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Attention-Deficit/Hyperactivity Disorder–like Phenotype in a Mouse Model with Impaired Actin Dynamics

Anika-Maria Zimmermann, Tanja Jene, Michael Wolf, Andreas Görlich, Christine B. Gurniak, Marco Sassoè-Pognetto, Walter Witke, Eckhard Friauf, and Marco B. Rust

ABSTRACT

BACKGROUND: Actin depolymerizing proteins of the actin depolymerizing factor (ADF)/cofilin family are essential for actin dynamics, which is critical for synaptic function. Two ADF/cofilin family members, ADF and n-cofilin, are highly abundant in the brain, where they are present in excitatory synapses. Previous studies demonstrated the relevance of n-cofilin for postsynaptic plasticity, associative learning, and anxiety. These studies also suggested overlapping functions for ADF and n-cofilin.

METHODS: We performed pharmacobehavioral, electrophysiologic, and electron microscopic studies on ADF and n-cofilin single mutants and double mutants (named ACC mice) to characterize the importance of ADF/cofilin activity for synapse physiology and mouse behavior.

RESULTS: The ACC mice, but not single mutants, exhibited hyperlocomotion, impulsivity, and impaired working memory. Hyperlocomotion and impulsive behavior were reversed by methylphenidate, a psychostimulant commonly used for the treatment of attention-deficit/hyperactivity disorder (ADHD). Also, ACC mice displayed a disturbed morphology of striatal excitatory synapses, accompanied by strongly increased glutamate release. Blockade of dopamine or glutamate transmission resulted in normal locomotion.

CONCLUSIONS: Our study reveals that ADHD can result from a disturbed balance between excitation and inhibition in striatal circuits, providing novel insights into the mechanisms underlying this neurobehavioral disorder. Our results link actin dynamics to ADHD, suggesting that mutations in actin regulatory proteins may contribute to the etiology of ADHD in humans.

Keywords: Actin dynamics, ADF, ADHD, Hyperactivity, Locomotion, n-cofilin

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Attention-deficit/hyperactivity disorder (ADHD) is defined by persistent and maladaptive symptoms of hyperactivity, impulsivity, and inattention (1). Among the most common psychiatric disorders, ADHD affects approximately 8% of school-age children worldwide and often persists into adolescence or adulthood (1,2). Twin, adoption, and segregation studies have revealed a high heritability of ADHD, and molecular genetics associated ADHD with dopamine-related genes, including the dopamine transporter (DAT) and dopamine receptors (3). Defects reminiscent of ADHD are displayed by DAT mutant mice, underlining the implication of dopamine dysfunction in the etiology of ADHD (4). Several ADHD susceptibility loci do not contain dopamine-related genes (5–9), and an increasing number of studies implicate corticostriatal network abnormalities in ADHD (10). These findings, together with the phenotypic heterogeneity of patients with ADHD, suggest the existence of diverse ADHD-associated genes and underlying mechanisms.

Actin is abundant in presynaptic terminals and postsynaptic spines (11), and numerous studies highlight the relevance of actin dynamics for postsynaptic plasticity (12). Pharmacologic studies and the subsynaptic distribution of actin filaments also suggest a role for actin in neurotransmitter release (13,14). Actin depolymerizing proteins of the actin depolymerizing factor (ADF)/cofilin family are essential regulators of actin dynamics (15). Two family members, ADF and n-cofilin, are highly abundant in the adult brain and present in excitatory synapses (16–18). Analyses of gene-targeted mice revealed the relevance of n-cofilin for postsynaptic plasticity, learning, and anxiety (19,20). However, these analyses also suggested an overlapping function for ADF and n-cofilin.

We report an ADHD-like phenotype in double mutant mice lacking ADF and n-cofilin (named ACC mice) that lack ADF systemically and n-cofilin specifically in the adult telencephalon. Neurobehavioral abnormalities included hyperlocomotion, impulsivity, and impaired working memory. These abnormalities were not present in single mutants. In ACC mice, locomotion was normalized, and impulsive behavior was reduced by the ADHD medication methylphenidate (1). We also found an altered morphology of excitatory synapses and increased glutamate release in the striatum of ACC mice. Additionally, locomotion of ACC mice was normalized by

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blockade of glutamate transmission. Our data suggest that enhanced glutamate release caused by impaired actin dynamics results in dopamine deregulation and, ultimately, in an ADHD-like phenotype.

METHODS AND MATERIALS

Mice

Double mutant mice lacking ADF and n-cofilin (ACC mice; $ADF^{-/-}$/n-$Cof^{flx/flx,CamKII\text{-}cre}$) were generated by intercrossing mutants with a single ADF allele and a single floxed n-cofilin allele (16). One of the breeding animals additionally expressed Cre recombinase under control of the CaMKIIα promoter (21). $ADF^{+/+}$/n-$Cof^{flx/flx}$ mice served as control animals. The CaMKII-Cre mice were also intercrossed with Rosa26-Cre reporter mice to determine Cre activity (22). All mice were housed in the animal facility of the University of Kaiserslautern on 12-hour dark-light cycles with food and water available ad libitum. Behavioral experiments were conducted during the light cycle. Treatment of mice was in accordance with the German law for conducting animal experiments and followed the guidelines for the care and use of laboratory animals of the U.S. National Institutes of Health. Behavioral experiments and killing of mice for tissue analysis were approved by the Landesuntersuchungsamt Rheinland-Pfalz (references: 23177–07/G09-2-001, 23177-07/G12-2-077, and A2010_01_15UniKL). Mouse husbandry and breeding was approved by the City of Kaiserslautern, Referat Umweltschutz.

Behavior

With the exception of home cage behavior, all experiments were conducted on mice 35–42 days old. A detailed description of the methods is provided in Supplement 1.

Electron Microscopy

Small specimens taken from the striatum (bregma .98–.14) of male mice 40–42 days old were used for ultrastructure analyses. Experiments were performed as described previously (17). Detailed information is provided in Supplement 1.

Histology

Histology was performed on brains from mice 38–40 days old. Detailed information is provided in Supplement 1.

Electrophysiology

Electrophysiology was performed on coronal sections from mice 4–6 weeks old. Detailed information is provided in Supplement 1.

DARPP-32 Phosphorylation

Striatal protein lysates from mice 38–40 days old were used to determine DARPP-32 (dopamine- and adenosine monophosphate–regulated phosphoprotein of 32 kDa) phosphorylation levels. Detailed information is provided in Supplement 1.

Statistics

Sets of data are presented by mean values and SEM. When comparing two data sets, an unpaired two-tailed Student *t* test was used. A paired Student *t* test was used when comparing the effect of a drug with the effect of saline in the same animal.

RESULTS

Hyperlocomotion, Reduced Response Habituation, and Impaired Nesting Behavior in ACC Mice

We noted altered behavior in ACC mice, which lack ADF systemically and n-cofilin specifically in the adult telencephalon (16,20). The mice appeared more active compared with controls or single mutants, for example, on opening of the cage and removal of nest material (Supplemental Video S1 in Supplement 2). In contrast to control or single mutants, which rapidly adapted to the new situation, ACC mice showed increased activity levels 10 min after opening the cage (Supplemental Video S2 in Supplement 3).

Beginning at postnatal day 32, home cage activity was strongly increased in male ACC mice (Figure 1A). Activity counts at postnatal day 39 amounted to 182% of control levels (Figure S1A in Supplement 1). Activity was increased in ACC mice during the night (light off) but not during the day (Figure 1B; Figure S1A in Supplement 1). Increased activity during the night resulted from less resting time and increased numbers and amplitudes of activity peaks (Figure S1B–D in Supplement 1). Very similar results were obtained from female mice (data not shown). No changes were observed in single mutants.

When testing for activity in the open field for 1 hour, horizontal activity (locomotion) of male ACC mice was increased fourfold (Figure 1C). Conversely, vertical activity (rearing) was unchanged in ACC mice (data not shown). Although locomotion was unchanged in ADF mutants, it was increased by 35% in n-cofilin mutants. Plotting locomotion over time revealed increased levels in ACC mice during the entire recording period (Figure 1D). In contrast to control mice and single mutants, which habituated to the novel environment and showed reduced locomotion during the last (12th) 5-min interval compared with the first interval, locomotion in ACC mice did not differ between both intervals (Figure S1E in Supplement 1). Very similar results were obtained in female mice (data not shown).

Apart from their hyperlocomotion, ACC mice were indistinguishable by observation alone from control mice or single mutants. They appeared healthy and showed normal posture and grooming behavior. The ACC mice did not display stereotypy or any other bizarre behavior, such as circling, waltzing, repetitive sniffing, or vertical leaping. Also, the mice did not display tremors or any obvious motor impairment. However, we noted impaired nesting behavior of ACC mice. After 24 hours of single housing, all tested control mice or single mutants had organized the bedding material into a nest, whereas none of the four ACC mice tested did so (Figure 1E).

Taken together, ACC mice showed strongly increased locomotion in familiar and novel environments, but, apart from

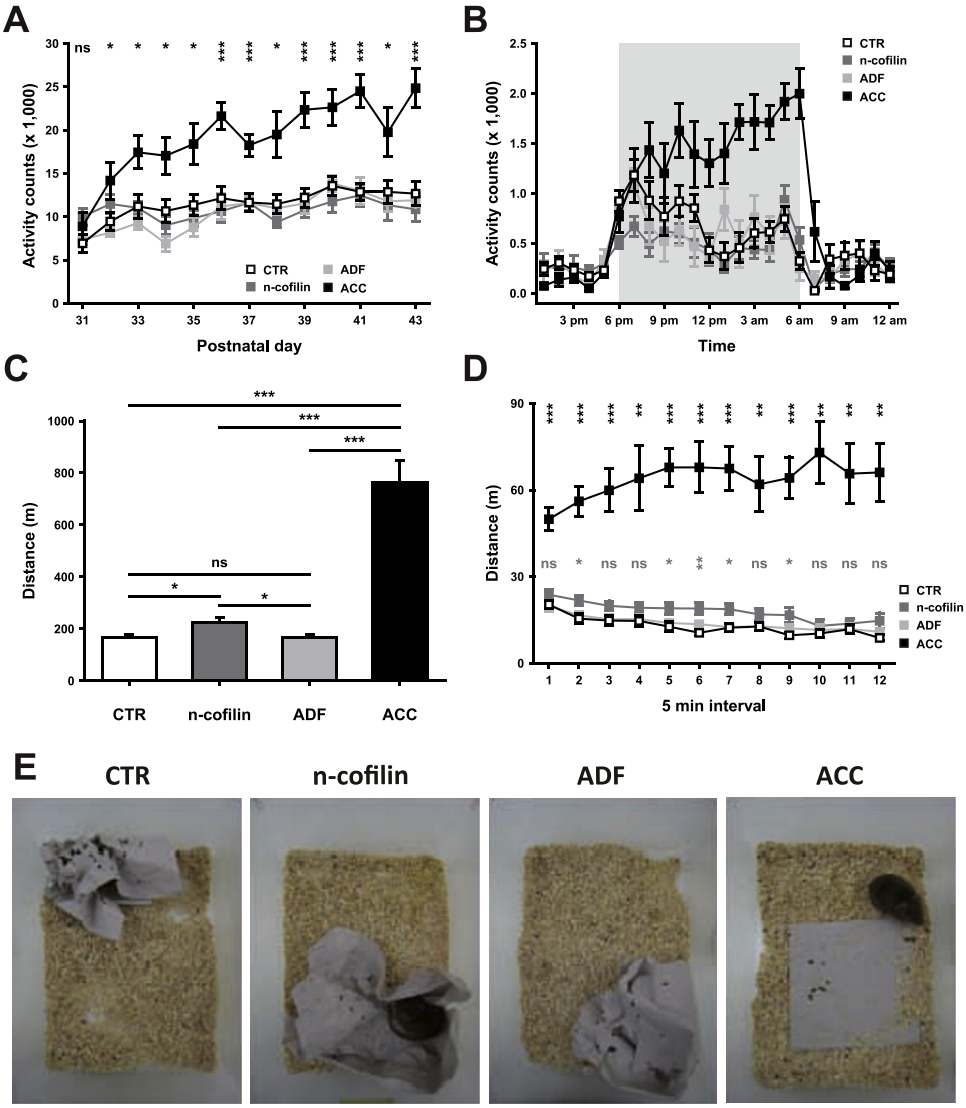

Figure 1. Increased locomotion and impaired nesting behavior in double mutant (ACC) mice. **(A)** Beginning at postnatal day 32, ACC mice, but not actin-depolymerizing factor (ADF; $n = 6$) or n-cofilin single mutants, showed increased locomotion in home cages (control [CTR], $n = 14$; n-cofilin, $n = 8$; ADF, $n = 6$; ACC, $n = 9$). Asterisks and ns, CTR-ACC comparison. **(B)** Activity plots at hourly intervals revealed increased activity of postnatal day 39 ACC mice during the night (gray box), but not during the day. **(C)** In the open field, locomotion was increased fourfold in ACC mice (CTR, 165.1 ± 12.6 m, $n = 10$; ACC, 662.2 ± 99.0 m, $n = 9$; $p \leq .001$). Although locomotion was also increased by 35% in n-cofilin mutants (223.0 ± 21.8 m, $n = 10$; $p \leq .05$), it was unchanged in ADF mutants (165.9 ± 13.1 m, $n = 7$; $p = .964$). **(D)** Locomotion plots in 5-min intervals revealed enhanced locomotion of ACC mice during the entire recording period. Black asterisks, CTR-ACC comparison; gray asterisks and ns, CTR-n-cofilin comparison. **(E)** After 24 hours of single housing, CTR mice and ADF and n-cofilin mutants organized bedding material into a nest, whereas ACC mice did not do so. ns, not significant; $^{*}p \leq .05$; $^{**}p \leq .01$; $^{***}p \leq .001$.

this, they were indistinguishable from control mice. Locomotion was unchanged in ADF mutants but slightly increased in n-cofilin mutants when exposed to a novel environment. The ACC mice, but not the single mutants, showed impaired nesting behavior and reduced response habituation to novelty.

Normal Locomotion in ACC Mice on Blockade of Dopamine Transmission

Hyperlocomotion is often associated with impaired dopamine function (23–27). To test whether this association also held true for ACC mice, we analyzed locomotion on modulation of dopamine transmission (Figure 2A). Injection of the D_1-like dopamine receptor antagonist SCH23390 reduced locomotion in all groups (Figure 2B). The reduction was much stronger in ACC mice compared with control mice (control, −70.9 ± 6.3 m, $n = 11$; ACC, −568.0 ± 41.7 m, $n = 5$; $p \leq .001$) (Figure S2A in Supplement 1). After SCH23390 treatment, locomotion did not differ between the four groups. Similarly, blocking D_2-like dopamine receptors by haloperidol reduced locomotion in ACC mice to a much greater degree than it did in control mice (control, −43.0 ± 6.8 m, $n = 10$; ACC, −568.3 ± 82.6 m, $n = 5$; $p \leq .001$) (Figure 2C and Figure S2B in Supplement 1). These findings strongly suggest that increased locomotion in ACC mice is due to impaired dopamine function. The scenario would be consistent with elevated dopamine activity or increased dopamine responsiveness.

Calming Effect of Psychostimulants in ACC Mice

To test whether dopamine responsiveness was changed in ACC mice, we blocked DAT, which removes dopamine from the synaptic cleft and terminates dopamine activity (23). Cocaine, a nonselective monoamine reuptake inhibitor, increased locomotion by 180% in control mice (Figure 2D; Figure S2C in Supplement 1). Conversely, it reduced locomotion by 34% in ACC mice. Likewise, the selective DAT inhibitor vanoxerine tripled locomotion in control mice but reduced

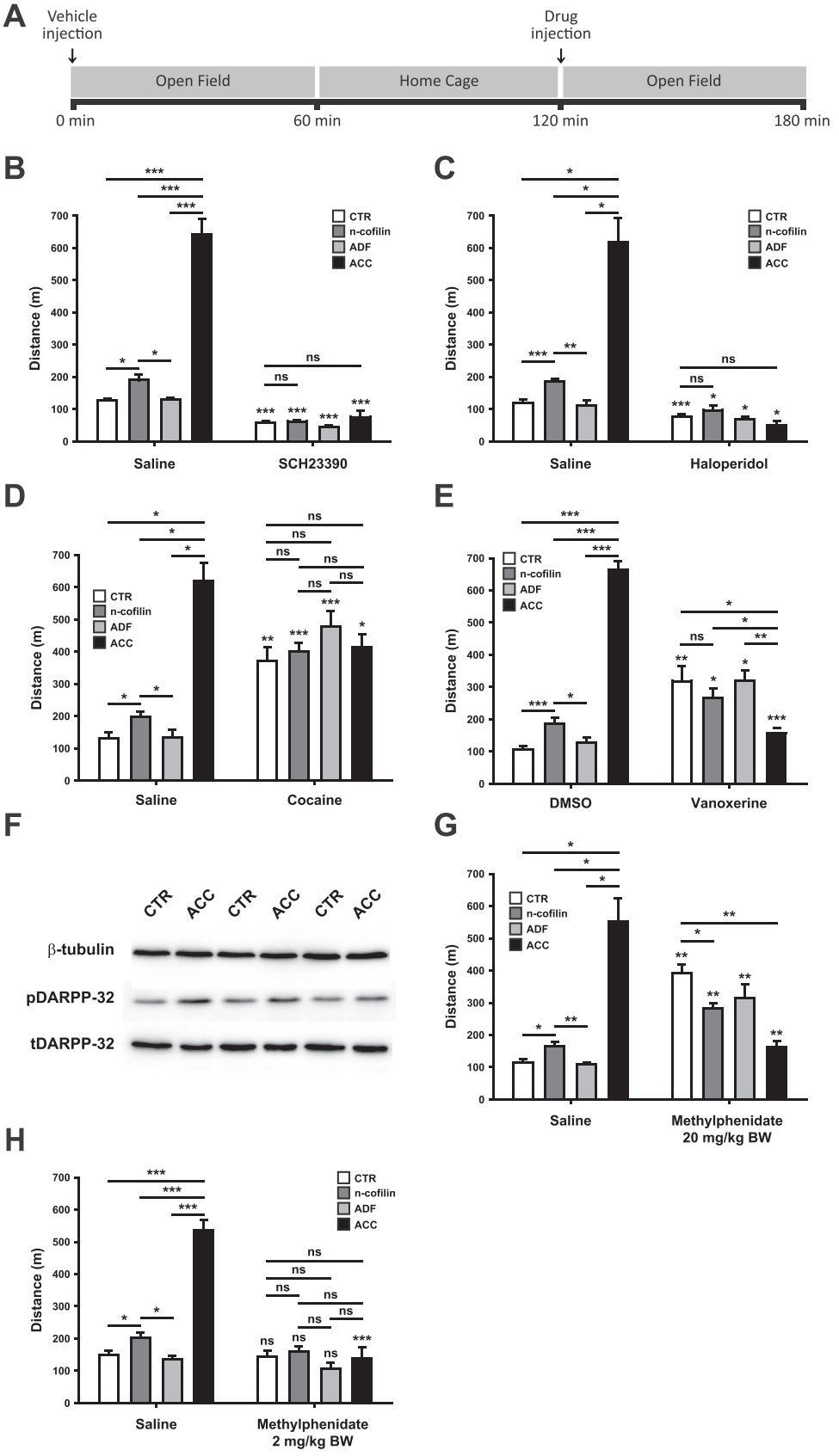

Figure 2. Blockade of dopamine receptors and dopamine transporter normalized locomotion in double mutant (ACC) mice. **(A)** Scheme of pharmacobehavioral experiments. On vehicle injection, locomotion was recorded for 60 min in the open field. Thereafter, mice were kept in their home cages for 60 min before drugs were injected, and locomotion was scored for another 60 min in the open field. **(B)** Blockade of D_1-like dopamine receptor by SCH23390 reduced locomotion in all groups compared with saline (marked by asterisks directly above the SCH23390 bars; control [CTR], 127.9 ± 5.0 m vs. 57.0 ± 6.0 m, $n = 11$, $p \leq .001$; n-cofilin, 190.6 ± 17.7 m vs. 61.9 ± 3.9 m, $n = 6$, $p \leq .001$; actin depolymerizing factor [ADF], 129.5 ± 5.6 m vs. 44.3 ± 6.2 m, $n = 8$, $p \leq .001$; ACC, 643.3 ± 47.1 m vs. 75.3 ± 19.1 m, $n = 5$, $p \leq .001$). With SCH23390 treatment, locomotion of mutant mice was similar to CTR mice (n-cofilin, $p = .579$; ACC, $p = .251$). **(C)** Likewise, blockade of D_2-like dopamine receptors by haloperidol reduced locomotion in all groups (CTR, 119.9 ± 9.3 m vs. 76.9 ± 7.8 m, $n = 10$, $p \leq .01$; n-cofilin, 186.5 ± 8.6 m vs. 96.7 ± 15.3 m, $n = 5$, $p \leq .001$; ADF, 110.4 ± 16.9 m vs. 69.5 ± 6.1 m, $n = 8$, $p \leq .001$; ACC, 618.4 ± 74.8 m vs. 50.1 ± 12.2 m, $n = 5$, $p \leq .05$). With haloperidol treatment, locomotion of mutant mice was similar to CTR mice (n-cofilin, $p = .217$; ACC, $p = .076$). **(D)** Cocaine treatment increased locomotion in CTR mice and single mutants (CTR, 132.4 ± 17.0 m vs. 371.9 ± 42.1 m, $n = 8$, $p \leq .01$; n-cofilin, 197.4 ± 17.3 m vs. 401.9 ± 27.0 m, $n = 9$, $p \leq .001$; ADF, 133.4 ± 24.9 m vs. 477.3 ± 48.4 m, $n = 6$, $p \leq .001$). In contrast, cocaine reduced locomotion in ACC mice (620.7 ± 55.0 m vs. 413.2 ± 39.7 m, $n = 5$, $p \leq .05$). With cocaine treatment, locomotion of mutant mice was similar to CTR mice (n-cofilin, $p = .548$; ACC, $p = .520$). **(E)** Dopamine transporter inhibition by vanoxerine increased locomotion in CTR mice and single mutants (CTR, 106.3 ± 9.7 m vs. 318.4 ± 45.8 m, $n = 10$, $p \leq .01$; n-cofilin, 187.3 ± 17.5 m vs. 266.7 ± 29.7 m, $n = 9$, $p \leq .05$; ADF, 128.2 ± 14.6 m vs. 320.6 ± 30.6 m, $n = 7$, $p \leq .05$). In contrast, vanoxerine reduced locomotion in ACC mice (664.6 ± 25.6 m vs. 157.9 ± 15.2 m, $n = 5$, $p \leq .001$). On vanoxerine injection, locomotion was similar to CTR mice in n-cofilin mutants ($p = .369$) but reduced in ACC mice ($p \leq .05$). **(F)** Compared with CTR mice, phosphorylation of DARPP-32 (dopamine- and adenosine monophosphate–regulated

locomotion by 76% in ACC mice (Figure 2E; Figure S2D in Supplement 1). With vanoxerine treatment, locomotion in ACC mice was similar to locomotion in nontreated control mice. These data demonstrate that increased dopamine activity, and not increased dopamine responsiveness, is responsible for augmented locomotion in ACC mice. This conclusion is further supported by increased phosphorylation levels of DARPP-32 in the striatum of ACC mice (Figure 2F). DARPP-32 is a protein phosphatase inhibitor that is highly expressed in striatal medium spiny neurons (28) and becomes phosphorylated at threonine 34 in response to dopamine (29).

ADHD Medication Normalizes Locomotion in ACC Mice

Hyperlocomotion and calming effects of psychostimulants are hallmarks of ADHD (1). The dopamine/norepinephrine reuptake inhibitor methylphenidate is widely used for ADHD treatment and normalizes locomotion in ADHD mouse models (1,4,27,30,31). We analyzed whether methylphenidate has similar effects in ACC mice. At a high concentration (20.0 mg/kg body weight), methylphenidate increased locomotion 2.3-fold in control mice but reduced locomotion by 70% in ACC mice (Figure 2G; Figure S2E in Supplement 1). At a lower concentration (2.0 mg/kg body weight), methylphenidate reduced locomotion by 74% in ACC mice (Figure 2H). However, it did not affect locomotion in control mice. In both experiments, methylphenidate reduced locomotion in ACC mice to levels similar to nontreated control mice.

Impaired Working Memory in ACC Mice

We noted altered exploratory behavior of ACC mice in the Y-maze. Compared with control mice, the relative number of alternate arm returns was 1.6-fold increased in ACC mice, whereas the relative numbers of spontaneous alternation performances or same arm returns were unchanged (Figure 3A). No such changes were noted when comparing single mutants with control mice. Altered exploratory behavior of ACC mice in the Y-maze suggests impaired working memory (32). To study working memory in an independent approach, we tested the mice on an eight-arm radial maze using a win-shift rule (33). Food-deprived mice were allowed to explore the maze for a maximum of 10 min or until all baits were eaten. Only the first entry into each arm was , and every further visit was counted as working memory error (WME). In the first trial, the performance was equally poor in control and ACC mice as deduced from similar WME numbers, WME/total arm visit ratios, and numbers of correct entries before the first WME (Figure 3B–D). As training proceeded, control mice improved and ultimately performed better in the fifth trial than in the first trial. No such improvement was present in ACC mice. In the fifth trial, performance of ACC mice was weaker compared with control mice; WME numbers and WME/total arm visit ratio were increased by 260% and 135%, respectively, and the number of correct entries before the first WME was reduced by 32%. Conversely, single mutants performed similarly to control mice throughout the experiment. Working memory was intact in single mutants but impaired in ACC mice.

ACC Mice Display Impulsive Behavior That Is Rescued by Methylphenidate

ACC mice also behaved differently from control mice or single mutants in the elevated plus maze. All control mice or single mutants remained on the maze for the entire testing period (300 sec). However, 89% of ACC mice jumped off the 0.5 m high maze (Figure 4A), reducing the average retention time on the maze to 111.2 ± 32.2 sec (Figure 4B). Of the nine tested ACC mice, three remained on the maze for a minimum of 180 sec, such that we could analyze their exploratory behavior within this time frame. Control mice predominantly visited closed arms of the elevated plus maze, and their relative distance on closed arms was higher than on open arms (Figure 4C–D). Conversely, ACC mice visited open arms more often, and the relative distance was longer on open than on closed arms. Exploratory behavior of single mutants did not differ from behavior of control mice.

In another cohort of ACC mice, we tested whether methylphenidate normalized behavior in the elevated plus maze. On methylphenidate injection (2.0 mg/kg body weight), all control mice or single mutants remained on the maze for 300 sec and showed a clear preference for the closed arms, very similar to nontreated mice (Figure 4E,F). Methylphenidate almost doubled the retention time of ACC mice on the maze (Figure 4B). Of 15 ACC mice, 5 remained on the maze for 300 sec, and 9 remained for a minimum of 180 sec (Figure 4A). In contrast to nontreated ACC mice, methylphenidate-treated ACC mice showed no preference for open arms; the treated ACC mice visited closed and open arms equally often, and the relative distance did not differ between closed and open arms. These data reveal an impulsive behavior in ACC mice that is partially rescued by methylphenidate.

N-Cofilin Is Deleted in Only a Small Fraction of Dopamine Neurons in Substantia Nigra Pars Compacta

The present data are consistent with increased activity of dopamine neurons in the substantia nigra pars compacta (SNc) that innervate striatal medium spiny neurons (34). In ACC mice, n-cofilin is deleted in a tissue-specific manner controlled by the expression of a CaMKII-Cre transgene (21). We next tested whether CaMKII-Cre is active in SNc dopamine neurons. To do

phosphoprotein of 32 kDa) at threonine 34 (pDARPP-32) was increased in the striatum of ACC mice. Conversely, total DARPP-32 (tDARPP-32) levels were unchanged. Lysates from three CTR and ACC mice are shown. β-Tubulin was used as a loading control. **(G)** Injection of methylphenidate (20 mg/kg body weight) increased locomotion in CTR mice and single mutants (CTR, 115.7 ± 10.7 m vs. 391.3 ± 27.7 m, $n = 8$, $p \leq .01$; n-cofilin, 165.2 ± 14.1 m vs. 282.4 ± 17.0 m, $n = 10$, $p \leq .01$; ADF, 108.8 ± 6.9 m vs. 314.8 ± 42.0 m, $n = 7$, $p \leq .05$). In contrast, methylphenidate reduced locomotion in ACC mice (552.8 ± 71.5 m vs. 163.1 ± 19.4 m, $n = 5$, $p \leq .01$). Compared with CTR mice, locomotion was reduced in n-cofilin mutants and in ACC mice (n-cofilin, $p \leq .05$; ACC, $p \leq .01$). **(H)** At a lower concentration (2 mg/kg body weight), methylphenidate reduced locomotion in ACC mice (535.5 ± 32.5 m vs. 138.9 ± 33.8, $n = 5$, $p \leq .001$), but it did not alter locomotion in CTR mice or single mutants (CTR, 149.7 ± 13.1 m vs. 144.6 ± 19.0, $n = 9$, $p = .828$; n-cofilin, 192.3 ± 15.5 m vs. 141.2 ± 12.7, $n = 10$, $p = .069$; ADF, 135.0 ± 12.3 m vs. 106.5 ± 17.6, $n = 6$, $p = .213$). With methylphenidate treatment, locomotion of mutant mice was similar to CTR mice (n-cofilin, $p = .572$; ACC, $p = .874$). BW, body weight; DMSO, dimethyl sulfoxide; ns, not significant; $^*p \leq .05$; $^{**}p \leq .01$; $^{***}p \leq .001$.

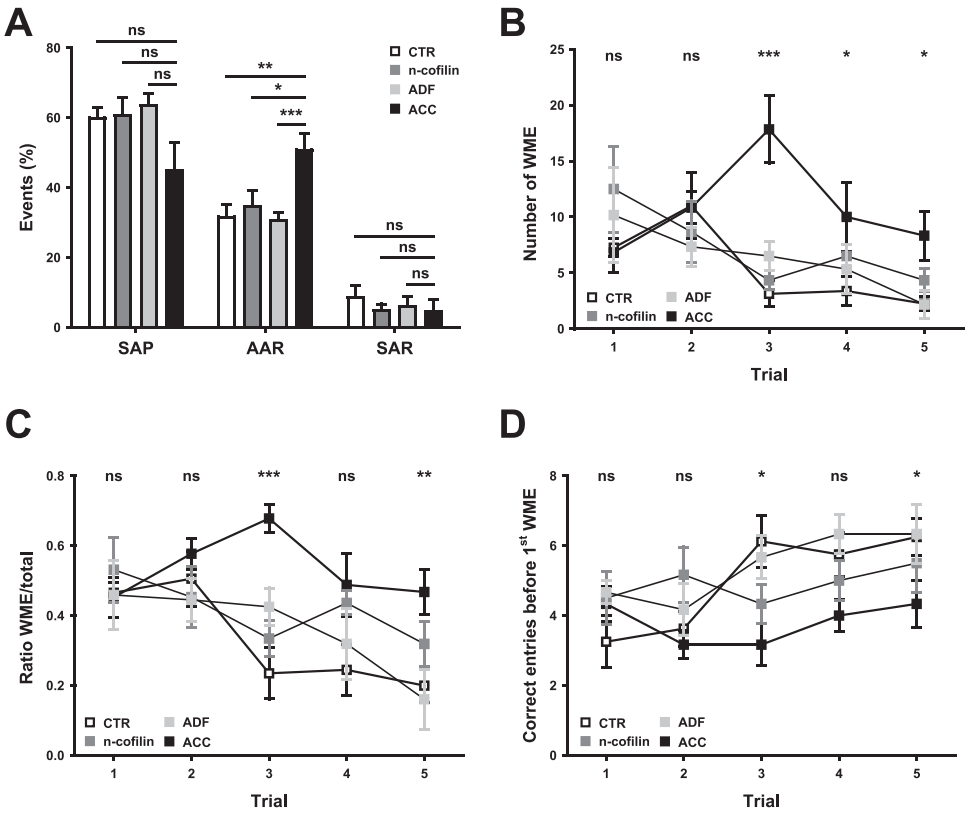

Figure 3. Impaired working memory in double mutant (ACC) mice. **(A)** In ACC mice, the relative number of alternate arm returns (AAR) was increased during a 5-min testing period in the Y-maze (control [CTR], 31.6% ± 3.6%, $n = 10$; ACC, 50.7% ± 4.8%, $n = 7$; $p \leq .01$), whereas the relative numbers of spontaneous alternation performances (SAP) and same arm returns (SAR) were unchanged (SAP—CTR, 59.9% ± 3.0%; ACC, 44.8% ± 8.1%; $p = .065$; SAR—CTR, 8.5% ± 3.5%; ACC, 4.4% ± 3.5%; $p = .438$). Conversely, n-cofilin and ADF mutants displayed normal exploratory behavior in the Y-maze. **(B)** Compared with CTR mice, the numbers of working memory errors (WME) were increased in trial 3 to trial 5 in ACC mice in the eight-arm radial maze (CTR—trial 3, 3.1 ± 1.2; trial 4, 3.1 ± 1.2; trial 5, 2.3 ± .6, $n = 8$; ACC—trial 3, 17.8 ± 3.0, $p \leq .001$; trial 4, 10.0 ± 3.1, $p \leq .05$; trial 5, 8.3 ± 2.2, $p \leq .001$, $n = 6$). The WME numbers were unchanged in the single mutants. **(C)** Ratios of WME to total arm visit (WME/total ratio) were increased in ACC mice in trial 3 and trial 5 compared with CTR mice (CTR—trial 3, .23 ± .07; trial 5, .20 ± .05; ACC—trial 3, .68 ± .04, $p \leq .001$; trial 5, .47 ± .07, $p \leq .01$). In trial 4, the increase in WME/total ratio did not reach statistical significance (CTR, .24 ± .07; ACC, .49 ± .09; $p = .056$). **(D)** Likewise, the numbers of correct entries before first WME were decreased in ACC mice in trial 3 and trial 5 (CTR—trial 3, 6.1 ± .7; trial 5, 6.3 ± .5; ACC—trial 3, 3.2 ± .6, $p \leq .05$; trial 5, 4.3 ± .7, $p \leq .05$). The decrease in trial 4 did not reach statistical significance (CTR, 5.8 ± .6; ACC, 4.0 ± .4; $p = .054$). The WME/total ratios and numbers of correct entries before first WME were unchanged in single mutants. **(B–D)** Asterisks and ns, CTR-ACC comparison; ns, not significant; $^{*}p \leq .05$; $^{**}p \leq .01$; $^{***}p \leq .001$.

so, we intercrossed CaMKII-Cre mice with Rosa26 reporter mice that express beta-galactosidase on Cre activation (22). X-Gal staining of double transgenic mice confirmed broad Cre activity in the telencephalon of CaMKII-Cre mice (Figure 5A–C), including the striatum, in which Cre was active in 84.2% ± 2.4% of all neurons ($n = 6$ sections from 3 mice) (Figure 5D). Conversely, only sparse beta-galactosidase expression was detectable in SNc (Figure 5B,C). Immunostaining against beta-galactosidase and tyrosine hydroxylase confirmed the absence of Cre activity from most SNc dopamine neurons (94.5% ± 1.2%, $n = 5$ sections from 3 mice) (Figure 5E,F). From these data, we conclude that altered dopamine activity in ACC mice is not caused by cell autonomous defects.

Altered Density and Morphology of Striatal Excitatory Synapses in ACC Mice

Medium spiny neurons are inhibitory projection neurons that amount to ~95% of all neurons in the striatum (35). Medium spiny neurons integrate glutamatergic inputs from the cerebral cortex and thalamus as well as dopaminergic inputs from SNc, and they play a key role in controlling locomotion (34). A reciprocal modulation of dopamine and glutamate has been described in the striatum, where glutamate facilitates dopamine release (36,37). Deregulation of actin dynamics can enhance glutamate release in striatal excitatory synapses, accompanied by alterations in synapse ultrastructure (38). To assess whether impaired ADF/cofilin activity causes similar alterations, we first quantified the density of striatal excitatory synapses by electron microscopy and found it decreased by 16% in ACC mice (Figure 6). The morphology of striatal excitatory synapses was altered in ACC mice—the presynaptic bouton area was increased by 33%, the dendritic spine area was increased by 39%, and the postsynaptic density length was increased by 21% (Figure 7A–H). Additionally, the number of docked vesicles per active zone was increased by 17% (Figure 7I), although this effect did not reach statistical significance ($p = .083$). In contrast to ACC mice, the numbers of excitatory synapses were unaltered in the striatum of n-cofilin and ADF mutants, and their morphologies were grossly preserved except for a slight increase in spine size in n-cofilin mutants (Figures 6 and 7). Overall, these changes demonstrated a crucial role of ADF/cofilin for the ultrastructure of striatal excitatory synapses, which became evident only on inactivation of both ADF/cofilin proteins.

Blockade of Glutamate Transmission Normalizes Locomotion in ACC Mice

To test whether the alterations in synapse ultrastructure may be paralleled by altered glutamate release in the striatum, we recorded spontaneous excitatory postsynaptic

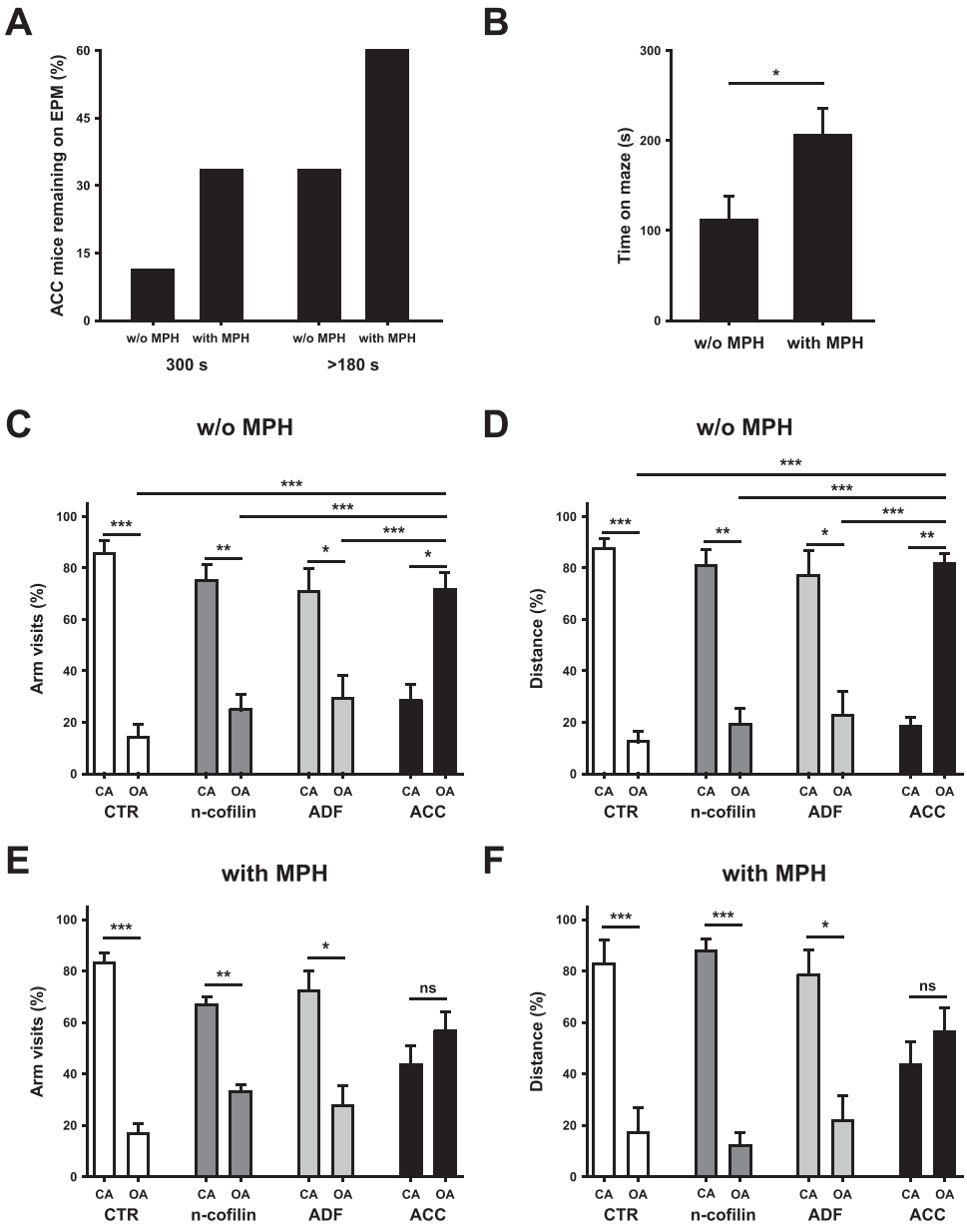

Figure 4. Methylphenidate (MPH) partially rescued impulsive behavior in double mutant (ACC) mice. **(A)** Relative number of nontreated (w/o MPH; $n = 9$) and MPH-injected (with MPH; $n = 15$) ACC mice that remained on the elevated plus maze (EPM) either for the entire duration of recording (300 sec) or for a minimum of 180 sec. **(B)** Average time that nontreated or MPH-injected ACC mice remained on the EPM (w/o MPH, 111.2 ± 32.2 sec; with MPH, 205.9 ± 26.8 sec; $p \leq .05$). **(C)** During the first 180 sec of recording, control (CTR) mice visited closed arms (CA) more often than open arms (OA) (CA, 85.8% ± 4.6%; OA, 14.2% ± 4.6%; $n = 9$, $p \leq .001$), whereas ACC mice visited CA less often (CA, 28.3% ± 5.9%; OA, 71.7% ± 5.9%; $n = 3$, $p \leq .05$). **(D)** The relative distance in the CA was much higher than that in the OA in CTR mice (CA, 87.5% ± 3.8%; OA, 12.5% ± 3.8%; $p \leq .001$). The opposite was found for ACC mice (CA, 18.3% ± 3.4%; OA, 81.7% ± 3.4%; $p \leq .01$). Single mutants displayed unaltered exploratory behavior. **(E)** Relative numbers of arm visits on MPH treatment. The CTR mice visited CA more often (CA, 83.1% ± 3.5%; OA, 16.9% ± 3.5%; $n = 12$, $p \leq .001$), whereas CA visits were similar to OA visits in ACC mice (CA, 43.4% ± 7.6%; OA, 56.6% ± 7.6%; $n = 9$, $p = .204$). **(F)** Likewise, relative distance in CA was higher in CTR mice with MPH treatment (CA, 82.7% ± 9.1%; OA, 17.3% ± 9.1%; $p \leq .001$), whereas in ACC mice, the relative distances were similar in both arms (CA, 43.8% ± 8.9%; OA, 56.2% ± 8.9%; $p = .256$). With MPH treatment, exploratory behavior of single mutants was similar to CTR mice. ns, not significant; $^{*}p \leq .05$; $^{**}p \leq .01$; $^{***}p \leq .001$.

currents from patch-clamped medium spiny neurons (Figure 8A). We found unchanged spontaneous excitatory postsynaptic current amplitudes in ACC mice, indicative of normal vesicular glutamate load and postsynaptic sensitivity (Figure 8B). In contrast, the interevent interval was reduced by 69% in ACC mice (Figure 8C). Pharmacologic blockade of glutamate transmission by CNQX, a competitive antagonist of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid and kainate receptors, strongly reduced the amplitudes and increased the interevent interval of spontaneous excitatory postsynaptic currents recorded from medium spiny neurons in ACC mice, confirming that spontaneous excitatory postsynaptic currents are mediated by glutamate (data not shown). Together, these data demonstrate strongly elevated glutamate release in the striatum of ACC mice.

We next tested whether the hyperlocomotion in ACC mice is caused by high glutamate activity. The use of CNQX in behavioral experiments is impractical because of the massive disturbances expected from blocking the bulk of fast excitatory transmission. We assessed locomotion after injection of dizocilpine, a noncompetitive antagonist of *N*-methyl-D-aspartate receptors that colocalize with alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (39). Dizocilpine increased locomotion by 64% in control mice. Conversely, it reduced locomotion by 66% in ACC mice (Figure 8D; Figure S2G in Supplement 1). On dizocilpine injection, locomotion of ACC mice did not differ from nontreated control mice.

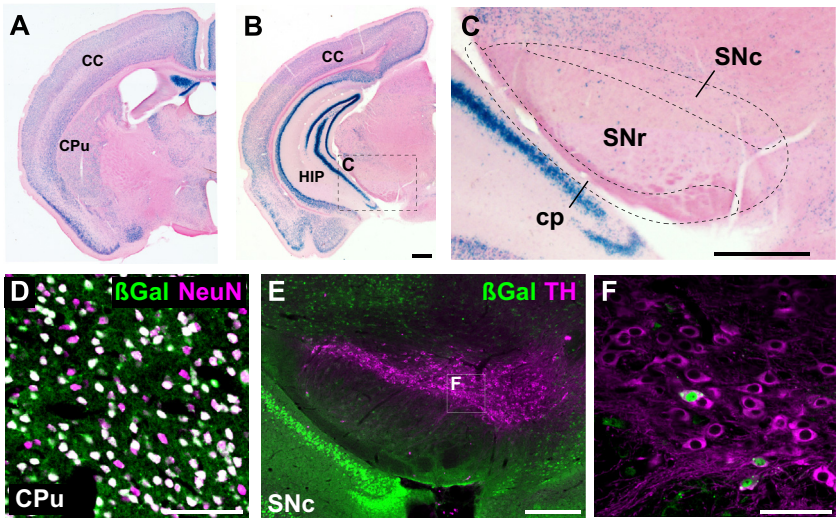

Figure 5. Cell type–specific inactivation of n-cofilin. **(A, B)** X-gal staining of CaMKII-cre/Rosa26 reporter mice confirmed active Cre in the telencephalon of adult mice, for instance, in the cerebral cortex (CC), nucleus caudatus and putamen (CPu) of the striatum, and hippocampus (HIP). Framed area in **(B)** is depicted in **(C)** at higher magnification. Scale bar = 500 μm. **(C)** In the substantia nigra pars compacta (SNc), only a few neurons showed X-gal staining. Scale bar = 500 μm. **(D)** Double-immunostaining against beta-galactosidase (βGal; green) and neuron-specific nuclear protein (NeuN; magenta) revealed active Cre in most CPu neurons. Scale bar = 100 μm. **(E)** Double-immunostaining against βGal (green) and tyrosine hydroxylase (TH; magenta) that was used as a marker for dopamine neurons in SNc. Framed area is depicted in **(F)** at higher magnification. Scale bar = 100 μm. **(F)** Only a small fraction of dopamine neurons in the SNc expressed βGal (whitish green cells). Scale bar = 20 μm. cp, cerebral peduncle; SNr, substantia nigra pars reticulata.

Blocking glutamate transmission normalizes locomotion in ACC mice, demonstrating that increased glutamate activity causes hyperlocomotion in these animals.

DISCUSSION

In the present study, we report abnormal behavior in ACC mice lacking the actin depolymerizing proteins ADF and n-cofilin. Increased locomotion, impulsivity, and impaired working memory were displayed by ACC mice. These defects were corrected by psychostimulants, resembling the situation observed in patients with ADHD. We noted markedly increased glutamate transmission in the striatum that is likely to be responsible for the deregulation of dopamine in ACC mice. Normal locomotion on blockade of dopamine or glutamate activity suggests that the ADHD-like phenotype is caused by elevated glutamate activity and disturbed dopaminergic transmission.

By exploiting gene targeted mice, we have unraveled the relevance of actin depolymerizing proteins of the ADF/cofilin family for synapse physiology and mouse behavior. We have shown previously that inactivating n-cofilin in the adult telencephalon impairs postsynaptic plasticity, associative learning, and anxiety (19,20), whereas synapse physiology and behavior are preserved in systemic ADF mutants (17). In the present study, we report neurobehavioral abnormalities in ACC mice that are not present in single mutants, providing evidence for overlapping functions of ADF and n-cofilin in the brain. The neurobehavioral abnormalities in ACC mice include hyperlocomotion, impulsivity, impaired working memory, and missing nesting behavior. In ACC mice, hyperlocomotion was more pronounced in a novel environment than in a familiar one, indicating increased responses to novelty. Additionally, ACC mice showed decreased response habituation to novelty, a trait that is implicated in attention deficits in humans (40,41). Psychostimulants, including the ADHD medication methylphenidate, ameliorated the behavioral defects in ACC mice. However, methylphenidate normalized locomotion yet only partially

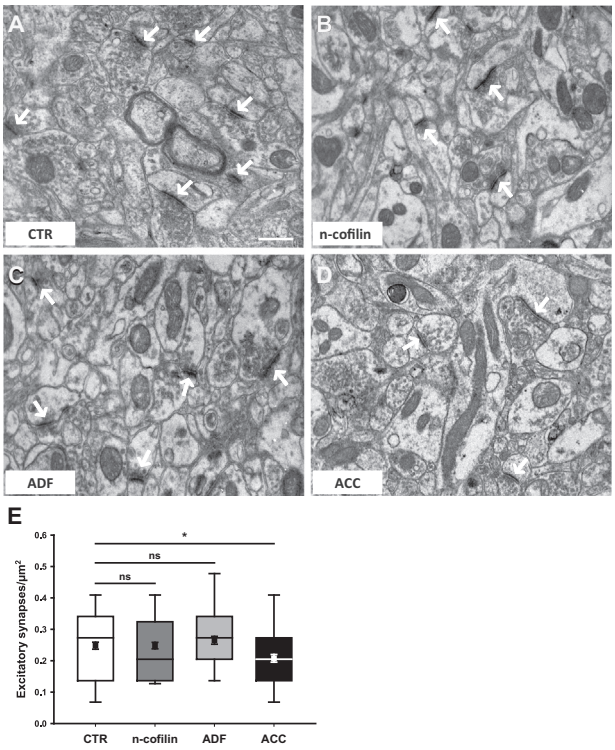

Figure 6. Reduced density of striatal excitatory synapses in double mutant (ACC) mice. **(A–D)** Representative electron microscopy micrographs as used for determination of synapse density. Arrows indicate excitatory synapses. **(A,** Scale bar = 200 nm.) **(E)** In ACC mice, the density of striatal excitatory synapses was reduced (control [CTR]—.247 ± .011 synapses/μm^2; total analyzed area, 1715.22 μm^2 in three mice; ACC—.207 ± .012 synapses/μm^2; total analyzed area, 1319.40 μm^2 in three mice; $p \leq .05$). No such reduction was seen in n-cofilin or actin depolymerizing factor (ADF) mutants (n-cofilin—.248 ± .010 synapses/μm^2; total analyzed area, 1700.56 μm^2 in three mice; $p = .983$; ADF—.265 ± .012 synapses/μm^2; total analyzed area, 1539.30 μm^2 in three mice; $p = .447$). Apart from mean values ± SEM, the median and the 1%, 25%, 75%, and 99% values are given in the box plots (same in Figure 7). ns, not significant; *$p \leq .05$.

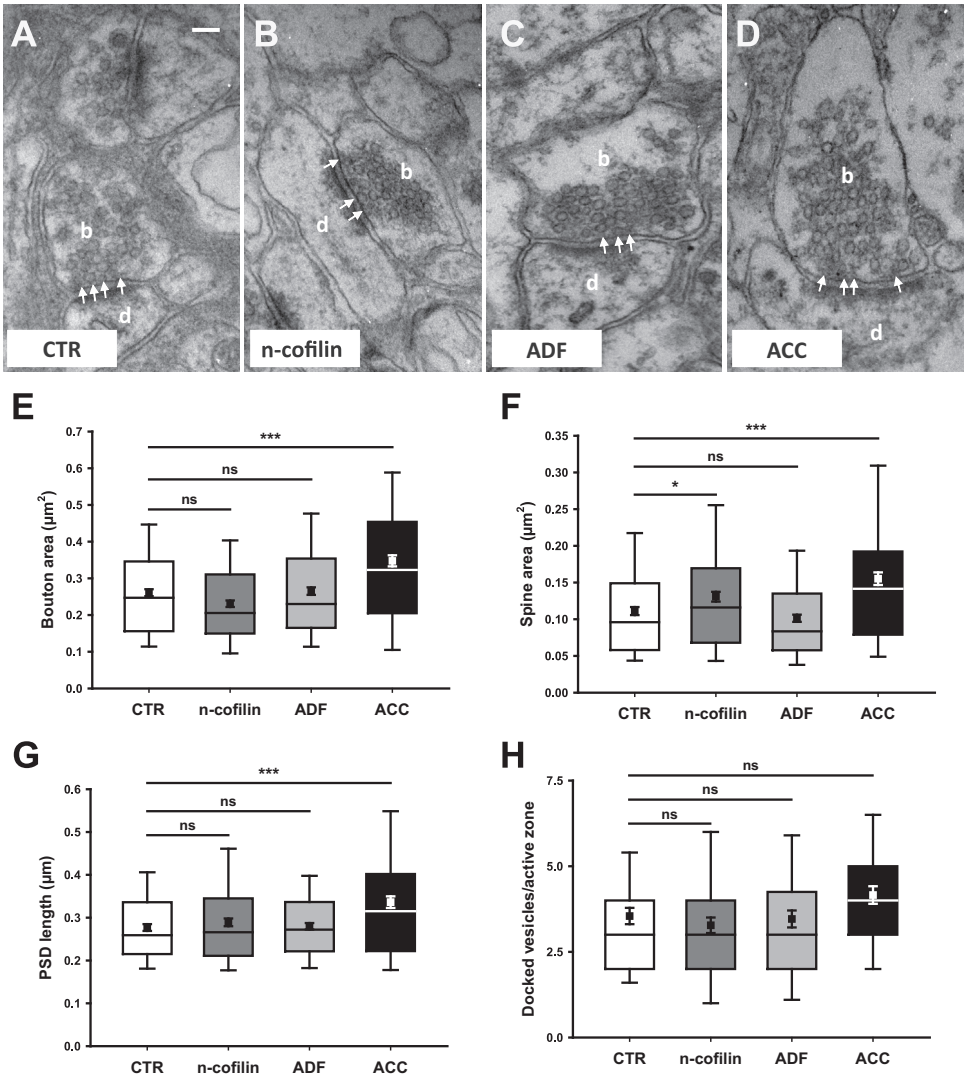

Figure 7. Altered morphology of striatal excitatory synapses in double mutant (ACC) mice. **(A–D)** Representative electron microscopy micrographs as used for the analysis of synapse morphology. b, presynaptic bouton; d, dendritic spine; arrows indicate docked vesicles. (**A,** Scale bar = 100 nm.) **(E)** Box plot showing enlarged bouton areas in ACC mice (control [CTR], .261 ± .009 μm^2; ACC, .348 ± .015 μm^2; $p \leq .001$). No such alteration was noted in n-cofilin or actin depolymerizing factor (ADF) mutants (n-cofilin, .231 ± .009 μm^2, $p = .071$; ADF, .265 ± .010 μm^2, $p = .962$). **(F, G)** Dendritic spine area (CTR, .111 ± .005 μm^2; ACC, .155 ± .008 μm^2; $p \leq .001$) and length of the postsynaptic density (PSD). (CTR, .277 ± .008 μm; ACC, .336 ± .013 μm; $p \leq .001$) were also increased in ACC mice. Dendritic spine area, but not PSD length, was increased in n-cofilin mutants (spine area, .130 ± .007 μm^2, $p \leq .05$; PSD length, .289 ± .009 μm, $p = .316$). No changes were noted in ADF mutants (spine area, .101 ± .004 μm^2, $p = .248$; PSD length, .279 ± .007 μm, $p = .788$). **(H)** The number of docked vesicles was increased by 17% in ACC mice (CTR, 3.55 ± .24 docked vesicles/active zone, $n = 55/3$; ACC, 4.16 ± .25 docked vesicles/active zone, $n = 44/3$). However, this increase did not reach statistical significance ($p = .083$). No such increase was noted for n-cofilin or ADF mutants (n-cofilin, 3.28 ± .25 docked vesicles/active zone, −8%, $n = 58/3$, $p = .357$; ADF, 3.460 ± .25 docked vesicles/active zone, −3%, $n = 50/3$, $p = .804$). ns, not significant; *$p \leq .05$; ***$p \leq .001$.

rescued the impulsive behavior of ACC mice in the elevated plus maze, suggesting that defects in certain neurotransmitter systems or neuronal circuits or both contribute differentially to the behavioral defects. A similar observation has been made for coloboma mice (42), an established ADHD model (43,44). Together, the neurobehavioral defects of ACC mice strongly resemble the symptoms observed in patients with ADHD (1). The neurobehavioral defects of ACC mice are very similar to defects described in ADHD mouse models, including DAT knockout or knockdown mice (4), GIT1 (G protein–coupled receptor kinase–interacting protein-1) knockout mice (31), or mice overexpressing casein kinase 1-δ (CK1δ) (27). Associative learning was impaired in DAT and GIT1 mutants (31,45), and anxiety was reduced in CK1δ-overexpressing mice (27), similar to what we reported previously for n-cofilin mutants (19,20). The behavioral abnormalities in ACC mice include all hallmarks of ADHD, suggesting that ACC mice are a valuable tool to study the mechanisms underlying ADHD.

How might inactivation of ADF and n-cofilin cause ADHD? Human genetics and mouse models suggest dopamine deregulation as causative for ADHD (3,4). Normal locomotion on blockade of dopamine receptors revealed a dopamine deregulation in ACC mice as well. In these mutants, ADF is systemically deleted, whereas deletion of n-cofilin is controlled by the expression of a CaMKII-Cre transgene (21). In these transgenic mice, Cre is not expressed during brain development, and expression in adults is largely restricted to the telencephalon. We found that Cre is not active in most SNc dopamine neurons, and we can conclude that altered behavior of ACC mice is not caused by developmental defects and most likely not caused by impaired function of SNc dopamine neurons. The fact that Cre expression does not start before the third postnatal week is likely to be responsible for the relatively late onset of hyperlocomotion in ACC mice.

How does loss of ADF and n-cofilin lead to a deregulation of dopamine? Our ultrastructural analyses revealed an altered density and morphology of striatal excitatory synapses in ACC

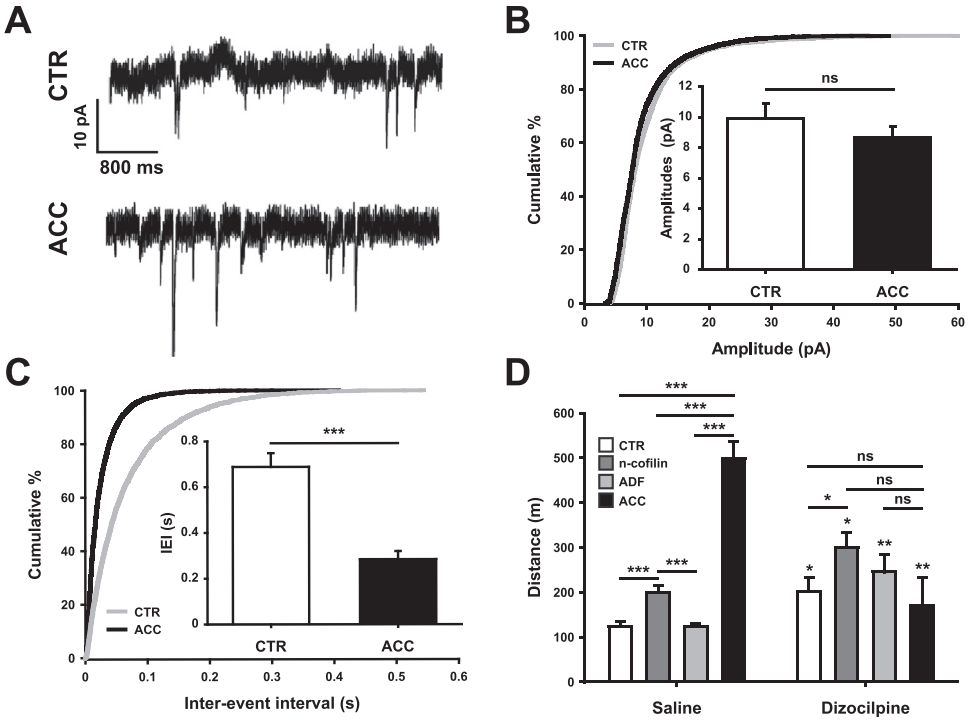

Figure 8. Blockade of glutamate transmission rescues locomotion in double mutant (ACC) mice. **(A)** Representative traces show spontaneous excitatory postsynaptic currents recorded from striatal medium spiny neurons of a control (CTR) and an ACC mouse. **(B)** Cumulative curves and mean values (inset) of spontaneous excitatory postsynaptic current amplitudes. No difference was found between CTR and ACC mice (CTR, 9.91 ± 1.01 pA, n = 8 neurons from 5 mice; ACC, 8.68 ± .72 pA, n = 7/3; p = .175). **(C)** Cumulative curves and mean values (inset) demonstrate a reduction in the interevent intervals of spontaneous excitatory postsynaptic currents in ACC mice (CTR, .688 ± .060 msec; ACC, .284 ± .035 msec; $p \leq .001$). **(D)** Injection of the *N*-methyl-D-aspartate receptor antagonist dizocilpine increased locomotion in both CTR and single mutants (CTR, 123.6 ± 10.4 m vs. 202.5 ± 31.6 m, n = 10, $p \leq .05$; n-cofilin, 200.2 ± 15.2 m vs. 303.1 ± 37.5 m, n = 10, $p \leq .05$; ADF, 124.0 ± 7.3 m vs. 245.5 ± 39.6 m, n = 8, $p \leq .01$) but reduced locomotion in ACC mice (498.2 ± 37.6 m vs. 171.3 ± 61.6 m, n = 5, $p \leq .01$). After treatment, locomotion was comparable between CTR and ACC mice but slightly increased in n-cofilin mutants. ns, not significant; *$p \leq .05$; **$p \leq .01$; ***$p \leq .001$.

mice. Specifically, we found a reduced density of excitatory synapses, together with enlarged presynaptic and postsynaptic structures, and elevated synaptic vesicle counts. Synapse density and morphology were unchanged in ADF mutants, and spine size was increased in n-cofilin mutants, similar to what we reported previously for hippocampal synapses (17,20). However, in contrast to in the hippocampus, synapse density, bouton size, and postsynaptic density length were unchanged in the striatum of n-cofilin mutants. This discrepancy could be explained either by region-specific functions of n-cofilin or by different levels of Cre activation in both brain regions. Although Cre was active in only 84% of all striatal neurons, we found active Cre in >97% of all CA1 pyramidal cells (M. Wolf, unpublished data, April 4, 2014). In ACC mice, the structural defects were accompanied by increased striatal glutamatergic activity in ACC mice, suggesting that ADF and n-cofilin have a crucial role in the control of synaptic transmission in central excitatory synapses. Glutamate and dopamine intimately interact at the level of medium spiny neurons (34), and the interaction is important for the control of behavior, including locomotion (46,47). Specifically, glutamate facilitates striatal dopamine release (36,37). It is plausible that the enhanced glutamate activity in the striatum of ACC mice facilitates dopamine release and ultimately results in the observed behavioral defects. In very good agreement with this idea, we found normalized locomotion on blockade of *N*-methyl-D-aspartate receptors in ACC mice. The idea of altered dopamine activity in ACC mice is further supported by the phenotypic similarities to DAT mutants (4), in which DAT inactivation results in a state of "functional hyperdopaminergia" that is responsible for behavioral impairment (48).

The activity of inhibitory neurons was unchanged in ACC mice, as indicated by unchanged amplitudes and interevent intervals of miniature inhibitory postsynaptic currents recorded from CA1 pyramidal cells (A. Görlich, PhD, unpublished data, April 4, 2014), a finding that is in very good agreement with the absence of Cre activity in inhibitory neurons of CaMKII-Cre transgenic mice (21). In ACC mice, the balance between excitation and inhibition (E/I balance) is shifted toward excitation. Maintenance of the E/I balance is crucial for the functionality of the brain (49), and E/I imbalance is postulated to underlie the pathogenesis of neuropsychiatric disorders, such as autism, schizophrenia or Tourette's syndrome (50–52). More recent studies have associated E/I imbalance with ADHD (31,53–55). In these studies, it is suggested that reduced neuronal inhibition contributes to ADHD pathogenesis. Our data not only support this idea but further suggest that an E/I imbalance can be causative for ADHD and that ADHD can result from increased excitation.

In conclusion, our study is the first to link ADHD to actin dynamics. Use of ACC mice allows novel insights into the mechanisms that cause ADHD. Our data also allow us to hypothesize that mutations in actin regulatory proteins contribute to the etiology of ADHD in humans.

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AG is currently affiliated with the Laboratory of Molecular Biology, Rockefeller University, New York, New York.

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ARTICLE INFORMATION

From the Neurobiology/Neurophysiology Group (A-MZ, TJ, MW, AG, MBR), University of Kaiserslautern, Kaiserslautern, Germany; Animal Physiology Group (EF), University of Kaiserslautern, Kaiserslautern, Germany; Institute of Genetics (CBG, WW), University of Bonn, Bonn, Germany; Department of Neuroscience (MS-P), University of Turin, Turin, Italy; and Institute of Physiological Chemistry (MBR), University of Marburg, Marburg, Germany

Address correspondence to Marco B. Rust, Ph.D., Molecular Neurobiology Group, Institute of Physiological Chemistry, University of Marburg, 35032 Marburg, Germany. E-mail: marco.rust@staff.uni-marburg.de.

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REFERENCES

1. Antshel KM, Hargrave TM, Simonescu M, Kaul P, Hendricks K, Faraone SV (2011): Advances in understanding and treating ADHD. BMC Med 9:72.
2. Biederman J, Faraone SV (2005): Attention-deficit hyperactivity disorder. Lancet 366:237–248.
3. Franke B, Faraone SV, Asherson P, Buitelaar J, Bau CH, Ramos-Quiroga JA, *et al*. (2012): The genetics of attention deficit/hyperactivity disorder in adults, a review. Mol Psychiatry 17:960–987.
4. Gainetdinov RR (2010): Strengths and limitations of genetic models of ADHD. Atten Defic Hyperact Disord 2:21–30.
5. Bakker SC, van der Meulen EM, Buitelaar JK, Sandkuijl LA, Pauls DL, Monsuur AJ, *et al*. (2003): A whole-genome scan in 164 Dutch sib pairs with attention-deficit/hyperactivity disorder: Suggestive evidence for linkage on chromosomes 7p and 15q. Am J Hum Genet 72: 1251–1260.
6. Fisher SE, Francks C, McCracken JT, McGough JJ, Marlow AJ, MacPhie IL, *et al*. (2002): A genomewide scan for loci involved in attention-deficit/hyperactivity disorder. Am J Hum Genet 70: 1183–1196.
7. Ogdie MN, Fisher SE, Yang M, Ishii J, Francks C, Loo SK, *et al*. (2004): Attention deficit hyperactivity disorder: Fine mapping supports linkage to 5p13, 6q12, 16p13, and 17p11. Am J Hum Genet 75:661–668.
8. Ogdie MN, Macphie IL, Minassian SL, Yang M, Fisher SE, Francks C, *et al*. (2003): A genomewide scan for attention-deficit/hyperactivity disorder in an extended sample: Suggestive linkage on 17p11. Am J Hum Genet 72:1268–1279.
9. Zhou K, Dempfle A, Arcos-Burgos M, Bakker SC, Banaschewski T, Biederman J, *et al*. (2008): Meta-analysis of genome-wide linkage scans of attention deficit hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 147B:1392–1398.
10. Bush G (2010): Attention-deficit/hyperactivity disorder and attention networks. Neuropsychopharmacology 35:278–300.
11. Cingolani LA, Goda Y (2008): Actin in action: The interplay between the actin cytoskeleton and synaptic efficacy. Nat Rev Neurosci 9: 344–356.
12. Rex CS, Chen LY, Sharma A, Liu J, Babayan AH, Gall CM, Lynch G (2009): Different Rho GTPase-dependent signaling pathways initiate sequential steps in consolidation of long-term potentiation. J Cell Biol 186:85–97.
13. Korobova F, Svitkina T (2010): Molecular architecture of synaptic actin cytoskeleton in hippocampal neurons reveals a mechanism of dendritic spine morphogenesis. Mol Biol Cell 21:165–176.
14. Morales M, Colicos MA, Goda Y (2000): Actin-dependent regulation of neurotransmitter release at central synapses. Neuron 27:539–550.
15. Bernstein BW, Bamburg JR (2010): ADF/cofilin: A functional node in cell biology. Trends Cell Biol 20:187–195.
16. Bellenchi GC, Gurniak CB, Perlas E, Middei S, Ammassari-Teule M, Witke W (2007): N-cofilin is associated with neuronal migration disorders and cell cycle control in the cerebral cortex. Genes Dev 21: 2347–2357.
17. Görlich A, Wolf M, Zimmermann AM, Gurniak CB, Al Banchaabouchi M, Sassoe-Pognetto M, *et al*. (2011): N-cofilin can compensate for the loss of ADF in excitatory synapses. PloS One 6:e26789.
18. Racz B, Weinberg RJ (2006): Spatial organization of cofilin in dendritic spines. Neuroscience 138:447–456.
19. Goodson M, Rust MB, Witke W, Bannerman D, Mott R, Ponting CP, *et al*. (2012): Cofilin-1: A modulator of anxiety in mice. PLoS Genet 8: e1002970.
20. Rust MB, Gurniak CB, Renner M, Vara H, Morando L, Gorlich A, *et al*. (2010): Learning, AMPA receptor mobility and synaptic plasticity depend on n-cofilin-mediated actin dynamics. EMBO J 29:1889–1902.
21. Minichiello L, Korte M, Wolfer D, Kuhn R, Unsicker K, Cestari V, *et al*. (1999): Essential role for TrkB receptors in hippocampus-mediated learning. Neuron 24:401–414.
22. Soriano P (1999): Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat Genet 21:70–71.
23. Giros B, Jaber M, Jones SR, Wightman RM, Caron MG (1996): Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379:606–612.
24. Zhuang X, Oosting RS, Jones SR, Gainetdinov RR, Miller GW, Caron MG, *et al*. (2001): Hyperactivity and impaired response habituation in hyperdopaminergic mice. Proc Natl Acad Sci U S A 98:1982–1987.
25. Fadda P, Bedogni F, Fresu A, Collu M, Racagni G, Riva MA (2007): Reduction of corticostriatal glutamatergic fibers in basic fibroblast growth factor deficient mice is associated with hyperactivity and enhanced dopaminergic transmission. Biol Psychiatry 62:235–242.
26. Salahpour A, Ramsey AJ, Medvedev IO, Kile B, Sotnikova TD, Holmstrand E, *et al*. (2008): Increased amphetamine-induced hyperactivity and reward in mice overexpressing the dopamine transporter. Proc Natl Acad Sci U S A 105:4405–4410.
27. Zhou M, Rebholz H, Brocia C, Warner-Schmidt JL, Fienberg AA, Nairn AC, *et al*. (2010): Forebrain overexpression of CK1delta leads to down-regulation of dopamine receptors and altered locomotor activity reminiscent of ADHD. Proc Natl Acad Sci U S A 107:4401–4406.
28. Walaas SI, Greengard P (1984): DARPP-32, a dopamine- and adenosine 3′:5′-monophosphate-regulated phosphoprotein enriched in dopamine-innervated brain regions. I. Regional and cellular distribution in the rat brain. J Neurosci 4:84–98.
29. Le Novere N, Li L, Girault JA (2008): DARPP-32: Molecular integration of phosphorylation potential. Cell Mol Life Sci 65:2125–2127.
30. Avale ME, Falzone TL, Gelman DM, Low MJ, Grandy DK, Rubinstein M (2004): The dopamine D4 receptor is essential for hyperactivity and impaired behavioral inhibition in a mouse model of attention deficit/hyperactivity disorder. Mol Psychiatry 9:718–726.
31. Won H, Mah W, Kim E, Kim JW, Hahm EK, Kim MH, *et al*. (2011): GIT1 is associated with ADHD in humans and ADHD-like behaviors in mice. Nat Med 17:566–572.
32. Chen Q, Kogan JH, Gross AK, Zhou Y, Walton NM, Shin R, *et al*. (2012): SREB2/GPR85, a schizophrenia risk factor, negatively regulates hippocampal adult neurogenesis and neurogenesis-dependent learning and memory. Eur J Neurosci 36:2597–2608.
33. Gainetdinov RR, Wetsel WC, Jones SR, Levin ED, Jaber M, Caron MG (1999): Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. Science 283:397–401.
34. Girault JA (2012): Integrating neurotransmission in striatal medium spiny neurons. Adv Exp Med Biol 970:407–429.
35. Tepper JM, Tecuapetla F, Koos T, Ibanez-Sandoval O (2010): Heterogeneity and diversity of striatal GABAergic interneurons. Front Neuroanat 4:150.
36. West AR, Floresco SB, Charara A, Rosenkranz JA, Grace AA (2003): Electrophysiological interactions between striatal glutamatergic and dopaminergic systems. Ann N Y Acad Sci 1003:53–74.

37. David HN, Ansseau M, Abraini JH (2005): Dopamine-glutamate reciprocal modulation of release and motor responses in the rat caudate-putamen and nucleus accumbens of "intact" animals. Brain Res Brain Res Rev 50:336–360.
38. Pilo Boyl P, Di Nardo A, Mulle C, Sassoe-Pognetto M, Panzanelli P, Mele A, *et al*. (2007): Profilin2 contributes to synaptic vesicle exocytosis, neuronal excitability, and novelty-seeking behavior. EMBO J 26:2991–3002.
39. Kovacic P, Somanathan R (2010): Clinical physiology and mechanism of dizocilpine (MK-801): Electron transfer, radicals, redox metabolites and bioactivity. Oxid Med Cell Longev 3:13–22.
40. Tipper SP, Bourque TA, Anderson SH, Brehaut JC (1989): Mechanisms of attention: A developmental study. J Exp Child Psychol 48: 353–378.
41. Cohen RA, Sparling-Cohen YA (1993): Contributions of Behavioral Psychology to the Study of Attention. In: Cohen RA, editor. The Neuropsychology of Attention. NY: Plenum, 75–93.
42. Bruno KJ, Freet CS, Twining RC, Egami K, Grigson PS, Hess EJ (2007): Abnormal latent inhibition and impulsivity in coloboma mice, a model of ADHD. Neurobiol Dis 25:206–216.
43. Russell VA, Sagvolden T, Johansen EB (2005): Animal models of attention-deficit hyperactivity disorder. Behav Brain Funct 1:9.
44. Arime Y, Kubo Y, Sora I (2011): Animal models of attention-deficit/hyperactivity disorder. Biol Pharm Bull 34:1373–1376.
45. Morice E, Billard JM, Denis C, Mathieu F, Betancur C, Epelbaum J, *et al*. (2007): Parallel loss of hippocampal LTD and cognitive flexibility in a genetic model of hyperdopaminergia. Neuropsychopharmacology 32:2108–2116.
46. Mohn AR, Gainetdinov RR, Caron MG, Koller BH (1999): Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. Cell 98:427–436.
47. Gainetdinov RR, Mohn AR, Bohn LM, Caron MG (2001): Glutamatergic modulation of hyperactivity in mice lacking the dopamine transporter. Proc Natl Acad Sci U S A 98:11047–11054.
48. Gainetdinov RR, Jones SR, Caron MG (1999): Functional hyperdopaminergia in dopamine transporter knock-out mice. Biol Psychiatry 46:303–311.
49. Sudhof TC (2008): Neuroligins and neurexins link synaptic function to cognitive disease. Nature 455:903–911.
50. Deutsch SI, Rosse RB, Schwartz BL, Mastropaolo J (2001): A revised excitotoxic hypothesis of schizophrenia: Therapeutic implications. Clin Neuropharmacol 24:43–49.
51. Kalanithi PS, Zheng W, Kataoka Y, DiFiglia M, Grantz H, Saper CB, *et al*. (2005): Altered parvalbumin-positive neuron distribution in basal ganglia of individuals with Tourette syndrome. Proc Natl Acad Sci U S A 102:13307–13312.
52. Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, *et al*. (2007): A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science 318:71–76.
53. Moll GH, Heinrich H, Trott G, Wirth S, Rothenberger A (2000): Deficient intracortical inhibition in drug-naive children with attention-deficit hyperactivity disorder is enhanced by methylphenidate. Neurosci Lett 284:121–125.
54. Moll GH, Heinrich H, Rothenberger A (2003): Methylphenidate and intracortical excitability: Opposite effects in healthy subjects and attention-deficit hyperactivity disorder. Acta Psychiatr Scand 107:69–72.
55. Richter MM, Ehlis AC, Jacob CP, Fallgatter AJ (2007): Cortical excitability in adult patients with attention-deficit/hyperactivity disorder (ADHD). Neurosci Lett 419:137–141.