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Cdh13 and *AdipoQ* gene knockout alter instrumental and Pavlovian drug conditioning

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Genome-wide association studies in humans have suggested that variants of the cadherin-13 (*CDH13*) gene are associated with substance use disorder, subjective response to amphetamine, and attention deficit hyperactivity disorder. To examine the role of the *Cdh13* and its peptide ligand adiponectin (*AdipoQ*) in addiction-related behaviors, we assessed *Cdh13* knockout (KO) rats and *AdipoQ* KO mice using intravenous cocaine self-administration and conditioned place preference (CPP) paradigms. During intravenous cocaine self-administration, male *Cdh13* heterozygous (+/−) and KO (−/−) rats showed increased cue-induced reinstatement compared with wild-type (WT) rats when presented with a cocaine-paired stimulus, whereas female *Cdh13* rats showed no differences across genotype. *Cdh13* −/− rats showed higher responding for a saccharin reinforcer and learned the choice reaction time (RT) task more slowly than WTs. However, we found no differences between *Cdh13* −/− and +/+ rats in responding for sensory reinforcement, number of premature responses in the RT task, tendency to approach a Pavlovian food cue, CPP and locomotor activation to cocaine (10 or 20 mg/kg). In *AdipoQ* −/− mice, there was a significant increase in CPP to methamphetamine (1 mg/kg) but not to a range of d-amphetamine doses (0.5, 1, 2 and 4 mg/kg). Taken together, these data suggest that *Cdh13* and *AdipoQ* regulate sensitivity to psychomotor stimulants and palatable rewards without producing major changes in other behaviors. In humans, these two genes may regulate sensitivity to natural and drug rewards, thus influencing susceptibility to the conditioned drug effects and relapse.

Keywords: Action impulsivity, associative learning, autoshaping, feeding behavior, incentive sensitization, motivation, operant conditioning, psychomotor stimulants, substance use disorder, T-cadherin

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Many psychiatric disorders show substantial heritability (Sullivan *et al.* 2012), including attention deficit hyperactivity disorder (ADHD) (Faraone *et al.* 2005; Hinney *et al.* 2011; Larsson *et al.* 2013; Morrison & Stewart 1971; Stergiakouli *et al.* 2015) and substance use disorders (SUDs) (Bienvenu *et al.* 2011; Goldman *et al.* 2005; Kendler *et al.* 2003, 2012; Verhulst *et al.* 2015). Genome-wide association studies (GWAS) have been used to identify specific genetic loci associated with these disorders. Once identified, rodent models can be used to confirm the importance of implicated genes and can also be used to identify the biological and psychological mechanisms by which these genes influence behavior.

Recently the *CDH13* gene, which codes for the cell-adhesion molecule cadherin-13 (Ranscht & Dours-Zimmermann 1991) or T-Cadherin, has received attention for its relationship to substance abuse (Johnson *et al.* 2011; Liu *et al.* 2006; Uhl *et al.* 2008a) and smoking cessation outcomes (Drgon *et al.* 2009). GWASs have also identified associations between several single nucleotide polymorphisms in *CDH13* and multiple behavioral disorders as well, including ADHD (Lesch *et al.* 2008; Mavroconstanti *et al.* 2013; Neale *et al.* 2010; Salatino-Oliveira *et al.* 2015), bipolar disorder symptoms (Cho *et al.* 2015), schizophrenia (Borglum *et al.* 2014), violent behavior (Tiihonen *et al.* 2015), and alterations in working memory performance (Arias-Vásquez *et al.* 2011). *CDH13* is also associated with subjective response to amphetamine in non-addicted subjects (Hart *et al.* 2012b; Leventhal *et al.* 2017), which may be an intermediate phenotype of drug abuse. Thus, multiple lines of evidence suggest that various alleles of *CDH13* pleiotropically affect a wide array of behaviors; however, the neural and psychological mechanisms through which these behaviors are modified remain open questions.

CDH13 is expressed throughout the adult brain (Takeuchi *et al.* 2000), where it is thought to act as a negative regulator of neural proliferation, including regions known to be involved in drug abuse and behavioral regulation such as cortex and midbrain. In addition to its regulation of neural cell growth, cadherin-13 also acts as a receptor for the peptide hormone adiponectin (Hug *et al.* 2004). Serum adiponectin levels in ADHD patients are inversely correlated with psychiatric symptoms (Mavroconstanti *et al.* 2014), and

can reduce depression-related behaviors in rodents when administered intracerebroventricularly (Liu *et al.* 2012). Thus, *CDH13* actions on psychiatric symptoms may operate in part through its interaction with adiponectin.

Previous studies have shown that *Cdh13* knockout (KO) mice show deficits in spatial learning and fear conditioning (Rivero *et al.* 2015), which may represent a rodent ADHD endophenotype. Furthermore, these mice are more sensitive to the motivational properties of cocaine at low doses (Drgonova *et al.* 2016; Uhl *et al.* 2014), such that the dose-response curve for reinforcing effects of drugs such as cocaine is shifted leftward. These observations suggest that alterations in cadherin-13 function can modify learning and drug reward sensitivity in rodents. In light of these findings, we sought to determine whether disrupting *Cdh13* function in rats would similarly modify drug-taking and drug-conditioning, as well as alter features of behavioral regulation associated with ADHD and SUD.

To this end, we tested *Cdh13* KO rats in several relevant behavioral paradigms: (1) intravenous cocaine self-administration, which measures the motivational properties cocaine and its associated cues (Bossert *et al.* 2013; Saunders & Robinson 2010), (2) cocaine conditioned cue preference, which measures individuals' approach to drug-paired stimuli (Cunningham *et al.* 2006), (3) saccharin reinforcement, which is used to examine sensitivity to reward and anhedonia (Pijlman *et al.* 2003; Pucilowski *et al.* 1993) and (4) choice reaction time (CRT) task, which measures attention and action impulsivity by requiring subject to withhold responding and maintain attention (Bari & Robbins 2013; Richards *et al.* 2013; Robbins 2002). We additionally measured the effect of adiponectin deletion in an *AdipoQ* KO line of mice on conditioned place preferences (CPPs) induced by methamphetamine and d-amphetamine. Additional subjects were tested for (1) sensory reinforcement, which is thought to reflect aspects of sensation seeking and predict drug self-administration acquisition (Gancarz *et al.* 2012a,2012b), and (2) Pavlovian conditioned approach, which evaluates the propensity of Pavlovian food cues to elicit approach (Robinson *et al.* 2014) (see Appendices S1 and S2, Supporting Information).

Materials and methods

Animals

Subjects were an inbred line of salt-sensitive Dahl rats (SS-*Cdh13*^{em1Mcwi}) that were polymorphic at the *Cdh13* locus for either a KO or wild-type (WT) allele. This KO line SS-Cdh13^{em1Mcwi} (http://rgd.mcw.edu/rgdweb/report/strain/main.html?id=5131922) was supplied by Drs. Aron Geurts and Howard Jacob at the Medical College of Wisconsin as part of the Genome Editing Rat Resource Center (http://rgd.mcw.edu/wg/gerrc). They were generated by targeting the *Cdh13* gene with zinc finger nucleases, resulting in an 8 bp frameshift deletion in exon 1. Rats were either bred from heterozygotes (HET) at the Psychology Department at the University at Buffalo or shipped directly to the Research Institute on Addictions in Buffalo, NY. All rats were tested as littermates and pair-housed as same sex pairs in plastic cages (42.5 × 22.5 × 19.25 cm), except during self-administration in which subjects were single housed. Cages were lined with bedding (Aspen Shavings) and kept in a temperature controlled environment (22 ± 1°C). Water and food (Harlan Teklad Laboratory Diet #8604, Harlan Inc., Indianapolis, IN, USA) were available *ad lib* except during CRT training when access to water was restricted beginning the week prior to the onset of testing. In this case, rats were given water for 20 min following daily testing. Rats were housed on a 12 h reverse light/dark cycle (lights off at 0730–0800 h), and all testing occurred during the dark phase at least 1 h following lights off. For all experiments, subjects were tested in blocks of 16 for *Cdh13* rats. Run order for all subjects was held constant, such that testing occurred at the same time of day throughout testing. When applicable, random assignment was used for all variables, including apparatus assignment. All procedures were approved by the University at Buffalo's Institutional Animal Care and Use Committee.

Cdh13 rats were tested in separate groups for each of the six test procedures: First, subjects ages 75–85 days were tested in intravenous cocaine self-administration ($n = 49$; 12–13 +/+ and –/–, 25 +/–) (Table S1a). Separate subjects underwent conditioned cue preference (ages 76–101 days), to either a 10 mg/kg ($n = 33$; 5–15 –/– and +/+, 13 +/–) or 20 mg/kg dose of cocaine ($n = 38$; 14–15 +/+ and –/–, 9 +/–) (Table S1a). For the behavioral regulation tasks, subjects were tested sequentially on sensory and saccharin reinforcement, followed by a CRT task ($n = 19$; 12 +/+ and 7 –/–) (Table S1a).

We used the software program GPower to determine that for a fixed effects ANOVA, a large effect size (Cohen's $f = 0.5$) and 6 groups (male × female, WT × HET × KO), a target sample size of 42 would be needed to obtain this effect with a power level of 0.8. Sex differences and heterozygous subjects were not analyzed for the behavioral regulation tasks because of the low sample sizes. Finally, a separate group of subjects underwent Pavlovian conditioned approach ($n = 77$; 14–24 +/+ and –/–, 39 +/–) (see Table S1a for a more detailed breakdown of subject groups). Mean ages at the beginning of testing for Cdh13 rats were 63 days (263 g) at the start of the self-administration, 114 days (279 g) at the beginning of CPP, and 89 days (~270 g) old at the beginning of the behavioral regulation experiments. Each experimenter was blind to each subject's genotype during behavioral testing.

AdipoQ KO mice were tested in two CPP experiments using methamphetamine and d-amphetamine. B6.129-*Adipoq*^{tm1Chan}/J mice were obtained from the Jackson Laboratory. Separate groups of male and female KO and WT subjects underwent conditioning to methamphetamine ($n = 58$; 29 +/+ and –/–), or one of four doses of d-amphetamine ($n = 113$; 9–18 per +/+ and –/– group) (Table S1b). Mean ages at the beginning of testing for *AdipoQ* mice were 66 days (20.3 g). For all experiments, subjects were tested in blocks of 10 for the *AdipoQ* mice. All procedures were approved by the University at Chicago's Institutional Animal Care and Use Committee. Results were prepared using the reporting standards outlined by Kilkenny *et al.* 2010 and Steckler *et al.* 2016.

Intravenous cocaine self-administration

Apparatus

Testing occurred in modular test chambers (20.5 × 24.1 cm floor area, 29.2 cm high; Med Associates Inc., St. Albans, VT, USA) inside sound attenuating cubicles equipped with ventilation fans (A&B Displays, Bay City, MI, USA). Each chamber contained two eye-level nose poke holes on both the left and right side, outfitted with infrared photobeams and stimulus lights. A red houselight was located on the rear panel of the chamber (27 cm high). Chambers were also outfitted with metal tethers with modified plastic threaded screw-caps (PlasticsOne, Roanoke, VA, USA), and each tether was attached to a weighted swivel (Instech, Plymouth Meeting, PA, USA). Tethers and swivels were attached syringe pumps (Med Associates Inc.) which delivered drug solution through a polyethylene tubing (Tygon, Akron, OH, USA) that attached to surgically implanted catheter lines (see below). All testing data was collected using MED-PC IV software.

Procedure

Rats were first surgically implanted with catheters in the right jugular vein (described in Crombag *et al.* 2005) under ketamine [70 mg/kg intraperitoneal (i.p.); Patterson Veterinary, Mt. Joy, PA, USA] and xylazine (5 mg/kg i.p.) anesthesia. Each catheter line (Braintree Scientific Inc., Braintree, MA, USA) was connected to a threaded 22-gauge

guide cannula (PlasticsOne) which was embedded into a mesh disc (2.5 cm diameter) using dental acrylic. When implanted, this cannula exited the dorsal side of the rat, posterior to the shoulder blades. Following surgery, rats were treated with carprofen (5 mg/kg) for a minimum of 3 days, and flushed daily with 0.1 ml of heparinized saline and enrofloxacin (Baytril, Bayer HealthCare LLC, Shawnee Mission, KS, USA). During self-administration, catheters were flushed immediately before and following the session. Once a week during non-testing days, catheter patency was verified using 0.05 ml of diluted ketamine (10 mg/ml). Rats that became ataxic within 5 seconds were considered patent and were included in the study.

Following recovery from surgery, rats underwent five daily self-administration sessions per week. During self-administration, rats were presented with two nose poke holes. Nose pokes into the active hole resulted in a ~3-second infusion of 0.2 mg/kg/infusion cocaine on an FR1 schedule. Infusion lengths were adjusted for body weight to deliver a volume that would produce a 0.2 mg/kg dose. Rats were weighed every other test session and infusion length was adjusted accordingly. The nose poke port stimulus light was illuminated simultaneously with the infusion, lasted for 4 seconds, and initiated a timeout period in which further responses produced no effect. Responses into the inactive hole were recorded but had no programmed consequences. Subjects were randomly assigned to either left or right active nose poke holes. Sessions lasted for 3 h, or until subjects reached infusion criterion. For sessions 1–3, an infusion criterion of 10 was used, such that once subjects reached 10 infusions, testing ended. For sessions 4–6, the infusion criterion was increased to 20, and then finally for sessions 7–10 the criterion was increased to 40. In this manner, WT, HET and KO rats received equal number of cocaine infusions.

Following the initial 10 sessions of self-administration, rats were tested in a progressive ratio paradigm on sessions 11 and 12. Nose pokes were reinforced according to the following exponential progression [1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, etc., derived from the formula $((5 \times e^{0.2n}) - 5)$] (Saunders & Robinson 2011). Rats were reinforced with 0.2 mg/kg/infusion during session 11, and with 0.5 mg/kg/infusion during session 12. The 0.5 mg/kg/infusion dose was produced by increasing the length of the infusion rather than by modifying the concentration of cocaine given. Sessions terminated after 3 h, or if subjects received no reinforcement for a period of 1 h. All other features of the testing environment were identical to sessions 1–10.

Following progressive ratio, rats were reestablished on an FR1 schedule during sessions 13–15. Then, rats were placed under extinction for sessions 16–23. During these eight sessions, there were no programmed consequences in either the active or the inactive nose poke hole. Sessions lasted for 3 h. Rats were then left undisturbed in their home cages for 7 days, and then were tested in a single cue-induced reinstatement session, in which active port nose pokes were reinforced with the cocaine-paired light cue. No drug was delivered during this session.

During testing, session time and nose pokes into the active and inactive ports were recorded using Med-PC IV. The number of reinforced nose pokes was also collected throughout testing, except for extinction during which there was no reinforcer contingency present. During the infusion criterion sessions, dividing the number of nose pokes by session time yielded the rate of responding for drug.

Statistical analysis

All statistics for all experiments were computed using Statistica 13 (Dell Inc., Tulsa, OK, USA). For cocaine self-administration, nose poke responses, rates of reinforcement and progressive ratio breakpoints were analyzed using repeated-measures ANOVA, with genotype (WT, HET and KO) and sex (male and female) as between-groups factors and session (1–10, 13–15 or 16–23), port (active, inactive) and dose (0.2 mg/kg, 0.5 mg/kg) as the within-subjects factors when appropriate. For the cue-induced reinstatement test, only the first 10 min, during which peak responding occurs, were analyzed.

All ANOVA analyses for all experiments reported here that yielded a significant result was tested for homoscedasticity by using Levene's Test for Homogeneity of Variances. A violation of homogeneity of variance was established when the result of this test yielded $P < 0.05$

Planned comparisons

For main effects and interactions, we used an *a priori* planned comparison of least square means (contrast analysis). It has been shown that *Cdh13* gene KO in mice results in more robust CPP (Drgonova *et al.* 2016), and variants in *Cdh13* in humans alter the euphorigenic properties of amphetamine (Hart *et al.* 2012a) suggesting increases in reward sensitivity following gene KO. For this reason, we hypothesized that when compared with WT subjects, KOs and heterozygous subjects would be more sensitive to the reinforcing properties of both drug and food rewards across experiments. Therefore, each planned comparison sought to evaluate increases in reward sensitivity in heterozygous and KO subjects when contrasted with WT subjects. Specifically, we predicted that KO subjects would should more robust progressive ratio responding for cocaine, and larger cue-induced reinstatement of responding for cocaine following extinction when compared with WT subjects.

We tested planned comparisons separately in males and females whenever we detected a main effect or interaction ($P \leq 0.05$) that included sex because sensitivity to drug reward and relapse are thought to be different between males and females (Bobzean *et al.* 2014). To directly evaluate sex differences, we compared only WT males to WT females. Tukey's honest significant difference (HSD) was used to probe any additional significant main effects or interactions. Significance was set at $P \leq 0.05$ for all analyses across all experiments where Tukey's HSD is reported.

Conditioned cue preference in* Cdh13 *KO rats

Apparatus

Rats were tested in black acrylic chambers (47 cm length × 19 cm width × 30 cm height) with black spray-painted textured floors that were either 'grid' or 'hole'. Textured floors were placed on top of a smooth black matte floor underneath the testing chamber. The entire chamber was spray-painted black to maximize video contrast for video capture via infrared cameras connected to a 16-channel DVR (Swann Communications, Inc., Santa Fe Springs, CA, USA). Locomotor data and side preference were analyzed using Topscan video tracking software (Clever Sys., Inc., Reston, VA, USA; Flagel & Robinson 2007; Meyer *et al.* 2012b). Chambers were housed in custom-made light-proof, sound attenuating chambers. Cocaine was prepared by dissolving powder from cocaine HCl (National Institute on Drug Abuse, Bethesda, MD, USA) into saline at a concentration of either 10 or 20 mg/ml to produce solutions for the 10 or 20 mg/kg conditions, respectively.

Procedure

On each day of testing, rats were weighed, placed into individual transport containers (Sterilite Corporation, Townsend, MA, USA), transferred to the testing room, and left for a 15-min period before testing began. On the first day of testing (habituation), rats were injected with saline and placed into the chamber with a smooth black matte floor for 30 min, to allow the rats to acclimate to the chamber. On the subsequent day (pretest) rats were injected with saline and placed into chambers containing 'hole' and 'grid' floor halves. Subjects were randomly assigned which side of the apparatus the grid and hole floors were situated during the pretest. The least preferred floor (i.e. the floor the subject spent the least amount of time on) for each subject was assigned as the cocaine-paired floor. Subjects were then conditioned for the following 8 days, in which cocaine and saline were paired with the least and most preferred floor, respectively. Rats were injected with cocaine (either 10 mg/kg, i.p. or 20 mg/kg i.p.) or saline on alternating days immediately before being placed into the chamber. The chamber contained only one floor type on these days. Each pair of cocaine-paired and saline-paired days was termed a trial. Thus, there were four trials during the 8-day conditioning period. A posttest was administered on the final day, where rats were given a saline injection and placed into the chamber with both floor types. Each test session lasted 30 min.

The time spent on the cocaine-paired side was recorded in seconds during the pretest and posttest days. Change in time spent on the cocaine-paired floor after conditioning was calculated by subtracting the pretest time spent on the cocaine-paired floor from the posttest

time. Locomotor activity (mm traveled) was recorded on all test days using TopScan.

Statistical analysis

Subjects were analyzed using a repeated-measures ANOVA. For locomotor activity analysis, the between-subjects factors were genotype (WT, HET and KO), sex (male and female) and dose (10 and 20 mg/kg). The within-subjects factors across conditioning days were trial (1–4) and drug (saline and cocaine). For preference analysis, we examined time spent on the cocaine-paired side using the between-subjects factors: genotype (WT, HET, KO), sex (male, female), and drug dose (10 and 20 mg/kg). The within-subjects factor was test day (pretest, posttest). Tukey's HSD was used to probe significant main effects or interactions.

Conditioned place preference in adiponectin KO mice

Apparatus

The testing apparatus and procedure used in this experiment has been described previously (Bryant *et al.* 2012b,2012a). In brief, 37.5 cm × 37.5 cm open field chambers (AccuScan Instruments, Columbus, OH, USA) were divided in half to form two compartments. Each half of the open field was separated by a 30 cm tall black divider, which contained a 5 cm × 5 cm entryway between each side. Both sides of the compartment were distinguished with separate visual and tactile stimuli. The left compartment contained white horizontally stripped walls and a smooth floor, whereas the right compartment contained black vertically stripped walls and a pointed floor. During conditioning, the divider was turned upside down so that subjects could not pass between compartments. The drug-paired compartment was randomly assigned to either left or right, and counterbalanced such that each compartment was equally represented. Time spent on the drug-paired side and locomotor activity was recorded and analyzed using Versamax software (AccuScan Instruments version 4.12-125E).

Procedure

We tested WT and KO animals in an 8-day CPP paradigm. For the first CPP experiment, subjects were treated with either saline or methamphetamine HCl (1 mg/kg i.p.) (Sigma-Aldrich, St. Louis, MO, USA). For the second set of experiments, subjects were pretreated with either saline or one of four doses of d-amphetamine sulfate (0.5, 1.0, 2.0 or 4.0 mg/kg i.p.) (Sigma-Aldrich).

For the first group of subjects, mice underwent methamphetamine CPP. On Day 1 (pretest), animals received a saline injection and were placed into the CPP chamber, which was separated into two distinct sides by a divider; animals had access to both sides of the chamber. On Days 2–7, animals received alternating injections of either saline or methamphetamine and were confined to one drug-paired side of the chamber. On Day 8 (posttest), animals were treated with saline and allowed to access both sides of the chamber and time on the methamphetamine-paired side was recorded. The drug-paired side was randomized between subjects.

For the second group of subjects, mice underwent d-amphetamine CPP. Subjects were randomly assigned to receive the 0.5, 1.0, 2.0 or 4.0-mg/kg dose of d-amphetamine throughout conditioning. The CPP testing procedure was identical to the methamphetamine procedure for all d-amphetamine doses.

Statistical analysis

Subjects were analyzed for time spent on the drug-paired side of the conditioning apparatus using a repeated-measures ANOVA, with test day as the within-subjects factor (Days 1 and 8) and genotype as the between-subjects factor (WT, KO). For d-amphetamine conditioning, an additional between-subjects factor for dose (0.5, 1, 2 and 4 mg/kg) was included in the repeated-measures ANOVA. Because adiponectin acts as a ligand for the CDH13 protein (Hug *et al.* 2004), we hypothesized that AdipoQ KO would recapitulate the effects of Cdh13 KO, specifically by increasing reward sensitivity, and thus increasing CPP for methamphetamine and d-amphetamine compared with WT subjects. We ran a planned comparison between WT and KO for the posttest time spent on the methamphetamine-paired floor. Tukey's HSD was used to probe any additional significant main effects or interactions.

Saccharin reinforcement

Apparatus

Rats were tested in locally constructed experimental chambers (Lloyd *et al.* 2012), with stainless steel grid floors, enclosed in light and sound attenuating boxes. The test panel contained two liquid feeder ports. Each liquid feeder port was equipped with an infrared photo beam that detected snout entries. Syringe pumps (PHM-100; MED Associates, East Fairfield, VT, USA) were used to deliver water or saccharin into the liquid feeder ports. Either the left or right liquid feeder port was assigned as the active port, and was counterbalanced between subjects.

Procedure

Rats were tested 3 times on each of 2 consecutive days for a total of 6 test sessions. Individual test sessions lasted 18 min with a 1-h interval between the start of each test. On the first day, active port responses were reinforced with water. On the second day, the rats' active port responses were reinforced with 30 μl of 0.1% saccharin (w/v). The first response after 5 seconds had elapsed since the last reinforcer delivery produced a 30 μl amount of fluid (water or saccharin).

The primary dependent measures were number of nose pokes in the active and inactive holes, defined as the number of infrared photobeam interruptions for each session. Each entry counted only once. Data were collected using the same The MED-PC IV software package as described previously.

Statistical analysis

Responding was analyzed using repeated-measures ANOVA with session (1–3) and fluid (water, saccharin) as within-subjects factors and genotype (WT, KO) as a between-subjects factor. Heterozygotes were excluded from analysis. For significant genotype effects or interactions, we tested planned comparison between WT and KO subjects, hypothesizing that KO subjects would show more responses for a palatable saccharin reward compared with WT subjects. We also compared the responses of these two genotypes when working for a water reinforcer, with the hypothesis that there would be no difference when the fluid was a non-reward.

CRT task

Apparatus

The apparatus has been described previously (Richards *et al.* 2013). In brief, the test panel of each test chamber had two water dispensers located on either side of a centrally located nose poke hole. Stimulus lights were mounted above the two water dispensers and the center nose poke hole. Sonalert tone generators were mounted above the left and right stimulus lights. The left Sonalert emitted a continuous pure tone at 2.9 kHz and the right Sonalert emitted a pulsed 1.9 kHz tone. The water dispenser and stimulus lights were arranged so that they were at the level with the rat's eyes when the rat's snout interrupted an infrared beam in the center snout-poke hole. Nose pokes into the water dispensers were monitored with infrared detectors. Precise amounts of water were delivered by syringe pumps (PHM-100; MED Associates).

Procedure

Testing occurred such that rats could initiate a trial by holding its snout in the center nose poke hole until either the left stimulus light was activated (*imperative stimulus*). The rat was required to maintain holding in the center nose poke hole, for a duration defined as the *hold time*. After the hold time was met, an imperative stimulus signaled availability of a water reinforcer (30 μl) in the left feeder hole. Following the onset of the imperative stimulus, subjects had 3 seconds

to enter into the correct feeder hole. The amount of time elapsed before entering the correct feeder hole is the subject's *reaction time* (*RT*). Responses into the right feeder hole were counted as *incorrect responses*. If no feeder hole entry occurred within 3 seconds, the trial terminated and was counted as an *omission*. Responses into the feeder hole prior to the onset of the imperative stimulus were counted as *premature responses*. Only the left stimulus light and water feeder were used to measure RT. If the rat made a correct response, the rat received a water reinforcer and the trial ended. If the rat made an incorrect response, the trial ended without reinforcement. The stimulus lights were the only sources of illumination in the test chamber. Training occurred 5 days a week, and each session lasted until the subject either completed 100 trials, or 15 min elapsed.

Rats first underwent 25 training sessions, in which the hold time was held constant at 0.25 seconds. The hold time was then increased to 0.5 seconds during sessions 26–30, 1.0 seconds during sessions 31–35 and 2.0 seconds during sessions 36–40. The calculation of hold time was cumulative; e.g. a rat could meet a 2 seconds hold time requirement by holding its snout in the center hole for 1 second on two separate instances. A 2.9 kHz tone was turned on for the duration of each snout poke into the center hole. This feedback tone/stimulus occurred independently of any other contingency and was in effect for the duration of the test session.

The primary dependent measures were the total number completed trials per session, RT, omissions, incorrect responses and premature responses. Data were collected using Med-PC IV as described in previous sections. For analysis, number of premature responses was divided by the total number of trials completed to provide an estimate of premature responses that was not biased by the number of trials completed.

Statistical analysis

During acquisition, number of completed trials was analyzed using repeated-measures ANOVA with session block (1–5, 6–10, 11–15, 16–20, 21–25) as the within-subjects factor, and denotype (WT, KO) as the between-subjects variable. Following acquisition, RT and premature responses were analyzed with hold time (0.5, 1.0, 2.0 seconds) included as a within-subjects factor. One KO rat failed to maintain responding when the hold time was increased and were therefore dropped from the study following acquisition. Heterozygotes were not included in the analysis.

Results

Cocaine intravenous self-administration

Cdh13 KO, heterozygous and WT rats learned to self-administer cocaine over 10 sessions. During the acquisition of cocaine self-administration, subjects directed their nose poking toward the cocaine-paired active port [main effect of port: ($F_{1,39}$) = 144.9, $P < 0.001$] and subjects increased their rate of responding as training progressed [main effect of session: ($F_{9,351}$) = 20.6, $P < 0.001$; session × port interaction: ($F_{9,351}$) = 20.2, $P < 0.001$] (Fig. 1a,b). However, there were no significant effects or interactions with either genotype or sex on responding throughout acquisition of self-administration (Fig. 1a,b).

During progressive ratio, the number of required nose pokes to obtain cocaine delivery increased following each infusion throughout the session. Subjects directed their nose poking to the active port [main effect of port: ($F_{1,43}$) = 143.92, $P < 0.001$] and active responding was larger when the dose of cocaine was increased from 0.2 to 0.5 mg/kg/infusion [main effect of dose: ($F_{1,43}$) = 226.61, $P < 0.001$; dose × port interaction ($F_{1,43}$) = 195.60, $P < 0.001$]. Consequently, subjects received more infusions when the dose was increased

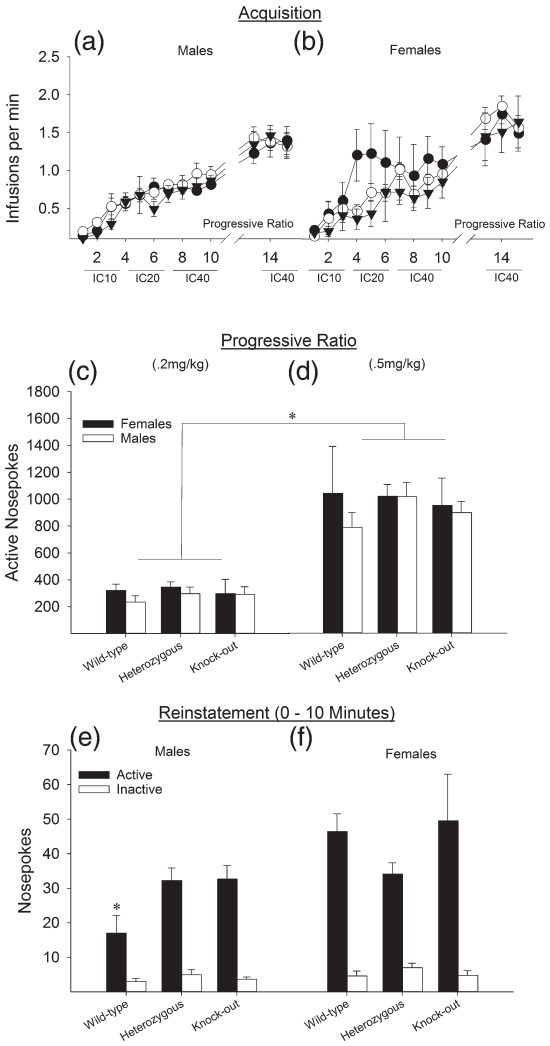

Figure 1: Cocaine self-administration and reinstatement. Subjects learned to nose poke for intravenous infusions of cocaine across multiple sessions. Although there was a main effect of session in responding for cocaine, all genotypes and sexes increased rate of responding for cocaine similarly (a, b). Subjects self-administered cocaine on a progressive ratio schedule of reinforcement, in which the requirement for each successive infusion of cocaine increased within session (c, d). Male and female subjects increased responding for the larger dose of cocaine (0.5 mg/kg/infusion) (d) compared with the smaller dose (0.2 mg/kg/infusion) (c) across all genotypes. Following self-administration, subjects showed similar extinction across eight sessions (see S.1) and then tested for cue-induced reinstatement of responding for cocaine. (e) Male heterozygous and knockout subjects showed larger cue-induced reinstatement of drug-seeking in the active port during the first 10 min of reinstatement. (f) In females, there were no differences across genotype, although wild-type females responded more strongly than wild-type males. There were no differences between any groups in responding on the inactive port. Data are presented as means ± SEM. Asterisks (*) denote significant differences between groups ($P < 0.05$).

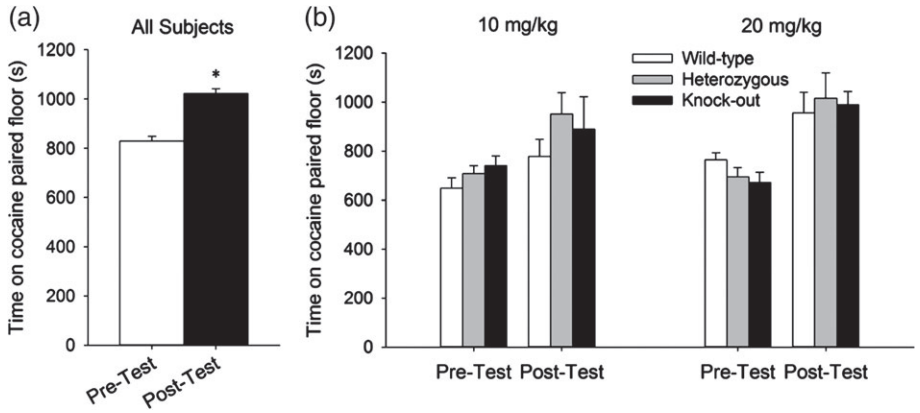

Figure 2: Time spent on cocaine-paired floor during *Cdh13* conditioned place preference. Time spent on the cocaine-paired floor changed following conditioning to repeated cocaine-floor pairings. (a) There was a main effect of conditioning across all subjects, in which time spent on the cocaine-paired floor increased as a result of conditioning. (b) There were no differences in magnitude of conditioning between the 10 and 20-mg/kg dose of cocaine, nor were there differences between sexes or genotype. Data are presented as means ± SEM, and are collapsed across sex in (b). Asterisk (*) denotes significant increase relative to the pre-test ($P < 0.05$).

to 0.5 mg/kg/infusion [main effect of dose: ($F_{1,41}$) = 159.00, $P < 0.001$]. However, there were no main effects of sex or genotype during progressive ratio ($P > 0.05$), and subjects responded similarly as the reinforcement requirement was increased during these test days (Fig. 1c,d).

Following progressive ratio, subjects were returned to an FR1 schedule of reinforcement and allowed to self-administer up to 40 infusions of 0.2 mg/kg cocaine [main effect of port: ($F_{1,43}$) = 6.1, $P < 0.05$] (Fig. 1a). There were no effects or interactions with genotype following progressive ratio ($P > 0.05$). After subjects completed these three sessions, subjects showed a reduction in responding during extinction [port × session interaction: ($F_{7,301}$) = 42.90, $P < 0.001$] (Fig. S1a). *Post hoc* analysis using Tukey's HSD indicated that subjects significantly reduced responding on the active and inactive ports by the final day of extinction. There were no effects or interactions with sex or genotype ($P > 0.05$) (Fig. S1b,c).

During the first 10 min of reinstatement, subjects largely responded in the active nose poke hole [main effect of port: ($F_{1,41}$) = 205.9, $P < 0.001$] (Fig. 1e,f), and this effect was larger in females compared with males [main effect of sex: ($F_{1,41}$) = 15.4, $P < 0.001$]. Performance during reinstatement interacted with genotype [port × sex × genotype interaction: ($F_{2,41}$) = 4.2, $P < 0.05$]. We found that our interaction between port, sex and genotype yielded a partial eta-squared of 0.17, and thus a Cohen's *f* of 0.45. Planned comparisons showed that male heterozygous and KO subjects responded more than WTs (Fig. 1e) (*Ps* < 0.05). In females, however, we found that WT subjects did not differ from either heterozygous or KO subjects (Fig. 1f), and thus the effect of genotype was different between males and females. Finally, planned comparisons indicated that in WT subjects, females showed stronger reinstatement than males ($P < 0.05$).

Conditioned cue preference in CDH13 *KO rats*

The *Cdh13* KO line of rats learned the association between cocaine and a textured floor type over repeated drug-floor pairings. Throughout conditioning, locomotor activity was examined in response to the four cocaine injections. We found that, compared with saline, cocaine injections produced robust increases in locomotor activity at 10 mg/kg [main effect of drug ($F_{1,63}$) = 66.55, $P < 0.001$] (Fig. S2a). This locomotor activation was larger in the subjects who underwent conditioning to the 20 mg/kg dose [dose × drug interaction ($F_{1,63}$) = 5.63, $P < 0.05$] (Fig. S2a,b). However, the locomotor activating effect of cocaine at either dose yielded no main effects or interactions with either sex or genotype ($P > 0.05$) (Fig. S2c,d). Thus, locomotor activation in response to cocaine was similar between males and females for all genotypes.

We also examined the propensity for the cocaine-paired floor to elicit approach behavior following conditioning to the non-preferred floor. Subjects showed an increased amount of time spent on the cocaine-paired floor stimulus following conditioning [main effect of test: ($F_{1,59}$) = 38.78, $P < 0.001$] (Fig. 2a). However, unlike locomotor activation, there was no effect of dose, and thus conditioning was similar at both 10 and 20 mg/kg doses (Fig. 2b). In addition, there were no main effects or interactions with either genotype or sex ($P > 0.05$).

Conditioned place preference in AdipoQ *KO mice*

During CPP, *AdipoQ* KO and WT mice learned the association between methamphetamine treatment and a drug-paired compartment. Following drug conditioning, subjects spent more time on the drug-paired side [main effect of test: ($F_{1,54}$) = 109.63, $P < 0.001$]. The degree of conditioning varied between WT and KO subjects [genotype × test interaction: ($F_{1,54}$) = 4.81, $P < 0.05$], and this test × genotype interaction yielded a partial eta-squared of 0.08, and thus a *Cohen's f* of 0.30. We used a planned comparison analysis and showed that on the posttest, KO subjects spent more time on the methamphetamine-paired floor ($P = 0.05$) (Fig. 3a). There were no differences between groups on time spent on the drug-paired floor during the pretest, and thus this difference

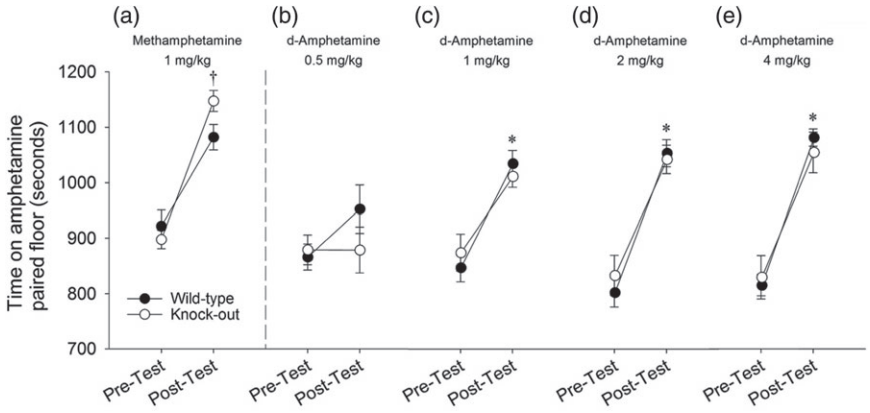

Figure 3: Time spent on drug-paired floor during *AdipoQ* conditioned place preference. Time spent on a methamphetamine and d-amphetamine-paired floor increased following conditioning. Daggers (†) denote significant differences between genotypes ($P < 0.05$) and asterisk (*) denote significant differences within-subjects ($P < 0.05$). (a) *AdipoQ* knockout subjects showed increased time spent on the methamphetamine-paired floor following conditioning compared with wild-type subjects. (b) The 0.5-mg/kg dose of d-amphetamine did not result in a change in time spent on the drug-paired floor, although the 1, 2 and 4 mg/kg doses (c–e) did produce a similar magnitude of conditioning. There were no main effects of sex or genotype across d-amphetamine doses. Data are presented as means ± SEM, and are collapsed across sex.

was driven by a change in time following conditioning. There were no main effects or interactions with sex, so we did not run a comparison analysis. Thus, males and females showed similar behavioral responses to methamphetamine treatment.

Separate *AdipoQ* KO and WT mice were also conditioned to one of four doses of d-amphetamine using an identical procedure. The conditioning procedure increased the amount of time spent on the drug-paired floor [main effect of test: ($F_{1,97}$) = 150.62, $P < 0.001$]. However, the degree of conditioning varied depending on dose of d-amphetamine used for conditioning [dose × test interaction: ($F_{3,97}$) = 5.92, $P < 0.05$]. Specifically, although 1, 2 and 4 mg/kg produced similar increases in time spent on the drug-paired side, *post hoc* indicated that there was no change in time spent on the drug-paired side for the 0.5 mg/kg subjects (Fig. 3b–e).

In contrast to methamphetamine conditioning, there was also a main effect of sex [($F_{1,97}$) = 4.00, $P < 0.05$], with females showing an overall increase in time spent on the drug-paired floor following conditioning. Also, in contrast to methamphetamine conditioning, the only effect of genotype was a four-way interaction [test × sex × genotype × dose interaction: ($F_{3,97}$) = 3.08, $P < 0.05$], however, *post hoc* analysis indicated that there were no genotype effects within dose and sex as a result of this interaction. Thus, although conditioning to methamphetamine and d-amphetamine increased time spent on the drug-paired floor, the effect of *AdipoQ* KO was selective to methamphetamine.

Operant responding for saccharin

Cdh13 KO and WT rats performed operant responses into liquid feeder ports for water and saccharin across three sessions. Responding for saccharin was higher in comparison to water [main effect of fluid: ($F_{1,16}$) = 234.89, $P < 0.01$] (Fig. 4a,b), indicating saccharin was an overall stronger

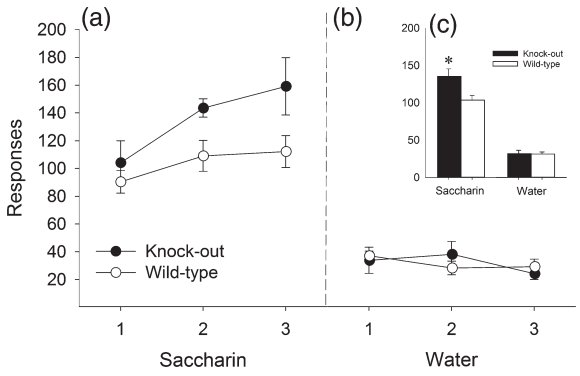

Figure 4: Saccharin reinforcement. *Cdh13* knockout and wild-type subjects nose poked for water and saccharin reinforcement over six sessions. Asterisk (*) denote significant differences between genotypes ($P < 0.05$). (a) Both wild-type and knockout subjects performed nose poke responses when reinforced with saccharin. (b) However, the number of responses made was lower for a water reinforcer. (c) The number of responses for the saccharin reinforcer was larger in knockout subjects compared with wild-type when collapsing across day ($P < 0.05$), but there were no genotypes effects on responding for water. Data are presented as means ± SEM, and are collapsed across sex.

reinforcer for all subjects. This effect of fluid, however, was informed by genotype [fluid × genotype interaction: ($F_{1,16}$) = 7.43, $P < 0.05$]. This interaction for saccharin reinforcement yielded a partial eta-squared of 0.32 and thus a Cohen's *f* of 0.68. We ran a planned comparison analysis between KO and WT subjects on responses for saccharin, and found that KO subjects made significantly more responses ($P < 0.05$). In contrast, to saccharin we found that

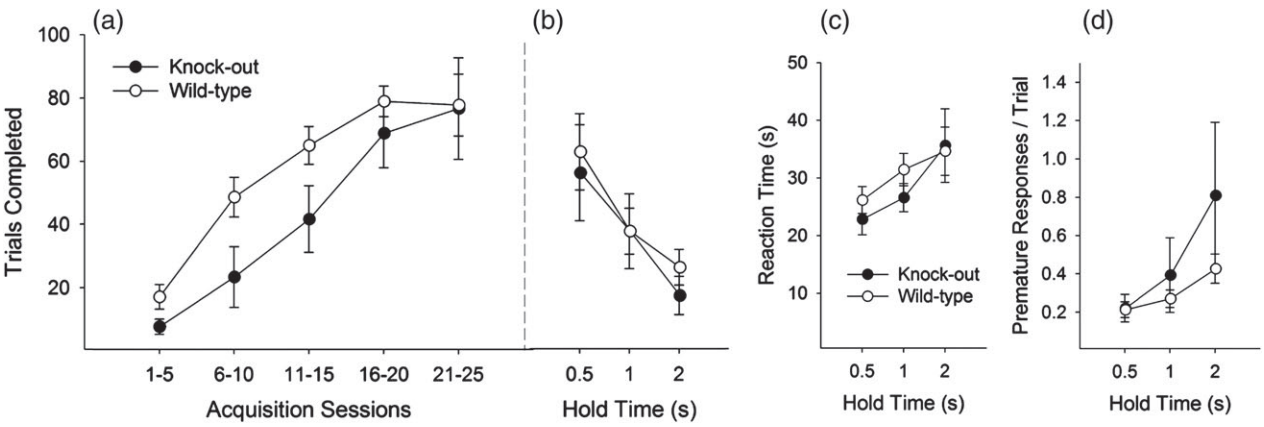

Figure 5: Choice reaction time task. Subjects were trained on a choice reaction time task. (a) Subjects learned to maintain a hold time in a center nose poke hole to receive water reinforcement in an adjacent water magazine. Data are plotted across blocks of five sessions. Knockout subjects completed less trials compared with wild-types across the initial 25 acquisition sessions. (b) Following the acquisition sessions, the hold time was increased from 0.25 to 0.5, 1.0 and 2.0 seconds. All subjects showed a similar decrease in the number of successfully completed trials. (c) Although all subjects had slower reaction time as the hold time requirement was increased, there was no effect of genotype. (d) There was also an increase in premature responding per trial as the hold time was increased, but this effect was similar between wild-type and knockout subjects. Data are presented as means ± SEM, and are collapsed across sex.

WT and KO subjects showed similar responses for water ($P > 0.05$).

CRT task

Rats were successfully completed more trials throughout training [main effect of session: ($F_{4,68}$) = 25.86, $P < 0.05$]. Compared with KOs, however, WT animals completed more trials successfully across training [main effect of genotype: ($F_{1,17}$) = 4.56, $P < 0.05$] (Fig. 5a). Following acquisition, increasing the hold time from 0.25 to 0.5, 1.0 and 2.0 seconds decreased the number of successfully completed trials similarly in both genotypes [main effect of hold time: ($F_{2,86}$) = 53.91, $P < 0.05$] (Fig. 5b).

Although subjects had slower RTs [main effect of hold time: ($F_{2,32}$) = 8.43, $P < 0.05$] and more premature responses [main effect of hold time: ($F_{2,32}$) = 19.17, $P < 0.05$] as the hold time increased, there were no main effects or interactions with genotype for either hold time (Fig. 5c) or premature responses per trial (Fig. 5d). Thus, genotype differences on this task are specific to the initial learning of task-performance and not premature responding or reaction following acquisition.

Discussion

Here, we show that cadherin-13 and adiponectin signaling are involved in several phenotypes related to SUD in rodents. Specifically, we found that *Cdh13* and *AdipoQ* KO altered several types of reward-directed behaviors, including cue-induced reinstatement of cocaine seeking, methamphetamine CPP, and responding for palatable food rewards. However, we did not find genotype differences in ongoing cocaine self-administration, CPP to cocaine and d-amphetamine, or differences in the locomotor activating effect of cocaine. Furthermore, genotype differences in other behavioral regulation tasks were also limited, including rate of habituation to a novel sensory reinforcer (Fig. S3 in Appendix S1), premature responding on the CRT, and finally conditioned approach to food cues (Fig. S4 in Appendix S2). Therefore, *Cdh13* regulates behaviors that are primarily related to reward sensitivity, but this effect is limited among different drug paradigms and psychomotor stimulant categories. *CDH13* is implicated in multiple behavioral disorders in humans, and the data presented here suggest that one avenue through which this gene can alter behavior may be through modifying sensitivity to rewards in the environment.

Intravenous cocaine self-administration

During intravenous self-administration of addictive compounds, the progressive ratio procedure can be used to determine the motivational strength of a drug reinforcer by requiring subjects to continually increase operant responding (Richardson & Roberts 1996). Here, we found that self-administration in rats missing cadherin-13 did not differ in either acquisition of self-administration or responding during progressive ratio. However, one of the hallmark features of addiction is propensity to relapse drug-taking in response to environmental cues. The presence of drug-cues facilitate ongoing self-administration (Schenk & Partridge 2001) and these cues can be utilized in reinstatement paradigms to prompt drug-seeking (Shaham *et al.* 2002). Indeed, cocaine cues in humans can elicit craving and conditioned physiological effects (Ehrman *et al.* 1992) and thus these models reflect one avenue to prompting drug-seeking behavior through drug stimuli. We show here that males with attenuated cadherin-13 function (e.g. heterozygous and KO subjects) showed a more robust reinstatement of cocaine seeking behavior compared with WT subjects.

However, compared with males, WT females showed significantly higher cue-induced reinstatement. This finding is consistent with previous studies showing that females show increased susceptibility to the reinforcing effect of stimulants and drug-primed reinstatement (Lynch & Carroll 2000; Roth *et al.* 2004) and some of these differences are thought to be due to modulatory effects of circulating ovarian hormones (Bobzean *et al.* 2014; Lynch 2006). The relationship between *Cdh13* and reinstatement also showed a different pattern of results in females compared with males. Instead, neither heterozygous nor KO females differed from WT during reinstatement. The reasoning for this is not entirely clear, although there are several possible explanations. One possibility is that the pathway from *Cdh13* function to behavior in females is similar to males, but for a variety of reasons was masked by factors that can influence drug sensitivity such as circulating hormone status (Becker 1999) or sex differences in the organization of the central nervous system during development (Hu *et al.* 2004). These sex-specific factors may therefore amplify responding during reinstatement in females, producing ceiling effects that negate the predisposing properties of *Cdh13* KO on reinstatement behavior. Furthermore, a variety of intervening factors could also modify the pathway from gene to behavior differently in males and females, such as alterations in epigenetic methylation status, differential effects on neural organization in development, and differences in neuroanatomical expression.

The *CDH13* locus has been identified for its importance in SUD in humans (Johnson *et al.* 2011; Uhl *et al.* 2007, 2008b), but currently the directionality of how specific changes in *CDH13* expression or function are related to clinical susceptibility is not completely understood. Here, we show that, in males, a reduction in *Cdh13* function is associated with the drug-relapse features of addiction, indicating that human individuals with dampened *CDH13* function may be especially susceptible to the motivational effects of abused substances. Further work will be needed to establish whether this effect is replicable in females and across other drug self-administration protocols. Additional work in this area will therefore be useful to determine whether these effects are preserved across other psychomotor stimulant compounds, or using alternative relapse models including drug- and stress-induced reinstatement.

Cocaine conditioned cue preference and place preference

Conditioned place preference traditionally measures approach and avoidance to a Pavlovian drug-paired context, and is thought to reflect rewarding and aversive properties of drugs (Bardo & Bevins 2000). Here, we use a modified version of the CPP paradigm in which the context is replaced with a discrete tactile floor cue. As suggested by Uhl *et al.* 2014, the dose-response curve for KO subjects may be shifted leftward. However, we did not detect any differences because of genotype at either the 10 or 20-mg/kg conditioning dose. One possibility is that the optimal conditioning dose for detecting genotype differences is lower than 10 mg/kg, where individual differences in susceptibility could be better exploited. Alternatively, in light of the findings from the first experiment, it may be that *Cdh13* is more strongly involved in energizing operant drug-seeking behavior, rather than being involved in eliciting approach to Pavlovian drug-cues by themselves. Interestingly, all genotypes appeared to be equally sensitive to cocaine's locomotor activating effects, suggesting that the immediate actions of cocaine treatment are similar between subjects.

In contrast to cocaine conditioned cue preference, mice lacking the *AdipoQ* gene spent more time on the methamphetamine side compared with WT animals, indicating that the methamphetamine-paired context elicited greater approach behavior. To date, this is the first demonstration that loss of adiponectin function alters sensitivity to drug conditioning. Importantly, it provides a second mechanism although which differences in *Cdh13* function might alter behavior. Given the evidence implicating *Cdh13* actions during development and organization of neural circuitry (Fredette *et al.* 1996; Takeuchi *et al.* 2000), it is interesting to note that eliminating AdipoQ's ligand effect on Cdh13 can replicate some features of SUD, perhaps independently of *Cdh13*'s involvement in cell–cell signaling and migration.

Despite this finding, no *AdipoQ* KO effect was observed when conditioning using d-amphetamine across a range of doses from 0.5 to 4 mg/kg. One explanation for these genotype differences in response to methamphetamine could be because of alterations in learning when *AdipoQ* is deleted. However, given that we found no differences between KO and WT subjects in contextual fear conditioning (not shown), it seems more likely that associative contextual learning is similar between genotypes. The relationship between *AdipoQ* and psychomotor stimulant conditioning instead may be fundamentally different between methamphetamine and d-amphetamine.

Operant responding for non-drug reinforcers

Our results indicate that the *Cdh13* deletion facilitates responding for saccharin, suggesting that the deletion of Cdh13 results in increased sensitivity to natural rewards in addition to drug rewards. In contrast, the responding for the water reinforcer during this task was similar between KO and WT rats, also suggesting that cadherin-13 signaling is specifically involved in processing reinforcers with hedonic components such as palatability. We further show that *Cdh13* KO rats do not differ in the efficacy of a sensory reinforcer to reinforce behavior (Fig. S3 in Appendix S1), and subjects habituate to sensory reinforcement at similar rates. These data are consistent with previous results with outbred rats indicating that responding for light onset rapidly habituates (Lloyd *et al.* 2012, 2014), and thus suggests that *Cdh13* is unlikely to be involved in sensation seeking and sensory habituation. Taken together, under normal circumstances *Cdh13* may regulate engagement with environmental rewards and behaviors directed toward acquiring them.

Behavioral regulation in CRT and PavCA

Given that *Cdh13* KO can modify reward sensitivity, we conducted a series of behavioral tasks meant to determine whether this gene influences other measures of behavioral

regulation. Premature responding for example has been interpreted as a measure of 'action' impulsivity during the CRT task (Bari & Robbins 2013), in which subjects are required to inhibit responding. Given the association between *CDH13* loci in humans and ADHD (Lesch *et al.* 2008; Salatino-Oliveira *et al.* 2015), we hypothesized that there would be differences in premature responding during the CRT between WT and KO subjects. However, this was not the case; thus it appears that expression of action impulsivity is not regulated by *Cdh13*. In humans, however, etiology of ADHD is heterogeneous and is likely driven by a wealth of factors (Evenden 1999), and may include engagement with tasks and rewards in the environment. In humans, *CDH13* may instead be involved in the response to these other factors, thereby producing ADHD symptomatology independently of measures of action impulsivity. Other methods of assessing ADHD-like phenotypes in rodents, such as delay discounting (Perry *et al.* 2005) or other versions of impulsivity paradigms such as the 5-choice serial RT task (Robbins 2002) may be required to detect these effects.

Furthermore, while we did not observe differences in action impulsivity, we did find that *Cdh13* KO rats were slower to learn the RT task compared with WT rats. However, given that there was similar performance following task acquisition, and given that there were no differences in learning to respond for a water or light reinforcer, it is unlikely that group differences are due to global performance deficits on the task or the general reinforcing value of fluid used within the CRT itself. Instead differences in acquisition of this task may reflect differences in attention to novel stimuli, or reflect an underlying deficit in initial memory formation. These learning deficits would be consistent with previous results examining maze learning in *Cdh13* KOs (Rivero *et al.* 2015).

Finally, we examined whether there were differences in tendency to attribute incentive salience to a food-cue using the Pavlovian conditioned approach paradigm (Figs. S4, S5 in Appendix S2). This task assesses sensitivity to cues in the environment, by measuring the ability of those cues to elicit approach behavior (Meyer *et al.* 2012a; Robinson *et al.* 2014). Response to Pavlovian reward cues have been further associated with response to drug-cues (Saunders & Robinson 2010; Versaggi *et al.* 2016) and action impulsivity (King *et al.* 2016; Lovic *et al.* 2011). Response to environmental cues may explain one mechanism by which drug-associated stimuli come to prompt maladaptive behavior or drug seeking (Childress *et al.* 2008; Franklin *et al.* 2011). Given the existing links between *CDH13* and ADHD, as well as the findings described here that reinstatement is larger in heterozygous and KO subjects, we predicted tendency to approach the Pavlovian lever cue would be greater in KO subjects. Our results, however, indicated that differences in *Cdh13* KO did not affect Pavlovian conditioned approach behaviors (Figs. S4, S5 in Appendix S2). This finding is interesting given that cue-induced reinstatement of cocaine seeking was greater in heterozygous and KO subjects compared with WTs. Therefore, while *Cdh13* KO increases sensitivity to drug cues, this effect does not extend to cues associated with natural reward stimuli.

Summary and future directions

In summary, we show that *Cdh13* and *AdipoQ* loss of function alter responsiveness to cocaine and methamphetamine, as well as saccharin, which suggests that these two genes can modify sensitivity to rewards more generally. However, this effect was not robust across drugs or testing paradigms. Despite differences in reinstatement to cocaine seeking, there were no differences in cocaine conditioned cue preference, and there were no genotype effects in conditioning to d-amphetamine. Thus, our data suggest a limited role for *Cdh13-AdipoQ* system in altering sensitivity to rewards which may depend on drug classes or the behavioral paradigm used. Although there were differences in speed at which the CRT was acquired, there were no differences in other behaviors including habituation to sensory reinforcer, premature responses, and tendency to learn and form conditioned responses to a food-cue. We therefore conclude that the effects of *Cdh13* and *AdipoQ* gene deletion are relatively specific to rewards, and gene KO can potentiate sensitivity to reward under some circumstances. However, the effect sizes presented here when taken together suggest that behavioral paradigms that exploit operant conditioning (e.g. the cocaine self-administration and saccharin conditioning) may be more sensitive to *Cdh13* induced changes in motivation than associative conditioning paradigms (e.g. CPP and Pavlovian conditioned approach). We therefore suggest that future studies on this topic may benefit from using approaches that more directly ascertain reward-directed motivation by introducing instrumental contingencies.

In humans, the development of SUD is influenced by the response to drugs (Chen *et al.* 2003; Le Strat *et al.* 2009) and drug cues that elicit drug-seeking (Robinson & Berridge 1993, 2003). During early drug exposure, these responses may be pivotal in promoting future intake. In light of differences in sensitivity to rewards, it may be the case that *Cdh13* potentiates susceptibility to SUD, ADHD and other attentional disorders by altering in engagement with rewards in the environment. The rodent *Cdh13* KO model may therefore prove useful in future studies targeting direct biological mechanisms of *Cdh13* on behavior. In particular, because of the widespread expression profile of *Cdh13* and *AdipoQ*, and because expression of these genes appear to be involved in neuronal development and cell-migration, future work will need to address specifically where these genes operate in the nervous system to modify behavior, determine how they alter functional circuitry, and finally explain which gene variants in the human population map onto these fundamental biological processes that alter behavior.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Table S1: Subject counts for *Cdh13* rats and *AdipoQ* mice for each study. Males and females are separated where appropriate. +/+ indicate wild-type subjects, +/– indicate heterozygous subjects, and –/– indicate knock-out subjects.

Figure S1: Following self-administration, subjects were extinguished across eight sessions and then tested for cue-induced reinstatement of responding for cocaine. (a) All subjects significantly decreased responding across sessions. (b) Males and females showed similar rates of extinction across all genotypes.

Figure S2: Subjects received four cocaine injections across conditioning that produced increases in locomotor activation. (a) The 10 mg/kg injections of cocaine produced significant locomotor activation compared to saline in all subjects. (b) The 20 mg/kg injections of cocaine produced the largest increases in locomotor activation. (c) There were no differences in locomotor activity between genotypes to either 10 mg/kg or (d) 20 mg/kg. Data are presented as means ± SEM.

Appendix S1. Sensory reinforcement.

Appendix S2. Pavlovian conditioned approach.