Published in final edited form as:

J Mol Neurosci. 2014 July ; 53(3): 409–416. doi:10.1007/s12031-013-0130-5.

Preclinical Studies on Neurobehavioral and Neuromuscular Effects of Cocaine Hydrolase Gene Therapy in Mice

Vishakantha Murthy, Yang Gao, Liyi Geng, Nathan LeBrasseur, Thomas White, and Stephen Brimijoin

Mayo Clinic, Rochester, MN, USA

Abstract

Cocaine hydrolase gene transfer of mutated human butyrylcholinesterase (BChE) is evolving as a promising therapy for cocaine addiction. BChE levels after gene transfer can be 1,500-fold above those in untreated mice, making this enzyme the second most abundant plasma protein. Because mutated BChE is approximately 70 % as efficient in hydro-lyzing acetylcholine as wild-type enzyme, it is important to examine the impact on cholinergic function. Here, we focused on memory and cognition (Stone T-maze), basic neuromuscular function (treadmill endurance and grip strength), and coordination (Rotarod). BALB/c mice were given adeno-associated virus vector or helper-dependent adenoviral vector encoding mouse or human BChE optimized for cocaine. Age-matched controls received saline or luciferase vector. Despite high doses (up to 10^{13} particles per mouse) and high transgene expression (1,000-fold above baseline), no deleterious effects of vector treatment were seen in neurobehavioral functions. The vector-treated mice performed as saline-treated and luciferase controls in maze studies and strength tests, and their Rotarod and treadmill performance decreased less with age. Thus, neither the viral vectors nor the large excess of BChE caused observable toxic effects on the motor and cognitive systems investigated. This outcome justifies further steps toward an eventual clinical trial of vector-based gene transfer for cocaine abuse.

Keywords

Cocaine; Addiction; Gene therapy; Butyrylcholinesterase; Adeno-associated viral vector; Helper-dependent adenoviral vector

Introduction

The National Institute on Drug Abuse (NIDA) reports that approximately 2.1 million in the USA are cocaine addicts, and according to the Office of National Drug Control Policy, over a million new users every year are at risk. Despite efforts to develop therapeutic interventions, there are still no effective treatments for cocaine abuse, but one promising strategy on the horizon is gene therapy with an enzyme derived from human

butyrylcholinesterase (BChE). In this approach, cocaine is rapidly hydrolyzed in the bloodstream, preventing its access to reward centers in the brain, while the resulting metabolites, ecgonine methyl ester and benzoic acid, lack rewarding actions. BChE mutagenesis to enhance this reaction has resulted in the development of a cocaine hydrolase (CocH) with ~1,500-fold increase in catalytic efficiency for cocaine (Yang et al. 2010; Zheng et al. 2008). Our gene transfer studies on mice with adeno-associated vector (AAV) or helper-dependent adenoviral vector (hdAD) have shown that high vector doses can elevate CocH activity as much as 1,000,000-fold without apparent ill effect, while reactions to otherwise lethal amounts of cocaine are obliterated (Brimijoin et al. 2013; Gao et al. 2013; Geng et al. 2013).

These positive results encourage us to contemplate an eventual clinical trial of CocH gene transfer for addiction therapy. With that goal in mind, a thorough and systematic evaluation of the safety of vector treatment assumes great importance. In particular, because CocH retains substantial ability to hydrolyze acetylcholine, potential effects of abundant mutated BChE on cholinergic function in the peripheral and central nervous systems deserve close attention.

Several safety and efficacy studies on exogenous BChE in mice, rats, and rhesus monkey have been reported recently (Saxena et al. 2011, 2005; Weber et al. 2011) with encouragingly negative results regarding toxicity. This is not surprising, because cholinergic synapses in the brain should be protected by the blood–brain barrier, and the neuromuscular junction is also a restricted compartment. Nonetheless, further exploration is warranted to evaluate potential adverse effects from high levels of CocH in multiple systems. In the present study, we used mice to examine possible effects of CocH gene transfer on the following neurobehavioral tasks: memory and cognition in the “Stone T-maze,” motor function on Rotarod tests, and basic neuromuscular function (forced maximal endurance with treadmill and grip strength).

Materials and Methods

Drug and Biological Sources

Cocaine HCl was obtained from the National Institute of Drug Abuse (Research Triangle Institute, Research Triangle Park, NC, USA). This drug was freshly dissolved in 0.9 % NaCl for each mouse experiment at a concentration that allowed delivery of 200 µl per 30 g (typical subject weight).

Animals—BALB/c male mice obtained at 6–7 weeks of age from Harlan Sprague Dawley (Madison, WI) were housed in plastic cages with free access to water and food (Purina Laboratory Chow, Purina Mills, Minneapolis, MN, USA) in rooms controlled for temperature (24 °C), humidity (40–50 %), and light (light/dark, 12/12-h with lights on at 6:00 a.m.). The animal protocols (A9812 and A59111) were approved by the Mayo Clinic Institutional Animal Care and Use Committee. All experiments were conducted in accordance with the Principles of Laboratory Animal Care in AAALAC-accredited laboratories.

Viral Gene Transfer

Some gene transfer experiments used a vector based on adeno-associated virus assembled by methods previously described (Miyagi et al. 2008; Schirmer et al. 2007; Waehler et al. 2007). Wild-type mouse BChE cDNA (“mBChE wt,” provided by S. Camp and P. Taylor, UCSD) was cloned into adeno-associated viral vector (AAV with cytomegalovirus (CMV) promoter), and a Kozak consensus sequence (GCCACC) was introduced before the translational start site. With AAV-mBChE wt as template and using primers with specific base-pair alterations, site-directed mutagenesis generated the AAV-mBChE mutant (A199S/S227A/S287G/A328W/Y332G). To construct AAV-VIP-plasmids for the equivalent mutant human BChE (A199S/F227A/S287G/A328W/Y332G/E441D), recognition sites for the enzymes “Not I” and “BamH I” were introduced into mBChE DNA directly before the Kozak consensus sequence and after the stop codon, respectively. Subsequently, the BChE sequence was ligated into pAAV-VIP vector (Balazs et al. 2012) between Not I and BamH I. To produce and purify the AAV8-mBChE virus, the AAV-CMV-mBChE (wt or mutant) or the AAV-VIP-mBChE mutant plasmid was co-transfected into human embryonic kidney cells (HEK) 293T cells with helper vectors, pHelper and pAAV2/8, by using a FuGENE HD Transfection Reagent (Roche). Seventy-two hours later, AAV virus was purified from the cell lysates by ultracentrifugation against Optiprep Density Gradient Medium Iodixanol (Sigma-Aldrich, St Louis, MO). The concentration of viral particles was subsequently determined by real-time quantitative PCR (QPCR). Other gene transfer experiments used hdAD that contained cDNA for CocH under regulation by a human ApoE hepatic control region (Kim et al. 2001), with a bovine growth hormone polyadenylation sequence cloned into a derivative of the p28lacZ hdAD backbone plasmid. The vector was propagated using the AdNG163 helper virus, as described (Parks et al. 1996), and particle titers were determined by optical density at 260 nm. Helper virus contamination, determined by plaque assay on HEK293 cells, was approximately 0.2 % for both loaded and empty vectors. Vector (200 μ l, in doses from 10^{11} to 10^{13} viral particles) was delivered to 6–7-week-old mice by rapid tail vein injection followed by a 200- μ l flush of 0.9 % sterile NaCl.

Blood Collection and Enzyme Assay—Blood samples (<0.1 ml) were taken from the mouse tail vein by using a 21-gauge mouse bleeding lancet. A sterile gauze pad was then applied with slight pressure for about a minute to stop bleeding. Blood samples were centrifuged for 15 min in serum separator tubes (Becton Dickinson, Franklin Lakes, NJ, USA) at 8,000 g, and the sera were stored at -20°C until analysis. Cocaine hydro-lase activity in 50- μ l aliquots of plasma or brain supernatant was assayed in a solvent-partitioning assay with ^3H -cocaine (50 nCi, 18 μM) as substrate as previously described (Brimijoin et al. 2002). Native mouse BChE activity was assayed by a radiometric method using 1 mM ^3H -acetylcho-line as substrate and 10^{-5} M BW284c51 as an acetylcholinesterase inhibitor (Johnson and Russell 1975).

Cocaine-Induced Locomotor Activity—Cocaine-induced locomotion in mice was assessed in sound-insulated rectangular activity chambers from Med Associates Inc., St Albans, VT, USA (27-cm W $\times$ 27-cm L $\times$ 20-cm D) with continually running fans and infrared lasers and sensors. Beam breaks were assessed in 2-min bins over 60 min, converted automatically to distance travelled (centimeters) and recorded on a computer with Med-PC

software Version 4.0. The mice were acclimatized to the chamber for 2 days. On the test day, following an acclimatization for an hour, the mice were weighed and injected i.p. with 40-mg/kg dose of cocaine (time-zero) and immediately introduced in to the chambers. The recording was done for 60 min.

Cognition and Memory Analysis with Stone T-maze—A Stone T-maze (“STM”) as described previously (Pistell and Ingram 2010) was constructed by Med Associates according to specifications reported from the Nutritional Neuroscience and Aging Laboratory at the National Institute on Aging, NIH. A 69-cm straight tunnel was used for pretraining (exclusion test) on the day before maze training and testing. The water level for the pretraining session was 2.2 cm. On the test day, the STM was used for acquisition training with the water level at 3.3 cm, which forced the mice to swim rather than walk. Because our BALB/c mice showed limited desire to escape from water, in contrast with male C57BL/6J mice (Pistell and Ingram 2010), we modified the procedure to add positive reinforcement (food pellet reward following overnight fasting).

On day 1, the mice underwent straight-run training to establish the contingency that moving forward would allow escape from water into the dry box with food pellet. The time limit was 15 s per trial. Mice that failed to complete 8/10 trials on this criterion were excluded from further maze testing. In each experiment, eight to ten mice received all ten pretrials in a single day. All mice completed trial 1 before the first one entered trial 2, resulting in an intertrial interval of 5–10 min. Each time the mouse reached the dry box, it received a reward of ~100-mg high-fat food and was allowed to relish it while the next mouse began its trial. Afterward, the mice were lifted out of the box, patted dry with a paper towel, and returned to their home cage with a heating pad while awaiting subsequent turns.

Mice that met the criteria were fasted overnight with access to water and given STM acquisition training the next day (typically six to eight mice at a time). The intertrial interval was ~30 min in earlier trials and ~8 min in later trials. Again, on reaching the dry box, the mice received ~100 mg of food while the mouse next in line completed its trial. Following this, the mice were patted dry and returned to their cage with a heat pad. Subsequent trials followed the same procedure.

The STM trials were recorded using a video tracking system (Watchport/V3 USB Camera, noninterlaced model from Digi International, Minnetonka, MN, USA) and later reviewed for analysis of learning as indicated by (a) latency to reach the dry box and (b) number of errors committed. An error was defined as complete entry of the mouse’s head into an incorrect path. The acquisition time limit for each trial was 6 min. If a mouse failed to reach the dry box within 6 min, the trial was termed a failure. Any mouse failing to achieve 2/9 acquisition trials was removed from the study. The experimenter was blinded to treatment conditions throughout the testing and analysis process.

Motor Behavior on Rotarod—Motor coordination and balance were tested on an accelerating multilane Rotarod (Med Associates, St. Albans, VT, USA). Mice were acclimated to the experimental room for 2 days before testing. On the test day, they were in the room at least 15 min before starting the procedure. The Rotarod was set at 4 rpm. The

mice were placed on their respective lanes and were trained to walk forward and balance on the rod for an initial 30 s to 1 min. Next, the machine moved to acceleration mode (start of test) from 4 to 40 rpm over a 5-min period. The latency at which each mouse fell off the rod was automatically recorded. If a mouse clung to the rod completing a full passive rotation, the timer on that lane was stopped, and the time was recorded as “latency.” Three trials with an intertrial interval of 15 min were performed.

Treadmill and Grip Strength—Forced maximal endurance was characterized by measuring running time, velocity, and work using a motorized treadmill (Columbus Instruments, Columbus, OH, USA). In brief, the mice ($n=8$ to 12 per group) were acclimated to the experimental room for 2 days before exposure to the treadmill protocol. They were then acclimated to the treadmill for three consecutive days from 4:00–6:00 p.m. on a 5° incline at incremental speeds of 8 m/min for 2 min, 10 m/min for 2 min, and 12 m/min for 1 min. A shock grid mounted at the back of the treadmill delivered a 3.0-mA current to provide motivation for remaining on the treadmill. On the fourth day, the test day, animals were weighed and exercised on the treadmill at the same incline, starting at a speed of 10 m/min for 5 min, which then increased incrementally by 2 m/min every 2 min until the mice ran to exhaustion. Exhaustion was defined as inability to remain on the treadmill despite the shock stimulus and mechanical prodding. Running time was recorded, and running distance (product of time and treadmill speed) and work (product of body weight [kilograms], gravity [9.81 m/s^2], vertical speed [meters per second $\times$ angle], and time [seconds]) were calculated in units of kilojoules (kJ).

Grip strength was measured using a meter (BIOSEB, model GS3, Vitrolles Cedex, France). The mice were held by the tail above the wire grid of the apparatus, lowered until all four legs grasped the grid, and then pulled away along the horizontal axis. Maximal achieved force was displayed and recorded. The procedure was repeated three times on each test day, and mean peak force was used for statistical analysis.

Statistics—Data were analyzed using either single-factor (group) analysis of variance (ANOVA) or two-way ANOVA with Holm-Sidak test for post hoc analyses. Statistical significance was accepted as $p < 0.05$. The analyses were conducted using the GraphPad Prism Statistical Software version 6.0 (San Diego, CA, USA).

Results

Overview

Towards our primary goal of testing the safety of Coch gene transfer with regard to cholinergic function, we examined cognition and memory, balance and coordination, muscular strength, and physical strength. The results detailed below indicated that no deficit or adverse effect could be attributed either to the viral vectors or to the high levels of recombinant enzyme transduced. In fact, some older mice expressing BChE for over 1 year performed significantly better on the Rotarod and treadmill tests than saline-treated age-matched controls.

Enzyme Expression Levels

As shown in Fig 1, cocaine hydro-lysis activity in plasma rose to high levels after transduction with a large dose of either murine or human versions of CocH with the AAV and hdAD vectors. These levels, about 200,000-fold above baseline, were well sustained and remained substantial even after 8 to 15 months.

Locomotor Activity and Grip Strength

Spontaneous locomotor activity, tested at approximately 3 months of age, was at control levels in vector-treated mice (Fig 2), but cocaine-induced locomotion was completely suppressed, as previously reported (Geng et al. 2013). Grip strength tested at two separate ages (7 weeks and 13 months) was completely unaffected by vector treatment (Table 1).

Cognitive Performance

Learning and memory were examined in a “Stone T-water maze” with “latency” (time from entry until exit into the dry platform) and errors as indices of cognitive function (Fig. 3). Tested groups were untreated controls, luciferase vector controls, and three different active vector groups (AAV mouse CocH, AAV human CocH, and hdAD mouse CocH). All groups exhibited learning (significant reduction of latency and errors) as determined by a grouped two-way ANOVA.

The grouped two-way ANOVA on latency data (Fig. 3a) indicated a significant main effect for trials ($F(2, 90)=59.17, p<0.0001$), but no significant effect for treatments ($F(4, 45)=1.529, p=0.21$) and no significant interaction ($F(8, 90)=1.818, p=0.083$). Within-treatment comparisons of trial blocks 1–3 vs. 7–9 confirmed highly significant decreases of latency in every tested group, although the luciferase vector controls performed significantly less well than other groups.

The grouped two-way ANOVA for errors (Fig. 3b) indicated a significant main effect for both trial ($F(2, 90)=60.60, p<0.0001$) and interaction ($F(8, 90)=2.977, p=0.0053$), but again no significant effect for treatments ($F(4, 45)=1.261, p=0.29$). Likewise, comparisons within treatments, comparing trial block 1–3 vs. 7–9 showed a highly significant effect for all groups, again with the luciferase controls performing less well than the others.

Rotarod Performance

In order to evaluate the effect of BChE expression on motor coordination and balance, mice were subjected to an accelerating Rotarod task at two different stages, first as “young adults” (aged 3–4 months) and then (in selected groups) as older adults (aged 12–14 months). With the younger animals (Fig. 4a), all mice, regardless of treatment, exhibited significant improvement across trials in the Rotarod task as indicated by increase in run times (latency to fall off the accelerating Rotarod). Grouped two-way ANOVA indicated significant main effects for trials ($F(2, 206)=23.54, p<0.0001$) and interaction ($F(6, 106)=17.79, p<0.0001$), but no significant effect for treatments ($F(3, 53)=1.899, p=0.14$). With the older animals (Fig. 4b), there were again significant effects for trials ($F(2, 96)=5.400, p=0.006$) and treatments ($F(2, 96)=14.95, p<0.0001$), but no significant interaction ($F(4, 96)=1.120,$

$p=0.35$). In post hoc testing, the hdAD vector group showed significantly better performance than controls ($p=0.007$).

Treadmill

Mice with all different treatments were also tested for forced maximal endurance on a treadmill, again at two separate ages (5–7 and 14–17 months). The results in younger mice (Fig. 5a) show no significant difference among treatments in terms of work output (kJ) by the animals ($F(4, 49)=1.493$, $p=0.22$). As with the Rotarod tests, there was a significant treatment effect in the older mice ($F(2, 31)=17.84$, $p<0.0001$), and by this measure, post hoc testing showed that both active vector treatments were associated with improved performance ($p<0.0001$ for the AAV group and $p=0.02$ for the hdAD group).

Discussion

In this study, we report that a gene therapy based on modified versions of BChE lacks detectable adverse effects on the cholinergic nervous system in mice, at least as regards the basic motor and cognitive function. This claim is supported by consistent negative results from learning and memory tests in a structured maze, from treadmill tests of physical endurance, from grip strength monitoring, and from evaluations of motor coordination in a Rotarod paradigm. In each category of observation, the performance of mice examined a few weeks after enzyme vector treatment did not differ significantly from untreated controls or mice that received an irrelevant (luciferase) gene transfer. That outcome arose despite the high dose of viral vector (up to 10^{13} genome copies per mouse, or 3×10^{14} copies/kg), which generated plasma cocaine hydrolase activity of 20 U/ml, representing a ~1,000-fold increase in circulating levels of BChE (Geng et al. 2013). Cholinergic dysfunction was also absent in older mice that had retained high-level BChE expression for a year or more. We actually noted an increase in treadmill performance in each of two experimental groups, one transduced with AAV-mutant human BChE vector and one transduced with hdAD-mutant mouse BChE vector. The same two groups also showed enhanced Rotarod performance, but this was statistically significant only in the latter.

These unexpected improvements in motor performance by older mice have no ready explanation. On subjective observation, the animals in these groups appeared identical to controls during the first few months after vector treatment, but after 6 or 12 months, they seemed to have remained somewhat healthier and more robust. At this stage, it would be premature to suggest that the treatment had somehow enhanced their physical condition or delayed its decline, and there is no obvious or plausible mechanism for such an effect. Pending confirmation in a subsequent study, we draw only two modest conclusions at present. First, BChE is a physiologically benign enzyme that has little influence on cholinergic tone (Saxena et al. 2005; Weber et al. 2011). Second, the viral vectors tested here do not cause even minor physical disability or generate a sustained inflammatory state that would lead to impaired motor function or neurological disability. This assertion is in line with other results from our laboratory, indicating no elevation of the inflammatory cytokine IL-6 in animal sera tested at multiple intervals after vector treatment (Liyi Geng, unpublished data).

For several years, BChE was regarded as a metabolic enzyme, protecting against toxic esters in the diet, but lacking in physiological relevance in view of its minor contribution toward the pool of cholinesterase activity in the muscle, brain, and other cholinergic tissues (Anglister et al. 1998; Silver, 1974). This view was in line with observations that humans with null mutations of BChE and lacking all BChE activity still live a healthy and normal life (Manoharan et al. 2007, 2006) as do BChE knockout mice (Duysen et al. 2007; Li et al. 2008). However, recent reports suggest that BChE may have important functions in cholinergic neurotransmission and in the central nervous system (Li et al. 2000). In addition, the enzyme conceivably offers some protection against certain neuropathological conditions. For example, there is evidence suggesting that the presence of the less stable BChE-K variant, leading to lower serum BChE activity, is a risk factor for stroke (Ben Assayag et al. 2010). In addition, BChE's ability to serve as a prophylactic bio-scavenger to sequester toxic organophosphorus chemical nerve agents and pesticides has gained attention in agricultural and military arenas (Lenz et al. 2005). Our study provides further information on the safety profile of BChE, and the outcome encourages further work along those lines.

For the present cognition and memory studies, we chose the Stone T-maze over a "traditional" Morris water maze because of advantages such as the lack of need for visual cues, the reduced amount of water required, and the much smaller space requirements. To the best of our knowledge, there have been no prior reports on BALB/c mice in the Stone T-maze, which, surprisingly, pilot studies have revealed to be very strain-sensitive. The general essence of the paradigm relies on an innate instinct to escape water, even if shallow. Since BALB/c mice in our hands proved weak in this regard, we modified the protocol to require swimming instead of walking, as originally intended. To enhance the incentive and motivate mice to perform a sufficient number of acquisition trials, positive reinforcement with a high fat reward was also introduced. This proved most effective when mice were fasted overnight after a straight-run training session, and it was incorporated into the standard protocol. We suspect that these or similar modifications would facilitate maze learning studies with any strain of mice in the Stone T-maze.

Mice receiving the AAV-luciferase control vector alone did lag slightly in learning performance, but the performance of mice given with active enzyme vector, which was on a par with saline-treated controls, strongly implies unimpaired cognitive function. In our view, the data clearly show that neither viral vector per se nor a large pool of circulating BChE is able to alter cognition and memory circuits in the brain. A similar conclusion can be drawn from the Rotarod studies since balance, motor coordination, and motor learning require functional integration of the frontoparietal and motor cortex, cerebellum, and striatal circuitry (Hikosaka et al. 2002; Jueptner et al. 1997; Massaquoi and Hallett 1998). Studies are now underway to determine whether the vector-treated mice actually do retain a healthy phenotype longer than untreated littermates. Meanwhile, our results indicate that the gene therapy in the development for cocaine abuse lacks neurobehavioral and neuromuscular side effects in a murine model and may deserve an eventual clinical trial of CocH gene transfer for cocaine abuse.

Acknowledgments

This work was supported by NIH-NIDA grants RO1DA23979 and DP1DA31340.

References

- Anglister L, Eichler J, Szabo M, Haesaert B, Salpeter MM. 125I-labeled fasciculin 2: a new tool for quantitation of acetylcholinesterase densities at synaptic sites by EM-autoradiography. *J Neurosci Methods*. 1998; 81:63–71. [PubMed: 9696311]
- Balazs AB, Chen J, Hong CM, Rao DS, Yang L, Baltimore D. Antibody-based protection against HIV infection by vectored immunoprophylaxis. *Nature*. 2012; 481:81–84. [PubMed: 22139420]
- Ben Assayag E, Shenhar-Tsarfaty S, Ofek K, Soreq L, Bova I, Shopin L, Berg RMG, Berliner S, Shapira I, Bornstein NM, Soreq H. Serum cholinesterase activities distinguish between stroke patients and controls and predict 12-month mortality. *Mol Med*. 2010; 16:276–286.
- Brimijoin S, Shen ML, Sun H. Radiometric solvent-partitioning assay for screening cocaine hydrolases and measuring cocaine levels in milligram tissue samples. *Anal Biochem*. 2002; 309:200–205. [PubMed: 12413452]
- Brimijoin S, Orson F, Kosten TR, Kinsey B, Shen XY, White SJ, Gao Y. Anti-cocaine antibody and butyrylcholinesterase-derived cocaine hydro-lase exert cooperative effects on cocaine pharmacokinetics and cocaine-induced locomotor activity in mice. *Chem-Biol Interact*. 2013; 203:212–216. [PubMed: 22960160]
- Duysen EG, Li B, Darvesh S, Lockridge O. Sensitivity of butyrylcholinesterase knockout mice to (–)-huperzine A and donepezil suggests humans with butyrylcholinesterase deficiency may not tolerate these Alzheimer's disease drugs and indicates butyrylcholinesterase function in neurotransmission. *Toxicology*. 2007; 233:60–69. [PubMed: 17194517]
- Gao Y, Geng L, Orson F, Kinsey B, Kosten TR, Shen X, Brimijoin S. Effects of anti-cocaine vaccine and viral gene transfer of cocaine hydrolase in mice on cocaine toxicity including motor strength and liver damage. *Chem-Biol Interact*. 2013; 203:208–211. [PubMed: 22935511]
- Geng L, Gao Y, Chen X, Hou S, Zhan CG, Radic Z, Parks RJ, Russell SJ, Pham L, Brimijoin S. Gene transfer of mutant mouse cholinesterase provides high lifetime expression and reduced cocaine responses with no evident toxicity. *PLoS One*. 2013; 8:e67446. [PubMed: 23840704]
- Hikosaka O, Nakamura K, Sakai K, Nakahara H. Central mechanisms of motor skill learning. *Curr Opin Neurobiol*. 2002; 12:217–222. [PubMed: 12015240]
- Johnson CD, Russell RL. A rapid simple radiometric assay for cholinesterase, suitable for multiple determinations. *Anal Biochem*. 1975; 64:229–238. [PubMed: 237428]
- Jueptner M, Frith CD, Brooks DJ, Frackowiak RS, Passingham RE. Anatomy of motor learning. II. Subcortical structures and learning by trial and error. *J Neurophysiol*. 1997; 77:1325–1337. [PubMed: 9084600]
- Kim IH, Jozkowicz A, Piedra PA, Oka K, Chan L. Lifetime correction of genetic deficiency in mice with a single injection of helper-dependent adenoviral vector. *Proc Natl Acad Sci U S A*. 2001; 98:13282–13287. [PubMed: 11687662]
- Lenz DE, Maxwell DM, Koplovitz I, Clark CR, Capacio BR, Cerasoli DM, Federko JM, Luo C, Saxena A, Doctor BP, Olson C. Protection against soman or VX poisoning by human butyrylcholinesterase in guinea pigs and cynomolgus monkeys. *Chem-Biol Interact*. 2005; 157-158:205–210. [PubMed: 16289064]
- Li B, Stribley JA, Ticu A, Xie W, Schopfer LM, Hammond P, Brimijoin S, Hinrichs SH, Lockridge O. Abundant tissue butyrylcholinesterase and its possible function in the acetylcholinesterase knockout mouse. *J Neurochem*. 2000; 75:1320–1331. [PubMed: 10936216]
- Li B, Duysen EG, Carlson M, Lockridge O. The butyrylcholinesterase knockout mouse as a model for human butyrylcholinesterase deficiency. *J Pharmacol Exp Ther*. 2008; 324:1146–1154. [PubMed: 18056867]
- Manoharan I, Wieseler S, Layer PG, Lockridge O, Boopathy R. Naturally occurring mutation Leu307Pro of human butyrylcholinesterase in the Vysya community of India. *Pharmacogenet Genomics*. 2006; 16:461–468. [PubMed: 16788378]

- Manoharan I, Boopathy R, Darvesh S, Lockridge O. A medical health report on individuals with silent butyrylcholinesterase in the Vysya community of India. *Clin Chim Acta*. 2007; 378:128–135. [PubMed: 17182021]
- Massaquoi, SG.; Hallett, M. Parkinson's disease and movement disorders. Williams & Wilkins; Baltimore: 1998. Ataxia and other cerebellar syndromes; p. 623-86.
- Miyagi N, Rao VP, Ricci D, Du Z, Byrne GW, Bailey KR, Nakai H, Russell SJ, McGregor CG. Efficient and durable gene transfer to transplanted heart using adeno-associated virus 9 vector. *J Heart Lung Transplant*. 2008; 27:554–560. The Official Publication of the International Society for Heart Transplantation. [PubMed: 18442723]
- Parks RJ, Chen L, Anton M, Sankar U, Rudnicki MA, Graham FL. A helper-dependent adenovirus vector system: removal of helper virus by Cre-mediated excision of the viral packaging signal. *Proc Natl Acad Sci U S A*. 1996; 93:13565–13570. [PubMed: 8942974]
- Pistell PJ, Ingram DK. Development of a water-escape motivated version of the Stone T-maze for mice. *Neuroscience*. 2010; 166:61–72. [PubMed: 20026250]
- Saxena A, Sun W, Luo C, Doctor BP. Human serum butyrylcholinesterase: in vitro and in vivo stability, pharmacokinetics, and safety in mice. *Chem-Biol Interact*. 2005; 157-158:199–203. [PubMed: 16263104]
- Saxena A, Sun W, Fedorko JM, Koplovitz I, Doctor BP. Prophylaxis with human serum butyrylcholinesterase protects guinea pigs exposed to multiple lethal doses of soman or VX. *Biochem Pharmacol*. 2011; 81:164–169. [PubMed: 20846507]
- Schirmer JM, Miyagi N, Rao VP, Ricci D, Federspiel MJ, Kotin RM, Russell SJ, McGregor CG. Recombinant adeno-associated virus vector for gene transfer to the transplanted rat heart. *Transplant International*. 2007; 20:550–557. Official Journal of the European Society for Organ Transplantation. [PubMed: 17403107]
- Silver, A. The biology of cholinesterases. Elsevier-North Holland; Amsterdam: 1974. p. 67-98.
- Waehler R, Russell SJ, Curiel DT. Engineering targeted viral vectors for gene therapy. *Nat Rev Genet*. 2007; 8:573–587. [PubMed: 17607305]
- Weber A, Butterweck H, Mais-Paul U, Teschner W, Lei L, Muchitsch EM, Kolarich D, Altmann F, Ehrlich HJ, Schwarz HP. Biochemical, molecular and preclinical characterization of a double-virus-reduced human butyrylcholinesterase preparation designed for clinical use. *Vox Sanguinis*. 2011; 100:285–297. [PubMed: 20946535]
- Yang W, Xue L, Fang L, Chen X, Zhan CG. Characterization of a high-activity mutant of human butyrylcholinesterase against (–)-cocaine. *Chem-Biol Interact*. 2010; 187:148–152. [PubMed: 20060817]
- Zheng F, Yang W, Ko MC, Liu J, Cho H, Gao D, Tong M, Tai HH, Woods JH, Zhan CG. Most efficient cocaine hydrolase designed by virtual screening of transition states. *J Am Chem Soc*. 2008; 130:12148–12155. [PubMed: 18710224]

Fig. 1.

Cocaine hydrolase activity across time after vector transduction. **a** Mice received viral vector (AAV human CocH, $n = 14$, closed circles; hdAD mouse CocH, $n = 10$, open circles) at approximately 8 weeks of age and were followed for over 1 year. CocH activity in plasma samples is expressed in units of micromoles cocaine hydrolysis per minute. Baseline activity (pretreatment levels) was typically less than 0.001 U. **b** Mice received viral vector (AAV human CocH, $n = 8$) at 8 weeks and were followed for 9 months. CocH activity in plasma samples is expressed in milliunits of micromoles per minute. Baseline activity (pretreatment levels) was typically less than 0.001

Fig. 2.

Suppression of cocaine-induced locomotor activity. Mice ($n=8-12$) were tested in locomotor chambers 4–6 weeks after receiving viral vectors. Controls were previously untreated animals. Shown are the means and SEM of locomotor activity (centimeters travelled per 2 min bin) from hour-long sessions on consecutive days beginning immediately after injection of saline (SAL) on the first hour of day 1 and was followed by cocaine (COC) at 40 mg/kg in the second hour. In the first hour on day 2, saline was given and followed by D-amphetamine (AMP) of 5 mg/kg in the second hour. One-way analysis of variance showed highly significant effects of cocaine in controls but not in vector-treated mice, while amphetamine increased locomotion regardless of pretreatment (** $p < 0.0001$; * $p = 0.014$)

Fig. 3.

Cognition and memory analysis in Stone T-maze. Test groups: AAV human CocH, AAV mouse CocH, and hdAD mouse CocH. Control groups: AAV LucZ vector and saline. **a** Mean latency ($\pm$ SEM) in three trial blocks during acquisition. **b** Mean errors ($\pm$ SEM) in three trial blocks during acquisition. *** $p < 0.0001$ (compared to average performance on acquisition trials 1–3)

Fig. 4.

Motor performance on Rotarod. **a** Mean latency ($\pm$ SEM) to fall from accelerating Rotarod in test groups (AAV human CocH, AAV mouse CocH, hdAD mouse CocH) and control groups (AAV LucZ vector and saline controls). Mice were tested at 3–4 months of age. *** p < 0.0001 (vs. acquisition trial 1). **b** Older mice tested at 12–14 months of age. Only trial 3 is shown. * p = 0.007 (vs. saline controls)

Fig. 5. Physical function (forced maximal endurance) on treadmill. Calculated mean total work (kJ) in test groups expressing mutated mouse and human BChE and controls treated with saline or AAV LucZ vector. **a** Mice tested at 5–7 months of age. **b** Older mice tested at 14–17 months of age. Statistical significance is shown (* $p=0.02$; **** $p<0.0001$)

Table 1**Grip strength**

Age	Control	AAV human Coch	hdAD mouse Coch
7 weeks	268±14 (n =8)	243 ±5 (n =9)	238±14 (n =5)
13 months	276±9(n =19)	266±10(n =8)	280±13 (n =7)

Grip strength was assessed in test groups AAV human Coch, hdAD mouse Coch, and age-matched saline-treated controls at 7 weeks and 13 months of age. Results shown are average of three trials in grams ($\pm$ SEM). Compared to controls, there was no significant effect in the test groups at both ages