Overexpression of ASIC1A in the nucleus accumbens of rats potentiates cocaine-seeking behavior

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Abstract
Acid-sensing ion channels (ASICs) are abundantly expressed in the nucleus accumbens core (NAcore), a region of the mesolimbocortical system that has an established role in regulating drug-seeking behavior. Previous work shows that a single dose of cocaine reduced the AMPA-to-NMDA ratio in Asic1a−/− mice, an effect observed after withdrawal in wild-type mice, whereas ASIC1A overexpression in the NAcore of rats decreases cocaine self-administration. However, whether ASIC1A overexpression in the NAcore alters measures of drug-seeking behavior after the self-administration period is unknown. To examine this issue, the ASIC1A subunit was overexpressed in male Sprague-Dawley rats by injecting them with adeno-associated virus, targeted at the NAcore, after completion of 2 weeks of cocaine or food self-administration. After 21 days of homecage abstinence, rats underwent a cue-/context-driven drug/food-seeking test, followed by extinction training and then drug/food-primed, cued, and cued + drug/food-primed reinstatement tests. The results indicate that ASIC1A overexpression in the NAcore enhanced cue-/context-driven cocaine seeking, cocaine-primed reinstatement, and cued + cocaine-primed reinstatement but had no effect on food-seeking behavior, indicating a selective effect for ASIC1A in the processes underlying extinction and cocaine-seeking behavior.

KEYWORDS
abstinence, incubation, reinstatement, self-administration, withdrawal

1 INTRODUCTION
Chronic exposure to drugs of abuse, including cocaine, causes a variety of synaptic and molecular alterations in the nucleus accumbens (NA), many of which contribute to the potential for relapse, as assessed in models of drug-seeking behavior. Studies, including our own, indicate that the NA abundantly expresses acid-sensing ion channels (ASICs),1,2 which provide a depolarizing current when activated by decreases in extracellular pH. ASICs include multiple channel subunits, and our prior work suggests that the ASIC1A subunit plays a critical role in drug-related behaviors, including for cocaine. Deletion of the Asic1a gene in mice enhances cocaine-conditioned place preference, an effect reversed by rescue of ASIC1A in the NA.3 Local deletion of Asic1a in the NA also enhances cocaine cocaine-conditioned place preference. Conversely, overexpression of ASIC1A in the NA of rats decreases the number of infusions taken during cocaine self-administration and produces a rightward shift in the cocaine dose-response curve during self-administration.3 Together, these findings suggest that ASIC1A negatively regulates the reinforcing properties of cocaine.

On a physiological level, ASIC1A disruption appears to produce long-lasting synaptic changes similar to those observed following withdrawal from chronic cocaine administration. Previous findings indicate that Asic1a−/− mice exhibit an increased AMPA/NMDA receptor ratio, akin to cocaine-experienced rats and mice following a period of abstinence.3-5 Asic1a−/− mice also have an increased rectification...
index in NA medium-spiny neurons, which is thought to reflect insertion of GluA2-lacking AMPA receptors and mirrors observations made in the NA of rodents following abstinence from cocaine self-administration. Evidence suggests that experimenter-administered cocaine produces diminished motor responses in Asic1a−/− mice compared with that observed in wild-type mice. Together, the prior findings indicate that ASICs, and particularly the ASIC1A subunit, contribute to a variety of cocaine-related behaviors and synaptic changes in the NA related to cocaine exposure.

Of note, one study reported that 5 days of experimenter-administered cocaine followed by 14 days of abstinence caused the upregulation of ASIC1 protein in the striatum, including the nucleus accumbens core (NAcore). However, this work does not reveal whether increased ASIC1A expression opposes or mediates the long-term effects of cocaine exposure. Moreover, prior work did not investigate how the ASIC1A subunit influences measures of relapse and craving following the drug self-administration period. Therefore, the present study investigated how ASIC1A overexpression, targeted at the NAcore of rats, alters cocaine-seeking behavior following the self-administration period. Rats underwent cocaine self-administration for at least 12 days and, following the last day of cocaine administration, received an intra-NA injection of an adeno-associated virus (AAV) overexpressing ASIC1A. After 21 days of homecage abstinence, the effects of ASIC1A overexpression on cue-/context-driven drug/food seeking, extinction, and reinstatement were tested. Behavioral effects of ASIC1A overexpression following food self-administration were also examined for comparison purposes.

2 | MATERIALS AND METHODS

2.1 | Subjects

Male Sprague-Dawley rats (Charles River, ~300 g at the time of surgery, n = 28) were individually housed and maintained on a 12-hour reverse light-dark cycle. Rats were given ~20 g of food per day except during the 21-day homecage period following self-administration, when they remained in their homecages and were given ad libitum food. Methods were approved by the University of Iowa Institutional Animal Care and Use Committee and were in compliance with NIH guidelines for the care of laboratory animals.

2.2 | Surgery

Rats underwent surgery for double-barreled cannulae targeted to the NAcore and, in some cases, implantation of intravenous jugular catheter. Surgical details are provided in the Supporting Information.

2.3 | Self-administration

Self-administration sessions were carried out 6 days per week in standard operant boxes housed within sound-attenuating chambers (Med Associates, Fairfield, VT). Operant boxes were equipped with a central reward receptacle flanked by two levers. A cue light was positioned above each lever, and a 4500-Hz Sonalert module was used as the tone generator. A house light was positioned on the wall opposite the levers and was illuminated during the behavioral sessions. In Experiments 1, rats were food deprived for 24 hours and then trained during a 15-hour overnight session to lever press on a FR1 schedule of reinforcement for 45-mg food pellets (Bio-serve Dustless Precision Pellets, Flemington, NJ). One day after food training, rats began training 6 days per week on cocaine self-administration (Experiment 1). Rats in Experiment 2 did not undergo overnight food training but instead entered daily 2-hour food self-administration sessions (described more below).

During self-administration sessions, a lever press on the active (right) lever resulted in drug or food delivery and the presentation of light and tone cues. A 20-second time out period followed each cocaine or food pellet reward, during which active lever presses had no consequence. In all experiments, lever presses on the inactive (left) lever had no consequence. All rats underwent at least 12 days of self-administration, followed by a virus microinjection, and 21 days spent in the homecage.

2.4 | Microinjections and homecage period

On the day immediately following the final day of self-administration, AAV2/1-CMV-ASIC1A(mouse)-eGFP or AAV2/1-CMV-eGFP (0.3-μL infused at a rate of 0.1 μL/min) was infused via injectors that extended 2 mm beyond the cannulae. Injectors were left in place for 2 minutes to allow the virus to diffuse. Rats were counterbalanced so that GFP and ASIC1A groups earned equivalent levels of cocaine or food pellets on the last 3 days of self-administration. Rats were kept in their home cage for a 21-day homecage period to allow for robust ASIC1A overexpression prior to the tests measuring relapse-like behavior. This time period has previously been shown to be sufficient to induce incubation of craving following 2-hour daily self-administration sessions. Food restriction (~20 g/day) was resumed 1 day prior to the first drug/food-seeking test after the homecage period (described for each experiment below).

2.5 | Experiment 1: Cocaine self-administration

This experiment examined whether ASIC1A overexpression in the NAcore affects the first cue-/context-driven drug-seeking test after the homecage period, the extinction of cocaine seeking, and the reinstatement of cocaine-seeking behavior following extinction training. Rats underwent daily 2 hours self-administration sessions in which active lever presses produced a 50-μL intravenous cocaine infusion (200-μg cocaine per infusion, dissolved in 0.9% sterile saline; cocaine kindly provided by the National Institute on Drug Abuse). Cocaine infusions were capped at 35 during the first 3 days of self-administration. Rats self-administered cocaine for at least 12 days, and the criteria for entering the homecage phase were at least 10 days of greater than 10 infusions, with greater than 15 infusions on each of the final 2 days.

Following the homecage period, rats underwent a 2-hour cue-/context-driven drug-seeking test, during which active lever presses resulted in the delivery of light and tone cues but no cocaine infusion. Rats then underwent at least 7 days of 2-hour extinction sessions, during which
both active and inactive lever presses had no consequences. Rats were required to make fewer than 25 active lever presses on the final 2 days of extinction training in order to begin the reinstatement tests. Rats underwent the three reinstatement tests in the following order: food primed, cued, cocaine-primed, and cued + cocaine-primed. During cocaine-primed reinstatement, rats received an injection of cocaine immediately before the session (10 mg/kg, i.p.), and active lever presses had no consequences. During cued reinstatement, active lever presses resulted in the presentation of light and tone cues. During cued + cocaine-primed reinstatement, rats received the cocaine prime prior to the session and underwent the cued reinstatement session described above. During both the cue-context-driven drug-seeking test and the reinstatement tests, active lever pressing served as a measure of cocaine-seeking behavior. At least 3 days of extinction training were included between reinstatement tests, and rats were required to make fewer than 25 active lever presses during the two extinction sessions immediately preceding a reinstatement test.

2.6 | Experiment 2: Food self-administration

This experiment examined whether ASIC1A overexpression in the NAcore affects the cue-context-driven food-seeking test after the homecage period, extinction learning, and the reinstatement of food-seeking behavior following extinction. During daily 2-hour sessions, rats were trained to lever press for a 45-mg food pellet (described above) on an FR1 schedule of reinforcement. Rats were required to earn ≥100 pellets per session for at least 3 days in order to advance to an FR3 schedule of reinforcement. Rats self-administered food for at least 12 days, and the criteria for entering the homecage phase was at least 3 days of self-administration on the FR3 schedule, with ≥100 pellets earned on each of these days.

Following the 21-day homecage period, rats underwent a 2-hour cue-context-driven food-seeking test, as described for Experiment 1. Rats were then trained to extinguish active lever pressing during at least 7 days of 2-hour extinction sessions. The criterion for reinstatement testing was 2 consecutive days of extinction training during which rats executed fewer than 10% of the average number of lever presses made during the last 2 days of food self-administration. Reinstatement tests occurred in the following order: food primed, cued, and cued + food primed. During the food-primed reinstatement test, one 45-mg pellet was passively delivered every 2 minutes for the first 30 minutes of the session, but active lever presses had no consequence. During the cued reinstatement test, active lever presses resulted in the delivery of the tone and light cue previously associated with the food pellet. During cued + food-primed reinstatement, rats received the food primes for the first 30 minutes of the session and tone and light cue presentations following active lever presses throughout the entire session.

2.7 | Histological analysis

Brains were removed and analyzed using immunohistochemistry to confirm expression of the EGFP virus in the NAcore. Details of histological analysis are provided in the Supporting Information.

2.8 | Statistical analysis

Unpaired t-tests were used to examine the rewards earned on the last 3 days of self-administration and the lever presses during the initial post-homecage drug/food-seeking tests. Within-session lever pressing was examined during the test by dividing the session into 15-minute bins and performing a two-way repeated measures ANOVA with bin as the within-subjects variable and group (GFP or ASIC1A) as the between-subjects variable. For reinstatement tests, lever pressing during the extinction baseline (an average of the 2 days immediately preceding the reinstatement) and reinstatement test were analyzed using two-way ANOVAs with day as a repeated measure. Holm-Sidak’s multiple comparisons test was used for all post-hoc analyses.

3 | RESULTS

Figure 1A shows the experimental timeline used for all experiments. Figure 1B shows a representative immunohistochemistry image with virus expression in the NAcore. Figure 1C shows the observed minimum and maximum virus spread. As pictured, spread of the virus occurred in some rats, and as such, it cannot be ruled out that ASIC1A overexpression in nearby striatal regions contributed to the observed results. In particular, two rats showed evidence of expression in the lateral NAShell, and many rats showed spread into the portions of the dorsal striatum closest to the NAcore.

3.1 | Experiment 1: Cocaine-self administration

Figure 2 shows the results from Experiment 1, which examined the effect of ASIC1A overexpression in the NAcore on cue-context-driven cocaine-seeking behavior following 21 days in the homecage, the extinction of cocaine seeking, and the reinstatement of cocaine-seeking behavior following extinction training. A t-test examining cocaine infusions averaged across the last 3 days of self-administration revealed no difference between GFP and ASIC1A groups (Figure 2A, \(t_{(12)} = 0.33, P > 0.05\)). A t-test comparing inactive lever presses averaged across the last 3 days of self-administration similarly revealed no difference between the groups (\(t_{(12)} = 0.33, P > 0.05\)). A t-test for the initial cue-context-driven cocaine-seeking test found that following 21 days of abstinence, active lever pressing of rats overexpressing ASIC1A was significantly higher than that of GFP-control rats (Figure 2B, \(t_{(12)} = 6.02, P < 0.0001\)). A t-test of inactive lever presses during the initial cue-context-driven cocaine-seeking test likewise revealed significantly higher levels of lever pressing for ASIC1A-overexpressing rats compared with those of the GFP-control rats (Figure 2C, \(t_{(12)} = 2.97, P < 0.05\)).

Lever pressing during the initial cue-context-driven cocaine-seeking test was broken down into 15-minute bins and examined to determine whether ASIC1A overexpression altered lever pressing in a time-dependent manner. A two-way repeated measures ANOVA examining within-session active lever presses during the test revealed a significant main effect of group (Figure 2D, \(F_{(1, 12)} = 36.21, P < 0.0001\)), a significant effect of day (\(F_{(7, 84)} = 4.78, P < 0.001\)), and a significant interaction (\(F_{(7, 84)} = 2.34, P < 0.05\)). Post-hoc tests
indicated a significant difference in active lever presses between GFP and ASIC1A-overexpressing groups for the first 90 minutes of the session ($P < 0.001$ for 0 to 15-minute and 31 to 45-minute bins; $P < 0.01$ for 16 to 30-minute, 45 to 60 minute, and 61 to 75-minute bins; $P < 0.05$ for 76 to 90-minute bin), with ASIC1A-overexpressing rats showing higher lever pressing during that time compared with their control counterparts. Within-session analysis of inactive lever pressing during the cocaine-seeking test revealed a main effect of group (Figure 2D, $F(1,12) = 8.94$, $P < 0.05$) and a main effect of day ($F(7, 84) = 7.37$, $P < 0.0001$) but no interaction ($F(7, 84) = 1.20$, $P > 0.05$). Post-hoc tests indicated that ASIC1A-overexpressing rats had significantly higher inactive lever pressing during the 0 to 15 minute bin ($P < 0.05$) and marginally higher during the 16 to 30 and 45 to 60-minute bins ($P < 0.08$) compared with their control counterparts.

A two-way repeated measures ANOVA of active lever presses during extinction revealed a main effect of group (Figure 2E, $F_{(1,11)} = 3.30, P < 0.1$), a significant main effect of day ($F_{(6, 66)} = 16.79, P < 0.0001$), and no significant interaction ($F_{(6, 66)} = 1.38, P > 0.05$). A two-way repeated measures ANOVA of inactive lever presses during the same extinction training revealed a main effect of group (Figure 2F, $F_{(1,11)} = 5.53, P < 0.05$), no main effect of day ($F_{(6, 66)} = 1.44, P > 0.05$), and no interaction ($F_{(6, 66)} = 1.14, P > 0.05$). Post-hoc tests indicated that GFP and ASIC1A groups significantly differed in inactive lever presses on the second day of extinction ($P < 0.05$). Note that only the first 7 days of extinction training are shown on the graph. Although the ASIC1A group required more days than the GFP group to reach reinstatement criteria ($7.17 \pm 0.17$ for GFP vs $8.14 \pm 0.59$ for ASIC1A), a $t$-test comparing the number of days found no between-group differences ($t(11) = 1.47, P > 0.05$).

Figure 2F-H shows active lever presses for the tests of reinstatement of cocaine seeking. Two rats from each group were excluded from one or more reinstatement tests due to death before the test was completed. A two-way repeated measures ANOVA of active lever presses during the cocaine-primed reinstatement revealed a significant effect of day (Figure 2F, $F_{(1,9)} = 29.18, P < 0.001$), a strong trend for an effect of group ($F_{(1,9)} = 5.01, P = 0.05$), and a significant interaction ($F_{(1,9)} = 7.02, P < 0.05$). Post-hoc analysis indicated the GFP group marginally reinstated cocaine seeking relative to extinction baseline ($P < 0.1$) and that ASIC1A-overexpressing rats significantly reinstated cocaine seeking ($P < 0.001$). Moreover, the ASIC1A-overexpressing group had significantly greater active lever presses relative to GFP controls on the cocaine-primed reinstatement day ($P < 0.01$). A two-way repeated measures ANOVA of active lever presses during the cued reinstatement indicated a significant main effect of day (Figure 2G, $F_{(1,9)} = 55.30, P < 0.0001$), no main effect of group ($F_{(1,9)} = 1.23, P > 0.05$), and no interaction ($F_{(1,9)} = 2.07, P > 0.05$).
Post-hoc tests revealed that both groups significantly reinstated cocaine seeking relative to extinction baseline (P < 0.01 for GFP and P < 0.001 for ASIC1A). Although ASIC1A overexpression increased active lever pressing during the cued reinstatement test compared with that of the GFP control group, the effect was not statistically significant (P > 0.05). A power analysis, assuming a desired power of 0.8, indicated that an n of ~25 per group would be required to observe statistical significance in the difference between the groups during the cued reinstatement test. A two-way repeated measures ANOVA of active lever presses during cued + cocaine-primed reinstatement revealed a significant main effect of day (Figure 2H, F_{1,8} = 50.79, P < 0.0001), a significant effect of group (F_{1,8} = 7.32, P < 0.05), and a significant interaction (F_{1,8} = 6.32, P < 0.05).
on the reinstatement day relative to the extinction day ($P < 0.05$ for GFP and $P < 0.001$ for ASIC1A), and the ASIC1A group had significantly more active lever presses relative to the GFP group on the cued + cocaine-primed reinstatement day ($P < 0.01$).

Figure 2I-K shows inactive lever presses for the same sessions. A two-way repeated measures ANOVA of inactive lever pressing during the cocaine-primed reinstatement revealed no effect of group (Figure 2I, $F_{(1,9)} = 0.48, P > 0.05$), no effect of day ($F_{(1,9)} = 0.64, P > 0.05$), and no interaction ($F_{(1,9)} = 0.86, P > 0.05$). A two-way repeated measures ANOVA of inactive lever pressing during the cued reinstatement showed no effect of group (Figure 2J, $F_{(1,9)} = 1.81, P > 0.05$), no effect of day ($F_{(1,9)} = 0.86, P > 0.05$), and no interaction ($F_{(1,9)} = 0.49, P > 0.05$). Similarly, a two-way repeated measures ANOVA for inactive lever pressing during cued + cocaine-primed reinstatement indicated no main effect of group (Figure 2K, $F_{(1,9)} = 0.21, P > 0.05$), no main effect of day ($F_{(1,9)} = 0.50, P > 0.05$), and no interaction ($F_{(1,9)} = 0.03, P > 0.05$). Taken together, Experiment 1 showed that ASIC1A overexpression in the NAcore increased cocaine seeking during tests for cue-driven cocaine seeking after the homecage period, the extinction of cocaine seeking, and the cocaine-primed and cued + cocaine-primed reinstatement of cocaine-seeking behavior after extinction.

### 3.2 Experiment 2: Food self-administration

Figure 3 shows the results from Experiment 2, which examined the effect of ASIC1A overexpression in the NAcore on the cue-/context-driven food-seeking behavior following 21 days in the homecage, the extinction of food seeking, and the reinstatement of food seeking after extinction training. This experiment tested whether the results from Experiment 1 generalize to non-drug rewards. A t-test examining food pellets earned, averaged across the last 3 days of self-administration, revealed no difference between GFP and ASIC1A-overexpressing rats (Figure 3A, $t_{(12)} = 0.95, P > 0.05$). A t-test comparing active lever presses after the 21-day homecage period showed no between-group differences (Figure 3B, $t_{(12)} = 0.94, P > 0.05$). A two-way repeated measures ANOVA of active lever presses during the extinction of food-seeking behavior revealed no main effect of group (Figure 3C, $F_{(1, 12)} = 1.01, P > 0.05$), a significant main effect of day
inactive lever presses during food seeking signs a significant effect of day (Figure 3G, F(1,12) = 1.97, P < 0.09), and no interaction (F(6,72) = 0.76, P > 0.05).

Figure 3D-F shows active lever presses for the reinstatement of food-seeking tests. A two-way repeated measures ANOVA of active lever presses during the food-primed reinstatement revealed a significant main effect of day (Figure 3D, F(1,12) = 13.24, P < 0.01), no effect of group (F(1,12) = 0.11, P > 0.05), and no interaction (F(1,12) = 0.22, P > 0.05). Post-hoc tests indicated that both groups significantly reinstated cocaine seeking relative to baseline (P < 0.05 for both) but did not differ from one another (P > 0.05). A two-way repeated measures ANOVA of active lever pressing during cued reinstatement indicated a significant main effect of day (Figure 3E, F(1,12) = 23.29, P < 0.001) but no significant main effect of group (F(1,12) = 0.19, P > 0.05) and no interaction (F(1,12) = 0.17, P > 0.05). Post-hoc tests showed both ASIC1A-overexpressing rats and GFP rats significantly reinstated cocaine seeking (P < 0.01) but did not differ from each other (P > 0.05). A two-way repeated measures ANOVA examining active lever pressing during cued + food-primed reinstatement showed a significant main effect of day (Figure 3F, F(1,12) = 29.48, P < 0.001) but no effect of group (F(1,12) = 0.95, P > 0.05) and no interaction (F(1,12) = 1.28, P > 0.05). Post-hoc tests indicated rats in both groups reinstated food-seeking behavior (P < 0.01) but did not differ from one another (P > 0.05). Figure 3G-I shows inactive lever presses for the same sessions. A two-way repeated measures ANOVA examining inactive lever presses during food-primed reinstatement revealed no significant effect of day (Figure 3G, F(1,12) = 1.97, P > 0.05), no effect of group (F(1,12) = 0.62, P > 0.05), and no interaction (F(1,12) = 0.87, P > 0.05). A two-way repeated measures ANOVA of inactive lever pressing during cued reinstatement indicated no significant effect of day (Figure 3H, F(1,12) = 0.12, P > 0.05), no effect of group (F(1,12) = 0.04, P > 0.05), and no interaction (F(1,12) = 0.25, P > 0.05). Similarly, a two-way repeated measures ANOVA of inactive lever pressing during cued + food-primed reinstatement revealed no significant effect of day (Figure 3I, F(1,12) = 3.23, P > 0.05), no effect of group (F(1,12) = 0.52, P > 0.05), and no interaction (F(1,12) = 0.0061, P > 0.05).

4 | DISCUSSION

The present findings indicate that ASIC1A overexpression targeted at the NAc core following the completion of cocaine self-administration increased cocaine seeking during the cue-/context-driven test following the homecage abstinence period, marginally impaired the extinction of cocaine seeking, and increased cocaine-primed and cued + cocaine-primed reinstatement. In contrast, the current work found that ASIC1A overexpression did not alter food-seeking behavior. Together, the present findings indicate a selective role for ASIC1A in the NA in regulating relapse-like behavior following a period of abstinence from cocaine self-administration.

Our prior work reported that ASIC1A overexpression decreases the number of cocaine infusions and shifts the dose-response curve to the right during self-administration. As a result, it is unclear whether the present results would differ if the ASIC1A overexpression was present throughout the self-administration and abstinence phases, as such an experiment would be inherently confounded by the differences in cocaine self-administration levels. Although the current results would appear to be in contrast to those with overexpression during self-administration, a few important differences exist between the studies that likely account for the apparent discrepancies. First, different neural systems appear to mediate self-administration vs. relapse-like behaviors. During cocaine self-administration, blocking dopamine receptors in the NA alters the number of cocaine infusions. In contrast, during cued and cocaine-primed reinstatement tests, evidence suggests that blocking AMPA glutamate receptors, but not dopamine receptors, in the NAc core reduces cocaine seeking. Together, these results suggest that the neurobiological substrates mediating the reinforcing properties of cocaine differ from those required to drive reinstatement of cocaine seeking following self-administration. Moreover, Kreple et al. overexpressed ASIC1A prior to cocaine self-administration, whereas the present study overexpressed ASIC1A on the first day of abstinence. A 3-week period was required in the current experiment in order to achieve the desired expression of ASIC1A. Because the transduction and upregulation of ASIC1A occurred during the same time period as abstinence from cocaine self-administration, it is possible that changes in ASIC1A signaling interacted with the molecular mechanisms underlying abstinence in an unforeseen manner.

Prior findings indicate that relapse-like behavior for cocaine seeking depends on glutamatergic transmission in the NAc core. As ASICs contribute to excitatory synaptic transmission in the NAc core and elsewhere, the present findings may result from alterations in excitatory signaling as a consequence of changes in ASIC1A expression or functioning. Such changes in excitatory transmission may cause structural remodeling in ASIC1A-overexpressing rats that contributes to enhanced cocaine-seeking behavior. Consistent with this possibility, ASIC1A-null mice show increased spine density in the NAc core, and therefore ASIC1A-overexpressing rats may have decreased spine density. A prior study using 3D imaging and analysis showed that cocaine self-administration followed by abstinence reduces spine density in the mPFC. Taken together, these studies suggest that ASIC1A-mediated changes in spine density in the mPFC-to-NA pathway may contribute to enhanced cocaine-seeking behavior in the present experiment. (Of note, due to viral spread, overexpression was observed outside the NAc core as well and, therefore, overexpression in nearby striatal regions may have contributed to the present findings.)

In support of the involvement of NAc core glutamate transmission, ASIC1A-null mice show increased expression of GluA2-lacking AMPA receptors in the NA, suggesting that, in this model, ASIC1A negatively regulates the expression of these receptors. GluA2-lacking AMPA receptors are inserted into the NA during withdrawal and mediate the incubation of cocaine craving. Moreover, disruption of ASIC1A increases AMPA/NMDA receptor ratio in mice. Evidence suggests this neural adaptation contributes to the incubation of craving, as rats exposed to 2 hours per day cocaine self-administration sessions followed by a period of abstinence also showed an enhanced
localized to dendrites and dendritic spines. It therefore remains to within the synapse is unknown, endogenous ASIC1A is preferentially cocain seeking. Whereas the spatial expression of AAV increased extrasynaptic ASIC1A expression and the consequent ing by promoting firing of these neurons. Another possibility is that increased ASIC1A expression in the NAcore could drive cocaine seeking by promoting firing of these neurons. Another possibility is that increased extrasynaptic ASIC1A expression and the consequent increase in ASIC1A currents may place cells in a more depolarized state, making them more sensitive to glutamatergic inputs promoting cocaine seeking. Whereas the spatial expression of AAV-ASIC1A within the synapse is unknown, endogenous ASIC1A is preferentially localized to dendrites and dendritic spines. Therefore it remains to be determined whether disrupting endogenous ASIC1A signaling, rather than overexpressing ASIC1A, would reduce cocaine-seeking behavior after abstinence. Moreover, it is unknown whether ASIC1A overexpression causes an increase in ASIC1A heteromeric channels (contain ASIC2A and/or ASIC2B) or ASIC1A homomeric channels. Future studies examining sensitivity to the ASIC1A-specific blocker Psalmotoxin-1 (PcTx1) could be used to discriminate between these possibilities.

CMV is a general promoter, and evidence suggests that this promoter may target non-neuronal cells with giall morphology. Previous work shows that depolarization of perineuronal oligodendrocytes can decrease the firing latency of neighboring neurons, whereas activation of perineuronal astrocytes suppresses the activity of neighboring interneurons. Moreover, astrocytes influence synaptic functioning and efficacy, for example, by regulating extrasynaptic glutamate levels and activation of presynaptic glutamate receptors, which, in turn, influence drug-seeking behavior. Therefore, the present findings may result from changes not only in ASIC1A expression in neurons but also alterations in glial cell functioning.

Cocaine self-administration followed by abstinence may increase ASIC1A expression or acid-evoked currents, and if ASIC1A contributes to drug seeking as reported here, then these data may suggest an ASIC1A-mediated feedforward loop that perpetuates addiction. Indeed, previous work reported that 5 days of experimenter-administered cocaine followed by 14 days of abstinence enhances ASIC1 protein expression in the NA of mice. However, this previous study did not examine a causative relationship between ASIC1A expression and cocaine-seeking behavior, and ASIC1A could act as a compensatory mechanism to oppose cocaine seeking.

ASIC1A overexpression increased responding on the inactive lever during the first cocaine seeking test after the homecage period and the extinction training. This can likely be accounted for by rats engaging in alternative drug-seeking behavior. It is possible that overexpression of ASIC1A caused a loss of discrimination capacity between the active and inactive levers, although this seems unlikely because the number of active lever presses is still higher than inactive lever presses in all of the tests. Another possibility is that ASIC1A overexpression caused a general increase in motor behavior. This seems unlikely, however, because GFP and ASIC1A groups showed equivalent levels of both active and inactive lever pressing during the cue-/context-driven food-seeking test in Experiment 2 (food seeking).

Together with previous findings, the present study suggests that ASIC1A has a dynamic role in cocaine-taking and cocaine-seeking behavior, such that it negatively regulates cocaine taking but promotes cocaine seeking. However, it appears these findings are not generalizable to all types of reward-seeking behavior, as ASIC1A overexpression in the NAc core had no effect on food-seeking behavior.

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AUTHOR CONTRIBUTIONS

R.T.L. and M.F.N. designed the experiments. A.L.G., C.V.C., M.F.N., and W.R.W. conducted the experiments. A.L.G. analyzed the data. A.L.G. and R.T.L. wrote the manuscript. All authors critically reviewed the content and approved the final version for publication.

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