Cadherins mediate cocaine-induced synaptic plasticity and behavioral conditioning

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Drugs of abuse alter synaptic connections in the reward circuitry of the brain, which leads to long-lasting behavioral changes that underlie addiction. Here we show that cadherin adhesion molecules play a critical role in mediating synaptic plasticity and behavioral changes driven by cocaine. We demonstrate that cadherin is essential for long-term potentiation in the ventral tegmental area and is recruited to the synaptic membranes of excitatory synapses onto dopaminergic neurons following cocaine-mediated behavioral conditioning. Furthermore, we show that stabilization of cadherin at the membrane of these synapses blocks cocaine-induced synaptic plasticity, leading to a reduction in conditioned place preference induced by cocaine. Our findings identify cadherins and associated molecules as targets of interest for understanding pathological plasticity associated with addiction.

Drugs of abuse induce widespread alterations to the neural circuits that mediate reward learning in the brain. Cocaine exposure drives the strengthening of excitatory inputs onto dopaminergic neurons of the ventral tegmental area (VTA) and causes increased release of dopamine from the VTA onto corticostriatal structures, including the nucleus accumbens, the prefrontal cortex and the dorsal striatum. Drug-evoked synaptic plasticity in the VTA is believed to underlie behavioral changes that lead to addiction. The potentiation of excitatory inputs to dopaminergic neurons is increased following associative learning of reward-predicting cues, and intact glutamatergic synapse function in the VTA is required for the formation of cocaine-induced conditioned place preference (CPP), indicating that plasticity at these synapses may contribute to the learned association between environmental cues and the rewarding effects of cocaine. Electrophysiological studies have shown that cocaine-induced potentiation of VTA synapses is mediated by the insertion of Ca2+-permeable AMPA receptors (AMPARs) lacking the GluA2 subunit to the synaptic membrane. To determine how drugs of abuse alter synapses in the reward circuitry and cause behavioral changes underlying addiction, it is important to further understand the molecular mechanisms that mediate drug-induced plasticity at synapses in the VTA.

Cadherin adhesion molecules have been shown to play a critical role in synaptic plasticity underlying different forms of learning and memory. Cadherins mediate adhesion at synapses through homophilic trans interactions across the synaptic membrane and associate with AMPARs through direct and indirect interactions with both GluA1 and GluA2 AMPAR subunits. In the hippocampus, cadherins are essential for long-term potentiation (LTP) and long-term depression (LTD). Following enhanced activity, cadherins are increasingly localized to the synaptic membrane, leading to increased synaptic stability and the stabilization of AMPAR at the synaptic membrane. Disruption of trans-synaptic cadherin interaction in the hippocampus has been shown to abolish the acquisition of context-dependent memory formation while aberrant increases in cadherin stability at the membrane lead to impaired behavioral flexibility on hippocampus-dependent tasks.

Because they regulate AMPAR trafficking and stability at synapses, cadherins are strong candidate molecules for mediating plasticity in the VTA underlying behavioral changes driven by drugs of abuse. In the context of addiction, genome-wide association studies have identified mutations in cadherin adhesion-complex proteins as risk factors for substance abuse. However, very little is known about the expression of cadherins in the VTA, and their potential function in synaptic plasticity in this region has not been examined.

Here we show that cadherin plays a key role in synaptic plasticity in the VTA and behavioral changes driven by cocaine. We demonstrate that cadherins are widely expressed in dopaminergic neurons and are essential for LTD of synapses in the VTA. We further demonstrate that recruitment of cadherin to excitatory inputs onto dopaminergic neurons is correlated with cocaine-mediated CPP. Finally, we demonstrate that stabilization of cadherin at the synaptic membrane of synapses onto dopaminergic neurons can completely block cocaine-induced changes in AMPAR localization and LTD, and greatly reduce behavioral conditioning driven by cocaine.

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RESULTS
Cadherins are expressed in dopaminergic neurons and required for LTP

We first examined the expression of several classical cadherins and found that cadherins are widely expressed in both dopaminergic and nondopaminergic neurons in the VTA (Fig. 1a and Supplementary Fig. 1). Nearly all dopaminergic cells were immunopositive for N-cadherin, R-cadherin, cadherin-7, cadherin-8 and cadherin-11, isoforms whose mRNA had previously been detected in this region22,23.

We next investigated the function of cadherins in activity-induced potentiation of excitatory synapses onto dopaminergic neurons in the VTA. We used an antagonistic peptide containing an HAV (His-Ala-Val) motif that blocks cadherin interactions in trans to reduce cadherin stability at the synaptic membrane. It has previously been shown that disrupting cadherin trans interactions significantly attenuates cadherin membrane stability24. Treatment of VTA slices with the HAV peptide abolished spike-timing-dependent (STD) LTP at excitatory synapses onto dopaminergic neurons in the VTA (Fig. 1b).

Like potentiation of VTA synapses induced by cocaine, STD LTP is mediated by the insertion of Ca$^{2+}$-permeable GluA1 homomers3,5,25. As cadherin can stabilize AMPARs through its association with GluA1 (ref. 11) and/or GluA2 (refs. 12,13) subunits, this suggested that peptide treatment disrupted STD LTP by decreasing cadherin membrane stability, thus preventing the stabilization of newly inserted GluA1 homomers at the synaptic membrane. We therefore next sought to investigate the relationship between cadherin localization and AMPAR trafficking in synaptic plasticity and behavioral conditioning driven by cocaine.

Cadherins are required for LTP

Cues driven by drugs of abuse 26. In other brain regions, enhanced synaptic activity is associated with increased recruitment of cadherins to the synapse15,16, as well as increased stability of cadherin at the synaptic membrane18. Intact cadherin adhesion at synapses is also required for the acquisition of new memories20. We therefore hypothesized that the acquisition of cocaine-induced CPP is associated with increased insertion of cadherin to the synaptic membrane of VTA synapses. We used immunogold electron microscopy (EM) (validated in Supplementary Fig. 2) to examine nanometer-scale changes in the localization of cadherins and AMPAR subunits at synapses of the VTA following cocaine-induced CPP.

CPP was induced in a three-chamber apparatus (Fig. 2a), producing a robust increase in preference for the cocaine-paired, conditioned chamber (Fig. 2b). Mice were then sacrificed and the VTA isolated by microdissection for immunogold EM (Fig. 2c). We found that cocaine-induced CPP resulted in a striking redistribution of cadherin at excitatory synapses onto dopaminergic neurons in the VTA (Fig. 2d). Indeed, the proportion of cadherin localized to the synaptic membrane increased by 86% after CPP (number of immunolabeled beads within 40 nm of the synaptic membrane divided by total beads in the pre- and postsynaptic compartments; Fig. 2e), although no changes in total levels of cadherin at synaptic compartments were detected (Supplementary Fig. 3). Moreover, analysis of individual mice demonstrated a strong positive correlation between the amount of cadherin at the synaptic membrane and time spent in the cocaine-paired conditioned chamber ($r = 0.66$; Fig. 2f). The increased localization of cadherin to the synaptic membrane was observed at both pre- and postsynaptic compartments following cocaine CPP (Supplementary Fig. 4), which is consistent with increased trans-synaptic adhesion between cadherins.

Notably, we saw no significant change in cadherin localization in control mice which received the same schedule of cocaine and saline administration in their home cages rather than in a novel environment (Fig. 2e and Supplementary Fig. 5) or in mice where CPP was induced using palatable food rewards instead of cocaine (Fig. 2e and Supplementary Fig. 6). This suggested that the effects observed after cocaine CPP were not due to general effects of cocaine or nonspecific learning-induced plasticity, but were specifically attributable to the

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**Figure 1** Cadherins are expressed in dopaminergic neurons and are essential for LTP in the VTA. (a) VTA neurons coimmunostained for cadherins (green), dopamine transporter (DAT; magenta) and DAPI (blue). Arrowheads indicate neurons positive for both cadherin and DAT; asterisks indicate neurons positive for cadherin but not DAT. Scale bars, 10 μm. (b) STD LTP in the VTA was abolished by treatment with a peptide containing an HAV motif that disrupts N-cadherin extracellular interactions ($F_{3,78,624} = 4.037$. *$P < 0.05$, Bonferroni’s test post hoc, n = 8 cells in 8 mice vehicle, 6 cells in 6 mice HAV, 5 cells in 5 mice scrambled peptide). Vehicle-only and scrambled peptide (HAV-S) had no effect on LTP. Data shown as mean ± s.e.m.
Cocaine-induced CPP leads to recruitment of cadherin and GluA1 to excitatory synapses onto dopaminergic neurons in the VTA. (a) Experimental schedule for CPP and extinction experiments. Hab., habituation. (b) Cocaine administration produced robust CPP (*P < 0.001, significant interaction between treatment and test day, two-way repeated-measures ANOVA, F(6,60) = 4.422, **P < 0.01, Bonferroni's test post hoc) that extinguished over 5 d. (c) EM of VTA synapse showing immunogold-labeled DAT, PSD-95 and cadherin (scale bar, 100 nm). (d) Cadherin shifted to the synaptic membrane of excitatory synapses following cocaine CPP (20 nm bins). Pre, presynaptic; Post, postsynaptic. (e) The relative percentage of cadherin at the synaptic membrane at excitatory synapses ([number of cadherin beads within 40 nm of the pre and postsynaptic membrane]/[number of beads within 500 nm of the synaptic membrane]) was significantly increased at excitatory synapses following cocaine CPP (linear regression, percentage of cadherin localized to the synaptic membrane at excitatory synapses was significantly correlated with time spent in conditioned chamber (NF: no food, PF: palatable food; see also Supplementary Fig. 6). (f) Cocaine administration produced robust CPP (P < 0.01, significant interaction, two-way ANOVA, F(4,41) = 4.999; **P = 0.0026; n.s., P > 0.9999; Bonferroni's test post hoc), but not in home cage controls, following extinction of CPP, following return to home cage for 6 d after CPP (CPP + HC), or following food CPP (NF: no food; PF: palatable food; see also Supplementary Fig. 6). CPP: n = 6 mice saline, 6 mice cocaine; HC: n = 4 mice saline, 3 mice cocaine; CPP + extinction: n = 5 mice saline, 6 mice cocaine; CPP + HC: n = 6 mice saline, 5 mice cocaine; food CPP: n = 4 mice NF, 6 mice PF. (g) The percentage of cadherin localized to the synaptic membrane at excitatory synapses was significantly correlated with time spent in conditioned chamber following cocaine CPP (linear regression, R² = 0.118, P = 0.78), 6 mice per condition. (h) No change in cadherin localization was observed at inhibitory synapses following cocaine CPP. (i) The relative percentage of cadherin at the synaptic membrane was not changed at inhibitory synapses following cocaine CPP, home cage controls, extinction of CPP, or food CPP (no significant interaction, P = 0.8399, two-way ANOVA, F(4,40) = 0.3537; n.s., P > 0.9999, Bonferroni's test post hoc; CPP: n = 6 mice saline, 6 mice cocaine; HC: n = 4 mice saline, 3 mice cocaine; CPP + extinction: n = 4 mice saline, 5 mice cocaine; CPP + HC: n = 6 mice saline, 6 mice cocaine; food CPP: n = 4 mice NF, 6 mice PF). (j) Cadherin localization to synaptic membrane at inhibitory synapses was not correlated with time spent in conditioned chamber following CPP (b) GluA1 localization shifted toward the PSD membrane following cocaine CPP. (k) The relative percentage of GluA1 at the PSD membrane ([number of GluA1 beads within 30 nm of the PSD membrane]/[number of beads within 500 nm of the PSD membrane]), expressed as a percent relative to saline controls, was significantly increased at excitatory synapses following cocaine-induced CPP (P < 0.01, significant interaction, two-way ANOVA, F(4,44) = 4.049; **P = 0.0041; n.s., P > 0.9999; Bonferroni's test post hoc), but not in home cage controls, following extinction of CPP, following return to home cage for 6 d after CPP, or following food CPP. CPP: n = 6 mice saline, 6 mice cocaine; HC: n = 4 mice saline, 3 mice cocaine; CPP + extinction: n = 6 mice saline, 6 mice cocaine; CPP + HC: n = 6 mice saline, 6 mice cocaine; food CPP: n = 6 mice NF, 5 mice PF. (l) Percentage of GluA1 localized to the PSD membrane was significantly correlated with time spent in conditioned chamber following cocaine-induced CPP (linear regression, R² = 0.661, P = 0.019), 6 mice per condition; >100 synapses were analyzed per group. Data shown as mean ± s.e.m. with data for individual mice (circles) overlaid.
formation of drug-associated memories. Together, these findings indicate that insertion of cadherin into the synaptic membrane specifically occurs following the learned association between contextual cues and the effects of cocaine during behavioral conditioning. These changes in cadherin localization were also transient, returning to baseline following active extinction of CPP or return of mice to home cages for an equivalent period of time without re-exposure to the CPP apparatus (Fig. 2e). At inhibitory synapses, we observed no changes in cadherin distribution following cocaine CPP, extinction of cocaine CPP or food CPP, or in home cage controls (Fig. 2g–i and Supplementary Figs. 5 and 6). We also observed no changes in cadherin localization at excitatory or inhibitory synapses onto glutamatergic or GABAergic neurons in the VTA following CPP (Supplementary Fig. 7), indicating that the increase in cadherin localization to the synaptic membrane was specific to excitatory synapses onto dopaminergic neurons in the VTA.

GluA1 is recruited to VTA synapses during cocaine CPP

We then used immunogold labeling to identify GluA1-containing AMPARs. We found that they exhibited the same pattern of insertion and removal from the synaptic membrane as cadherin in each of the behavioral conditions (Fig. 2j). The proportion of GluA1-containing AMPARs localized to the postsynaptic density (PSD) membrane was significantly increased following CPP (121% increase, Fig. 2k), though total levels of GluA1 were unchanged at synaptic compartments (Supplementary Fig. 3). As with cadherin, there was a strong positive correlation between the amount of GluA1 at the synaptic membrane and time spent in the cocaine-paired chamber for individual mice (r = 0.66, Fig. 2l), and GluA1-containing AMPAR localization was also unchanged compared to saline controls following extinction of CPP, in home cage controls, or following CPP using palatable food (Fig. 2k and Supplementary Figs. 5 and 6). These data directly demonstrate that cocaine CPP drives the insertion of GluA1-containing AMPARs to the PSD membrane, which is thought to contribute to the cocaine-mediated increase in AMPA:NMDA receptor (AMPA:NMDAR) ratio at VTA synapses previously observed using electrophysiological techniques.1,9,27 We also examined communolabeling of GluA1 and cadherin together at individual VTA synapses following cocaine CPP and found that individual synapses with a greater

Figure 3 Stabilization of cadherin by β-catenin at synapses in the VTA reduces cocaine-induced CPP. (a) β-catenin levels were significantly increased in DAT+ neurons (arrowheads) in the VTA of DAT-Cre;β-catenin mice compared to adjacent DAT− cells (asterisks) and to DAT+ neurons in littermate control mice (P < 0.001, significant interaction between genotype and cell type, two-way ANOVA, F(1,48) = 38.13, ***P < 0.001 Bonferroni’s test post hoc, littermate control: n = 14 cells non-DAT, 10 cells DAT+, DAT-Cre;β-catenin: 16 cells DAT−, 12 cells DAT+). Scale bars, 10 μm. (b) Cocaine-induced CPP was significantly reduced in DAT-Cre;β-catenin mice compared to controls (P = 0.0440, significant interaction between genotype and test day, two-way repeated-measures ANOVA, F(6,264) = 2.194; *P = 0.0488, Bonferroni’s test post hoc, n = 23 mice per genotype). Preference for the cocaine-paired chamber returned to baseline after 3 d of extinction in control mice and 1 d of extinction in DAT-Cre;β-catenin mice (*P < 0.01, **P < 0.001, significantly different from day 1, Dunnett’s test post hoc). Hab., habituation. (c,d) DAT-Cre;β-catenin mice showed no differences in average speed (c) in the three-chamber CPP apparatus during habituation or after CPP (no significant interaction, two-way repeated-measures ANOVA, F(6,154) = 0.2211; n.s., P = 0.9695; n = 10 mice control, 14 mice DAT-Cre;β-catenin), and no differences in locomotor sensitization to cocaine (d) compared to littermate controls (no significant interaction, two-way repeated-measures ANOVA, F(7,240) = 0.5123; n.s., P = 0.8249; n = 16 mice control, 16 mice DAT-Cre;β-catenin). (e) DAT-Cre;β-catenin mice showed normal coordination and motor learning on an accelerating rotorod task (no significant interaction, two-way repeated-measures ANOVA, F(9,260) = 0.3601; n.s., P = 0.9529; n = 13 mice control, 15 mice DAT-Cre;β-catenin). (f) DAT-Cre;β-catenin mice showed no change in the acquisition of contextual fear memory following a foot shock in a novel environment compared to littermate controls (no significant interaction, two-way ANOVA F(2,90) = 1.348; n.s., P = 0.2650; n = 16 mice control, 16 mice DAT-Cre;β-catenin) (g) DAT-Cre;β-catenin mice showed no impairments in CPP driven by palatable food rewards (no significant interaction, two-way ANOVA F(1,27) = 0.1161; n.s., P = 0.7360; n = 17 mice control, 12 mice DAT-Cre;β-catenin). Data shown as mean ± s.e.m.
proportion of cadherin localized to the synaptic membrane also had more GluA1 localized to the membrane (Supplementary Fig. 8). This correlation between cadherin and GluA1 levels at individual synapses provided further support that cadherin acts to stabilize GluA1 homomers at potentiated synapses, consistent with our electrophysiological data demonstrating that cadherin stability was essential for STD LTP at VTA synapses (Fig. 1b).

**Cadherin stabilization at VTA synapses reduces cocaine CPP**

Given the strong correlation between CPP acquisition and cadherin localization at excitatory synaptic membranes in the VTA, we hypothesized that subcellular changes in cadherin localization may regulate cocaine-induced synaptic plasticity and behavioral conditioning. To test this, we increased cadherin at the synaptic membrane using a transgenic mouse line in which β-catenin levels are increased in dopaminergic neurons (Slc6a3:Cre; Ctnnb1\(^{lox/ex3}/lox/ex3\) mice, termed hereafter DAT-Cre;β-catenin\(^{ex3}\) mice) (Fig. 3a)\(^{28,29}\). β-catenin is the major intracellular binding partner of all classical cadherins, and we have previously shown that elevating β-catenin levels using this approach significantly increases the stabilization of cadherin and AMPARs at the synaptic membrane in hippocampal neurons in vivo\(^{20}\).

DAT-Cre;β-catenin\(^{ex3}\) mice exhibited a 48% reduction in cocaine-induced CPP compared to control mice at day 8 (Fig. 3b). There was no significant difference between groups in the rate of extinction from day 8 to 9 (P = 0.5527, unpaired t-test, t(44) = 0.5983, n = 23 mice per condition). However, due to the decreased magnitude of CPP, DAT-Cre;β-catenin\(^{ex3}\) mice returned to baseline levels of preference for the conditioned chamber after 1 d of extinction (day 9), compared to 3 d in controls (day 11). Behavioral changes in these mice were specific to cocaine-mediated CPP; DAT-Cre;β-catenin\(^{ex3}\) mice appeared phenotypically normal, exhibited no changes in exploratory behavior or basal locomotion (Fig. 3c), and showed intact locomotor sensitization to repeated cocaine administration (Fig. 3d). DAT-Cre;β-catenin\(^{ex3}\) mice also showed no change in motor learning (Fig. 3e), contextual fear conditioning (Fig. 3f), food consumption (Supplementary Fig. 9), or CPP driven by food rewards (Fig. 3g). The lack of change in tasks which require intact recognition of a novel context (contextual fear learning and food CPP) also indicated that impairments in spatial memory were not responsible for the reduction in cocaine CPP.
observed in DAT-Cre;β-catΔmex3 mice. We also verified that, following β-catenin stabilization in DAT-Cre;β-catΔmex3 mice, no subsequent changes in Wnt pathway targets were observed in dopaminergic neurons in the VTA, indicating that the observed effects on CPP were not due to alterations in Wnt signaling (Supplementary Fig. 10).

**Cadherin stabilization at VTA synapses blocks synaptic plasticity**

To determine why there was a marked attenuation of cocaine CPP in DAT-Cre;β-catΔmex3 mice, we used immunogold EM to examine the distribution of cadherin, GluA1 and GluA2 at excitatory synapses onto VTA dopaminergic neurons after CPP. We found that the proportion of cadherin localized to the synaptic membrane was significantly increased in DAT-Cre;β-catΔmex3 mice under basal conditions (−77% increase) (Fig. 4a,b). However, unlike control mice, DAT-Cre;β-catΔmex3 mice did not recruit more cadherin to the synaptic membrane during cocaine-mediated CPP. Additionally, both the removal of GluA2-containing AMPARs and the insertion of GluA1-containing AMPARs driven by cocaine CPP were blocked in DAT-Cre;β-catΔmex3 mice (Fig. 4c–f). There was also no change in total levels of cadherin (P = 0.3567), GluA1 (P = 0.8557) or GluA2 (P = 0.5683) in DAT-Cre;β-catΔmex3 mice (Supplementary Fig. 10c–e).

In addition to interacting with GluA1 (ref. 11), cadherins can also interact directly and indirectly with the GluA2 subunit of AMPARs[12,13]. Consequently, these data indicate that aberrantly increasing cadherin localization to the synaptic membrane before CPP stabilizes GluA1/2 heteromers present at VTA synapses under basal conditions, which blocks cocaine-induced insertion of GluA1 homomers and results in reduced cocaine CPP.

To functionally verify these changes in AMPAR trafficking, we also examined STD LTP at synapses in the VTA. We found that this form of LTP was abolished in DAT-Cre;β-catΔmex3 mice (Fig. 5a). Additionally, treatment with NASPM (1-naphthyl acetyl spermine...
Ca2+-permeable GluA1 homomers into AMPA receptor ‘slots’ in the PSD 32,46. Enhanced synaptic activity also leads to increased levels of cadherin owing to their stabilization by increased synaptic cadherin in β-catenin 11–13,45. Cadherins regulate the dynamic localization of AMPARs through direct and indirect interactions with GluA1 and GluA2 (refs. 11–13,45). Preventing the insertion of GluA1-containing AMPARs and the potentiation of these synapses. Thus, stabilizing synaptic cadherin in β-catenin promotes the stability of cadherin, resulting in an increase in cadherin localized to the synaptic membrane. Cadherins are then situated to associate with and stabilize GluA1 homomers at the synaptic membrane, contributing to the potentiation of these synapses underlying behavioral changes in CPP. Under basal conditions, elevated levels of β-catenin promote the stability of cadherin, resulting in an increase in cadherin localized to the synaptic membrane. Cadherins associate with GluA1/2 heteromers 11–13, enhancing their stability at the synaptic membrane. During cocaine-mediated CPP, the removal of GluA1/2 heteromers is prevented owing to their stabilization by increased synaptic cadherin in DAT-Cre;β-catenin Δex3 mice. GluA1/2 heteromers are retained in available AMPAR slots, preventing the insertion of GluA1-containing AMPARs and the potentiation of these synapses. Thus, stabilizing synaptic cadherin in DAT-Cre;β-catenin Δex3 mice disrupts the cocaine-induced switch in AMPAR composition and reduces CPP.

Figure 6 Model of changes in cadherin and AMPAR subunit localization in wild type and DAT-Cre;β-catenin Δex3 mice during CPP. (a.b) Wild-type mice. (a) Under basal conditions, the population of AMPARs at excitatory inputs to dopaminergic neurons is composed of GluA1/2 heteromers. Cadherins regulate the dynamic localization of AMPARs through direct and indirect interactions with GluA1 and GluA2 (refs. 11–13,45). (b) During cocaine-mediated CPP, activity is enhanced at excitatory inputs to dopaminergic neurons, driving the removal of GluA1/2 heteromers and the insertion of Ca2+-permeable GluA1 homomers into AMPA receptor ‘slots’ in the PSD 32,46. Enhanced synaptic activity also leads to increased levels of cadherin at the synaptic membrane. Cadherins are then situated to associate with and stabilize GluA1 homomers at the synaptic membrane, contributing to the potentiation of these synapses underlying behavioral changes in CPP. (c.d) DAT-Cre;β-catenin Δex3 mice. (c) Under basal conditions, elevated levels of β-catenin promote the stability of cadherin, resulting in an increase in cadherin localized to the synaptic membrane. Cadherins associate with GluA1/2 heteromers 11–13, enhancing their stability at the synaptic membrane. (d) During cocaine-mediated CPP, the removal of GluA1/2 heteromers is prevented owing to their stabilization by increased synaptic cadherin in DAT-Cre;β-catenin Δex3 mice. GluA1/2 heteromers are retained in available AMPAR slots, preventing the insertion of GluA1-containing AMPARs and the potentiation of these synapses. Thus, stabilizing synaptic cadherin in DAT-Cre;β-catenin Δex3 mice disrupts the cocaine-induced switch in AMPAR composition and reduces CPP.

trihydrochloride), a selective antagonist of GluA2-lacking AMPARs, reduced excitatory postsynaptic potential (EPSP) amplitude back to basal levels in control mice, indicating that the enhanced EPSP amplitude observed following LTD induction was the result of the insertion of GluA2-lacking AMPARs. In contrast, NASPM treatment had no effect on EPSP amplitude in DAT-Cre;β-catenin Δex3 mice, demonstrating that GluA2-lacking AMPARs were not recruited to these synapses following the STD LTD protocol (Fig. 5a). These data confirmed that increasing cadherin at the synaptic membrane in DAT-Cre;β-catenin Δex3 mice results in the retention of GluA2-containing AMPARs at the membrane and prevents the insertion of GluA2-lacking AMPARs, which are crucial for the strengthening of these synapses.

We then examined changes in AMPAR:NMDAR ratio and inward rectification of AMPA EPSCs 24 h after cocaine administration in control and DAT-Cre;β-catenin Δex3 mice. In control mice we found, consistent with previous studies, that cocaine administration caused a significant increase in AMPAR:NMDAR ratio (Fig. 5b) and inward rectification of AMPA EPSCs (Fig. 5c) indicating increased insertion of GluA2-lacking AMPARs at synapses onto VTA DA neurons 30. However, these increases were completely absent in DAT-Cre;β-catenin Δex3 mice (Fig. 5b,c), consistent with our immuno-EM data showing that GluA1 at the synaptic membrane is increased in control mice but not DAT-Cre;β-catenin Δex3 mice following cocaine CPP. To confirm that these changes were not due to earlier, developmental disruptions, we also examined the morphology, density and function and VTA synapses in DAT-Cre;β-catenin Δex3 mice compared to controls (Supplementary Fig. 11). We also found no differences in the frequency or amplitude of miniature excitatory postsynaptic currents and miniature inhibitory postsynaptic currents onto VTA dopaminergic neurons in DAT-Cre;β-catenin Δex3 mice compared to those in control mice (Fig. 5d,e). Together, these data demonstrate that increasing cadherin at the synaptic membrane before behavioral training results in a reduction in cocaine CPP through the aberrant retention of GluA1/2 heteromers and the prevention of GluA1 homomer membrane insertion to VTA synapses (Fig. 6).

DISCUSSION
Here we demonstrate that cadherin plays a critical role in synaptic plasticity in the VTA and behavioral conditioning driven by cocaine.
We show that cadherins are widely expressed in the VTA and are essential for the potentiation of excitatory synapses onto dopaminergic neurons. Using immunogold EM, we observed a strong correlation between cocaine-induced CPP in wild-type mice and the insertion of cadherin and GluA1-containing AMPARs into the synaptic membrane of these synapses. These changes in cadherin and AMPAR localization were specific to cocaine-induced CPP, and were not observed when mice were given the same schedule of cocaine and saline administration in their home cage, nor following CPP induced by palatable food rewards. In DAT-Cre; β-cat∆ex3 mice, we found that stabilization of cadherin at VTA synapses was sufficient to reduce the magnitude of cocaine-induced CPP. This behavioral effect was associated with disruptions in the plasticity of excitatory synapses formed onto dopaminergic neurons; stabilization of cadherin led to the abolishment of LTP induced by both cocaine administration and STD stimulation in these mice. Indeed, our immunogold EM data demonstrated that cocaine-induced internalization of GluA2-containing AMPARs and the subsequent insertion of GluA2-lacking AMPARs into the membrane was blocked in DAT-Cre; β-cat∆ex3 mice.

Our findings suggest that, in wild-type mice, increased cadherin at the synaptic membrane acts to stabilize newly inserted GluA1 homomers during cocaine CPP. This is supported by evidence that cadherin interacts with the GluA1 subunit and stabilizes GluA1-containing AMPARs11, as well as by our data showing that intact cadherin adhesion is required for STD LTP at VTA synapses, which is also mediated by insertion of GluA1-homomers, as in potentiation of these synapses by cocaine. Cadherin-mediated stabilization of GluA1 homomers may therefore contribute to increased strength of excitatory synapses onto dopaminergic neurons, as well as prolonged postsynaptic Ca2+ influx through these AMPARs. Potentiation of these synapses is also likely to enhance the activity of dopaminergic neurons and increase dopamine release onto target structures of the mesocorticolimbic system, leading to further downstream changes in synaptic plasticity and circuit activity that contribute to addiction.

Excitatory synapses onto dopaminergic neurons have previously been implicated in contextual conditioning and reward learning27–28, and our data demonstrate a strong relationship between increased cadherin at these synapses and drug-induced behavioral changes in wild-type mice. Increased cadherin at the synaptic membrane was correlated with the magnitude of CPP in individual mice, which suggests that increased cadherin adhesion may be a mechanism that contributes to drug-induced increases in the stability and potentiation of VTA synapses, promoting the ‘hard-wiring’ of synaptic traces of drug-associated memories and behaviors. We speculate that increased strength and stability of VTA synapses mediated by cadherin adhesion may also contribute to sensitization to drug-associated cues, which can trigger relapse both in humans and animal models of addiction even after extended periods of abstinence from drug-taking35.

In DAT-Cre; β-cat∆ex3 mice, stabilization of cadherin at VTA synapses before cocaine administration prevented the removal of GluA2-containing AMPARs and blocked the insertion of GluA1-containing AMPARs. This disruption of cocaine-induced changes in AMPAR localization was associated with attenuated CPP and the abolishment of STD LTP. These impairments contrast with observations in the hippocampus, where stabilization of cadherin leads to impairments in LTD and behavioral flexibility but has no effect on LTP or acquisition of spatial memory20. The key difference in plasticity between these regions appears to be the requirement for removal of GluA2-containing AMPARs for LTP at VTA synapses. It has been proposed that the number of AMPARs at synapses onto dopaminergic neurons is limited by the number of ‘slots’ where receptors can associate with scaffold proteins in the PSD32,33. Indeed, inhibiting the internalization of GluA2-containing AMPARs by disrupting GluA2–PICK1 (protein interacting with C kinase-1) interactions abolishes cocaine-induced increases in AMPAR:NMDAR ratio that are typically mediated by the insertion of GluA2-lacking AMPARs32. Since cadherin can stabilize AMPARs at synapses through interaction with both the GluA1 and GluA2 subunits11–13, this suggests that in DAT-Cre; β-cat∆ex3 mice increased cadherin at the synaptic membrane stabilized GluA2-containing AMPARs that occupied available slots in the PSD and prevented the insertion of GluA1 homomers. Our electrophysiological data confirmed the functional identity of AMPARs at VTA synapses in DAT-Cre; β-cat∆ex3 mice, demonstrating that GluA2-containing AMPARs were indeed retained at these synapses and insertion of GluA2-lacking AMPARs was blocked, leading to abolishment of LTP.

Our findings also show that the timing of cadherin stabilization at the synaptic membrane had a critical effect on AMPAR trafficking and plasticity at VTA synapses. In wild-type mice, increased cadherin localization to the synaptic membrane during CPP resulted in the stabilization of GluA1 homomers that were also inserted during CPP. However, in DAT-Cre; β-cat∆ex3 mice, increased cadherin localization to the synaptic membrane occurred before CPP, which resulted in the stabilization of GluA1/2 heteromers present under basal conditions. In both cases, the effect of cadherin was consistent: increased cadherin at the synaptic membrane was found to stabilize the AMPARs that were present at that time. However, the differences in timing of when cadherin was stabilized and the type of AMPAR subunits present at each time led to different effects in LTP and CPP observed between DAT-Cre; β-cat∆ex3 mice and controls.

Notably, while CPP was reduced in DAT-Cre; β-cat∆ex3 mice, we observed no changes in cocaine-induced locomotor sensitization in these mice compared with controls. This finding is consistent with studies showing that CPP and sensitization are not necessarily directly correlated and may be mediated by distinct circuits and neural adaptations in the brain driven by cocaine. Disruption of the dopaminergic projections from the substantia nigra, but not VTA, has been shown to abolish locomotor sensitization to cocaine while leaving CPP intact34. Additionally, an analysis of CPP and locomotor sensitization in different inbred mouse lines found no correlation between the two behaviors, with some strains exhibiting robust CPP but no locomotor sensitization, or vice versa35. Finally, no correlation was found to exist between CPP and sensitization within individual mice in a behavioral task designed to test both parameters simultaneously36.

Our findings also demonstrate interesting differences in CPP driven by cocaine and food rewards. Cocaine CPP, but not food CPP, increased both cadherin and GluA1 localization to the synaptic membrane at excitatory synapses onto dopaminergic neurons in the VTA. Consistent with this, stabilization of cadherin (and GluA1/2 heteromers) at the synaptic membrane of these synapses in DAT-Cre; β-cat∆ex3 mice markedly decreased the magnitude of cocaine CPP, but had no effect on food CPP. These findings are consistent with a number of studies indicating that changes in dopaminergic neuron activity and behavior driven by food rewards may be mediated by mechanisms other than increased LTP at VTA synapses. Food-related peptide hormones have been shown to play a major role in mediating behavioral changes driven by food37, in some cases by acting directly on VTA dopamine neurons to regulate their activity38. Additionally, pharmacological disruption of NMDARs and metabotropic glutamate receptors have both been shown to have opposite effects on CPP driven by food rewards and CPP driven by drugs of abuse39–40, which further suggests that major differences exist in the mechanisms underlying these different forms of CPP.
What are the molecular mechanisms responsible for the changes in cadherin localization observed following CPP? In hippocampal neurons, cadherins have been shown to undergo constitutive turnover at the synaptic membrane under basal conditions. We have previously shown that synaptic activity leads to post-translational changes in the cadherin-binding protein δ-catenin, increasing its association with cadherin and augmenting cadherin’s retention at the synaptic membrane. This result in increased trans-synaptic adhesion and the stabilization of postsynaptic AMPARs by cadherin, causing long-lasting increases in synapse strength, size and stability over time. An important direction for future research will be to determine whether these same mechanisms drive changes in cadherin localization during activity- and drug-induced plasticity at synapses in the VTA.

The finding that cadherins regulate AMPAR trafficking at synapses in the VTA supports an emerging view that structural and scaffolding proteins may be of central importance in synaptic plasticity due to their role in mediating the recruitment and removal of postsynaptic AMPARs. Since the finding that LTP can be induced at synapses as long as extrasynaptic glutamate receptors are present, there has been a shift of focus to identify the proteins that control the insertion, removal and stabilization of AMPARs during different forms of synaptic plasticity. The present study provides further evidence that cadherins are key molecules that control AMPAR trafficking during plasticity underlying different forms of learning and memory. Additionally, our findings demonstrate that cadherins play a critical role in synaptic plasticity outside of the hippocampus, where they have typically been studied, and likely have specialized functions in mediating different forms of synaptic plasticity throughout the CNS. Another important goal for future studies will be determining the specific cadherin subtypes responsible for mediating different forms of synaptic plasticity throughout the brain. In the VTA, N-cadherin likely plays a major role in activity and cocaine-induced plasticity examined in the present study, as the HAV peptide used to block STD LTP at VTA synapses has been shown to specifically disrupt N-cadherin trans interactions, and as N-cadherin is critical for plasticity at excitatory synapses in other brain regions such as the hippocampus. However, cadherins have highly similar structure and functional redundancy, and other cadherin subtypes expressed in dopaminergic neurons may therefore contribute as well.

Finally, our study provides mechanistic insight into how mutations in cadherin adhesion complex proteins could contribute to susceptibility or resilience to addiction. Genome-wide association studies in substance abusers have identified increased prevalence of clustered single nucleotide polymorphisms in δ-catenin, which stabilizes cadherin at synapses, and α-catenin, which tethers the cadherin adhesion complex to the actin cytoskeleton. While the functional consequences of these polymorphisms are unknown, we speculate that changes in the function or expression levels of δ-catenin or α-catenin could affect the stabilization of cadherin and AMPARs at the synaptic membrane of VTA synapses, which our findings show is sufficient to alter drug-induced synaptic plasticity and behavior. Cadherin adhesion-complex proteins may therefore be targets of interest for future studies investigating genetic risk factors for addiction.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

F.M. and A.K.G. performed all behavioral, immunogold electron microscopy, and immunohistochemistry experiments. S.L. performed all electrophysiological experiments under S.L.B’s supervision. C.M.C. assisted with EM sample processing and immunoelectron microscopy and performed biochemical experiments. M.M. assisted with data analysis and genotyping of mice. A.G.P. provided experimental reagents. F.M., A.K.G. and S.X.B. designed all experiments, interpreted the results and wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Animals. Male C57BL/6 mice 6–8 weeks old were used in all experiments, unless stated otherwise. For experiments the effects of cadherin stabilization of cocaine-induced plasticity and behavioral conditioning, we used mice Sema3a-Cre+/++;Ctnnb1lox(ex3) mice (termed DAT-Cre;β-cat;ex3 mice for brevity), which are homozygous for a loxP-flanked exon-3 transgene28 and express Cre recombinase in dopaminergic neurons29. Littermates lacking the Sema3a-Cre+ transgene (+/++;Ctnnb1lox(ex3);Ctnnb1lox(ex3) mice) were used as controls. Mice were housed in reverse day/night cycle and given ad libitum access to food and water. Experimental procedures and animal housing conditions were approved by the UBC Animal Care Committee and were in accordance with Canadian Council on Animal Care (CCAC) guidelines. All mice were housed with littermates in groups of two to five and were used for only one behavioral test apiece, unless otherwise noted.

Immunoblot analysis. Mice were killed by cervical dislocation, and their brains were quickly removed and then sliced into 300-µm-thick horizontal sections by vibratome. The VTA was dissected from the slices, with the VTAs of 5 mice being pooled and homogenized in lysis buffer (20 mM Tris pH 7.4, 137 mM NaCl, 0.5% NP-40, 10% glycerol) with protease and phosphatase inhibitor tablets (Roche) and cleared by centrifugation at 14,000g for 40 min at 4 °C. VTA lysates were separated by SDS-PAGE and probed with antibodies against N-cadherin (mouse, BD Transduction, cat. no. 610920, predicted band size 130 kDa, 1:1,000), R-cadherin (rabbit, Novus, cat. no. NB2-27372, predicted band size 130 kDa, 1:1,000), cadherin-7 (rabbit, Santa Cruz, cat. sc-68422, predicted band size 90 kDa, 1:500), cadherin-8 (rabbit, Abcam, cat. no. ab97268, predicted band sizes 150 kDa precursor and 90 kDa protein, 1:1,000) and cadherin-11 (mouse, Invitrogen, cat. no. 321700, predicted band size 110 kDa, 1:1,000). Proteins were visualized by chemiluminescence on a Bio-Rad Versadoc 4000 (Bio-Rad Laboratories (Canada) Ltd., Mississauga, ON). Immunoblot experiments were conducted twice to ensure reproducibility.

Immunohistochemistry. Mice were anesthetized with sodium pentobarbital (120 mg/kg) and transcardially perfused with PBS followed by 4% paraformaldehyde (PFA) in PBS. Brains were removed and postfixed in 4% PFA for 2 h, then cryoprotected by saturation with 30% sucrose, frozen, and sliced into 20-µm-thick coronal sections by cryostat. For immunolabeling of target proteins, sections were first placed in a blocking buffer containing 10% goat serum, 0.1% bovine serum albumin and 0.1% Triton-X-100 in PBS. Primary antibodies against DAT (dopamine transporter) (rat, Millipore, cat. no. MAB369, 1:500), N-cadherin (mouse, BD Transduction, cat. no. 610920, 1:250), R-cadherin (rabbit, Novus, cat. no. NB2-27372, 1:250), cadherin-7 (rabbit, Santa Cruz, cat. sc-68422, 1:250), cadherin-8 (rabbit, Abcam, cat. no. ab97268, 1:250), cadherin-11 (rabbit, Santa Cruz, cat. no. sc-28643, 1:250), β-catenin (mouse, BD Transduction, cat. no. 610153, 1:250), Axin2, (rabbit, Abcam, cat. no. ab109307, 1:250), LEF1 (rabbit, Abcam, cat. no. ab137872, 1:250), c-Myc (rabbit, Cell Signaling, cat. no. 5605S, 1:250), c-Jun (rabbit, Cell Signaling, cat. no. 9165P, 1:250), and GAD67 (rabbit, Abcam, cat. no. ab75712, 1:250) were diluted in this buffer, added to sections and incubated overnight at 4 °C. The following day, samples were washed three times with PBS, and secondary antibodies diluted in the blocking buffer were added to sections and incubated for 1–2 h at room temperature. Slides were washed again with PBS and stained with DAPI (0.5 µg/mL). Sections were mounted with ProLong Gold (Life Technologies, Carlsbad, CA), and were imaged on an Olympus Fluoview FV1000 confocal microscope using Fluoview software (Olympus, Melville, NY).

Immunostaining experiments were conducted twice to ensure reproducibility. The brightness and contrast of entire images was judiciously adjusted using Photoshop (Adobe Systems Canada, Toronto, ON) following recommended, scientifically acceptable procedures, and no information was obscured or eliminated from the original images. Immunohistochemical experiments were repeated on brain slices from at least 2 mice per genotype to ensure reproducibility.

Electrophysiology. Electrophysiological recordings were taken from dopaminergic cells in the VTA of male and female mice ranging from postnatal day 21 (P21) to P30. Horizontal slices of mouse midbrain were cut with a vibratome (Leica, Nussloch, Germany), and slices (250 µm) were equilibrated in artificial cerebrospinal fluid (aCSF) containing (in mM) 126 NaCl, 1.6 KCl, 1.1 NaH2PO4, 1.4 MgCl2, 2.4 CaCl2, 26 NaHCO3, 11 glucose (32–34 °C) and saturated with 95% O2/5% CO2. Cells were visualized using infrared differential contrast video microscopy and whole-cell voltage-clamp recordings were made using a MultiClamp 700B amplifier (Axon Instruments, Union City, CA). Putative dopaminergic cells were identified by the presence of a large hyperpolarization-activated cation current (iHPA)57, fusiform shape and location in the lateral VTA proximal to the medial terminals of the optic nucleus59. In this VTA subregion, iHPA is a reliable predictor of dopamine neurons30–32. A subset of dopaminergic neurons was further confirmed by post hoc immunostaining for tyrosine hydroxylase after recording as described previously53. 99 of 131 post hoc stained neurons were identified as TH positive. The remaining 32 were unable to be recovered. In the spike-timing-dependent plasticity experiments, neurons were patch clamped in current-clamp mode with electrodes containing (in mM) potassium methanesulfonate 125 mM, KCl 5 mM, HEPES 0.2 mM, MgCl2 2 mM, 2.5 mg/ml Mg-ATP, 0.25 mg/ml GTP, and 0.2% bicyton, pH 7.2–7.4, 275–285 mOsm and picrotoxin (100 µM) in the external aCSF solution. Slices were preincubated in 200 µM HEPV, 200 µM scrambled control peptide, or vehicle. The spike-timing-dependent protocol for LTP induction was carried out as previously described54.

Briefly, the protocol consisted of 20 bursts of EPSP–spike pairs, with each burst consisting of five paired stimuli at 10 Hz (100 ms intervals), with an interburst interval of 5 s. Postsynaptic spikes were evoked by injection of depolarizing current pulses, with the onset of EPSPs preceding the peak of postsynaptic spikes by 5 ms. Evoked EPSPs were sampled at 0.1 Hz before and after LTP induction. In experiments where NASPM (1-naphthyl acetyl serine trihydrochloride, Tocris, Bristol, UK) was used, 100 µM NASPM in aCSF was added to slices 25 min after STDTP LTP induction to inhibit GluA2-lacking AMPA receptors. For the rest of experiments, electrodes (3–5 MQ) contained (in mM) 117 cesium methanesulfonate, 20 HEPES, 0.4 EGTA, 2.8 NaCl, 5 TEA chloride, 2.5 Mg-ATP and 0.25 Na-GTP (pH 7.2–7.3, 270–280 mOsm). To calculate AMPAR:NMDAR ratio, neurons were voltage clamped at 40 mV and an average of 30 EPSCs were measured before and after the application of 2-aminoo-5-phosphonopentanoate (AP-5) (50 µM) for 5 min. NMDAR responses were calculated by subtracting the average response in the presence of AP-5 (AMPAR-mediated only) from that recorded in its absence. The peak of the AMPAR EPSC was divided by the peak of the NMDAR EPSC in order to compute the AMPAR:NMDAR ratio.

Experiments measuring the I–V relationship were carried out in the presence of picrotoxin (100 µM) and, when indicated, in the presence of AP-5 (50 µM) to block GABA A and NMDA receptors, respectively. The holding potentials were −70 mV, 0 mV and 40 mV. Synaptic currents were evoked by stimuli at 0.1 Hz, and the rectification index was calculated by dividing the slope of the negative potentials by the gradient of the slope at positive potentials. Excitatory and inhibitory transmission were recorded in cells voltage-clamped at −67 mV for mEPSCs and 10 mV for mIPSCs in TTX (500 mM). AMPAR mEPSCs were selected on the basis of their amplitude (>12 pA), decay time (<3 ms) and rise time (<1 ms) using the Mini60 MiniAnalysis program (Synaptosoft). Similarly, GABA A mIPSCs were selected on the basis of amplitude (>12 pA), rise time (<4 ms) and decay time (<10 ms).

TH immunocytochemistry. Brain slices from patch-clamp recording were fixed overnight in cold 4% paraformaldehyde, rinsed in phosphate buffer solution (PBS), blocked in 10% normal donkey serum and incubated with monoclonal mouse anti-TH antibody (Sigma, Oakville, ON, 1:1,000, cat. no. T2928) for 48 h at 4 °C. Secondary donkey anti-mouse fluorescein isothiocyanate antibody (Cedarlane, Burlington, ON, cat. no. NBI20-6816, 1:500) was applied for 2 h at 4 °C. DyLight 594 streptavidin (Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania; 1:200) was applied overnight at 4 °C. Slices were mounted using Fluoromount (Sigma, Oakville, ON).

Conditioned place preference (CPP). CPP was induced using a standard three-chambered apparatus, consisting of two conditioning compartments and a middle, connecting compartment (Stoelting Co., Wood Dale, IL). The two conditioning compartments had distinct wall patterns and floor textures to allow mice to distinguish between them. Naïve mice were first allowed to habituate to the entire apparatus during a 30-min session on day 1. On day 2, mice in the conditioned group received a 15 mg/kg injection of cocaine and were placed in the conditioned chamber for 15 min. Mice were assigned to receive cocaine in one compartment or the other using an unbiased design. Individual mice were removed from the experiment if they showed a strong baseline preference (>70%)
Mice were placed in the condition -

Electron microscopy sample preparation. Mice were anesthetized with sodium pentobarbital (120 mg/kg), transcardially perfused by PBS followed by 4% paraformaldehyde (PFA) as described above, then postfixed in 4% PFA overnight. Brains were then cut into 250-µm-thick horizontal sections by vibrotome. Small pieces of VTA tissue (<1 mm in all dimensions) were dissected from these slices and cryoprotected in 30% glycerol overnight at 4 °C. Samples were then plunged in liquid ethane at −170 °C and transferred to a 1.5% uranyl acetate solution in 100% methanol, kept at −90 °C in a Leica EM AFS for 30 h. The temperature was increased to −45 °C over 11 h. Next, samples were rinsed in 100% methanol and infiltrated with HM-20 acrylic resin (Electron Microscopy Sciences, Hatfield, PA) by increasing the resin-to-methanol ratio in 2-h steps while maintaining the temperature at −45 °C. Samples were set up in capsules containing pure resin and polymerized under UV light for 24 h at −85 °C, after which the temperature was slowly increased to 0 °C. Tissue sections were cut at 85 nm using a Diatome diamond knife and a Leica ultramicrotome. Sections were collected on 300-mesh, formvar-coated nickel grids.

Immunogold electron microscopy. Grids were rinsed with distilled water and subsequently immersed in a bead of TTBS with 0.1% Triton-X with 0.1% sodium borohydride and 50 mM sodium glycine. The grids were then rinsed with TTBS with 0.1% Triton-X three times. Following this, nonspecific binding was blocked by immersing grids in a bead of 2% BSA in TTBS with 0.1% Triton-X for 10 min. Primary antibodies against DAT (dopamine transporter) (rat, Millipore, cat. no. MAB369), PSD-95 (rabbit, Frontier Institute, cat. no. Af628), pan-cadherin (mouse, Sigma, cat. no. C1821), GluA1 (rabbit, Millipore, cat. no. ABN241), GluA2 (rabbit, NeuroMab Antibodies Inc, cat. no. 75-002), GAD67 (mouse, Abcam, cat. no. ab75712), and VGLUT2 (guinea pig, Synaptic Systems, cat. no. 124014) were diluted in 2% BSA in TTBS with 0.1% Triton-X. Grids were immersed in 15-µl beads of diluted primary antibody overnight, at room temperature in a humidified chamber. The following day, grids were thoroughly rinsed by immersion in vials of TTBS with 0.1% Triton-X three times. Secondary antibodies were diluted in 2% BSA in TTBS with 0.1% Triton-X, and 0.05% polyethylene glycol (PEG) was added to prevent aggregation of gold beads. Grids were immersed in 15-µl beads of secondary antibody (Electron Microscopy Sciences, Hatfield, PA, goat-anti-rat 25 nm, cat. no. 25195; goat-anti-rabbit 15 nm, cat. no. 25112; goat-anti-rabbit 10 nm, cat. no. 25108; goat-anti-mouse 10 nm, cat. no. 25128; goat-anti-mouse 15 nm, cat. no. 25132; goat-anti-guinea pig 25 nm, cat. no. 25335; goat-anti-chicken 25 nm, cat. no. 25992) for 1.5 h. Following this step, grids were repeatedly rinsed in TTBS with 0.1% Triton-X and then rinsed in Milli-Q H2O and dried. Grids were then lightly counterstained with 2% uranyl acetate and Reynolds’s lead citrate. Images were collected at 98,000× magnification on a Tecnai G2 Spirit transmission electron microscope (FEI Company, Eindhoven, the Netherlands).

To analyze immunogold labeling, cell types were first identified by the presence of DAT, VGLUT2 or GAD67 markers, and synapse types were identified by the presence or absence of PSD-95 markers. The distance of all immunogold-labeled cadherin from the synaptic membrane or of all immunogold-labeled GluA1 or GluA2 to the postsynaptic active zone membrane was measured. Due to the sizes of proteins and reagents involved (see also Results and Supplementary Fig. 2) the maximum cutoff of immunogold particles considered to be labeling target proteins at the synaptic membrane was 40 nm for cadherin, 30 nm for GluA1 and 35 nm for GluA2. The percentage of immunogold particles localized to these regions was determined by [number of immunogold beads at target membrane]/[total number of immunogold beads within 500 nm of target membrane at pre and postsynaptic compartments] and was expressed as a percentage relative to saline-only controls that were processed and labeled in parallel. All images were acquired and analyzed blind to the genotype of each mouse. In each experiment, more than 100 synapses were analyzed for each condition.

Context-dependent fear conditioning. Mice were placed in the conditioning chamber for 3 min, and after 3 min they received an unconditioned foot shock stimulus (1 mA, 50 Hz) lasting 3 s. The next day mice were placed in the same chamber for 4 min, but did not receive an additional foot shock. Freezing behavior was determined by quantifying laser beam breaks in the conditioning chamber due to mouse activity, and total time percentage freezing was compared between groups. Experiments were performed during the dark (wake) cycle. Experimenters were blind to the genotype of the animal during testing and scoring. One cohort of mice completed the rotorod test, followed by context-depend -ent fear conditioning.

Food consumption testing. Mice were given preweighed pellets of low-fat (10% fat, Research Diets Inc, cat. no. D12450B) or high-fat (60% fat, Research Diets Inc, cat. no. D12492) food to consume over a 24-h period. The food given was in excess of what the mouse could consume in this period. Pellets were weighed after the 24-h period so that the amount of food consumed could be calculated. Experimenters were blind to the genotype of the animal during testing.

Statistical analysis. Unless otherwise noted, statistical analysis was done using unpaired Student’s t-test (two tailed) and two-way ANOVA. Data distribution was assumed to be normal, but this was not formally tested. Correlative data examining the relationship between behavioral data and immuno-EM data was analyzed
using linear regression. Data from STDP LTP electrophysiology experiments was analyzed by two-way repeated-measures ANOVA, and post hoc analysis was done using Bonferroni’s test. Data from CPP experiments was also analyzed by two-way repeated-measures ANOVA with genotype as the between-subjects factor and time as the within-subject factor. For comparisons between genotypes and within days, Bonferroni’s test was used. For comparisons to baseline within genotypes, Dunnett’s test was used. Correlative data examining the relationship between pre- and postsynaptic cadherin localization and CPP was analyzed using linear regression. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications. Results were considered significant when \( P < 0.05 \). Analysis was performed using GraphPad Prism (GraphPad Software, San Diego, CA). A Supplementary Methods Checklist is available.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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