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Selective knockout of the CK2 kinase in D1 medium spiny neurons controls dopaminergic function

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Abstract

Background—Dopamine, crucial for the regulation of motor function and reward, acts through receptors mainly expressed in striatum as well as cortex. Dysregulation of dopaminergic signaling is associated with various neuropsychiatric disorders. Consequently, dopamine-regulating drugs are effectively used in treating these disorders, such as L-DOPA for PD, methylphenidate for ADHD or antipsychotics for schizophrenia. As a result, there has been much interest in dissecting signaling networks in the two morphologically indistinguishable D1- and D2-receptor-expressing medium spiny neurons. Our previous results highlighted a role for CK2 kinase in the modulation of dopamine D1 receptor signaling in cells.

Methods—To study the importance of CK2 *in vivo*, we have selectively knocked out CK2, in either D1- or D2-MSNs and characterized the mice behaviorally and biochemically (N=4-18).

Results—The D1-MSN knockout mice exhibited distinct behavioral phenotypes including novelty-induced hyper-locomotion and exploratory behavior, defective motor control and motor learning. All of these behavioral traits are indicative of dysregulated dopamine signaling and the underlying mechanism appears to be an alteration of D1 receptor signaling. In support of this hypothesis, D1R levels were up-regulated in the knockout mice, as well as phosphorylation of DARPP-32, most of the behavioral phenotypes were abolished by the D1R antagonist, SCH23390, and the D2-MSN knockout mice displayed no obvious behavioral phenotype.

Conclusions—Thus a single kinase, CK2, in D1-MSNs significantly alters dopamine signaling, a finding which could have therapeutic implications for disorders characterized by dopamine imbalance such as Parkinson's disease (PD), attention deficit hyperactive disorder (ADHD) and schizophrenia.

Keywords

CK2; GPCR; Dopamine receptor; Medium spiny neurons; hyperactivity; dopamine

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Introduction

Dopamine (DA), a neurotransmitter present from vertebrates to *Drosophila melanogaster* and *Caenorhabditis elegans*, is involved in the regulation of movement, attention, reward and motivation (1, 2). DA neurons control the activity of the major output neurons from the striatum, the so-called medium spiny neurons (MSNs), which project either directly to the substantia nigra pars reticulata (direct striatonigral pathway), or indirectly to the substantia nigra via the pallidum and sub-thalamic nuclei (indirect striatopallidal pathway). The striatonigral pathway is characterized by expression of the D1 receptor (D1R) and its effectors, the G-proteins $G_{\alpha_{olf}}$, to a minor extent G_{α_s} , adenylyl cyclase and protein kinase A (PKA) which phosphorylates, amongst others, the striatal integrator DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of 32 kDa) (3-5). In contrast, D2-like receptor signaling in the striatopallidal pathway is mediated by G_{α_i} and G_{α_o} which inhibit adenylyl cyclase and reduce DARPP-32 signaling (6, 7). CK2 (casein kinase 2) is a ubiquitously expressed kinase present in high levels in brain (8, 9) and it appears to be constitutively active. However, its function in neurons is still poorly understood and, so far, there is no clear consensus about a potential mode for its regulation reviewed in (10).

We have previously studied the role of the CK2 in dopaminergic signaling (11-13). DARPP-32-Ser97 is one of several neuronal substrates for CK2 (11, 14), that also include alpha-synuclein, synphilin-1 (15) and tau (16). Using cell culture models, we have previously shown that CK2 negatively controls dopamine D1 receptor signaling (13). CK2 directly interacts with G_{α_s} or $G_{\alpha_{olf}}$ and inhibition or knockdown of CK2 leads to an elevated plasma membrane level of D1R, elevated cAMP generation, and PKA activation, in response to D1 agonists.

Studying CK2 *in vivo*, in particular in the brain, has however been difficult since even potent CK2 inhibitors (e.g. DMAT or TBB) are not adequately specific and do not permeate the blood-brain barrier. CK2 is a heterotetrameric enzyme consisting of two catalytic subunits (α and/or α') and two regulatory subunits (β) (17). Full knockout (KO) mice for CK2 α , α' and β isoforms have been made: CK2 α KO mice are embryonic lethal (18). CK2 β KO animals are also embryonic lethal (19). The CK2 α' full knockout is viable and does not exhibit obvious defects, with the exception of male sterility (20). Therefore, to study the role of CK2 in the brain and to address the function of CK2 in D1-MSNs versus D2-MSNs, specific conditional KO models were needed. Here we characterize, using biochemical, behavioral and pharmacological tools, knockout mouse lines where the catalytic α subunit of CK2 has been ablated in D1-MSNs or D2-MSNs. The *Drd1a*-Cre-CK2 α KO mice, in contrast to the *Drd2*-Cre-CK2 α KO mice, exhibit complex behavioral and biochemical changes indicative of a profound dopamine dysregulation.

Materials and Methods

Animals

We generated the floxed CK2 α and CK2 α' lines and crossed these with the *Gensat* *Drd1a*-Cre, *Drd*-Cre and *Drd1a*-EGFP mice as further described in Supplement 1. All animals were sacrificed using focused microwave irradiation. Animal use and procedures were in accordance with NIH guidelines and approved by the Rockefeller University IACUC.

Western blot analysis

Lysate preparation, Western blotting analysis as well as antibody manufacturers are described in Supplement 1.

cAMP assay

The concentration of cAMP from striatal lysates was determined according to the manufacturer's instructions (Assay Designs).

Preparation, incubation and processing of neostriatal slices

Experiments using neostriatal slices were performed as described (21) and also briefly described in Supplement 1.

Immunofluorescence microscopy

Mice were transcardially perfused with PBS, incubated overnight in sucrose, and sliced at a thickness of 40 μ m. Alexa Fluor labeled secondary antibodies were used to detect CK2 α -, GFP-, DARPP-32-antibodies as described in Supplementary Material. A Zeiss LSM510 microscope was used.

Behavioral assays

Locomotion, rotarod analysis, novelty-suppressed feeding, modified novel object and pole tests were performed as written in Supplementary Material. Tail suspension and forced swim tests were performed as published (22) and further described in Supplement 1.

Results

Generation of CK2 knockout mouse lines

We generated floxed CK2 α , CK2 α' , and CK2 α/α' mice which we first crossed with a mouse line in which Cre was expressed in the postnatal forebrain under the control of the CaMKII α promoter (23). However, neither the CaMKII α -Cre-CK2 $\alpha^{fl/fl}$ nor the CaMKII α -Cre-CK2 $\alpha^{fl/fl}/\alpha'^{fl/fl}$ mice were viable highlighting the importance of CK2 in the forebrain. Indeed, the mice died at birth or very shortly after birth. The CaMKII α -Cre-CK2 $\alpha'^{fl/fl}$ KO mice exhibited no obvious biochemical or behavioral phenotype, which is likely a reflection of the lower abundance of CK2 α' in brain compared to CK2 α (10). Cell-specific knockout was achieved by crossing the floxed animals with the Drd1a-Cre or the Drd2-Cre driver lines (24). Notably, the double KOs, homozygous for CK2 α and α' deletion, were not viable with any of the Cre-driver lines tested. However, the single KOs homozygous for CK2 α or α' isoforms were viable with both the Drd1a-Cre and the Drd2-Cre driver lines. Due to the high abundance ratio of CK2 α/α' in the brain (10), we chose to focus on genetic ablation of CK2 α in D1- and D2-MSNs.

The level of CK2 α protein expression in striatum from Drd1a-Cre-CK2 α KO mice was reduced by 59% compared to wildtype mice (Fig. 1A). The level of the regulatory β subunit was not affected by CK2 α KO (Fig. 1B). However, there was a small increase in expression of CK2 α' (~40%) in the Drd1a-Cre-CK2 α KO mice, indicating a compensatory mechanism in the absence of the CK2 α isoform (Fig. 1C). The level of CK2 α protein expression in striatum from Drd2-Cre-CK2 α KO mice was reduced by 50% (Fig. 1D). Similarly, the level of the regulatory β subunit was significantly reduced (by 58%) by CK2 α KO in the striatopallidal cells, indicating an adaptive downregulation of CK2 β in these cells but not in the cells of the direct pathway (Fig. 1E). In contrast, expression of CK2 α' was not altered in the Drd2-Cre-CK2 α KO mice (Fig. 1F). We also crossed the Drd1a-Cre-CK2 α conditional KO mice with a Drd1a-EGFP reporter line (24). Immunohistochemical analysis of coronal striatal slices confirmed the functionality of the Drd1a-Cre driver: while in the control animals, the GFP signal co-localized with the signal from CK2, in the Drd1a-Cre-CK2 α KO mice, the GFP-stained cells did not express CK2 α (Fig. 1G).

Drd1a-Cre-CK2 α knockout mice exhibit increased locomotor activity

Since the D1R pathway is strongly involved in locomotor control, we were particularly interested in testing the knockout mice behaviorally. First, basal locomotor activity of the Drd1a-Cre-CK2 α KO mice was recorded for 1 hr and analyzed in horizontal, vertical activity and stereotypy categories. The Drd1a-Cre-CK2 α KO mice exhibited hyperactivity under basal conditions compared to wildtype mice, especially in the first 30 min of a 60 min exposure to the open field arena (Fig. 2A). Stereotypy was also elevated in the Drd1a-Cre-CK2 α KO mice (Fig. 2B). Based on visual observation, the Drd1a-Cre-CK2 α KO mice exhibited repeated jumping (not shown); however overall vertical activity was unaltered in Drd1a-Cre-CK2 α KO mice (Fig. 2C). Thigmotaxis in these mice was normal, indicating that hyperlocomotion was not caused by changes in anxiety level (Fig. 2D). In line with this finding, the Drd1a-Cre-CK2 α KO mice behaved normally in elevated plus maze and light-dark choice tests (Fig. S1 in Supplement 1). Interestingly, overnight, after initial habitation to the new environment in the open field box, the KO mice were slightly less active than their control littermates (data not shown). This finding strengthens the fact that the elevated locomotor activity observed is due to the novel environment and not due to a general hyperlocomotive phenotype. In contrast, the Drd2-Cre-CK2 α KO mice did not exhibit changes in locomotor behaviour with the exception of briefly reduced horizontal activity in the first 5 min of exposure to the novel environment (Fig. 2E). Vertical activity, stereotypy and thigmotaxis were not altered in the Drd2-Cre-CK2 α KO mice (Fig. 2F, G, H).

The observed abnormal elevated locomotive behavior in the knockout mice could conceivably reflect an enhanced motor function or balance. Thus, we tested the mice in the rotarod test over three consecutive days. The Drd1a-Cre-CK2 α KO mice showed impaired or reduced function, both in basal motor function as well as in the ability to learn the accelerated rotarod task (Fig. 2I). In contrast, the Drd2-Cre-CK2 α KO mice did not exhibit significant altered performance on the accelerated rotarod test, indicating that the presence of CK2 in the D1-MSNs but not in the D2-MSNs is required for correct motor performance and learning (Fig. 2J). This finding was further confirmed in the pole test in which the knockout mice performed significantly worse than their control littermates (Fig. 2K, L). While it cannot be entirely excluded that the motor defects in the Drd1a-Cre-CK2 α KO are caused by the hyperactivity phenotype, we believe it is safe to assume, due to our findings that KO have a motor deficit, that the hyperactivity phenotype is not caused by changes in motor function.

Drd1a-Cre-CK2 α knockout mice exhibit increased exploratory behavior

In addition to controlling movement through its action on the dopamine receptors located in the dorsolateral striatum, dopamine has been linked to motivation, heightened responses to and a lack of habituation to novelty (25). This led us to explore the possibility of an exploratory phenotype underlying the hyperactivity of Drd1a-Cre-CK2 α KOs. One paradigm to test for exploratory behavior is a modified novel object test (26, 27). The Drd1-Cre;CK2 α KO mice spent significantly more time in the vicinity of the introduced novel object when compared to littermates which did not explore the novel object significantly (Fig. 3A). The strong locomotive response observed after introduction of the novel object clearly indicates that both genotypes noticed the presence of the novel object (Fig. 3B). In contrast, the Drd2-Cre-CK2 α KO mice did not exhibit any changes in exploratory behavior after introduction of the object compared to wildtype littermates (Fig. 3C, D).

Drd1a-Cre-CK2 α knockout mice exhibit reduced depression-like behavior

We next tested Drd1-Cre;CK2 α KO mice in the novelty suppressed feeding test. The knockout animals showed significantly reduced latency to feed when compared to wildtype

littermates (Fig. 4A). This was not due to an increase of appetite since food consumption after *ad libitum* access was not significantly changed (Fig. 4B).

In addition to addressing exploratory behavior, the novelty-suppressed feeding test is also a measure of anxiety and depression-like phenotypes (28, 29). Thus, we also tested the *Drd1a*-Cre-CK2 α KO mice in forced swim as well as the tail suspension tests of behavioral despair. Knockout mice exhibited significantly decreased immobility time in both tests (Fig. 4C, D). In contrast, the *Drd2*-Cre-CK2 α KO mice did not show altered immobility time in the forced swim test (Fig. 4E). We also tested knockout mice in paradigms that address anxiety phenotypes, such as thigmotaxis, elevated plus maze or light-dark choice tests and no difference was detected between the genotypes (Fig. S1A, B, C in Supplement 1). Taken together, the *Drd1a*-Cre-CK2 α KO mice show an anti-depressive phenotype with no changes in the anxiety level.

Elevated D1 receptor signaling in *Drd1a*-Cre-CK2 α knockout mice

To further assess the mechanisms involved in the altered behavioural responses seen in the *Drd1a*-Cre-CK2 α KO mice, we carried out a number of biochemical experiments to assess D1 receptor signalling. To examine whether in the *Drd1a*-Cre-CK2 α KOs, changes have occurred at the level of receptor protein, total striatal lysates from microwave-irradiated brains were used for western blotting analysis. We found that D1R levels were elevated in the D1RCre-CK2 α knockout mice (Fig. 5A) while D2R and G_{olf} levels were unaltered (Fig. 5B, C). Interestingly the level of A2aR is increased in the D2RCre-CK2 α knockout mice while D2R and G_{olf} are not affected (Fig. 5D, E, F). The change in D1R protein level must occur via a post-translational mechanism since RT-PCR indicated that D1R mRNA levels were not altered (Fig. 6A). The level of cAMP measured by ELISA exhibited a slight but significant elevation in the striata of D1RCre-CK2 α KO mice (Fig. 6B). However, despite the slight increase in cAMP, the basal level of pT34 DARPP-32 in striatum was not altered significantly *in vivo* (data not shown). Basal phosphorylation of T34-DARPP-32 was also unaffected in neostriatal slices, but pT34 DARPP-32 levels were significantly elevated in slices from *Drd1a*-Cre-CK2 α KO mice compared to wildtype mice after 5 min of incubation with the D1R agonist SKF81297 (Fig. 6C). Similar results were observed after 15 min incubation with SKF81297 (data not shown). Thus, the biochemical data indicated that the D1 receptor pathway is more sensitized in the *Drd1a*-Cre-CK2 α KO animals.

To ensure that CK2 activity was reduced *in-vivo*, in the striatum, we examined the DARPP-32 phosphorylation at S97, the site known to be phosphorylated by CK2. Our previous studies have shown that S97 phosphorylated to a high level by CK2 under basal conditions (14). In the striatum of *Drd1a*-Cre-CK2 α conditional KOs there was a strong reduction in phosphorylation of S97-DARPP-32 (Fig. 6D).

Altered behavioral phenotypes in *Drd1a*-Cre-CK2 α knockout mice are attenuated by the D1 receptor antagonist SCH23390

Taken together with the biochemical changes found in the *Drd1a*-Cre-CK2 α KO mice, the behavioural effects observed in the *Drd1a*-Cre-CK2 α KO mice may result from enhanced D1 receptor signaling in D1-MSNs. In support of this hypothesis, when mice were pre-treated with D1 antagonist SCH23390 (0.03 mg/kg) for 10 min before open field exposure, the hyperactivity was abolished and motor activity restored to wild type levels (Fig. 7A). In addition, SCH23390 injection abolished the difference between wild-type and KO animals seen in the time spent exploring a novel object (Fig. 7B). In the presence of SCH23390 the motor defect observed in KO animals were partially rescued (Fig. 7C). While the KO animals are still not able to learn significantly, the differences between the individual

experimental points for the two genotypes were abolished in the presence of SCH23390 (Fig. 7C vs. Fig. 2I).

It has been shown that D1R antagonist SCH23390 reduced swimming activity in the forced swim test (30) and also acts antagonistically towards the anti-depressive effect of enkephalins (31) and dopamine re-uptake inhibitors bupropion and nomifensine (32). We therefore tested the KO and wild-type littermates in the presence of SCH23390 in the forced swim and tail suspension tests. In both tests, the presence of SCH23390 did not affect immobility time of the wild-type animals but elevated it in the *Drd1a-Cre-CK2α* KO mice, thereby rescuing the difference between the genotypes (see Fig 4A, B).

We also assessed the responsiveness of the knockout animals to a D1R agonist. Several doses of SKF81297 (1, 2.5, 5 and 20 mg/kg, i.p. in 0.9% NaCl) were injected 10 min prior to exposure to the open field boxes. No enhanced locomotive response was detected at 1 mg/kg for either genotype. At 2.5 mg/kg a slight response was observed in both genotypes. At 5 mg/kg, the behavioral response to SKF81297 in the *Drd1a-Cre-CK2α* KOs was delayed, with no apparent change in the amplitude of the locomotive response compared to wild-type littermates (Fig. S2A in Supplement 1). This effect was also observed at a dose of 20 mg/kg (data not shown). Induction of stereotypy did not show this delayed response (Fig. S2B in Supplement 1).

To investigate whether A2aR signalling is altered in the *Drd2-Cre-CK2α* KO mice, we tested the behavioral response to caffeine, an A2aR antagonist. At 5mg/kg the *Drd2-Cre-CK2α* KO responded with significantly elevated horizontal activity when compared to wild type littermates (Fig. 7E). No significant difference was observed at 1mg/kg and 3mg/kg.

Since it had been shown that CK2 phosphorylates the NR2B subunit of the NMDA receptor (33) and interacts with the D1R (34), we also studied if glutamate receptor levels were altered in the *Drd1a-Cre-CK2α* KO mice. Neither NR2a, NR2B or NR1 subunit protein levels were significantly altered (Fig. S3A, B in Supplement 1).

We also tested the *Drd1a-Cre-CK2α* KO in a cocaine sensitization, withdrawal and challenge paradigm. While the KO mice are always more hyperactive in response to cocaine compared to their wildtype littermates, no changes in sensitization could be detected (Fig. S4 in Supplement 1). The ratio between cocaine-induced and basal activity is not changed in the KO mice.

Discussion

The availability of specific BAC-Cre mouse lines is greatly advancing our understanding of the functions of defined populations of neurons, as well as helping elucidate specific signaling pathways within these defined neuronal cell types (35-37). In the current study we took advantage of targeted expression of Cre recombinase to investigate the role of CK2 in the direct and indirect output neurons from the striatum, the MSNs that selectively express D1- or D2-type receptors, respectively.

CK2α is the predominant catalytic isoform in the brain. When knocked out in the D1R-MSN, we observed an upregulation of the alternate catalytic subunit CK2α' in striatal lysate. This did not occur in the *Drd2-Cre-CK2α* KO mice. However, in the *Drd2-Cre-CK2α* KO mice, the regulatory β subunit was downregulated as a response to the CK2α knockout. This indicates differences in the regulation of the subunit composition in the different striatal cell types. One could also speculate that substrates and binding partners of CK2 may be not completely overlapping. It has been shown that CK2β can act as monomer or as a regulatory

subunit to other kinases (38, 39), and therefore it may depend less or more on the presence of the catalytic CK2 α subunit in the different cell types.

When a wild-type animal is introduced to a non-familiar environment, an array of behavioral traits, such as walking, rearing or leanings against walls are triggered (40). Such exploratory behavior or heightened motivation is associated with elevated dopamine levels (41-43). Phenotypically, the Drd1a-Cre-CK2 α KO mice (but not the Drd2-Cre-CK2 α KO mice) displayed novelty-induced hyper-locomotion, exploratory behavioral differences (reduced latency to feed in the novelty-suppressed feeding test and heightened exploration in a modified novel object test). Thigmotaxis was normal in the Drd1a-Cre-CK2 α KO mice suggesting that the heightened locomotor response was not caused by changes in anxiety level. The Drd1a-Cre-CK2 α KO mice also exhibited motor deficiencies. Together, these various results suggest that the elevated locomotor activity observed in the Drd1a-Cre-CK2 α KO mice is due to the novel environment more than to a general hyperlocomotive phenotype. Interestingly, in a variety of other knockout or transgenic mice, where dopamine transduction and/or concentration is lowered, such as in tyrosine hydroxylase (TH), D1-, D2-, D4-KO mice, locomotor activity (horizontal or vertical) is reduced, possibly because of a decreased motivational level (1, 42, 44-46). Remarkable, the novelty-induced hyper-locomotion in the Drd1a-Cre-CK2 α KO mice is reminiscent of the phenotype of the dopamine transporter (DAT) knockdown mice in which reduced dopamine clearance is associated with hyperactivity and impaired response habituation in novel environments (47) but unaltered home cage activity. Thus, the observed phenotypes of the Drd1a-Cre-CK2 α KO mice could be indicative of a pre-synaptic mechanism, involving enhanced dopamine release or reduced clearance. Alternatively, the phenotypes could result from enhanced postsynaptic mechanisms where signaling downstream of the D1 receptor is activated.

Our previous *in vitro* studies have found that CK2 directly interacts with Gas or Gaolf, leading to a suppression of D1 receptor signaling (13). Consistent with these results, we now show that D1 receptor levels are increased in striatum in Drd1a-Cre-CK2 α KO mice. Furthermore, these KO mice exhibit elevated basal cAMP levels and increased signaling via PKA-mediated phosphorylation of DARPP-32 at T34. This suggest that selective KO of CK2 in D1-MSNs leads to changes in D1 signaling indicating that postsynaptic changes are most likely responsible in mediating the expression of the behavioral phenotypes.

To assess the role of increased D1 receptor signaling we analyzed the effects of exposure of the Drd1a-Cre-CK2 α KO mice to the D1R antagonist SCH23390. Notably, SCH23390 reversed all of the aforementioned behavioral phenotypes, confirming that the D1R pathway is hyper-activated in these mice and directly linked to the phenotypes described. However, this is perhaps less clear for the motor defects tested in the rotarod paradigm, due to the slight variation observed between the two wildtype cohorts in the two sets of experiments, especially for their capacity to learn in the earlier trials.

Drd1a-Cre-CK2 α KO mice displayed reduced immobility times in Porsolt's behavioral despair forced swim and tail suspension tests which are used to measure the effects of anti-depressant drugs. SCH23390 antagonized these anti-depressive-like phenotypes. Dopamine has been implicated in the etiology and treatment of depression (48). Depressed patients have reduced cerebrospinal levels of the major metabolite of dopamine, homovanillic acid (49, 50). Neuroimaging studies of medication-free depressed patients have found decreased ligand binding to the dopamine transporter and increased dopamine binding in the caudate and putamen, pointing towards a functional deficiency of synaptic dopamine in depressed patients (51). Wild-type mice exhibiting "learned helplessness" show striatal dopamine depletion (Dunlop and Nemeroff, 2007). In the forced swim test, immobility can be reduced by the dopamine/norepinephrine re-uptake inhibitor nomifensine as well as by tricyclic

antidepressants (32). How much the novelty-induced hyperactivity phenotype in the KO contributes to our findings in the Porsolt tests needs to be addressed in depth. Nevertheless, inhibition of CK2 may be a potential target for development of anti-depressant therapies.

Taken together, the data suggest that the phenotypes of hyperactivity, elevated exploration/motivation, motor performance deficits and perhaps the less depressive phenotype, too, are primarily caused by a hyper-activation of the D1R pathway in the Drd1a-Cre-CK2 α KO mice. Genetic ablation of CK2 using Cre recombinase under the control of the Drd1 promoter will lead to CK2 knockout in MSNs of the striatum, as well as in cortical layers 5 and 6. Therefore the behavioral phenotypes observed may be mediated through cortical and/or striatal involvement. We detected significant biochemical changes in D1 receptor signaling pathways in the striatum, supporting the hypothesis that alterations in D1-MSNs in striatum are responsible for the behavioral changes observed in Drd1a-Cre-CK2 α KO mice. It has been proposed that cortical D1Rs act antagonistically to striatal D1Rs, based on the findings that the D1 antagonist SCH23390, injected into the prefrontal cortex, enhances locomotion (52) and that in mice overexpressing D1R in the medial prefrontal cortex (PFC), SKF81297 inhibits locomotion (53). One further indication that D1R-MSNs, and not neurons of the PFC, mediate the effect, comes from the recent finding that diphtheria toxin mediated ablation of specific populations of striatonigral neurons reduces exploration of a novel object (54). Therefore, our results would support the conclusion that the striatum is primarily involved in causing the described phenotypes in the Drd1a-Cre-CK2 α knockout mice.

Altogether, this study highlights the importance of using cell-type specific knockout models. We find that loss of an ubiquitous kinase, CK2 α , in a single cell-type, the D1 MSNs (but not or much less in the D2 MSNs), has a profound impact *in vivo*. The described findings open new avenues for understanding the etiology and the clinical management of disorders characterized by dopamine imbalance such as Parkinson's disease or ADHD and point towards a possible therapeutic role CK2 for neuropsychiatric disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig.1. Biochemical characterization of conditional CK2α knockout mice

Total striatal protein lysates from Drd1a-Cre;CK2α^{fl/fl} knockout (KO), Drd2-Cre;CK2α^{fl/fl} knockout (KO) and their littermate CK2α^{fl/fl} control (WT) mice were separated by SDS/PAGE. Immunoblotting analysis with anti-CK2α antibody for the Drd1a-Cre;CK2α^{fl/fl} and Drd2-Cre;CK2α^{fl/fl} (A, D), anti-CK2β antibody (B, E) or anti-CK2α' (C, F), was performed. N= 8 (for Drd1a-Cre;CK2α^{fl/fl} and WT littermates) and N=7 for Drd2-Cre;CK2α^{fl/fl} and WT littermates; statistical analysis was performed using unpaired t test, error bars indicate SEM (***, $P < 0.001$; **, $P < 0.01$). (G) Conditional ablation of CK2α in D1 receptor expressing neurons. Neuron-specific ablation of CK2α was achieved by breeding CK2α^{fl/fl} mice with Drd1a-Cre mice. The resulting offspring were then crossed to a Drd1a-GFP reporter line to generate Drd1a-GFP;Drd1a-Cre;CK2α^{fl/fl} mice. Deficiency of CK2α in Drd1a-expressing neurons of the striatum was confirmed by comparative immunohistochemical analysis of CK2α (red) and GFP (green) protein expression in the striata of 3 to 4 months-old Drd1a-GFP;Drd1a-Cre;CK2α^{fl/fl} mice and control mice (Drd1a-GFP;CK2α^{fl/fl}). Arrows indicate D1R-expressing, GFP labeled cells.

Fig. 2. Locomotor performance of the *Drd1a-Cre;CK2α^{fl/fl}* knockout mice

Locomotor activity in 3-months-old *Drd1a-Cre;CK2α^{fl/fl}* (KO) or WT mice (A) or *Drd2-Cre;CK2α^{fl/fl}* (KO) or WT mice (E) was recorded using an open-field paradigm for 60 min (5 min bins per data point). (B, F) Stereotypy, (C, G) vertical activity, and (D, H) thigmotaxis is also shown for *Drd1a-Cre;CK2α^{fl/fl}* and WT animals. Graphs show the mean values $\pm$ SEM (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$), statistical analysis: 2-way ANOVA with Bonferroni posttests. $N=9$ for WT and $N=6$ for KO (A, C, D), $N=18$ for WT and $N=13$ for KO (B), $N=8$ for WT and KO (E-H).

In the rotarod test, the latency of *Drd1a-Cre;CK2α^{fl/fl}* and *Drd2-Cre;CK2α^{fl/fl}* knockout mice and control mice ($N=18$ for WT, $N=16$ for KO for *Drd1a-Cre;CK2α^{fl/fl}* and $N=13$ for WT, $N=11$ for KO for *Drd2-Cre;CK2α^{fl/fl}*) to fall off the rod (sec) during accelerated rotarod analysis for three consecutive days with three trials/day is shown (I, J). Graph shows the mean values $\pm$ SEM. Statistical analysis was performed using 2-way ANOVA with

Bonferroni posttests for all trials except for day 1(trial 1)/day 3 (trial 1) comparison where the unpaired t test was used (***, $P < 0.001$; **, $P < 0.01$, *, $P < 0.05$). The pole test was performed and time that Drd1a-Cre;CK2 $\alpha^{fl/fl}$ knockout mice and control mice require to land at the bottom of pole (**K**) and turn while on the pole (**L**) was recorded (N= 18 for both genotypes). Statistical analysis was performed using unpaired t test. Graphs show the mean values $\pm$ SEM (***, $P < 0.001$; **, $P < 0.01$, *, $P < 0.05$).

Fig. 3. Exploratory performance of the *Drd1a-Cre;CK2α^{fl/fl}* knockout mice

A modified novel object test was performed with *Drd1a-Cre;CK2α^{fl/fl}* or *Drd2-Cre;CK2α^{fl/fl}* mice and their respective WT controls, and analyzed as time spent in the vicinity of novel object location (**A, C**) and total distance traveled (**B, D**). Graphs show the mean values $\pm$ SEM (***, $P < 0.001$; **, $P < 0.01$, *, $P < 0.05$), 2-way ANOVA with Bonferroni posttests, $N=8$ for WT and KO (**A, B**), $N=8$ for WT, $N=10$ for KO (**C, D**).

Fig. 4. Performance of *Drd1a-Cre;CK2α^{fl/fl}* knockout mice in novelty-suppressed feeding test and in Porsolt's tests of behavioral despair

Novelty suppressed feeding tests in *Drd1a-Cre;CK2α^{fl/fl}* and WT mice are shown as latency to feed (A) and food intake after ad libitum access (B). N=11 for WT and N=12 for KO. (C) Forced swim test was performed with the *Drd1a-Cre;CK2α^{fl/fl}* KO in the absence or presence of SCH23390 (0.03 mg/kg) and the time spent immobile during a 4 min test period was recorded. (D) Tail suspension test was performed in the absence or presence of SCH23390 (0.03 mg/kg) and the time spent immobile during a 4 min test period was recorded. SCH23390 (0.03 mg/kg) was injected i.p. 10 min before testing. N=10 for each genotype and condition. (E) Forced swim test performed with the *Drd2-Cre;CK2α^{fl/fl}* KO mice. (N=18 for WT, N=12 for KO). Graphs show the mean values $\pm$ SEM (***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.05$), unpaired t test.

Fig. 5. D1 receptor levels are up-regulated in Drd1a-Cre;CK2α^{fl/fl} knockout mice
 Total striatal protein lysates from Drd1-Cre;CK2α^{fl/fl} (KO) and control CK2α^{fl/fl} (WT) or from Drd2-Cre;CK2α^{fl/fl} (KO) and control CK2α^{fl/fl} (WT) mice were separated by SDS-PAGE. Immunoblotting analysis was performed using the following antibodies: (A) anti-D1R, (B) anti-D2R, (C, F) anti-G_{off} antibody, (D) anti-A2aR or (E) anti-D2R. N= 8 for each genotype (a-c), and N=6-7 for each genotype (D-F), statistical analysis was performed using unpaired t test, error bars indicate SEM (**, $P < 0.01$).

Fig. 6. Signaling modules downstream of the D1R are up-regulated in *Drd1a-Cre;CK2 $\alpha^{fl/fl}$* knockout mice

(A) Real-time PCR gene expression analysis was performed for the D1 receptor (*Drd1*) using the Taqman assay (Invitrogen). CT (cycle threshold) value for *Drd1* normalized by CT value of GAPDH is shown. N=5 for WT and 7 for KO. Statistical analysis was performed using unpaired t test, error bars indicate SEM. (B) cAMP levels were compared between *Drd1-Cre;CK2 $\alpha^{fl/fl}$* and wild-type by ELISA using total striata from microwave-irradiated animals. N= 15 for each genotype, statistical analysis was performed using unpaired t test, error bars indicate SEM (**, $P < 0.01$). (C) Mouse neostriatal slices were incubated in Krebs buffer for 1 h before stimulation with D1 agonist, SKF81297 for 5 min. Immunoblotting analysis was performed using anti-pThr34 DARPP-32 and anti-total DARPP-32 antibodies. Data were normalized to total amounts of DARPP-32. Analysis of 5 individual experiments is shown. Statistical analysis was performed using 1-way ANOVA and Bonferroni posttest (***, $P < 0.001$; **, $P < 0.01$). (D) Immunoblotting analysis of total striatal lysates from *Drd1a-Cre;CK2 $\alpha^{fl/fl}$* knockout and WT mice using anti-pS97 DARPP-32 and anti-total DARPP-32 antibodies, N=5 for WT, N=4 for KO; statistical analysis was performed using unpaired t test, error bars indicate SEM (**, $P < 0.01$).

Fig. 7. The D1R antagonist SCH23390 rescues the complex behavioral phenotypes of Drd1a-Cre;CK2 $\alpha^{fl/fl}$ knockout mice

(A) Total horizontal activity in Drd1a-Cre;CK2 $\alpha^{fl/fl}$ knockout and WT mice was recorded for 60 min and the first 20 min are plotted. Data is represented in 1 min bins per data point. N=10 for each genotype and condition. Saline or SCH23390 (0.03 mg/kg) was pre-injected i.p. 10 min before WT and KO mice were exposed to the open field paradigm. (B) The modified novel object test was performed Drd1a-Cre;CK2 $\alpha^{fl/fl}$ knockout and WT mice in the presence of SCH23390 (0.03 mg/kg) and analyzed as time spent in the vicinity of novel object location. N=15 for each genotype. (C) The 3-day accelerated rotarod test was performed with Drd1a-Cre;CK2 $\alpha^{fl/fl}$ knockout and WT mice in the presence of SCH23390 (0.03 mg/kg) and latency to fall recorded, N=6 for WT, N=5 for KO. Graph shows the mean values $\pm$ SEM. Statistical analysis was performed using 2-way ANOVA with Bonferroni posttests for all trials except for day 1(trial1)/day 3 (trial1) comparison where the unpaired t test was used (*, $P < 0.05$). (D) Total horizontal activity in Drd2-Cre;CK2 $\alpha^{fl/fl}$ knockout and WT mice was recorded using an open-field paradigm for 60 min (5 min bins per data point). Caffeine (5 mg/kg) was injected i.p. after 30min as indicated. N=15 (WT), N=17 (KO).

Statistical analysis was performed using 2-way ANOVA with Bonferroni post test, error bars indicate SEM.