and throughout the remainder of the study. Monthly protracted reinstatement testing began with an i.p. injection of saline, and on the next day an i.p. injection of 10 mg/kg cocaine was given. After the final session, self-administration equipment (harnesses and tethers) was removed, a blood sample was taken, and rats were placed back in the plastic home cages until the next monthly testing. This cycle continued for 6 mo from the initial CocH vector or control treatment (Table 1). Finally, on the very last day of testing, rats received an i.p. injection of amphetamine (2 mg/kg) to assess the specificity of the CocH vector for cocaine-primed reinstatement and as a positive control for reinstatement responding.

Statistical Analysis

Graphs show means ($\pm$ SEM) for each dependent variable (lever responses, CocH plasma levels, or beam breaks). Dependent measures were analyzed using 2-way repeated-measures analyses of variance (ANOVA) with treatment group and priming condition or session as factors. Post-hoc tests were conducted using Bonferroni's correction procedure following a significant interaction between factors. Results were considered significant if $p < 0.05$.

Results

Enzyme expression and cocaine clearance after vector treatment

Most vector treated rats developed high levels of cocaine hydrolase activity in plasma within 2 weeks of the iv injection (see Methods) and retained substantial levels for the entire 6 months of observation. Cocaine hydrolase was also abundant in liver and spleen, which showed appreciable quantities of intact vector in a QPCR assay (respectively, 840 ± 85 and 410 ± 665 copies per ng of genomic DNA). Less activity was measured in pancreas, heart, and skeletal muscle, and least in brain. None of these other tissues showed detectable levels of vector, and the measured enzyme activity was assumed to reflect retained plasma.

The presence of transgene product was associated with a dramatic acceleration of cocaine clearance (Figure S1 in the Supplement). In untreated or control rats given empty vector (without hydrolase cDNA), plasma levels of ³H cocaine reached high levels immediately after i.v. injection and then declined with a terminal half-life of 40 to 60 min. In rats given active vector, virtually all cocaine was metabolized before the first sample was drawn, 30 seconds after injection. Overall, these results provided a strong basis for efforts to determine the long-term effects of cocaine hydrolase gene transfer on cocaine-stimulated drug-seeking behavior. More specifically, they predict that, when cocaine is delivered i.p. for tests of drug-primed reinstatement in vector-treated rats, very little of the drug will escape enzymatic destruction long enough to reach targets in the brain.

Maintenance, Extinction, and Initial Reinstatement Testing

Males and females responded similarly during the conditions of maintenance (Figure S2A and S2C in the Supplement), extinction (Fig. S2B and S2D in the Supplement), and initial reinstatement (Fig. S3A and S3B in the Supplement), and their levels of CocH expression

were not significantly different throughout the study (Fig. S3C and S3D in the Supplement). Likewise, there were no differences in responding between the controls treated with empty vector vs. saline during maintenance, extinction, or reinstatement. Thus, for analysis data were combined across sex and type of control treatment.

During the maintenance condition, responding for iv cocaine by rats designated for vector treatment did not differ from controls (Fig. S2A and S2C in the Supplement), and their extinction of cocaine seeking after vector treatment was also similar (Fig. S2B and S2D in the Supplement). Dramatic differences emerged, however, when the rats were tested for reinstatement responding over a period of 8 days (Fig. 1) by administering i.p. priming injections of saline (S) cocaine (5, 10, and 15 mg/kg in mixed order, C) and d-amphetamine (A). Results from the ANOVA indicated a significant main effect of treatment group ($F_{1,159} = 31.78$, $p < 0.0001$) and priming injection ($F_{3,159} = 17.45$, $p < 0.0001$) and a significant treatment group X priming injection interaction ($F_{3,159} = 7.60$, $p = 0.0001$). There was almost no responding after saline priming injections in CocH vector and control groups (Fig. 1). However, post hoc analyses revealed that reinstatement responding after cocaine-priming injections was robust and dose-dependent in the controls ($p < 0.01$) but minimal in the CocH vector-treated group. Furthermore, control rats responded significantly more than CocH vector-treated rats across each dose of cocaine ($ps < 0.01$). In contrast, a priming injection of amphetamine induced reinstatement of vigorous lever pressing (relative to saline priming injections) in both CocH vector and control groups ($p < 0.0001$) (Fig. 1). Thus, treatment with CocH vector specifically and consistently blocked reinstatement of responding following repeated priming injections of cocaine without interfering with responding elicited by a priming injection of amphetamine. Average CocH levels (mU/ml) during the extinction (final day of the extinction condition) and initial reinstatement conditions (final day of reinstatement testing) was $213 (\pm 90.9)$ and $220.1 (\pm 90.9)$, respectively.

Weekly and Monthly Reinstatement Testing

Over the next 4 weeks, saline- and cocaine-priming injections (10 mg/kg) were given every 7 days to determine how long the vector-driven attenuation of cocaine-primed reinstatement would last. There was a significant main effect of treatment group ($F_{1,319} = 25.56$, $p < 0.0001$), priming injection ($F_{7,319} = 19.59$, $p < 0.0001$), and a treatment group X priming injection interaction ($F_{7,319} = 8.69$, $p < 0.0001$). Post hoc analyses indicated that during this period, the CocH vector-treated rats continued to show negligible amounts of cocaine-primed reinstatement responding, while the control group's responding remained robust and undiminished from initial levels ($p < 0.01$). All groups showed minimal responding following saline administration and on the 5 intervening days between priming injection sessions (Fig. 2).

In view of these results we extended the time frame of observations. To optimize housing and chamber usage, the rats were moved to their home cages and then were returned to the operant chambers once monthly for retesting on cocaine-primed reinstatement (10 mg/kg). This pattern continued for 6 mo. There was a main effect of treatment group ($F_{1,303} = 23.23$, $p < 0.0001$) and priming injection ($F_{7,303} = 13.14$, $p < 0.0001$) and a significant treatment group X priming injection interaction ($F_{7,303} = 6.63$, $p < 0.0001$). Results from the post hoc analysis indicated that over these additional 4 reinstatement sessions control rats exhibited consistently robust and statistically significant reinstatement responding following cocaine vs. saline priming injections (Weeks 12, 20, 24: $p < 0.01$, Week 16: $p < 0.05$), with no appreciable diminution over time (Fig. 3A). However, the CocH vector-treated rats exhibited persistently low levels of cocaine-primed reinstatement of lever pressing. In contrast with this treatment-induced blockade of cocaine-primed reinstatement, there was no difference in the robust reinstatement responding after the final d-amphetamine priming injection at 6 mo between CocH-vector and control treated rats (Fig. 3A). Both groups

responded significantly more following priming injections of d-amphetamine than saline ($p < 0.0001$). It is important to note that enzyme levels in the vector treated rats did not diminish greatly over time, on average (Fig. 3B). Cocaine levels were not analyzed during actual reinstatement sessions, as sampling would have been intrusive, and previous work from our laboratories indicated that cocaine plasma half-life would be far too short for accurate measurements afterward (7).

Behavioral Specificity of CocH Vector

Analysis of responses on the *inactive* lever during weekly and monthly reinstatement testing indicated that CocH-vector treated rats had significantly fewer inactive responses than control rats ($F_{1,359} = 4.27$, $p = 0.0457$) (Fig. S4 in the Supplement), suggesting that differences in reinstatement could be attributed to non-specific behavioral depression. To further examine this possibility, CocH vector and control rats were compared in a separate experiment that measured general locomotor activity during three 45-min daily sessions. Between-group comparisons revealed no differences between the CocH-vector treated rats and control rats (Fig. S5 in the Supplement). Thus, the reduced inactive lever responding may have generalized from the enzyme-induced reduction in active-lever responding.

During monthly retesting of cocaine-primed reinstatement when rats were returned from home cages to the operant chambers they were allowed to rehabilitate to the chambers for a minimum of 3 days prior to the administration of saline and cocaine priming injections. Both the CocH vector and the control groups responded significantly on the previously active lever during the first day back in the operant chamber compared with the subsequent 2 days ($F_{7,295} = 11.96$, $p < 0.0001$), and the two groups did not differ across monthly retesting during this initial day (Fig. 4). Thus, the higher inactive lever responding in controls (vs. CocH vector-treated rats) suggested a generalized elevation of responding from the active to inactive lever (12).

Discussion

In previous work we demonstrated that systemic injection of Albu-CocH blocked the reinstatement of cocaine seeking effectively, though acutely (7). In the present study we extended these findings and used gene transfer of CocH (via adenoviral delivery) to drastically enhance the duration of CocH activity, for at least 6 mo. Elevated enzyme levels at the end of this period suggested that the expression could have lasted significantly longer if the rats had been left to continue. Our data provide evidence that a single delivery of CocH to rats via hdAD vector provides a sustained level of enzyme activity that selectively blocks reinstatement of cocaine-seeking behavior (relapse) for a prolonged period in both male and female rats. Thus, during each of 11 cocaine-primed reinstatement sessions that took place over 6 months of testing, responding by rats treated with CocH vector resembled responding following saline priming sessions, and it was significantly lower than that of controls (Figs. 1 – 3). This outcome can be regarded as evidence that CocH vector treatment resulted in lasting reduction in the reinforcing effects of cocaine. Further evidence was a significant reduction in the vector-treated (vs. control) group of reinstatement responding (Fig. S4 in the Supplement) that typically generalizes to the *inactive* lever when there is elevated reinstatement responding on the previously active lever (12). The present study is the first demonstration of a long-lasting treatment to prevent resumption of cocaine-seeking behavior in “cocaine experienced” animals for which cocaine self-administration access has been terminated.

The lack of sex differences in the present study contrasts with previous findings from our laboratories and others indicating that females outperform males on the maintenance, extinction, and reinstatement of cocaine-seeking behavior (10, 11). However, such sex

differences are not always consistent and depend on several factors such as dose of cocaine, durations of experimental sessions, and degree of challenge in the schedule of reinforcement (11). For example, previous work indicated that females self-administer greater amounts of cocaine than males at low doses of cocaine (e.g., 0.2 mg/kg), long session lengths (e.g., 6 hr), and more demanding requirements for reinforcement (e.g., progressive-ratio schedule) (11), whereas the present study used higher doses, shorter sessions, and simple schedules (FR1). Another interesting outcome of these experiments was that male and female rats treated with CocH vector showed a similar decrease in cocaine-primed reinstatement responding, whereas pharmacological treatments are usually more effective against reinstatement in females than males (10, 11). The failure to detect sex differences here might have been due to a floor effect produced by the CocH vector treatment or the limited number of male and female subjects used in the study. Nevertheless, to make an optimistic interpretation, the present results suggest that treatment with CocH vector would be equally effective in men and women.

Therapeutically efficacious treatments for cocaine relapse should be both pharmacologically and behaviorally specific in their action. In previous work we demonstrated that direct injection of Albu-CocH safely and specifically blocked cocaine-primed reinstatement without affecting amphetamine-primed responding, general locomotor activity, and food-maintained responding (7). Similarly, in the present study we demonstrated that the ability of CocH vector to block cocaine-primed reinstatement (relapse) was pharmacologically specific and did not reflect a general suppression of behavior motivated by a stimulant reward, as demonstrated by the vigorous amphetamine-primed reinstatement by CocH-vector treated rats either at week 3 (Fig. 1) or 24 (Fig. 3). In addition, the lack of an effect of CocH vector on the spontaneous recovery of reinstatement responding (Fig. 4) and open field locomotor activity (Fig. S5 in the Supplement) provides further evidence against a non-specific, debilitating “vector effect” on general behavior. Food and water intake by rats treated with CocH vector and controls were also similar following cocaine injections, indicating that CocH vector was well tolerated and without apparent adverse effects.

In our view, restricted drug access of cocaine to key centers in the brain is the most parsimonious hypothesis to account for the ability of CocH to reduce cocaine-driven behavior. Prior work from our laboratories has demonstrated extremely rapid elimination of cocaine in rats exhibiting plasma cocaine hydrolase activities comparable to those achieved in the present study. In fact, that elimination was so rapid that plasma drug half-life became too short to measure accurately (7). The rapid elimination of cocaine by viral delivered CocH was reconfirmed in the present study, which indicated that cocaine plasma levels remained near baseline throughout a 2-hr period following a 3.5 mg/kg i.v. injection of drug (Fig. S1 in the Supplement). Further evidence for effective pharmacological interception of cocaine by gene transfer of CocH comes from a study in progress, where a similar treatment effectively blocked the locomotor response to acute cocaine, 10 mg/kg, i.p.

When the reinstatement responding in vector-treated rats was compared with CocH plasma levels, no simple linear relationship emerged. In particular, there was no indication that CocH enzyme levels were inversely proportional to the extent of reinstatement (relapse) responding. An inverse relationship might have been expected across time in a given animal if, as a result of falling enzyme levels in the later months, cocaine metabolism became too slow to reduce the drug's reward value. Two explanations deserve consideration. First, even the relatively low CocH expression in some rats at later stages may have still been adequate to blunt cocaine's central effects by metabolizing most drug before it entered the brain. Alternatively it is possible that, during protracted, high CocH expression, rats *unlearned* the association between cocaine intake and cocaine reward. Such an outcome is not implausible, even if the benzoic acid or ecgonine methyl ester produced by accelerated cocaine

metabolism had neutral properties. If either metabolite were mildly aversive, however, suppression of responses to cocaine priming might well outlast the high enzyme expression. Other explanations are also possible.

In addition to vector-based therapies, an additional approach to the long-term treatment of cocaine relapse by restricting cocaine's access to the brain involves the use of cocaine vaccines. Cocaine vaccines facilitate the production of antibodies that target blood-born cocaine and block cocaine-induced neurobiological and behavioral effects in rodents (9, 13, 14). The therapeutic efficacy of cocaine vaccines has been successfully demonstrated in Phase I trials in human cocaine addicts (9, 15, 16). The mechanisms by which hydrolases and anti-cocaine antibodies deactivate cocaine differ but are almost certainly not mutually exclusive. Whereas BChE-like enzymes such as CocH work at low protein levels to metabolize cocaine in blood plasma, anti-cocaine antibodies, present in higher molar concentrations, rapidly bind and sequester substantial quantities of drug. Recently, in an *in vitro* experiment Gao et al. (2010) (17) demonstrated that CocH relinquished cocaine from saturated anti-cocaine antibodies thereby synergistically enhancing cocaine clearance. One hypothesis is that enzyme-based gene therapies and cocaine vaccines may work in concert as a combination therapy with an additive power to prevent cocaine relapse (17). A combination therapy approach could be applied to the procedure used in the present study to determine additive treatment effects on cocaine-primed reinstatement.

The present experiments were terminated at 6 months in order to test other relevant behavioral parameters. We anticipate that the favorable effect observed could last considerably longer. This point deserves further study because it may have significant translational impact for human cocaine abuse. It will also be important in future work to examine the effectiveness of hydrolase vector treatment in other animal models of clinical relevance. Among these are paradigms in which animals are allowed to reacquire cocaine self-administration, a model of brief relapse in human addicts under treatment, or in those that provide an opportunity to escalate drug intake. Future work should also test CocH vector treatment in other species (e.g., nonhuman primates) and compare it with other pharmacological approaches to drug abuse or related disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Rats treated with CocH vector (N=14) responded significantly less than controls (N = 26) on the lever previously paired with iv cocaine self-administration following a 5, 10, and 15 mg/kg i.p. injection of cocaine (# $p < 0.01$) but not following a 2 mg/kg i.p. injection of d-amphetamine. Furthermore, compared to responding following i.p. injections of saline, control rats made more responses on the previously drug-paired lever following i.p. injections of cocaine (5, 10, and 15 mg/kg) (* $p < 0.01$) and d-amphetamine (2 mg/kg) (* $p < 0.0001$). CocH-treated rats also responded at the levels of control rats following the i.p. d-amphetamine injection (** $p < 0.0001$). Thus, the blockade of reinstatement of drug-seeking by CocH was specific to cocaine-primed reinstatement.

Fig. 2.

Responding following weekly i.p. injections of saline (S) or cocaine (C) 5 to 8 weeks post CocH vector or control injection. Lever responding following S or C did not differ in rats treated with CocH vector (N=14). In contrast, rats given control injections (N=26) (inactive vector or saline) responded significantly more following administration with cocaine than saline (* $p < 0.01$), and they responded more than CocH-vector treated rats across the 4 weeks of reinstatement testing († $p < 0.01$).

Fig. 3.

CocH vector expression suppressed cocaine-primed reinstatement for up to 24 weeks. **A.** CocH vector-treated rats (N=14) made significantly fewer responses (# $p < 0.01$; Week 16: * $p < 0.05$) on the lever previously paired with cocaine than control rats (N=26) following a 10 mg/kg cocaine i.p. priming injection during reinstatement testing sessions up to 24 weeks after vector or control injections. This effect was specific to cocaine, as 2 mg/kg d-amphetamine i.p. priming injections administered at 24 weeks post-vector injection elicited a similar number of responses in CocH vector-treated and control rats. **B.** Active CocH expression levels sampled up to 24 weeks post vector injection in rats treated with CocH vector. CocH expression was higher 3, 4, and 6 weeks post injection compared to weeks 16, 20, and 24 ($p < 0.05$) indicating that CocH levels diminished across weekly and monthly testing. Three of the 11 rats had values of over 200 mU/ml 24 weeks following injection with CocH vector while the remainder had relatively low levels of enzyme expression.

Fig. 4.

Spontaneous recovery of lever responding on the previously cocaine-active lever after rats were taken from the home cage and placed back in the operant chamber for monthly extinction and cocaine-primed reinstatement sessions. Rats treated with CocH vector (N=14) and saline/empty vector controls (N=26) responded similarly and made significantly more responses on the lever previously paired with iv cocaine self-administration on day 1 compared to the average of days 2 and 3 ($F_{7,295} = 11.96$, $p < 0.0001$).

Table 1

Summary of procedure.

Phase (days)	ACQ (~10)	MAINT (~10)	EXT (14)	Reinstatement			Protracted Reinstatement																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
				S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C

Phases of cocaine self-administration include acquisition (ACQ), maintenance (MAINT), extinction (EXT), and reinstatement. Reinstatement occurred over an initial 8 days then intermittently over 24 weeks. Immediately following the final session of cocaine self-administration, a single iv (tail vein) injection of CocH vector, empty vector, or saline was given (represented by the syringe). During reinstatement testing, ip priming injections of saline (S), cocaine (5, 10, or 15 mg/kg, C), or 2 mg/kg d-amphetamine (A) were given once at the beginning of the session.