Interneuron precursor transplants in adult hippocampus reverse psychosis-relevant features in a mouse model of hippocampal disinhibition

Ahmed I. Giliani\textsuperscript{a,b,1}, Muhammad O. Chohan\textsuperscript{a,c}, Melis Inan\textsuperscript{d,e}, Scott A. Schobel\textsuperscript{a,c,2}, Nashid H. Chaudhury\textsuperscript{a}, Samuel Paskewitz\textsuperscript{a}, Nao Chuhma\textsuperscript{a,c}, Sara Glickstein\textsuperscript{a}, Robert J. Merker\textsuperscript{a}, Qing Xu\textsuperscript{d}, Scott A. Small\textsuperscript{f}, Stewart A. Anderson\textsuperscript{d,3,4}, Margaret Elizabeth Ross\textsuperscript{e,4}, and Holly Moore\textsuperscript{a,c,4}

\textsuperscript{1}Present address: Children’s Hospital of Philadelphia, Philadelphia, PA 19104.
\textsuperscript{2}Present address: F. Hoffman-La Roche, Ltd., CH-4303 Kaiseraugst, Switzerland.
\textsuperscript{3}Present address: Department of Pathology, State University of New York Downstate Medical Center, Brooklyn, NY 11203.
\textsuperscript{4}To whom correspondence may be addressed. E-mail: hm2035@columbia.edu, or merr2005@med.cornell.edu.

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GABAergic interneuron hypofunction is hypothesized to underlie hippocampal dysfunction in schizophrenia. Here, we use the cyclin D2 knockout (Ccnd2\textsuperscript{−/−}) mouse model to test potential links between hippocampal interneuron deficits and psychosis-relevant neurobehavioral phenotypes. Ccnd2\textsuperscript{−/−} mice show cortical PV\textsuperscript{+} interneuron reductions, prominently in hippocampus, associated with deficits in synaptic inhibition, increased in vivo spike activity of projection neurons, and increased in vivo basal metabolic activity (assessed with fMRI) in hippocampus. Ccnd2\textsuperscript{−/−} mice show several neurophysiological and behavioral phenotypes that would be predicted to be produced by hippocampal disinhibition, including increased ventral tegmental area dopamine neuron population activity, behavioral hyperresponsiveness to amphetamine, and impairments in hippocampus-dependent cognition. Remarkably, transplantation of cells from the embryonic medial ganglionic eminence (the major origin of cerebral cortical interneurons) into the adult Ccnd2\textsuperscript{−/−} caudoventral hippocampus reverses these psychosis-relevant phenotypes. Surviving neurons from these transplants are 97% GABAergic and widely distributed within the hippocampus. Up to 6 mo after the transplants, in vivo hippocampal metabolic activity is lowered, context-dependent learning and memory is improved, and dopamine neuron activity and the behavioral response to amphetamine are normalized. These findings establish functional links between hippocampal GABA interneuron deficits and psychosis-relevant dopaminergic and cognitive phenotypes, and support a rationale for targeting limbic cortical interneuron function in the prevention and treatment of schizophrenia.

Precursors of most γ-aminobutyric acid (GABA)-releasing interneurons of the cerebral cortex and the hippocampus originate in the embryonic medial ganglionic eminence (MGE) (1–3). A subpopulation of MGE-derived cells differentiates into fast-spiking, parvalbumin-expressing (PV\textsuperscript{+}) interneurons that tightly regulate the activity and synchronization of cortical projection neurons (2, 4). Structural and functional deficits in PV\textsuperscript{+} interneurons are hypothesized as a pathophysiological mechanism in schizophrenia and psychotic disorders (4–6).

Although psychotic disorders are clearly heterogeneous in etiology, disinhibition within temporolimbic cortical circuits is postulated as a core pathophysiology underlying positive symptoms (e.g., delusions and hallucinations) and a subset of cognitive disturbances that manifest with psychosis (4, 5, 7). Postmortem studies of brains from individuals with psychotic disorders show reduced molecular markers of the number and/or function of PV\textsuperscript{+} interneurons in the hippocampus (6, 8). Consistent with these observations, basal metabolic activity in the hippocampus, as measured with functional magnetic resonance imaging (fMRI), is increased in schizophrenia, a phenotype that predicts psychosis and positive symptom severity (5, 7). This abnormal resting activity is postulated to underlie abnormal recruitment of hippocampal circuits during cognitive performance (5, 9). Striatal dopamine (DA) release capacity is also increased and correlated with positive symptoms in schizophrenia and its risk states (10, 11). Importantly, hippocampal hyperactivity may contribute to DA dysregulation (12), because rodent studies show that caudoventral hippocampal (in the primate, anterior hippocampal) efferents regulate the activity of DA neurons and medial striatal DA release (13, 14).

Thus, converging evidence implicates hippocampal disinhibition in the abnormal striatal DA transmission and cognitive impairment in schizophrenia. However, the role of hippocampal inhibitory interneurons in psychosis-relevant circuitry remains to be fully elucidated.

Significance

Hippocampal hyperactivity predicts psychosis and may disrupt aspects of cognition in schizophrenia. Here, we use interneuron precursor transplants in mice lacking cyclin D2 (Ccnd2) to test links between hippocampal GABAergic interneurons and psychosis-relevant phenotypes. Ccnd2-null mice show parvalbumin interneuron deficits and increased in vivo hippocampal excitatory neuron spiking and metabolic activity. This hippocampal disinhibition is associated with cognitive deficits and excess dopamine activity. Transplanting interneuron progenitors derived from the embryonic medial ganglionic eminence into adult hippocampus mitigates these abnormalities. This study thus provides a paradigm for elucidating mechanisms by which limbic cortical interneuron hypofunction may contribute to cognitive deficits and dopamine dysregulation in psychosis. The sustained efficacy of the transplants supports a rationale for targeting hippocampal GABAergic interneurons with novel therapies for psychosis.


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to be established. To this end, we used the cyclin D2 (Ccnd2) knockout mouse model (15), which displays a relatively selective deficit in cortical PV+ interneurons, and transplantation of interneuron precursors from the MGE to elucidate relationships between reduced hippocampal GABA interneuron function and multiple psychosis-relevant phenotypes, and to explore a novel treatment strategy for psychosis.

Results

PV+ Cell Density and Functional Deficits in Ccnd2−/− Hippocampus. CCND2 is a G1-phase active cell cycle protein expressed within the subventricular zone of the MGE. A loss-of-function mutation of Ccnd2 leads to reduced proliferation in the MGE and lower PV+ interneuron density in the cortex, while not affecting densities of glutamatergic projection neurons or other interneuron subtypes of the cerebral cortex (15, 16). To determine the impact of the PV+ neuron deficit on hippocampal output, we compared PV+ interneuron density, GABA-mediated synaptic inhibition, and in vivo activity of presumptive projection neurons in the hippocampus of Ccnd2−/− mice and their wild-type (Ccnd2+/+) littermates. Stereology-based quantification showed a significant reduction in PV+ interneuron density in hippocampus, particularly in CA1 (Fig. 1A and B and Fig. S1). The apparent PV+ neuron deficit in these Ccnd2−/− mice showed regional heterogeneity, with no significant differences between genotypes in the medial prefrontal cortex (mPFC) (Fig. S1). In parallel, GABA-mediated miniature inhibitory postsynaptic currents (mIPSC) frequencies at projection neurons were decreased in the Ccnd2−/− hippocampus (Fig. 1C and D) but not in mPFC (Fig. S1B). Indices of postsynaptic GABA function (e.g., mIPSC amplitude and kinetics) and excitatory synaptic transmission appeared unchanged in Ccnd2−/− mice (Fig. S2). Consistent with the loss of inhibitory input observed in vitro, a reduction in the density of spontaneously active presumptive inhibitory interneurons was observed in Ccnd2−/− mice in vivo, a deficit corresponding with increased in vivo spike activity of excitatory projection neurons (Fig. 1E–G and Fig. S3). Overall, these data suggest regional heterogeneity in the deficits in PV+ interneuron number and function in the Ccnd2−/− cortex, with a relatively greater deficit in hippocampus leading to disinhibition of hippocampal outputs in vivo.

fMRI Shows Increased in Vivo Basal Metabolic Activity in Ccnd2−/− Hippocampus. Whether reduced GABA-mediated inhibition increases hippocampal metabolic activity in vivo was assessed by using basal cerebral blood volume (CBV) maps of the mouse brain from gadodiamide-contrast fMRI (Fig. 1H and Fig. S4A). This method is used to characterize hippocampal hypermetabolism as a biomarker for psychosis (7). Relative to Ccnd2+/+ littermates, Ccnd2−/− mice showed increased basal CBV in the hippocampus (Fig. 1F). Basal CBV in the cerebellum was not different (Fig. S4B), despite an apparent deficit in cerebellar stellate neurons in the Ccnd2−/− mouse (17). We thus used the cerebellum as a reference region for analyses of additional brain regions and hippocampal subregions. Consistent with the regional variation in the cortical PV+ interneuron deficit, Ccnd2−/− mice showed increased basal CBV in the hippocampus but not in the mPFC or sensorimotor cortex (Fig. S4). Thus, whereas loss of Ccnd2 function has multiple developmental effects, decreases in PV+ interneuron function and increased hippocampal activity comprise a prominent phenotype cluster associated with this mutation.

Impaired Hippocampus-Mediated Cognition in Ccnd2−/− Mice. The increased basal or resting activity in hippocampus in schizophrenia patients may compromise recruitment of this region in response to cognitive demands (5). Thus, we evaluated contextual fear conditioning (18), a cognitive process that recruits and depends on the hippocampus in rodents. Conditioning took place in one distinct context (training context) by using five pairings of tones (conditioned stimulus, CS+) and shock (unconditioned stimulus, US+). Task parameters, including the use of highly contrasting contexts, were adjusted to produce robust context-dependent conditioned behavior in the wild-type mice (Fig. S5) and minimize contribution of the adult neurogenesis deficit in the dentate gyrus of Ccnd2−/− mice (19, 20). Twenty-four hours later, we measured freezing to the tone CS+ (without shock), an amygdala-dependent, largely hippocampus-independent response (18). During subsequent CS+ presentations in the novel context, we monitored posttone freezing, a behavior that is sensitive to hippocampal lesions (21). Six hours after the tone CS+ test, we tested
memory for the training context. Ccnd2+/− mice showed robust and selective deficits in the hippocampus-dependent components of learning and memory in this task (Fig. 2). This impairment was not associated with qualitative changes in sensory or motor functions or shock sensitivity (Table S1).

**Increases in Mesolimbic DA Neuron Population Activity and Responsiveness to Amphetamine in Ccnd2+/− Mice.** Projections from the ventral hippocampus to the basal ganglia regulate ventral tegmental area (VTA) DA neuron activity and responses to amphetamine (AMPH). Ventral hippocampal activation increases DA neuron population activity via disinhibitory circuits involving GABA neurons in the nucleus accumbens, ventral pallidum, and VTA (14, 22). We thus predicted that the hippocampal disinhibition in Ccnd2+/− mice would lead to increased VTA DA neuron activity. In vivo recordings within the VTA of anesthetized mice (Fig. 3 A and Fig. S6 A and B) revealed increases in spontaneously active DA neurons in Ccnd2+/− mice (Fig. 3 B).

By modulating activity within the limbic basal ganglia, ventral hippocampal activity can drive medial striatal DA release (13, 14) and behaviors mediated by this system, including the locomotor response to AMPH (23, 24). Consistent with this function of the hippocampus, Ccnd2−/− mice showed a dose-dependent increase in AMPH-induced locomotion (Fig. 3 C and D and Fig. S6 C), a difference blocked by systemic administration of a DA D2 receptor antagonist (Fig. S6 D). Importantly, this augmented response in the Ccnd2−/− mice was eliminated by partial lesions of the caudal (including caudoventral) hippocampus, but not by lesions of the overlying parietal cortex (Fig. S6 E). Together these data indicate that the augmented response to AMPH in the Ccnd2−/− mice is DA mediated and requires hippocampal activity.

**Normalizing Effects of MGE-Derived Interneuronal Precursors Transplanted into Adult Hippocampus.** The data above support the hypothesis that decreased PV+ interneuron function in the hippocampus contributes to the augmented DA system activity and cognitive impairment in Ccnd2−/− mice. However, because the Ccnd2 mutation exerts its effects early in development, one must manipulate hippocampal GABA interneuron population in adulthood to test this hypothesis. Previous studies have shown that MGE-derived cells transplanted into the postnatal cortex differentiate into GABA+ neurons with interneuron-like morphology. These new GABA neurons enhance synaptic inhibition of the surrounding neurons and attenuate seizures and related memory deficits (3, 25). We thus quantified the distribution and phenotypes of neurons derived from embryonic MGE cells transplanted into the hippocampus of adult Ccnd2−/− mice and examined their impact on hippocampal metabolic activity, hippocampus-mediated cognition, and VTA DA neuron activity. Cells were dissociated from the ventral MGE of green fluorescent protein (GFP)-expressing mouse embryos at embryonic day 15.5 (Fig. 4 A and B) and transplanted bilaterally into the ventral hippocampus of 6- to 8-week-old Ccnd2−/− mice. Control Ccnd2−/− mice received ventral hippocampal injections of killed (freeze-thawed) MGE cells.

At 4–6 mo after transplantation, brains with active (live-cell) MGE transplants contained an average of 3,706 ±573 (SEM); n = 4) surviving (GFP+) cells. These cells showed features of mature interneurons (Fig. 4 B–D) and were dispersed throughout the longitudinal axis of the hippocampus. Nearly all of the GFP+ cells in the hippocampus were GABAergic (97%), with ~56% and 35% expressing PV and somatostatin, respectively (Fig. 4 E). The new neurons were seen primarily in strata oriens and pyramidale of CA fields. Remarkably, MGE transplants normalized multiple aspects of the Ccnd2−/− phenotype. Ccnd2−/− mice with MGE transplants showed reduced hippocampal CBV, to levels similar to those observed in wild-type littermates (Fig. 5 A). Ccnd2−/− mice with active MGE transplants also showed improved hippocampus-dependent cognition (Fig. 5 B and C), and normalization of DA neuron population activity (Fig. 5 D) and the response to AMPH (Fig. 5 E and F).

Previous studies reported a neurogenesis deficit in the adult dentate gyros of Ccnd2−/− mice (26, 27). We thus examined the effects of the transplanted interneurons on newly born neurons in the adult Ccnd2−/− dentate by quantifying cells expressing double-cortin (DCX+), a marker of immature neurons. Relative to wild-type colonymates, both control- and active MGE-transplanted Ccnd2−/− mice showed a similarly marked reduction in DCX+ dentate gyrus neurons (Fig. 5 F). Thus, the effects of MGE-derived cells transplanted into the caudoventral hippocampus are not likely mediated by a change in adult hippocampal neurogenesis.

**Discussion.** The PV+ interneuron deficit in Ccnd2−/− mice is associated with adult neurobehavioral phenotypes relevant to psychosis, including increased hippocampal basal metabolic activity as assessed with fMRI, increased midbrain DA neuron activity,
GABAergic interneurons are important for a subset of the behavioral and neurophysiological effects of the Ccnd2 mutation that is particularly relevant to psychosis and point to the potential importance of mechanisms regulating cortical interneuron development in the pathogenesis of schizophrenia (6).

Although the Ccnd2<sup>−/−</sup> mutation has quite selective effects on brain structure and function, the potential contributions of neurophenotypes other than the hippocampal PV<sup>+</sup> interneuron deficit must be considered. Perhaps most relevant to the cognitive domain tested in this study is the neurogenesis deficit in the adult Ccnd2<sup>−/−</sup> dentate gyrus (26, 27) (Fig. S7). The significance of “adult neurogenesis” to behavioral health remains under intense investigation (20, 26, 31), and we do not rule out that this deficit may impact cognition in Ccnd2<sup>−/−</sup> mice (e.g., ref. 32). However, the present study indicates that adding new MGE-derived GABA interneurons to hippocampal CA fields of adult Ccnd2<sup>−/−</sup> mice can improve cognition without stimulating neurogenesis. Ccnd2<sup>−/−</sup> mice also show reduced cerebral cortical volume (16) and a deficit in cerebellar stellate neurons (17).

Although we expect these abnormalities to have effects on cognition and behavior, we and others have observed no evidence for qualitative sensory or motor (including cerebellar) phenotypes that would confound psychomotor or cognitive assessments used in this study. Notably, in schizophrenia, and in several rodent models of genetic susceptibility, hippocampal interneuron deficits occur in the context of cerebral cortical thinning, cerebellar pathology, and cell metabolic abnormalities (6, 28, 29, 33–35).
We thus consider the effects of the hippocampal-targeted transplants demonstrated in the Ccnd2−/− model to be of particular relevance to schizophrenia in part because they occur on a background of other schizophrenia-relevant neuropathologies (see also ref. 36).

Increased resting metabolic activity in the hippocampus and adjacent medial temporal cortex is characteristic of schizophrenia and its risk states (7). Interneuron dysfunction has been hypothesized to be both a potential driver and consequence of this abnormal state, in part through dysregulation of glutamatergic signaling (4, 5, 7, 37). Our findings are consistent with a deficit in PV+ interneurons driving abnormal hippocampal activity, and highlight a need for additional studies on mechanisms linking interneuron function to local cortical metabolism and hemodynamics. Our in vitro and in vivo electrophysiological data indicate that a disinhibition of hippocampal projection neurons may contribute to the MRI hypermetabolism phenotype in the Ccnd2−/− mouse. Although the potential effects of the Ccnd2 mutation on basal ganglia interneurons should also be studied, our current data argue for a model in which a deficit in the number or function in MGE-derived GABAergic interneurons in the hippocampus dysregulates hippocampal projections to limbic structures and the basal ganglia, leading to multiple phenotypes associated with schizophrenia including increased basal or resting hippocampal activity, increased striatal DA neurotransmission, and related psychomotor and cognitive disturbances (5–7, 9, 12) (Fig. S8).

In the Ccnd2−/− mouse model, psychosis-relevant phenotypes derived in part from a developmental PV+ interneuron deficit can be reversed in adulthood with transplantation of MGE-derived cells into the hippocampus. We hypothesize that the remarkable, sustained impact of the transplants observed here is related to the source and developmental stage of the transplanted cells: the ventral MGE at mouse embryonic day 15.5. Our findings extend previous studies showing that transplanted GABAergic interneurons in the hippocampus will lead to discovery of molecular and physiological changes induced by these transtherapeutics in schizophrenia (3, 6). Indeed, determining the GABA interneuron precursor-rich transplants in experimental connectivity and induce plasticity in the host cortex, and have a remarkable, sustained impact of the transplants observed here (Fig. S8).

In vivo fMRI. Design and procedures of imaging experiments were based on methods developed by Small and colleagues (cf. ref. 7). Briefly, four sets of axial T2-weighted images were acquired sequentially to generate 86 × 86 μm CBV maps, each set consisting of 24 images of 16 min each. Gadodiamide was injected (13 mmol/kg i.p.) after a precontrast set was acquired. CBV was mapped as the change in the transverse relaxation rate (R2) in duced by the contrast agent. CBV maps were measured from steady-state T2-weighted images as CBV R2 = ln(Spre/Spost)/TE, where TE is the effective echo time, Spre is the signal before the contrast administration, and Spost is the signal after the contrast agent reaches steady state. The derived maps were normalized to the maximum four-pixel signal value of the posterior cerebral artery. Standard atlases were used to identify anatomical landmarks and define ROIs (SI Materials and Methods). The hippocampal ROI included the CA fields, subiculum, and dentate gyrus. For additional genotype comparisons, CBV across brain regions and of active versus control MGE transplants, CBV in the target region was expressed as a ratio to basal c erbellar CBV (Fig. S4).

Materials and Methods. Animals and Treatments. All experiments were approved by the New York State Psychiatric Institute Animal Care and Use Committee in accordance with standards set by the National Institutes of Health Office of Laboratory Animal Welfare. Ccnd2 knockout mice (cf. ref. 15) were maintained on a C57BL/6J background; sex- and age-matched wild-type littermates (or colony-mates) were used as controls.

Immunohistochemistry and Neuron Quantification. Histological and cell quantification protocols are fully described in SI Materials and Methods. Briefly, paraformaldehyde-fixed brains were cryosectioned into 40-μm sections and collected serially into five equal sets according to the principles of systematic random sampling. Sets were processed with primary antibodies for parvalbumin (anti-mouse α-VP-235), somatostatin (anti-rat polyclonal; Chemicon/Millipore), GABA (anti-rabbit Sigma-Aldrich), doublecortin (anti-goat; Santa Cruz Biotechnology), and related psychomotor and cognitive disturbances (5–7, 9, 12) (Fig. S8).

In Vivo Slice Electrophysiology.Brains extracted from ketamine/xylazine-anesthetized Ccnd2−/− and sex-matched Ccnd2+/+ littermates, ages 3–6 wk, were processed for slice electrophysiology by using a standard protocol detailed in SI Materials and Methods. Whole-cell voltage-clamp recordings of identified pyramidal neurons used glass capillary pipettes (3-5 MΩ) filled with a buffered CsCl solution to record GABA currents or a buffered potassium gluconate to record excitatory currents. GABAergic interneuronal and GABAergic interneuron precursor-rich transplants in experimental connectivity and induce plasticity in the host cortex, and have a remarkable, sustained impact of the transplants observed here (Fig. S8).

In Vitro Hippocampal and VTA DA Neuron Recordings. Stereotoxic surgery and single-unit extracellular recording and neuron sampling methods were performed in chloral hydrate– or urethane-anesthetized mice by adapting published methods (14) as described in detail in SI Materials and Methods. Briefly, glass electrodes (4–10 MΩ) filled with 2 M NaCl were used to sample four tracks spaced 150 μm apart within the VTA (Fig. S4A and S5E) or six tracks at 200 μm apart in the caudal hippocampus (Fig. 1E). VTA DA neurons and hippocampal pyramidal and nonpyramidal neurons and the spontaneous activity thereof were quantified as described in SI Materials and Methods (Figs. S3 and S6).

Behavioral Experiments. Ccnd2−/− mice and sex-matched Ccnd2+/+ littermates 2.5–4 mo of age were used for behavioral testing. Mice were habituated to handling but were otherwise behaviorally naive for each experiment except for some transplanted mice, for which contextual fear conditioning followed locomotor testing by a few days. See SI Materials and Methods for full details. Spontaneous and amphetamine-induced locomotor activity was measured in 17 × 17-in open field boxes under standard lighting conditions. Mice were placed in open field for 30 min, after which, amphetamine (2 mg/kg dissolved in isotonic saline at 0.2 mg/mL) or saline was injected i.p. Activity (distance traveled) was measured for another 60 min. A mixed ANOVA design...
with genotype and drug as factors, and time (before or after injection) as the repeated measure, was used. This analysis was followed with planned Student t tests comparing within condition separately for baseline and postinjection locomotion. Contextual fear conditioning methods were adapted from previous studies (e.g., refs. 19 and 21 as detailed in SI Materials and Methods). Briefly, mice were acclimated to the testing room 1 h before training. The training/testing apparatus was a chamber with shock grid floors placed within a sound-attenuating chamber. The inner chamber featured a distinctive combination of visuospatial, tactile, and olfactory cues, which together defined the context. On the day of training, mice were placed in one context (“training context”) and the CS+ consisting of a tone (85 dB, 20 s duration, 4.5 kHz) was presented at 300, 470, 580, 670, and 840 s. During the last second of each tone, a 0.7-mA scrambled current was delivered through the floor grid (US). Mice were removed from the training context 140 s following the last CS-US presentation. Twenty-four hours later, mice were placed in a novel context and the tone CS+ was presented without shock at 300, 410, 580, 670, and 830 s. Six hours after the tone CS+ retrieval test, mice were placed in the training context for 600 s. Conditioned freezing, defined as absence of movement except for respiration, was quantified for the following epochs: (i) during the first presentation of the tone-CS+, (ii) for the 40–100 s following the offset of CS+ presentations 2–5 (posttone freezing: averaged for all five tones), and (iii) in the post CS+ data were analyzed with a mixed ANOVA with retrieval phase as the repeated measure and genotype as the between-subjects factor. Independent t tests were used for planned comparisons between genotypes.

Transplantation of Progenitor Cells from the MGE. Methods were adapted from those of Tyson and Anderson (3) as detailed in SI Materials and Methods.


Supporting Information

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SI Materials and Methods

Animals and Treatments. All experimental studies were approved by the New York State Psychiatric Institute Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals (1) and the regulations of the National Institutes of Health Office of Laboratory Animal Welfare. The cyclin D2 (Cnd2) knockout was produced through excision of exons I and II as described (2). Through heterozygote-heterozygote (het-het) breedings, the line was backcrossed onto a C57/BL/6 background and maintained with quarterly introduction of new C57/BL/6 wild-type breeders to the colony. Wild-type (Cnd2+/+) and homozygous null (Cnd2−/−) sex-matched siblings or colonymates obtained from het-het breedings were used for all experiments. Mice were housed under standard conditions in groups of three to five.

Tissue Processing and Immunohistochemistry. Mice were perfused with PBS, followed by phosphate-buffered 4% (wt/vol) paraformaldehyde (PFA). The brains were removed and postfixed in PFA for 1 h then cryoprotected by successive transfers to 10, 20, and 30% sucrose (in 0.1 M phosphate buffer) over 3 d at 4 °C. The brain was sectioned in its entirety according to the principles of systematic random sampling (3). Briefly, sectioning was initiated at a random starting point, 40-μm-thick sections were then collected serially into five equivalent sets (each with intersection thickness of 200 μm). Sets were stained as described below.

Primary antibodies included anti-mouse PV (Swant; 1:1,000), anti-rat somatostatin (Chemicon/Millipore; 1:200), anti-rabbit GABA (Sigma-Aldrich; 1:1,000), anti-rabbit or anti-chicken GFP (Molecular Probes/Invitrogen; 1:1,000), and anti-goat doublecortin (DCX), (1:500), Santa Cruz Biotechnology; SC 8,066). Staining was performed by using standard immunohistological staining protocols. Sections were washed in 0.1 M PBS and quenched in 0.3% H2O2 in 0.1 M PBS/CH3OH (1:1) for 15 min at room temperature, then washed and blocked in 10% normal donkey, 0.1% bovine, or 1–2% normal goat serum in 0.1 M PBS with 0.5% Triton X-100 for 30 min at room temperature. Sections were then incubated for 24–48 h at 4 °C (most primary antibodies) or slightly heated above room temperature for 24 h for DCX. Following rinsing with 0.1% M PBS, sections were incubated with a biotinylated secondary antibody. For most experiments, fluorescent secondary antibodies were used (Rhodamine Red, DyLight 488, DyLight 405, and DyLight 647; Jackson ImmunoResearch, diluted to 1:250 or 1:200). For a subset of brains, fluorescent secondary antibodies were used (Rhodamine Red, DyLight 488, DyLight 405, and DyLight 647; Jackson ImmunoResearch, diluted to 1:250 or 1:200). For a subset of brains stained for PV, a goat anti-mouse IgG (1:200 in PB containing 0.1% BSA and 0.25% Triton X-100) was used and revealed with a standard avidin-biotin-peroxidase reaction method (Vectastain Elite Kit; 1:100 in 0.1 M phosphate buffer; Vector Laboratories).

Quantification of Labeled Neurons. PV+ interneuron number and density were obtained by 2D modified unbiased sampling methods (3), using a Zeiss Axioslager M2 microscope with Apotome (Carl Zeiss) integrated components for semiautomated quantitative neuroanatomy (integrated system distributed by Microbrightfield Biosciences), including an X-Y motorized stage (Ludl Electronics) and high-resolution digital video monochrome (Hamamatsu) and color (Microbrightfield Biosciences) cameras optimized for epifluorescence. These components were interfaced with a PC workstation running Stereoinvestigator software (MicroBrightfield Biosciences) for quantitative neuroanatomy. Interneurons were counted in the hippocampus (HIPP), somatosensory/motor (SS-M), and prefrontal cortices (mPFC) by using boundaries as defined by Paxinos and Franklin (4). Cells were counted in every fifth stained section (one 40-μm section per 200 μm) (i) throughout the rostrocaudal extent of the HIPP starting at (relative to Bregma and dural surface) anterior-posterior (AP) −1.06 mm, medio-lateral (ML) 0–1.4 mm, dorso-ventral (DV) 0.6–2.2 mm, and spanning the entire CA regions and curving posteriorly through AP −3.88 mm, ML 2.0–3.0 mm, DV −2.0 to −4.0 mm. (ii) The mPFC ROI spanned anteriorly from the prelimbic and medial orbital cortex, starting at AP +2.96 mm, ML 0.0–0.8 mm, DV 0.0–2.5 mm, and extending posteriorly to include entire regions of cingulate cortex, areas 1 and 2, and infralimbic cortex, and ending at the posterior limit of the later at AP +1.34 mm, ML +0.8 mm, DV −3.2 mm. Medial and lateral borders were defined by the midline and medial border of forceps minor of the corpus callosum (posteriorly). (iii) For the region of interest (ROI) of the SS-M, ventralmedial border was defined by a perpendicular to the pial surface at 1.0 mm dorsal to rhinal fissure and the dorsomedial boundary was set at the lateral border of the cingulate cortex 0.8–1.0 mm lateral from the midline. Cortex within these boundaries was sampled from 2.46 mm anterior to Bregma to 1.94 mm posterior to Bregma, with the ROI staying dorsal to the external capsule. For each animal, cell counts were summed across all sections. Density was calculated by dividing the total cells counted by the average ROI area as determined with the Cavalieri estimator (3) and compared between genotypes by using independent (Student's) t tests.

In Vitro Slice Electrophysiology. Cnd2−/− and sex-matched Cnd2+/+ littersmates, ages 3–6 wk, were anesthetized with ketamine (90 mg/kg)/xylazine (10 mg/kg) mixture. After decapitation, brains were immediately transferred to ice cold solution (220 mM sucrose, 10 mM d-glucose, 2.5 mM KCl, 26 mM NaHCO3, 1.25 mM NaH2PO4, 2 mM CaCl2, and 2 mM MgCl2). Tissue block containing either the hippocampus or the PFC was mounted on a vibratome (Leica VT1200, Leica Microsystems), and 400-μm-thick horizontal (hippocampus) or coronal (PFC) slices were made. After 1 h preincubation at room temperature (24 °C) in oxygenated artificial cerebrospinal fluid containing (aCSF; 124 mM NaCl, 3 mM KCl, 26 mM NaHCO3, 1.25 mM NaH2PO4, 1 mM CaCl2, 1 mM MgSO4, and 10 mM d-glucose), each slice was transferred to the recording chamber, perfused with oxygenated ACSF at 2–3 mL/min with a gravity-fed system. Pyramidal cells in the PFC or the CA1 region of hippocampus were identified with Nomarski optics on an upright fixed-stage microscope (Zeiss Axioskop FS). Whole-cell voltage-clamp recordings were performed with an Axopatch 200B amplifier (Molecular Devices). Borosilicate glass capillary pipettes (1.2 mm outer diameter) with resistance of 3–5 MΩ were filled with CsCl solution (140 mM CsCl, 2 mM MgCl2, 0.1 mM CaCl2, 10 mM Hepes, and 1 mM EGTA at pH 7.3 adjusted with NaOH) to record GABA(A) events. For measurement of glutamate events, CsCl was replaced with 140 mM K+-glucuronate. The series resistance was measured by applying voltage steps of 5 mV at the beginning and the end of each recording session and was typically between 20 and 40 MΩ. The series resistance was compensated offline to avoid adding excessive baseline noise during recording.

To isolate GABAergic miniature inhibitory postsynaptic currents (mIPSCs), tetrodotoxin (TTX; 1 μM), 2-amino-5-phosphono-pentanoic acid (AP5; 50 μM), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 40 μM) were added to perfusion solution. GABA(A) mIPSCs were recorded using whole-cell current-clamp mode in cesium substitution.
were recorded at −70 mV. Because reversal potential of Cl− with CsCl solution was approximately 0 mV, GABA currents were recorded as inward. To isolate glutamatergic miniature excitatory postsynaptic currents (mEPSCs), TTX and gabazine were added to the perfusion solution, and glutamatergic nature was confirmed with application of CNQX and AP5. Glutamatergic mEPSCs were recorded at −80 mV. Liquid junction potential (~12 mV for K+-gluconate solution) was adjusted online.

Data were filtered at 5 kHz and digitized at 10 kHz. Analysis was done in MiniAnalysis software (Synaptosoft) by using a low-pass elliptic filter (1 kHz cutoff) to remove high frequency noise. Amplitude threshold for mPSC detection was set to three times the square root of mean of noise. Events were verified by visual inspection. Averages of mPSC frequency, amplitude, 10–90% rise time, and 10–90% decay time were calculated from at least 100 s recording for each cell, then averaged across cells for each subject. Statistical significance was tested with independent (Student’s) t tests. Multivariate analyses including frequency, amplitude, and decay times as variables yielded results consistent with the t tests.

In Vivo Functional Magnetic Resonance Imaging. Design and procedures of imaging experiments were based on methods developed by Small and coworkers (5, 6). Briefly, four sets of axial T2-weighted images were acquired sequentially to generate high-resolution (86 μm × 86 μm) cerebral blood volume (CBV) maps of the rodent brain. Each set consisted of 24 images, acquired over 16 min. The contrast agent gadodiamide was injected (15 mmol/kg i.p.) after a precontrast set was acquired. CBV was mapped as changes in the transverse relaxation rate (R2) induced by the contrast agent. CBV maps were measured from steady-state T2-weighted images as CBV R2 = ln(Spre/Spost)/TE, where TE is the effective echo time, Spre is the signal before the contrast administration, and Spost is the signal after the contrast agent reaches steady state. The derived maps were normalized to the maximum four-pixel signal value of the posterior cerebral artery. Visualized anatomical landmarks were used together with standard atlases (4) to define the ROI. The hippocampal region of interest included the CA fields, subiculum, and dentate gyrus with the ventral border approximated to be the rostrocaudal border of the medial entorhinal cortex. The borders of the PFC ROI extended laterally and posteriorly from the genu of the corpus callosum to the pial surface at approximately 1.5 mm lateral to the midline; slices at and dorsal to the caudate were used. For the cerebellum, the entire structure was included. For comparisons between live- versus killed-cell MGE transplanted mice, hippocampal CBV was expressed as a ratio to cerebellar basal CBV to control for nonspecific effects of the transplants. Independent (Student’s) t tests were used to compare each CBV measure across genotypes.

In Vivo Single-Unit Recordings of Dopamine and Hippocampal Neurons. Stereotaxic surgery and single-unit extracellular recording and neuron sampling methods were adapted from those described (7). For ventral tegmental area (VTA) dopamine (DA) neuron recordings, mice were anesthetized with chloral hydrate. Electrodes pulled from glass pipettes (tip diameter ~1 μm; impedance 4–10 kΩ) were filled with 2 M NaCl and stereotaxically lowered into the VTA. The VTA was systematically sampled by recording from four tracks spaced 0.15 mm apart with starting coordinates of 3.3 mm posterior, ±0.8 mm lateral and 3.6–4.2 mm ventral to Bregma at the dorsal surface. The electrode was lowered slowly through each track to detect and characterize spontaneously active neurons. The signals from individual neurons were processed as described (7). A coronal section from a representative case, showing histological evidence of several tracks at ~3.5 mm posterior to Bregma, is shown in Fig. S6A. DA neurons were identified by the long-duration, triphasic waveform with the “somatodendritic notch” on the positive phase and by their tonic irregular firing and intermittent bursts (Fig. S6B). Bursts were defined as per the criteria of Grace and Bunney (8) (see also ref. 9). The onset of bursts were marked by an interspike interval (ISI) less than 80 ms; the first spike thereafter preceded by an ISI of greater than 160 ms was considered the last spike in the burst. Within bursts on average, spike amplitudes decreased as ISI lengthened (Fig. S6D). DA neurons were defined as spontaneously active if spiking was detected before the electrode moving proximal to the neuron and if the firing rate of the neuron was stable for at least 2 min after discrimination. The number of spontaneously active DA neurons per track and the average firing rate and proportion of spikes fired within bursts for each DA neuron were quantified.

For hippocampal neurons, recordings were conducted in a separate cohort of mice anesthetized with urethane. Recording methods similar to those described above were used. The hippocampus was sampled with a grid of six tracks bounded by the following coordinates: 3.0–3.2 mm posterior; ±2.9–3.3 medial, and 1.5–5.0 ventral to Bregma at the dural surface. Spontaneously active neurons were detected and, following a stable recording of at least 2 min, each neuron was classified as presumptive pyramidal projection neurons or presumptive nonpyramidal neurons based on spike waveform duration and firing rate. Spike waveforms could be sorted into two distinct sets, one triphasic with spike waveform widths ≥2 ms and the other bi- or monophasic with spike waveform durations ≤1.2 ms. Units with waveform widths of ≥2 ms and firing rates between 0.1 and 5 Hz were classified as presumptive pyramidal neurons. The firing rates of neurons in the latter set ranged from 1.5 to 54 Hz; thus short (<1.2 ms) waveform units with firing rates >1.5 Hz were classified as nonpyramidal cells, presumptive interneurons. Although there is some overlap between pyramidal and nonpyramidal populations in these spike characteristics, these selection criteria reliably segregate pyramidal neurons from interneurons in hippocampus (10–12). Units with unstable or “borderline” waveform characteristics were not included to minimize misclassification. Electrode track locations were confirmed with histological methods as described for DA neuron recordings.

The number of spontaneously active DA or hippocampal pyramidal and nonpyramidal (presumed GABAergic interneurons) neurons per track, and firing rates and burst firing frequencies were compared between Ccnd2+/− mice and their Ccnd2−/− littermates by using independent t tests.

Behavioral Experiments. Ccnd2+/− mice and sex-matched Ccnd2−/− littermates 2.5–4 mo of age were used for behavioral testing. Mice were habituated to handling but were otherwise behaviorally naïve for each experiment except for MGE transplant mice, for which contextual fear conditioning followed a few days after locomotor response.

Contextual fear conditioning. The experimental design was adapted from methods developed by Fanselow and coworkers (13–16); context components and CS+ and US− intensities were based on Saxe et al. (17). Mice were acclimated to the testing room 1 h before the training/testing session. Two transparent plastic chambers with shock grid floors placed within a white melanine sound-attenuating chamber were used as training/testing apparatus. Each chamber featured a distinctive set of visuospatial, tactile, and odor cues, which together defined the context. For the training session, mice were placed in a distinct context (training context). Conditioned stimulus (CS+) consisting of pure tones (85 dB, 20 s duration, 4.5 kHz) were presented at 300, 470, 580, 670, and 840 s. During the last second of each tone, a 0.7-mA scrambled current was delivered through the floor grid. Animals were returned to their home cages 980 s after the start of the experiment. Twenty-four hours later, each mouse was placed in a novel context that...
was constructed with visual, tactile, and olfactory cues with a high level of contrast with training context. In novel context, mice were exposed to the conditioned tone (CS\textsuperscript{*}) only (without shock) at 300, 410, 580, 670, and 830 s. Six hours after being tested in the novel context, mice were placed in the training context for 290 s. During training and testing sessions, freezing was measured by using an automated video monitoring system (Med Associates) and was inspected visually to assure that the system was identifying freezing in animals that showed no movement except for respiration. Additional parameters for measuring freezing were bout duration of 0.25 s and motion index threshold between 2 and 5. Effects of increased exposure to training context on context learning were examined by systematically varying the duration (75 and 175 s) of context exposure (called placement to shock interval) before the first presentation of the US\textsuperscript{t}. Parameters in current study differ from those of Jaholkowski et al. (18) as follows: longer duration in the training-\textsuperscript{US}\textsuperscript{t} context before delivery of the first CS\textsuperscript{*}-US\textsuperscript{t} presentation (175 vs. 120 s); greater number of CS\textsuperscript{*}-US\textsuperscript{t} trials during training (5 vs. 3); shorter interval between training and the context retrieval test (30 vs. 48 h). These parameters interact with the integrity of the hippocampi to modulate context-dependent fear responding (13–15) and were set to achieve reliable context conditioning in the Ccnd\textsuperscript{2/-} mice.

An average percent time spent exhibiting the conditioned freezing response (the CR) was calculated for three types of conditioned responses: (i) tone-conditioned freezing, the percentage of time spent freezing during the first tone CS\textsuperscript{*} (20 s) in novel context, (ii) posttone freezing, percentage of time spent freezing during the 40–100 s following CS\textsuperscript{*} offset for CS\textsuperscript{*} presentations 2–5 (averaged across tones), and context-conditioned freezing, the percent time freezing in the training context, excluding the first 40 s For statistical analysis, a mixed ANOVA design was used with retrieval phase as the repeated measure and genotype as the between-subjects factor. Independent (Student’s) t tests were used for planned comparisons to test the effect of genotype on each conditioned response type.

**Open field locomotor activity.** Locomotor activity was measured in a 17 × 17 × 17 cm open field box with clear walls and white floor, fitted with computer-interfaced infrared motion sensing system (Med Associates). Mice were placed in open field for 30 min, after which, amphetamine (Study 1: 2 mg/kg dissolved in isotonic saline; Study 2: 2.4, or 8 mg/kg at 0.2–0.4 mg/mL) was injected i.p. and activity was measured for another 60 min. Total activity for successive 5-min bins was analyzed. Two separate cohorts were tested many months apart, and similar results were obtained. A mixed ANOVA design with genotype and drug as factors, and time (before or after injection) as the repeated measure, was used. This analysis was followed with planned Student t test comparisons of genotypes within drug condition separately for baseline and postinjection locomotion.

**Partial lesion of the caudal hippocampus or parietal cortex.** These lesions were induced as part of study to examine the impact on local guide cannula on hippocampal function. Mice were anesthetized with isoflurane anesthesia and placed mounted on a stereotaxic frame. Following aseptic preparation of the skin and skull, small burr holes were drilled through the skull. The coordinates used to target ventrocaudal CA1 in wild-type mice were (relative to Bregma and skull surface) AP = 3.0 mm, L ± 3.5 mm, DV = 2.9 mm. For the Ccnd\textsuperscript{2/-} mice, coordinates were modified to adjust for smaller brain size; the coordinates were AP = 2.9, L ± 3.3, DV = 2.7. For parietal cortex cannulations, the coordinates used were AP = 2.9, L ± 3.5, DV = 1.4 for Ccnd\textsuperscript{2/-} and AP = 2.9, L ± 3.3, DV = 1.25 for Ccnd\textsuperscript{2/-}. To induce partial lesions to cortical regions, custommade steel guide cannula were inserted (outer diameter 0.64 mm; BD Biosciences) and tissue was disrupted through movement and saline infusion through the internal cannula. The guide cannula was then left in place for 1 wk before behavioral testing. Postmortem histological analysis confirmed that this manipulation led to a consistent and lesion of the hippocampus and overlying parietal cortex or, in the control group, a lesion limited to the posterior parietal cortex.

The locomotor response to amphetamine was tested 4–10 d after recovery from surgery with the methods described above. The outcome measure used was average locomotion 10–60 min after amphetamine and was assessed with a 2 (lesion group) × 2 (genotype) ANOVA, with independent t tests used for planned comparisons of genotype within lesion group.

**Dissection, Dissociation, and Transplantation of Progenitor Cells from the MGE.** Methods of dissection, suspension, and transplantation of cells from the MGE were adapted from those described by Anderson and coworkers (19). Transgenic mice expressing green fluorescent protein (GFP), driven by chicken β-actin promoter (20) [FVB.Cg-Tg (CAG-EGFP) B5Nagy/J Stock no. 003516, maintained on a CD1 background], were obtained from Jackson Laboratories. Breeding pairs, a homozygous pan-GFP expressing (GFP\textsuperscript{+}) male and a wild-type female were placed in the mating cages at 1700 hours and separated the next morning. Dams were killed at E15.5 by cervical dislocation. GFP\textsuperscript{+} pups, identified by fluorescence under 488-nm light, were placed in Hanks’ balanced salt solution (Gibco/Invitrogen). The brains were removed and embedded in 4% (wt/vol) low melting point agarose (Invitrogen) in PBS and sliced at 250-μm-thick coronal sections on a vibrating microtome (Thermo Scientific HM650V). MGE was identified visually, and slabs of tissue corresponding to the ventral two-thirds of the MGE (Fig. 4A) were dissociated by using fine forceps. Samples obtained from both hemispheres of two slices from two embryos were combined and considered an individual experiment for statistical purposes. Donor cells were dissociated by trituration, centrifuged at 500 × g for 5 min, and resuspended in 15–30 μL of NB/B27 medium (Gibco/Invitrogen). A density of 30 × 10\textsuperscript{3} live cells per microliter was obtained. For killed cell control transplants, cells (obtained as described above) were killed by repeated freeze-thaw cycles (−80 °C/1 min × 3) immediately before transplantation. Killed-cell or live-cell suspensions were injected into the brain by using a glass pipette with a 50-μm outer tip diameter connected to a nanoinjector (Drummond Scientific). The glass pipette was then connected to the injector mounted on a stereotactic carrier. The suspended cells were transplanted bilaterally into the hippocampi of mice, aged 6–8 wk, with two injections per hemisphere (the second injection occurring 0.5 mm rostral to the first).

Mice were anesthetized with isoflurane anesthesia and placed on a stereotaxic frame. Following aseptic preparation of the skin and skull, small burr holes were drilled bilaterally through the skull. The coordinates used to target ventrocaudal CA1 in wild-type mice were (relative to Bregma and skull surface) AP = 3.0 mm, L ± 3.5 mm, DV = 2.9 mm. For the Ccnd\textsuperscript{2/-} mice, coordinates were modified to adjust for smaller brain size; the coordinates were AP = 2.9, L ± 3.3, DV = 2.7. The glass pipette was lowered slowly into the brain, taking several minutes to reach the target region, then a volume of 3.5 μL of the suspension was ejected over 5 min in 70-nL steps, for a total deposit of 2.0 × 10\textsuperscript{3} cells per hemisphere.

Statistical models are described for each experiment above. For all statistical tests, results are reported as “significant” if P < 0.05 and as “not significant (n.s.)" if P > 0.25; trends are reported individually.


**Fig. S1.** Parvalbumin-expressing (PV+) interneuron density in multiple cortical regions and corresponding data for GABA-mediated synaptic inhibition in mPFC in Ccn2+/+ and Ccn2−/− mice. (A) Ccn2−/− mice show a significant reduction in PV+ interneuron density in the HIPP (t6 = 2.9, P < 0.05) and a trend (t6 = 1.4, P < 0.1) in SS-M. PV+ interneuron density is not significantly reduced in the mPFC in the same animals. See SI Materials and Methods for descriptions of ROI contours. (B) Under conditions (and at a level of statistical power) sufficient to detect the decrease in hippocampal mIPSC frequency (Fig. 1D), mIPSC frequency in mPFC pyramidal cells is not significantly different in Ccn2−/− mice (t14 = 1.05, P > 0.25; n = 8). Although the failure to find a significant decrease in PV+ interneuron density or mIPSC frequency is not sufficient evidence to assume normal GABAergic neurotransmission in the mPFC, these results support the hypothesis that loss of CCND2 may have a relatively greater and more functional impact on PV+ interneurons in the hippocampus than in some other cortical regions. Box plots show interquartile range, with whiskers extending to the outermost data points within 1.5x of the interquartile range. Means are marked by dotted lines running through square markers, medians by solid lines, and outliers as dots outside the whiskers. *P < 0.05 in independent t tests (one-tailed).
Fig. S2. Characteristics of inhibitory and excitatory synaptic currents in hippocampal CA1 pyramidal cells of Ccnd2+/− mice. (A) Mean amplitude and decay time of mIPSCs in CA1 pyramidal neurons do not differ significantly between Ccnd2+/− cells (red bars) and Ccnd2+/+ cells (blue bars; n = 9–10; P > 0.25). (B) Characteristics of mEPSCs in CA1 neurons of Ccnd2+/− and Ccnd2+/+ mice. Left shows representative traces from whole-cell voltage clamp recordings; mEPSCs are inward (downward) current events (see Materials and Methods for recording conditions). Right three images show box plots of summary data for mEPSC frequency, amplitude, and decay time. There were no significant genotypic differences in these parameters of excitatory neurotransmission [n = 6–7, all independent t tests yielded nonsignificant (n.s.) results, P > 0.25]. Box plots show interquartile range, with whiskers extending to the outermost data points within 1.5× of the interquartile range. Means are marked by dotted lines running through square markers, medians by solid lines, and outliers as dots outside the whiskers. *P < 0.05, independent t tests (two-tailed).

Fig. S3. Spike waveform characteristics of single units classified as pyramidal neurons or nonpyramidal (interneurons) recorded extracellularly in vivo. (A) Representative trace showing waveforms of two different units detected by the glass electrode. The leftmost waveform in which the duration of the spike waveform (negative and positive phases) is approximately 1 ms is typical of units classified as nonpyramidal, presumed interneurons. The triphasic, longer-duration waveform at Right is typical of neurons classified as pyramidal (projection) neurons. (B) Detail of spike waveform and spontaneous firing rate of a representative nonpyramidal unit. (C) Detail of spike waveform and spontaneous firing rate of a representative pyramidal unit. The firing rate criteria applied to nonpyramidal versus pyramidal units was >1.5 Hz and <5 Hz, respectively (SI Materials and Methods).
Fig. S4. Basal CBV in various cortical regions and cerebellum in Ccn2<sup>+/+</sup> and Ccn2<sup>-/-</sup> mice. (A) Structural MRI image showing ROIs for mPFC (green outline), somatosensory-motor cortex (SS-Motor) (orange outline) and cerebellum (CBL) (yellow outline) in the horizontal plane at ~2.5 mm ventral to Bregma at the skull surface. See SI Materials and Methods for full description of ROIs. (B) Basal CBV (Normalized ΔR2) for CBL in Ccn2<sup>+/+</sup> and Ccn2<sup>-/-</sup> mice. The difference between genotypes is not significant (n = 7 per genotype; P > 0.25). (C) Basal CBV in the hippocampus (HIPP), SS-Motor, and mPFC expressed as a ratio of simultaneously-measured CBV in the CBL. Ccn2<sup>-/-</sup> mice show significantly higher CBV in the HIPP but not in SS-Motor or mPFC. (D) Basal CBV in hippocampal subregions. Basal CBV was significantly increased in CA1, CA3, and dentate gyrus (DG) but not in subiculum. Notably, the increase in basal CBV occurs in the context of a significant deficit in adult neurogenesis in the dentate gyrus (Fig. S7). Box plots show interquartile range, with whiskers extending to the outermost data points within 1.5x of the interquartile range. Means are marked by dotted lines running through square markers, medians by solid lines, and outliers as dots outside the whiskers. *P < 0.05, independent t tests (one-tailed).

Fig. S5. Conditioned behavioral freezing in a context associated with foot shock as a function of the duration of exposure to the context before experience of the foot shock. Training consisted of exposure to five tone (CS<sup>+</sup>)-footshock (US<sup>+</sup>) pairings a specific context. The time between the placement of the mouse in the context and delivery of the first tone-shock pair was varied between 75 and 175 s. The conditioned response to the context was tested 30 h after training. Similar to that previously reported (14), longer exposure to the context before the first foot shock leads a stronger conditioned fear response to the context. Similar to the pattern reported for dorsal hippocampus-lesioned rodents, Ccn2<sup>-/-</sup> mice show an overall deficit in context learning and a slight blunting of the learning slope across context exposure intervals.
Fig. S6. Ventral tegmental area (VTA) DA neuron activity and DA-mediated locomotor phenotype in Ccnd2−/− mice. (A) Representative coronal section showing electrode recording tracks through the central and lateral VTA at the approximate rostro-caudal level indicated by the bar in inset. (Scale bar: 1 mm, corrected for shrinkage.) (B) Representative trace from an extracellular recording of a single neuron. The unit exhibits waveform and burst-firing characteristics of a DA neuron (SI Materials and Methods). (C) Summary data from multiple cohorts of mice plotting average post-AMPH locomotion as a function of dose (plotted as log10 of dose with vehicle data shown at "0"). There is an “inverse U”-shaped dose-response function for the effect of AMPH on locomotion in both genotypes (effect of dose F4,112 = 33.9, P < 0.001) with Ccnd2−/− showing greater post-AMPH locomotion than Ccnd2+/+ mice (F1,112 = 4.9, P < 0.05) (dose group mean n: 14.5 (veh), 24 (2.0 mg/kg), 8.5 (4.0 mg/kg), 7 (8 mg/kg), and 7.5 (16.0 mg/kg)). (D) Average post-AMPH locomotion expressed as log10 of the mean Ccnd2+/+ baseline locomotion in mice administered saline or the dopamine D2 receptor antagonist raclopride (0.5 mg/kg, i.p.) 20 min before injection of AMPH (2.0 mg/kg, i.p.). Similar to the effect shown in Fig. 3D, Ccnd2−/− show greater post-AMPH locomotion than Ccnd2+/+ in the saline pretreatment condition. Pretreatment with raclopride blocks AMPH-induced locomotion in both genotypes, eliminating the genotypic difference. *P < 0.05, n.s. = P > 0.25 (Ccnd2−/− vs. Ccnd2+/+), independent t tests, n = 6–7. (E) Augmented response to amphetamine in Ccnd2−/− is eliminated by partial lesions of the caudoventral hippocampus (HIPP Lx). There is a strong trend for a genotype x lesion interaction (F1,30 = 3.9, P = 0.06). In mice with lesion of the overlying parietal cortex, Ccnd2−/− mice show greater post-AMPH locomotion than Ccnd2−/− mice (one-tailed t14 = 2.1, P < 0.05; n = 7–9). However, following lesions of the caudoventral hippocampus, the difference between genotypes is lost (one-tailed t19 = 0.77, P > 0.4; n = 10–11). This experiment did not include nonsurgically manipulated groups, thus does not address overall differences between lesioned and nonlesioned animals. Box plots in A and B show interquartile range, with whiskers extending to the outermost data points within 1.5x of the interquartile range. Means are marked by dotted lines running through square markers, medians by solid lines, and outliers as dots outside the whiskers. *P < 0.05, independent t tests (one-tailed).
Fig. S7. The deficit in neurogenesis in the adult dentate gyrus in Ccnd2−/− mice is not ameliorated by transplantation of MGE-derived cells into the caudoventral CA fields. (A) Images showing mitotic cells labeled with bromodeoxyuridine (BrdU) (Upper; bright-field images) and neural progenitors and immature neurons labeled with DCX (Lower; darkfield) in wild-type (Ccnd2+/+) and Ccnd2−/− adult mice. Ccnd2−/− mice show a marked reduction in neurogenesis in the adult dentate gyrus. (B) Neural progenitors and immature neurons labeled with DCX in the dentate gyrus of Ccnd2−/− mice 6 mo after transplantation of killed (control) or live (active) MGE-derived cells in caudoventral hippocampus. Transplants were targeted bilaterally at the caudoventral CA1. White arrows in Left point to DCX-labeled cells in the control transplant example. (C) Quantification of DCX+ cells in the dentate gyrus of Ccnd2−/− (sham surgery) and transplanted Ccnd2−/− adult mice. Relative to Ccnd2+/+ colony mates, Ccnd2−/− mice show significantly fewer DCX+ neurons in the dentate gyrus. This deficit is not affected by transplantation of active MGE-derived interneuron precursors into caudoventral CA1. (Scale bars: 0.5 mm.)
The circuitry hypothesized to underlie the impact of partial PV⁺ interneuron deficits on hippocampal function and the normalizing effects of MGE-derived interneuronal precursor transplants in adulthood. The blue-outlined box depicts local circuit effects of a partial loss of PV⁺ interneurons, such as that produced by a knockout of Ccnd2. Early developmental loss of PV⁺ interneurons in Ccnd2−/− mice leads to a persistent deficit in GABAergic synaptic contacts onto the perisomatic and axon hillock regions of glutamatergic projection neurons in the hippocampus. The GABA input deficit, in turn, is hypothesized to lead to disinhibition and disrupted temporal patterning (and synchronization) of signals transmitted from the ventral hippocampus to its cortical and basal forebrain targets, resulting in the disruption of cognitive operations modulated by hippocampal circuits (schematic left of blue line). The cognitive deficit observed in the present study, learning and memory of contextual information related to aversive events, is corruption of hippocampal signals to the amygdala and temporolimbic cortical regions, which, in turn, impairs learning adaptive responses to the aversive context. In parallel, the augmented signal from the hippocampus to the nucleus accumbens inhibits the activity of pallidal and tegmental GABAergic neurons that normally provide tonic inhibitory tone onto VTA DA neurons, thus disinhibiting this DA system. The increase in DA neuron activity augments ventral striatal DA-modulated behaviors including the locomotor response to AMPH. These downstream cognitive and psychomotor effects of functional deficits in hippocampal PV⁺ interneurons can be mitigated in adulthood by transplanting cells harvested from the medial ganglionic eminence (MGE) at the stage of embryonic development in which the MGE is enriched with PV⁺ interneuron progenitor cells.
Table S1. Qualitative assessment of sensorimotor and psychomotor behavior in Ccnd2<sup>+/−</sup> mice relative to Ccnd2<sup>+/+</sup> littermates

<table>
<thead>
<tr>
<th>Ability</th>
<th>Test description/Ccnd2&lt;sup&gt;+/+&lt;/sup&gt; mouse profile</th>
<th>Ccnd2&lt;sup&gt;−/−&lt;/sup&gt; relative to Ccnd2&lt;sup&gt;+/+&lt;/sup&gt; littermates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hearing</td>
<td>Orient or startles to click presented within home cage</td>
<td>Normal</td>
</tr>
<tr>
<td>Vision</td>
<td>When suspended by tail and lowered toward dark (high contrast) surface, mouse reaches for surface when 20 cm above the surface.</td>
<td>Intact, but do not reach until &lt;18 cm above surface</td>
</tr>
<tr>
<td>Tactile sense</td>
<td>Turning head toward tactile stimulus [touching hind limb with a wire (von Frey hair)].</td>
<td>Similar response but with increased latency</td>
</tr>
<tr>
<td>Olfaction</td>
<td>Obvious reaction (prolonged sniffing, digging) to new bedding; behavior</td>
<td>Show avoidance of new bedding</td>
</tr>
<tr>
<td>Posture</td>
<td>Animal is placed on all four feet, observed in standing and crouching positions, compared with Ccnd2&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>Normal profile</td>
</tr>
<tr>
<td>Muscle tone</td>
<td>Subjective rating: normal, rigid or flaccid</td>
<td>Normal</td>
</tr>
<tr>
<td>Ataxia/abnormal gait</td>
<td>Standing and ambulation without stumbling, weaving; uncoordinated ambulation; normal righting response</td>
<td>Normal gait; normal righting; no ataxia</td>
</tr>
<tr>
<td>Locomotor activity</td>
<td>Level of activity in novel environment: scale from 0–6 ranging from no movement (0) to continuous locomotion or stereotypy (6); show average score of 3 (intermittent to frequent locomotion)</td>
<td>Average score = 3.5 (SD = 1); may be slightly more active than Ccnd2&lt;sup&gt;+/+&lt;/sup&gt; in novel environment</td>
</tr>
<tr>
<td>Response to novelty</td>
<td>When exposed to novel bedding or novel materials, mice explore and incorporate materials into nest</td>
<td>Show strong tendency to avoid novel materials</td>
</tr>
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