



HAL
open science

Rapid synaptogenesis in the Nucleus Accumbens is induced by a single cocaine administration and stabilized by MAP Kinase interacting kinase 1 activity

Marc dos Santos, Marine Salery, Benoit Forget, Maria Alexandra Garcia Perez, Sandrine Betuing, Thomas Boudier, Peter Vanhoutte, Jocelyne Caboche, Nicolas Heck

► To cite this version:

Marc dos Santos, Marine Salery, Benoit Forget, Maria Alexandra Garcia Perez, Sandrine Betuing, et al.. Rapid synaptogenesis in the Nucleus Accumbens is induced by a single cocaine administration and stabilized by MAP Kinase interacting kinase 1 activity. *Biological Psychiatry*, 2017, 82 (11), pp.806-818. 10.1016/j.biopsych.2017.03.014 . hal-01500913

HAL Id: hal-01500913

<https://hal.sorbonne-universite.fr/hal-01500913>

Submitted on 3 Apr 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

Rapid synaptogenesis in the Nucleus Accumbens is induced by a single cocaine administration and stabilized by MAP Kinase interacting kinase 1 activity

Marc Dos Santos¹, Marine Salery¹, Benoit Forget¹, Maria Alexandra Garcia Perez¹, Sandrine Betuing¹, Thomas Boudier^{2,3}, Peter Vanhoutte¹, Jocelyne Caboche^{1,*,#} & Nicolas Heck^{1,#}

¹Sorbonne Universités, UPMC Univ Paris 06, INSERM, CNRS, Neurosciences Paris Seine - Institut de Biologie Paris Seine (NPS - IBPS), 75005 Paris, France

²Sorbonne Universités, UPMC Univ Paris 06, IPAL, CNRS, 75005 Paris France

³Bioinformatics Institute, Agency for science, technology and research (A*STAR), Singapore

Those authors contributed equally

* Corresponding author: jocelyne.caboche@upmc.fr; phone: +33 1 44 27 53 52; fax : +33 44 27 25 08.

Short title: ERK/MNK1 and rapid synaptogenesis induced by cocaine

Key words: Cocaine, dendritic spine, synaptogenesis, MAP Kinase Interacting Kinase (MNK), striatum, Extracellular-signal Regulated Kinase (ERK)

Abstract: 232 words; Text body: 3987 words; Figures: 8

Supplemental Materials include 6 figures and Methods and Materials

ABSTRACT

Background

Repeated cocaine exposure produces new spine formation in Striatal Projection Neurons (SPNs) of the Nucleus Accumbens (NAc). However, an acute exposure to cocaine can trigger long-lasting synaptic plasticity in SPN leading to behavioral alterations. This raises the intriguing question as to whether acute cocaine could modify enduringly striatal connectivity.

Methods

A 3D morphometric analysis of presynaptic glutamatergic boutons and dendritic spines was performed on SPN one hour and one week after a single cocaine administration. Time-lapse two-photon microscopy in adult slices was used to determine the precise molecular events sequence responsible for the rapid spine formation.

Results

A single injection triggered a rapid synaptogenesis and persistent increase in glutamatergic connectivity in SPN from the shell part of the NAc, specifically. Synapse formation occurred through clustered growth of active spines contacting pre-existing axonal boutons. Spine growth required ERK activation, while spine stabilization involved transcription-independent protein synthesis driven by MAP kinase interacting kinase-1 (MNK-1), downstream from ERK. The maintenance of new spines driven by MNK-1 was essential for long-term connectivity changes induced by cocaine *in vivo*.

Conclusions

Our study originally demonstrates that an acute administration of cocaine is able to induce stable synaptic rewiring in the NAc, which will likely influence responses to subsequent drug exposure. It also unravels a new functional role for cocaine-induced ERK pathway

independently of nuclear targets. Finally, it reveals that MNK-1 has a pivotal role in cocaine-induced connectivity.

ACCEPTED MANUSCRIPT

INTRODUCTION

Cocaine administration induces long-lasting changes in behaviors that result from synaptic plasticity and morphological changes in neurons of the brain's reward circuitry (1,2). Cocaine increases dopamine (DA) in the Nucleus Accumbens (NAc) (3), with dendritic spines from striatal projection neurons (SPN) receiving glutamate inputs informative of contextual information, together with DA inputs that code for reward signals (4, 5) and modulate the efficacy of glutamatergic synapses (6, 7). Most studies assessing structural changes in the striatum have focused on the observation of dendritic spine density changes induced by chronic cocaine in the adult striatum. However, in-depth studies of synaptogenesis and connectivity changes, which require the analysis of both pre and postsynaptic elements (8,9), remained largely unexplored until recently. To assess this issue we have set up a method that allows for 3D automated morphological analysis of synaptic connections onto SPN (10). Using VGLUT1^{Venus} mice, which express the vesicular glutamate transporter 1 (VGLUT1), a presynaptic protein present in afferents from cortices (neocortex, hippocampus and amygdala) fused to the fluorescent protein Venus (11), we showed that new spines formed after chronic exposure to cocaine were indeed connected to presynaptic cortical terminals (10). How and when these new glutamatergic synapses are formed within the striatum still remains a key issue, which has to be addressed to disentangle the cellular bases responsible for the initiation of neuronal plasticity mediated by cocaine. It was recently shown that DA gated glutamate driven spine enlargement in the NAc in a narrow time window, thereby providing a mechanism for reward-driven learning at the level of the dendritic spine(12). Furthermore, the synergistic interaction between D1R and glutamate NMDA receptor induces activation of the MAPkinase/ERK (extracellular-signal regulated kinase) pathway (13-15), which plays a key role in cocaine-induced neuronal and behavioral adaptations (16-19). The ERK pathway also regulates spine formation induced by chronic cocaine administration onto SPN expressing the

DA receptor of type 1 (D1-SPN) (20, 21). Classically, these long-term adaptations are thought to rely on gene transcription and epigenetic regulation (22). However, it was recently demonstrated that a single injection of cocaine was sufficient to cause an ERK-dependent synaptic plasticity and subsequent behavioral adaptations induced by a second administration of cocaine (23, 24). We therefore hypothesized that a single injection of cocaine was able to induce a rapid and stable modification of striatal connectivity. To address these issues, we investigated the changes in glutamatergic connectivity, along with the rules of synapse formation, as well as the associated molecular mechanisms. We bring evidence that an acute administration of cocaine is able to induce stable synaptic rewiring in the NAc and demonstrate that the ERK pathway plays a dual role in the growth and stabilization of new spines in D1-SPN.

METHODS AND MATERIALS

Animal care.

Animal care of wild-type C57BL/6 and VGLUT1-venus knock-in was conducted in accordance with standard ethical guidelines (NIH publication no. 85-23, revised 1985 and European Committee Guidelines on the Care and Use of Laboratory Animals 86/609/EEC), and the experiments were approved by the local ethic committee.

***In vivo* treatments, tissue preparation.**

Intraperitoneal injection of cocaine was performed. When indicated anisomycin (100 mg/kg) or SL327 (50 mg/kg) were injected intraperitoneally 30 min and 60 min, respectively, prior to cocaine. For CGP57380 treatment, cannulae were pre-implanted in the NAc shell in anesthetized 7-8 weeks-old mice. One week later, unilateral injections of 10 mM CGP57380 were performed, along with contralateral vehicle injection, followed by cocaine administration 15 min later. Mice brains were fixed *in situ* after intracardiac perfusion of paraformaldehyde (PFA) and coronal sections were cut with a vibratome. Dendrites were labeled with DiI using diolistic method.

Dendritic spine analysis, segmentation of presynaptic boutons and detection of synaptic contacts from confocal images.

Dendritic spine detection was performed using the Neuronstudio software and data were imported into ImageJ for custom 3D automated spine heads segmentation. Segmentation of VGLUT1 objects and detection of synaptic contacts were performed in 3D as described previously (10). Border to border distance between each spine head and all VGLUT1 objects was computed and the synaptic contact was decided for a distance of zero, meaning that the two objects overlap.

Viral infections and preparation of slices for two-photon microscopy.

The AAVrh9-PPTA-Cre viral construct contains an expression cassette consisting of the Cre recombinase driven by the promoter of PPTA (precursor of substance P) gene, which is specifically expressed in SPN expressing D1R (12). The virus was injected in the NAc of 4-week-old mice in combination with the AAVrh9-pCAG-flex-tdTomato-WRPE virus and when stated AAV1-hSyn-flex-GCaMP6S-WPRE virus. Coronal slices of 200-250 μm thickness of brains from 6 to 8 week-old mice were cut on a vibratome and maintained in equilibrated ACSF. Slices were stimulated with glutamate 3 μM and (R)-(+)-SKF-38393 10 μM in ACSF for 20 min, followed by 40 min in ACSF alone. Pharmacological inhibitors were applied 30 min before co-stimulation and washed 30 min after the end of the co-stimulation. Slices were either imaged in time-lapse or fixed with PFA 4% at RT for 60 min, processed for DiI dendrite labelling or immunofluorescence. For time lapse studies, slice imaging was performed in ACSF with two-photon upright microscope (SP5 MPE, Leica) equipped with water-immersion objective (25x, NA 0.95, Leica) and images were acquired with hybrid detector (Leica). Image stacks were sampled with voxels size of 100 nm in xy axis and z-step of 500 nm.

See Supplemental information for fully detailed experimental procedures

RESULTS

A single cocaine exposure induces a rapid and stable connectivity change in the NAc shell

We investigated spine formation and potential changes of striatal connectivity induced by a single administration of cocaine. Dendritic spine density of SPNs from the NAc shell was increased one hour after a single injection of cocaine (Figure 1A-C). A similar increase was observed at a lower dose (10 mg/kg, Supplemental Figure S1A), while no change was observed either in the NAc core, or in the dorsal striatum at higher doses (Supplemental Figure S1B, C). Upon cocaine administration, activated D1-SPNs express immediate early genes, including c-Fos (25). Spine density increase in SPNs expressing c-Fos was greater when compared to randomly picked SPN (Figure 1A-C), thus indicating that cocaine-induced spine formation occurred in activated SPNs. VGLUT1-positive boutons from glutamatergic axons were segmented, and synaptic contacts were identified by measurement of the 3D contacts between spine heads and boutons. About 60% of spines were connected to VGLUT1-positive boutons in saline conditions (Figure 1A, E), a result in agreement with electron microscopic studies (26). One hour after cocaine administration the number of spines in contact with a VGLUT1 bouton was increased (Figure 1A, D), indicating that acute cocaine rapidly increases glutamatergic connectivity in the NAc. Of note, the percentage of spines connected to VGLUT1 boutons remained unchanged, indicating that the increased connectivity did not modify the stoichiometry of VGLUT1 connections onto SPN (Figure 1E). We next assessed whether this rapid increase in connectivity was stable over time. One week after a single injection of cocaine, the increase in spine density and synaptic contacts were still present, while the percentage of VGLUT1-positive synapses remained stable (Figure 1F-I). An increased spine density was also found 28 days after a single administration of cocaine (Supplemental Figure S1D). Following a custom 3D procedure for spine heads segmentation (Figure 2A), the measurements of spine head volume and spine length showed

an increase one hour after cocaine administration, which was better revealed in c-Fos positive neurons (Figures 2B, C). One week after cocaine administration, spine head volume, but not spine length, was still larger in cocaine-treated mice as compared to saline controls (Figures 2D, E). The increased volume of spine heads corresponded to an increase in mushroom-shaped spine density that was significantly increased one hour and one week after a single cocaine administration (Figures 2F, G), indicating a rapid maturation of the newly-generated spines.

Synapse formation occurs in clusters with spines connecting pre-existing presynaptic boutons

The total density of VGLUT1-positive boutons in the NAc-shell was not increased either one hour or one week after cocaine administration (Figures 3A, B, left panels). Since synaptogenesis occurred, this indicates that new spines contact pre-existing presynaptic boutons. Accordingly, the occurrence of spines connected to a common VGLUT1-positive bouton –an observation reported in electron microscopy study (26)- was increased one hour and one week after cocaine injection (Figure 3A, B, right panels). The rules of synapse formation were further studied using live imaging in acute adult striatal slices. In order to recapitulate the effects of cocaine on SPN, we applied a co-stimulation with a low dose of glutamate together with a D1R agonist, as previously shown (13, 14). As expected, the co-stimulation, but not the application of each agonist independently, induced ERK activation and increased spine density and synaptic contacts in the NAc-shell (Supplemental Figure S2). Time-lapse experiments were performed on D1-SPN since cocaine induces ERK activation (25) and dendritic spine increase (21, 27) in this subpopulation. To this end, D1-SPN were sparsely labeled by injecting in the NAc from adult mice a combination of an adeno-associated virus (AAV) carrying tdTomato sequence flanked by loxP sites and a virus expressing Cre recombinase under the promoter of preprotachykinin A (PPTA), which is specifically expressed in D1-SPN (12), as confirmed by immunodetection of the D1R

(Supplemental Figure S3A, B). Striatal slices were performed two weeks after viral-infections, and time-lapse imaging of D1-SPN was performed by two-photon microscopy. Dendritic spine growth occurred as soon as 25 minutes after the beginning of the co-stimulation, and reached a plateau at 60 min (Figure 3C, D) and was absent when the selective D1R antagonist SCH23390 was applied (Supplemental Figure S3C). The amplitude of spine density increase at one hour was +21% (Fig. 3E).

A single cocaine administration occludes synaptic potentiation induced in striatal slices (23). We next tested whether previous cocaine administration could alleviate the spine growth induced by the co-stimulation in striatal slices. The spine density increase induced by cocaine *in vivo* was preserved in the slices before co-stimulation (Supplemental Figures S4A, B). A partial occlusion was observed in slices from mice acutely administrated with cocaine, since co-stimulation induced spine growth but the percentage of increase was not significant. On the other hand, chronic administration of cocaine led to complete occlusion (Supplemental Figures S4C-D). Those results are consistent with observation in the prefrontal cortex *in vivo* of progressive decline of spine density increase upon repeated injection of cocaine (28).

The connectivity and functionality of spines observed in time-lapse were then explored. In acute slices from VGLUT1^{venus} mice 62.8% of spines induced by the co-stimulation were in contact with a VGLUT1-positive bouton (5 mice, n = 35 spines). Newly-generated spines entered in contact with a VGLUT1-positive bouton that was in all cases present before spine growth (5 mice, n = 22 spines) (Figure 3F), which is consistent with our *in vivo* observation of increased density of synaptic contacts along with constant density of VGLUT1 boutons upon acute cocaine. In order to assess the functionality of the newly formed spines, the viruses AAV-PPTA::Cre, AAV-FLEX-tdTomato and AAV-FLEX-GCaMP6S were injected in the NAc. Spine growth and calcium events were analyzed in D1-SPN from striatal slices (Fig. 3G, left panel). Following co-stimulation, 6 out of 10 newly formed spines in the NAc

responded by an increase in calcium content. These functional spines had a bigger head diameter than the newly formed spines that did not respond to the stimulation (Figure 3G). This kinetics of maturation is in accordance with previous studies from other neuronal types (29-32). The fact that new spines contact axonal boutons already in place raised the question as to whether synaptogenesis could be driven by a stochastic spine growth. We generated an *in silico* simulation of synaptogenesis from random positions on the dendrite, and found that it did not reproduce the experimental data (Supplemental Figures S5A, B). Moreover, the analysis of spine distribution *in vivo* revealed a bimodal distribution with a high proportion of adjacent spine heads, which was increased after cocaine exposure (Supplemental Figures S5C, D). Hence, spine growth does not occur randomly but at locations where synapse clusters are generated. Taken together, our results indicate that new spines grow in clusters, are rapidly functional and contact pre-existing VGLUT1 boutons.

A single administration of cocaine induces the formation of thalamo-striatal synaptic contacts

Since a single administration of cocaine induced an increase in VGLUT1-positive synaptic contacts while keeping the ratio of spines connected to VGLUT1 afferents (see Figure 1), it was plausible that the unconnected spines would correspond to thalamo-striatal synapses, which are VGLUT2-positive (33). D1-SPN were labeled by *in vivo* viral infections (AAV-PPTA::Cre and AAV-FLEX-tdTomato), and the mice were treated with a single administration of cocaine. VGLUT2-positive boutons were immunolabeled and thalamo-striatal synaptic contacts were analysed (Figure 4). The density of VGLUT2-connected spines significantly increased upon cocaine administration, thus indicating that acute cocaine also induces the formation of thalamo-striatal synapses.

Dendritic spine growth and stabilization are regulated by ERK and MNK-1

We next investigated the signaling pathways involved in spine formation induced in striatal slices one hour after the co-stimulation. A pretreatment with U0126, a selective inhibitor of the ERK pathway, abolished the spine density increase induced by the co-stimulation (Figure 5A), revealing that the ERK pathway is necessary for spinogenesis. Similarly, rapamycin an inhibitor of the mammalian target of rapamycin (mTOR) pathway completely blocked the increase in spine density induced by co-stimulation in slices (Figure 5B). Since the ERK and mTOR pathways converge on the control of protein synthesis via the eIF complex, we investigated the possible implication of MNK-1, a cytoplasmic substrate of ERK, which activates the eIF complex to initiate protein translation (34)(Figure 5C). An increase in phosphorylated MNK-1 (pMNK-1) positive cells was measured by immunodetection in the NAc twenty minutes after co-stimulation (Figure 5D). Slices were then pre-treated with CGP57380, a specific inhibitor of MNK-1 activation, which totally inhibited the increase in spine density induced by the co- stimulation (Figure 5E).

In order to gain further insights into the specific role of ERK and MNK-1 in spine growth and maintenance, we used time-lapse imaging from striatal slices (Figure 6A, B). D1-SPN dendrites were labeled *in vivo* after intra-NAc infection with AAV PPTA::Cre and AAV-FLEX-tdTomato. Spine growth was absent in the presence of U0126, the inhibitor of the ERK pathway (Figure 6C), indicating that ERK activation is mandatory for the formation of new spines. In contrast, CGP57380 did not modify growth rate, clearly indicating that MNK-1 is not required for spine growth (Figure 6B, C). However, it strongly impacted the maintenance of the newly formed spines, with a loss of 59.1% and 91% of newly-formed spines, 30 minutes and 90 minutes after growth, respectively (Figure 6B, D). At this later time point, 67% of newly-formed spines remained stable in control conditions, as previously reported (27)(Figure 6B, D). Noteworthy, the inhibition of MNK-1 activation did not affect the stability of spines present before stimulation (Supplemental Figure S6). Hence these data

show that MNK-1 is mandatory for stabilization but not for spine growth. Since MNK-1 regulates protein synthesis, we interrogated the respective role of global transcription and translation for spine growth and maintenance. Inhibition of protein synthesis with anisomycin did not modify spine growth, but reduced spine stabilization, with identical kinetics and amplitude to that observed with the MNK-1 inhibitor (Figure 6B, D). By contrast, bath application of the transcription inhibitor actinomycin D did not alter either growth or stabilization of new spines (Figure 6B, D). Therefore, time-lapse analysis unraveled sequential molecular events involved in spine growth and stabilization. While ERK is essential for both steps of spine formation, MNK-1 activation and protein synthesis selectively control spine stabilization independently of transcription.

MNK-1 is involved in structural plasticity *in vivo*

We analyzed the impact of MNK-1 activation on long-lasting spinogenesis *in vivo*. An increase in pMNK-1-positive cells was observed in the NAc 10 minutes after a single cocaine administration, and the inhibition of ERK activation by SL327 completely abolished this increase (Figure 7A, B), thus confirming that cocaine-induced MNK-1 phosphorylation is strictly dependent on ERK activation. The MNK-1 phosphorylation state was transient, with return to basal levels 30 minutes after cocaine administration (Figure 7B). The dendritic localization of pMNK-1 in D1-SPN was shown by immunodetection of pMNK-1 in dendrites labeled with the PPTA::Cre and FLEX-tdTomato AAV. pMNK-1 positive punctates were detected in the dendritic shaft and in dendritic spines from D1-SPN (Figure 7C), further demonstrating the local activation of MNK-1. In agreement with the results from acute slices, inhibition of ERK or protein synthesis abolished spine formation one hour after cocaine administration *in vivo* (Figure 7D). The activation of MNK-1 was inhibited locally by intracranial injection of CGP57380 in the shell part of the NAc prior to either saline or

cocaine single administration. The local inhibition of MNK-1 did not affect basal spine density of SPN, but abolished the spine density increase observed 1 and 24 hours after cocaine administration (Figure 7E). Taken together, these results identify MNK-1 as a cytoplasmic target downstream from ERK activated by cocaine and posit MNK-1 and protein synthesis as essential for striatal structural plasticity *in vivo*.

ACCEPTED MANUSCRIPT

DISCUSSION

Our data show that a single administration of cocaine is sufficient to trigger long-lasting increase in glutamatergic connectivity in the NAc, with new spines growing in clusters and contacting pre-existing presynaptic boutons. We unravel the sequence of signaling events specifically involved in spine growth and stabilization. Spine growth was transcription and translation-independent, thus supporting a key role of ERK-induced phosphorylation of pre-existing proteins in this process. By contrast, spine stabilization required an ERK-mediated proteins synthesis via MNK-1, independently of transcription. Finally, we identify MNK-1 as essential for long-term connectivity changes induced by cocaine (Figure 8).

It has been long thought that persistent structural plasticity/morphology changes were selectively engaged following repeated administration of cocaine (10, 21, 28, 35, 36). Here we provide evidence that a single administration is sufficient to induce a rapid and persistent change in glutamatergic connectivity in the NAc. The rapid formation of mushroom-shaped spines with increased spine head volumes *in vivo*, and our calcium imaging in adult slices, both showed a rapid functional maturation of nascent spine. These observations fit with electrophysiological recordings performed after acute exposure to cocaine, showing an increase in the frequency of miniature currents in c-Fos positive SPN (37), and synaptic potentiation (23). Interestingly, repeated cocaine regimen leads to an increased spine density along with unchanged (10, 21) or decreased spine head size (36, 38), and the presence of immature spines is coherent with the observation of LTD (39) and generation of silent synapses (40, 41), which are not induced by acute cocaine injection (37). The shift from potentiated mature spines to silent immature spines is an active process, since chronic regimen recruits specific molecular pathways involved in dendritic spine remodeling, such as deltaFosB accumulation (42) and inactivation of Rac1 (43), which are not observed after

single cocaine injection. In this context, it is noteworthy that dendritic spine maturation and unsilencing of synapses are observed after a 3 weeks period of cocaine withdrawal (38, 44) and following a challenge injection (35, 39). Hence, the morphology of spines is dynamically regulated during the different stages of cocaine administration and addiction development (45), through the combined involvement of metaplasticity and homeostatic processes (46, 47). Whether VGLUT1 afferences arising from hippocampus, cortex or amygdala are similarly engaged onto new synapses remain to be established. On the other hand, we reveal that thalamic, VGLUT2-positive, synaptic contacts are generated after a single administration of cocaine. Along with previous work showing a plasticity of thalamo-striatal synapses upon chronic cocaine treatments (48, 49), our results pave the way for future study on the role of thalamic inputs on D1-SPN in long term adaptation induced by cocaine. In line with our results, it was previously observed that acute cocaine induces an increase in spine density in dopaminergic neurons (50) and in the prefrontal cortex (28), although those studies did not assess the connectivity of dendritic spines. Our results bring evidence that acute cocaine is a first essential step that primes the meso-cortico-limbic connectivity, which implies that subsequent cocaine action will operate on a modified network.

Our results also unravel the rules of synapse formation that leads to this connectivity change. Previous studies showed that new spines could make contact with pre-existing boutons (30, 51-54) and our *in vivo* as well as time-lapse observations in slices extend this mode of formation to striatal neurons (Figure 8). This mode of formation might imply a constraint on the location of spine growth. Accordingly, our modeling of synaptogenesis revealed the occurrence of clustered spines along the dendrite, indicating that the position of spine growth along the dendrite is actively regulated. The formation of dendritic spines in clusters has been studied in other brain structures (55-57) where they have implication on the integrative properties of the dendrite (58). Our study reinforces these observations, and further

show that newly-formed spines rapidly form synapses with pre-existing glutamatergic boutons. Moreover, we observed *in vivo* spine clusters in contact with a single bouton, which implies a temporal and spatial synchrony of the glutamatergic inputs on these spines. In conclusion, our observations of both pre and postsynaptic elements combined with a model of spinogenesis, which includes the constraint of synaptic contact formation, allows a better understanding of the rules of connectivity changes in the adult striatum.

It has been shown that dopamine gates glutamate-mediated spine enlargement in SPN via D1R (12). Along those lines, we have previously demonstrated that cocaine effects result from D1R gated NMDAR-mediated ERK activation (13-15). Here we observed that both a D1R agonist and Glu are required for ERK-dependent synaptogenesis in SPN downstream from D1R. Accordingly, it was recently shown that a single administration of cocaine can produce an ERK-dependent occlusion of synaptic potentiation on SPN of the NAc, and optogenetic inhibition of cocaine-induced plasticity prevented the behavioral adaptations induced by cocaine (23). Altogether, these data support that ERK-induced rapid synaptogenesis might be a critical cellular mechanism involved in the induction of long-term striatal plasticity and in the development of behavioral alterations induced by cocaine. We further demonstrate that synaptogenesis can be divided in different phases, with at least two distinct molecular mechanisms downstream the ERK pathway (Figure 8). We reveal a transcription and translation-independent role of ERK in the initiation of spine formation. While a previous study has shown that ERK activity is essential for spine morphogenesis in developing cortical neurons (32), we make the first demonstration of a functional role of cocaine-induced ERK activation independently of nuclear targets. It may likely imply the phosphorylation of pre-existing proteins, such as spinophilin, which upon phosphorylation by ERK, can increase spine protrusion density in hippocampal neurons (59).

We further show that spine stabilization requires MNK-1 activation and local protein synthesis independently of transcription. It is now clearly established that, downstream from ERK, MNK-1 phosphorylates initiation factors essential for the onset of local translation required for long-term plasticity (60, 61). Moreover, cocaine administration can induce synaptic plasticity via a mechanism that involves translation independently of transcription (62). Of interest, local synaptic translation also involves the mTOR signaling pathway, which is activated downstream Rap1b, a small GTPase essential for spine formation upon chronic cocaine administration (38). Several messengers are translated in dendrites (63) and could be involved in the stability of newly formed spines. The local translation of Arc is controlled by MNK-1 (64) and Arc proteins regulate spine density and morphology (65). Furthermore, the expression of Arc is induced in dendrites of D1R-SPN upon acute cocaine administration (66). PSD-95 is another candidate locally translated in response to intra-NAc injection of a D1R agonist (67), and involved in spine stabilization (68, 69). Importantly, we show that cocaine induces a rapid ERK-mediated phosphorylation of MNK-1 in dendrites of SPNs in the NAc, and its pharmacological inhibition leads to a loss of spine density increase observed one hour and 24 hours after a single administration of cocaine. Hence stabilization of newly-formed spines via MNK-1 is essential for long-term changes in glutamatergic striatal connectivity in the NAc.

ACKNOWLEDGEMENTS AND DISCLOSURES

We would like to thank Jean-Antoine Girault (INSERM, Paris, France) for critical reading of the manuscript. We wish to thank Dr. Haruo Kasai (Tokyo University, Japan) for providing the plasmid coding PPTA-Cre and Alexis Bemelmans (Molecular Imaging Research Center, CEA, France) for producing the AAV virus. The VGLUT2 antibody was a kind gift from Dr. Salah El Mestikawy (NPS, Paris, France). The tdTomato expressing virus was made available by Dr. Hongkui Zeng, of the Allen Institute for Brain Science. We wish to thank the Cellular Imaging facility of the IBPS (Institut de Biologie Paris-Seine) for expert assistance on microscopy and metrological control of the setup. This work was supported by Centre National pour la Recherche Scientifique (CNRS), Institut National de la Santé et de la Recherche Médicale (INSERM), University Pierre and Marie Curie (UPMC), Agence Nationale de la Recherche (France-Germany ANR-15-CE16-0017-01), Fondation pour la Recherche Médicale, Fondation Jérôme Lejeune and the Labex Bio-Psy cluster of excellence. M.D.S. was a recipient of a fellowship from French Ministry of Research and Labex Bio-Psy. The authors report no biomedical financial interests or potential conflicts of interest.

REFERENCES

1. Volkow ND, Morales M (2015): The brain on drugs: from reward to addiction. *Cell* 162: 712-725.
2. Russo SJ, Dietz DM, Dumitriu D, Morrison JH, Malenka RC, Nestler EJ (2010): The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends Neurosci.* 33: 267-276.
3. Di Chiara G, Imperato A (1988): Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci U S A* 85: 5274 –5278.
4. Schulz W (2002): Getting formal with dopamine and reward. *Neuron* 36: 241-263.
5. Steinberg EE, Keiflin R, Boivin JR, Witten IB, Deisseroth K, Janak PH (2013): A causal link between prediction errors, dopamine neurons and learning. *Nat. Neurosci.* 16: 966-973.
6. Nicola SM, Surmeier J, Malenka RC (2000): Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu Rev Neurosci.* 23: 185–215.
7. Hyman SE, Malenka RC, Nestler EJ (2006): Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu. Rev. Neurosci.* 29: 565-598.

8. Schoonover CE, Tapia JC, Schilling VC, Wimmer V, Blazeski R, Zhang W, Mason CA, Bruno RM (2014): Comparative strength and dendritic organization of halamocortical and corticocortical synapses onto excitatory layer 4 neurons. *J. Neurosci.* 34: 6746-6758.
9. Wierenga CJ, Becker N, Bonhoeffer T (2008): GABAergic synapses are formed without the involvement of dendritic protrusions. *Nat. Neurosci.* 11: 1044-1052.
10. Heck N, Dos Santos M, Amairi B, Salery M, Besnard A, Herzog E, *et al.* (2015): A new automated 3D detection of synaptic contacts reveals the formation of cortico-striatal synapses upon cocaine treatment in vivo. *Brain Struct. Funct.* 220: 2953-2966.
11. Herzog E, Nadrigny F, Silm K, Biesemann C, Heiling I, Bersot T, *et al.* (2011): In vivo imaging of intersynaptic vesicle exchange using VGLUT1 Venus knock-in mice. *J. Neurosci.* 31: 15544-15559.
12. Yagishita S, Hayashi-Takagi A, Ellis-Davies GC, Urakubo H, Ishii S, Kasai H (2014): A critical time window for dopamine actions on the structural plasticity of dendritic spines. *Science* 345: 1616-1620.
13. Pascoli V, Besnard A, Hervé D, Pagès C, Heck N, Girault JA, *et al.* (2011): Cyclic adenosine monophosphate-independent tyrosine phosphorylation of NR2B mediates cocaine-induced extracellular signal-regulated kinase activation. *Biol Psychiatry* 69: 218-227.

14. Cahill E, Pascoli V, Trifilieff P, Savoldi D, Kappès V, Lüscher C, *et al.* (2014b): D1R/GluN1 complexes in the striatum integrate dopamine and glutamate signalling to control synaptic plasticity and cocaine-induced responses. *Mol. Psychiatry* 19: 1295-1304.
15. Pascoli V, Cahill E, Bellivier F, Caboche J, Vanhoutte P (2014): Extracellular signal-regulated protein kinases 1 and 2 activation by addictive drugs: a signal toward pathological adaptation. *Biol Psychiatry* 76: 917-926.
16. Valjent E, Corvol JC, Pages C, Besson MJ, Maldonado R, Caboche J (2000): Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. *J Neurosci.* 20: 8701-8709.
17. Valjent E, Corvol JC, Trzaskos JM, Girault JA, Hervé D (2006): Role of the ERK pathway in psychostimulant-induced locomotor sensitization. *BMC Neurosci.* 7:20.
18. Miller CA, Marshall JF (2005): Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. *Neuron* 47: 873-884.
19. Lu L, Hope BT, Dempsey J, Liu SY, Bossert JM, Shaham Y (2005): Central amygdala ERK signaling pathway is critical to incubation of cocaine craving. *Nat Neurosci.* 8: 212-219.
20. Ren Z, Sun WL, Jiao H, Zhang D, Kong H, Wang X, Xu M (2010): Dopamine D1 and N-methyl-D-aspartate receptors and extracellular signal-regulated kinase mediate neuronal morphological changes induced by repeated cocaine administration. *Neuroscience* 168: 48-60.

21. Dobi A, Seabold GK, Christensen CH, Bock R, Alvarez VA (2011): Cocaine-induced plasticity in the nucleus accumbens is cell specific and develops without prolonged withdrawal. *J. Neurosci.* 31: 1895-1904.
22. Robison AJ, Nestler EJ (2011): Transcriptional and epigenetic mechanisms of addiction. *Nat. Rev. Neurosci.* 12: 623-637.
23. Pascoli V, Turiault M, Lüscher C (2011): Reversal of cocaine-evoked synaptic potentiation resets drug-induced adaptive behaviour. *Nature* 481: 71-75.
24. Valjent E, Bertran-Gonzales J, Aubier B, Greengard P, Hervé D, Girault JA (2010): Mechanisms of locomotor sensitization to drugs of abuse in a two-injection protocol. *Neuropsychopharmacology* 35: 401-415.
25. Bertran-Gonzalez J, Bosch C, Maroteaux M, Matamales M, Hervé D, Valjent E, Girault JA (2008): Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J. Neurosci.* 28: 5671-5685.
26. Doig NM, Moss J, Bolam JP (2010): Cortical and thalamic innervation of direct and indirect pathway medium-sized spiny neurons in mouse striatum. *J. Neurosci.* 30: 14610-14618.
27. Kim J, Park BH, Lee JH, Park SK, Kim JH (2011): Cell type-specific alterations in the nucleus accumbens by repeated exposures to cocaine. *Biol Psychiatry* 69: 1026-1034.

28. Muñoz-Cuevas FJ, Athilingam J, Piscopo D, Wilbrecht L (2013): Cocaine-induced structural plasticity in frontal cortex correlates with conditioned place preference. *Nat. Neurosci.* 16: 1367-1369.
29. Hill TC, Zito K (2013): LTP-induced long-term stabilization of individual nascent dendritic spines. *J Neurosci.* 33: 678-686.
30. Nägerl UV, Köstinger G, Anderson JC, Martin KA, Bonhoeffer T (2007): Protracted synaptogenesis after activity-dependent spinogenesis in hippocampal neurons. *J. Neurosci.* 27: 8149-8156.
31. Zito K, Scheuss V, Knott G, Hill T, Svoboda K (2009): Rapid functional maturation of nascent dendritic spines. *Neuron* 61: 247-258.
32. Kwon HB, Sabatini BL (2011): Glutamate induces de novo growth of functional spines in developing cortex. *Nature* 474: 100-104.
33. Fremeau RT Jr, Voglmaier S, Seal RP, Edwards RH (2004): VGLUTs define subsets of excitatory neurons and suggest novel roles for glutamate. *Trends Neurosci.* 27: 98-103.
34. Bhakar AL, Dölen G, Bear MF (2012): The pathophysiology of fragile X (and what it teaches us about synapses). *Annu. Rev. Neurosci.* 35: 417-443.

35. Shen HW, Toda S, Moussawi K, Bouknight A, Zahm DS, Kalivas PW (2009): Altered dendritic spine plasticity in cocaine-withdrawn rats. *J. Neurosci.* 29: 2876-2884.
36. Dumitriu D, Laplant Q, Grossman YS, Dias C, Janssen WG, Russo SJ, *et al.* (2012): Subregional, dendritic compartment, and spine subtype specificity in cocaine regulation of dendritic spines in the nucleus accumbens. *J Neurosci.* 32: 6957-6966.
37. Koya E, Cruz FC, Ator R, Golden SA, Hoffman AF, Lupica CR, Hope BT (2012): Silent synapses in selectively activated nucleus accumbens neurons following cocaine sensitization. *Nat. Neurosci.* 15: 1556-1562.
38. Cahill ME, Bagot RC, Gancarz AM, Walker DM, Sun H, Wang ZJ, *et al.* (2016): Structural plasticity after chronic cocaine administration occurs through Rap1 Small GTPase signaling. *Neuron* 89: 566-582.
39. Kourrich S, Rothwell PE, Klug, JR, Thomas MJ (2007): Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. *J Neurosci.* 27: 7921-7928.
40. Huang YH, Lin Y, Mu P, Lee BR, Brown TE, Wayman G, *et al.* (2009): In vivo cocaine experience generates silent synapses. *Neuron* 63: 40-47.
41. Graziane NM, Sun S, Wright WJ, Jang D, Liu Z, Huang YH, *et al.* (2016): Opposing mechanisms mediate morphine- and cocaine-induced generation of silent synapses. *Nat Neurosci.* 19: 915-925.

42. Maze I, Covington HE 3rd, Dietz DM, LaPlant Q, Renthal W, Russo SJ, *et al.* (2010): Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science* 327: 213-216.
43. Dietz DM, Sun H, Lobo MK, Cahill ME, Chadwick B, Gao V, Koo JW, *et al.* (2012): Rac1 is essential in cocaine-induced structural plasticity of nucleus accumbens neurons. *Nat Neurosci.* 15: 891-896.
44. Lee BR, Ma YY, Huang YH, Wang X, Otaka M, Ishikawa M, *et al.* (2013): Maturation of silent synapses in amygdala-accumbens projection contributes to incubation of cocaine craving. *Nat Neurosci.* 16: 1644-1651.
45. Dong Y, Nestler EJ (2014): The neural rejuvenation hypothesis of cocaine addiction. *Trends Pharmacol Sci.* 35: 374-383.
46. Lee B.R, Dong Y (2011): Cocaine-induced metaplasticity in the nucleus accumbens: silent synapse and beyond. *Neuropharmacology* 61: 1060-1069.
47. Kourrich S, Calu DJ, Bonci A (2015): Intrinsic plasticity: an emerging player in addiction. *Nat Rev Neurosci.* 16: 173-184.
48. Joffe ME, Grueter BA (2016): Cocaine Experience Enhances Thalamo-Accumbens N-Methyl-D-Aspartate Receptor Function. *Biol Psychiatry* 80: 671-681.

49. Neumann PA, Wang Y, Yan Y, Wang Y, Ishikawa M, Cui R, *et al.* (2016): Cocaine-Induced Synaptic Alterations in Thalamus to Nucleus Accumbens Projection. *Neuropsychopharmacology* 41: 2399-2410.
50. Sarti F, Borgland SL, Kharazia VN, Bonci A (2007): Acute cocaine exposure alters spine density and long-term potentiation in the ventral tegmental area. *Eur. J. Neurosci.* 26: 749-756.
51. Holtmaat A, Svoboda K (2009): Experience-dependent structural synaptic plasticity in the mammalian brain. *Nat. Rev. Neurosci.* 10: 647-658.
52. Knott GW, Holtmaat A, Wilbrecht L, Welker E, Svoboda K (2006): Spine growth precedes synapse formation in the adult neocortex in vivo. *Nat. Neurosci.* 9: 1117-1124.
53. Medvedev NI, Dallérac G, Popov VI, Rodriguez Arellano JJ, Davies HA, Kraev IV, *et al.* (2014): Multiple spine boutons are formed after long-lasting LTP in the awake rat. *Brain Struct. Funct.* 219: 407-414.
54. Yang Y, Liu DQ, Huang W, Deng J, Sun Y, Zuo Y, Poo MM (2016): Selective synaptic remodeling of amygdalocortical connections associated with fear memory. *Nat. Neurosci.* 19: 1348-1355.
55. De Roo M, Klauser P, Muller D (2008): LTP promotes a selective long-term stabilization and clustering of dendritic spines. *PLoS Biol.* 6: e219.

56. Chen JL, Villa KL, Cha JW, So PT, Kubota Y, Nedivi E (2012): Clustered dynamics of inhibitory synapses and dendritic spines in the adult neocortex. *Neuron* 74: 361-373.
57. Fu M, Yu X, Lu J, Zuo Y (2012): Repetitive motor learning induces coordinated formation of clustered dendritic spines in vivo. *Nature* 483: 92-95.
58. Lu J, Zuo Y (2017): Clustered structural and functional plasticity of dendritic spines. *Brain Res Bull.* 129: 18-22.
59. Futter M, Uematsu K, Bullock SA, Kim Y, Hemmings HC Jr, Nishi A, *et al.* (2005): Phosphorylation of spinophilin by ERK and cyclin-dependent PK 5 (Cdk5). *Proc. Natl. Acad. Sci. U. S. A.* 102: 3489-3494.
60. Kelleher RJ 3rd, Govindarajan A, Jung HY, Kang H, Tonegawa S (2004): Translational control by MAPK signaling in long-term synaptic plasticity and memory. *Cell* 116: 467-479.
61. Costa-Mattioli M, Sossin WS, Klann E, Sonenberg N (2009): Translational control of long-lasting synaptic plasticity and memory. *Neuron* 61: 10-26.
62. Scheyer AF, Wolf ME, Tseng KY (2014): A protein synthesis-dependent mechanism sustains calcium-permeable AMPA receptor transmission in nucleus accumbens synapses during withdrawal from cocaine self-administration. *J. Neurosci.* 34: 3095-3100.

63. Genheden M, Kenney JW, Johnston HE, Manousopoulou A, Garbis SD, Proud CG (2015): BDNF stimulation of protein synthesis in cortical neurons requires the MAP kinase-interacting kinase MNK1. *J. Neurosci.* 35: 972-984.
64. Panja D, Kenney JW, D'Andrea L, Zalfa F, Vedeler A, Wibrand K, *et al.* (2014): Two-stage translational control of dentate gyrus LTP consolidation is mediated by sustained BDNF-TrkB signaling to MNK. *Cell Rep.* 9: 1430-1445.
65. Peebles CL, Yoo J, Thwin MT, Palop JJ, Noebels JL, Finkbeiner S (2010): Arc regulates spine morphology and maintains network stability in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 107: 18173-18178.
66. Salery M, Dos Santos M, Saint-Jour E, Moumné L, Pagès C, Kappès V, *et al.* (2016): Activity-regulated cytoskeleton-associated protein accumulates in the nucleus in response to cocaine and acts as a brake on chromatin remodeling and long-term behavioral alterations. *Biol. Psychiatry* doi:10.1016/j.biopsych.2016.05.025.
67. Beckley JT, Laguesse S, Phamluong K, Morisot N, Wegner SA, Ron D (2016): The first alcohol drink triggers mTORC1-dependent synaptic plasticity in nucleus accumbens dopamine D1 receptor neurons. *J. Neurosci.* 36: 701-713.
68. Cane M, Maco B, Knott G, Holtmaat A (2014): The relationship between PSD-95 clustering and spine stability in vivo. *J. Neurosci.* 34: 2075-2086.

69. Meyer D, Bonhoeffer T, Scheuss V (2014): Balance and stability of synaptic structures during synaptic plasticity. *Neuron* 82: 430-443.

ACCEPTED MANUSCRIPT

FIGURE LEGENDS

Figure 1. A single administration of cocaine induces the rapid formation of stable glutamatergic synapses in the NAc

(A) Upper panel: time-course of the experiment. Following three days of habituation, VGLUT1^{venus} mice were injected with either saline or cocaine (ip, 20mg/kg) and perfused one hour later. Lower panel: 3D surface rendering of dendritic segments labeled by DiI (red) with the VGLUT1 boutons (green) in contact with spines. Spine heads were segmented and the associated boutons automatically selected using a 3D custom procedure implemented in ImageJ.

(B) Immunostaining for c-Fos following diolistic labeling of striatal neurons allowed the identification of SPN that responded to cocaine.

(C) Spine density of SPN from the NAc was measured one hour after a single exposure to saline or cocaine. Spine density was measured in randomly chosen SPN from saline and cocaine-treated mice and in c-Fos positive SPN from cocaine-treated mice.

(D) Synapse density, defined as the density of spines in contact with VGLUT1 boutons, is increased by acute cocaine.

(E) Percentage of spines connected to a VGLUT1 bouton is not altered by cocaine.

(F) Spine density from saline and cocaine-treated mice was measured one week after a single cocaine injection preceded by three days of habituation. Images illustrate the result of the 3D automated analysis of synaptic contacts.

(G) Measurements of spine density one week after a single exposure to cocaine.

(H) Density of VGLUT1-positive synapses was measured one week after a single injection of cocaine. Note the persistent connectivity change induced by cocaine.

(I) The percentage of VGLUT1-positive synapses remains constant one week after cocaine administration.

Scale bar in A and F = 5 microns, B = 10microns. Histograms represent mean \pm SEM with n=number of neurons analyzed from N = 4-5 mice in each condition. C-E: One-way ANOVA and Bonferroni post-test. G-I: T-test . *: $p < 0.05$, ***: $p < 0.001$. See supplemental figure S1 and S2.

Figure 2. Effect of a single cocaine exposure on spine morphology of SPN from NAc

(A) Results of the custom 3D segmentation of spine heads implemented in ImageJ software. A1: 3D volume rendering of a dendrite. A2: 3D volume rendering of the spine heads extracted with a custom procedure implemented in ImageJ, merged with 3D surface rendering of the dendrite. A3: 3D volume rendering of segmented headspine merged with 3D surface rendering of the original dendrite image.

(B-C) Cumulative frequency distribution of headspine volumes (B) and length (C) measured in neurons from mice perfused one hour after treatment with either saline or cocaine. When indicated (Cocaine c-Fos+) spines were quantified from neurons identified as SPN expressing c-Fos. Note that one hour after cocaine injection spines have a bigger head (B) and are longer (C) than in saline controls.

(D-E) Cumulative frequency distribution of headspine volumes (D) and length (E) measured in neurons from mice perfused one week after single injection of either saline or cocaine. One week after cocaine the spine heads volume (D) remained increased upon cocaine but spine length (E) was identical to saline controls.

(F-G) Dendritic spines were classified as thin or mushroom based on the diameter of the spine head. An increase in mushroom-shaped spine density is observed both one hour (F) and one week (G) after a single cocaine administration.

3900 to 6200 spines analyzed in $n = 29$ to 59 neurons from $N = 4$ mice in each condition. B-E: Kolmogorov-Smirnov test of distribution, F: One-way ANOVA and Bonferroni post-test, G: T-test. *: $p < 0.05$; **: $p < 0.01$, ***: $p < 0.001$.

Figure 3. Synapse formation through contact with pre-existing presynaptic boutons

(A-B) Density of VGLUT1^{Venus} boutons in the NAc, in a volume corresponding to a cube of 10 microns side. A single plane from confocal image stacks is shown (left panels). The density is similar in saline and cocaine-treated mice one hour (A), and one week (B) after a single cocaine injection (Mean \pm SEM, t-test). Right panels: Percentage of spines connected to a common VGLUT1 bouton (Median \pm Interquartiles, Mann Whitney test). N mice and n neurons are identical to figure 1.

(C) Maximum intensity projection of a dendritic segment imaged from tdTomato-labeled D1-SPN in acute striatal slice with two-photon microscopy. D1-SPN were labeled by in vivo co-infection with an AAV9 expressing Cre recombinase under PPTA promoter and an AAV9 carrying FLEX-tdTomato. Two weeks later, slices are stimulated with SKF38393 (10 μ M) and glutamate (3 μ M) for 20 minutes then washed with control ACSF. Green arrows indicate locations of newly-formed spines.

(D) Cumulative curve of spine growth represents the percentage of new spines using the number of spines present before the co-stimulation as reference. The increase in spine growth becomes significant between control and stimulated D1-SPN 25 minutes after the beginning of the stimulation. Two-way ANOVA and Bonferroni post-test.

(E) Comparison of spine density between 0 and 60 minutes time points confirms an increase in stimulated condition similar to *in vivo* conditions (see fig. 1). Data are expressed as mean \pm SEM, $n = 6$ neurons from $N = 4$ mice for each condition, T-test.

(F) Time-lapse analysis of synaptogenesis shows that new spines contact pre-existing VGLUT1-positive boutons. Single plane from 3D two-photon microscopy images.

(G) left panel : Nascent spines in D1-SPN are identified with tdTomato labeling and their synaptic activity is visualized using GCaMP6S calcium sensor during co-stimulation. To avoid fluorescence fluctuations independent of calcium variations, GCaMP6S signal is normalized using tdTomato signal. Nascent spines are considered as active if they present GCaMP6 fluorescent peaks ($\Delta F/F$) exceeding 0.3, compared to baseline. Maximum projections show active (green arrow) and silent (red arrow) nascent spines. The spine head diameter was measured from non-active and active spines (Fig. 3G, right panel). Data show values and mean \pm SEM, $n = 10$ spines from 3 independent neurons and $N = 3$ mice, T-test.

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. See supplemental figures S3 and S4. Scale bar in A, B = 5 microns, C = 4 microns, F and G = 1 micron.

Figure 4. A single administration of cocaine induces the rapid formation of thalamo-striatal synaptic contacts in the NAc

(A) D1-SPN were labelled through intra-NAc viral infection with viruses AAV-PPTA::Cre, AAV-FLEX-tdTomato (orange) and thalamic boutons were immunolabeled for VGLUT2 (cyan).

(B) One hour after a single administration of cocaine, the spine density is increased.

(C) Acute cocaine induces the rapid formation of VGLUT2-positive, thalamo-striatal synaptic contacts.

(D) Percentage of spines connected to a VGLUT2 bouton is not altered by cocaine. Note that the percentage is lower than for VGLUT1, owing to the difference in the efficiency of the labeling (knock-in mouse versus immunolabeling).

Scale bar = 2 microns. Histograms represent mean \pm SEM with n=number of neurons analyzed from N = 4 mice in each condition. B-D: T-test. **: $p < 0.01$, ***: $p < 0.001$.

Figure 5. Molecular mechanisms involved in spine density increase

Measurements of spine density from SPN labeled with DiI in striatal slices co-stimulated with low doses of glutamate and D1R agonist. The co-stimulation induces an increase in spine density.

(A) Inhibition of ERK pathway by U0126 (10 μ M) abolishes the spine density increase.

(B) Blockade of mTOR pathway with rapamycin (25 nM) in acute slices totally inhibited the increase in spine density elicited by co-stimulation.

(C) Scheme of the involved pathways showing the inhibitors used in the study. Both MNK-1 and mTOR activation are essential for the onset of translation.

(D) Measurement of the density of pMNK-1 positive cells in acute slice after co-stimulation.

(E) The inhibitor of MNK-1 activation, CGP57380 (10 μ M), cancels the increase in spine density induced by co-stimulation of SPN from striatal slices.

Histograms show mean \pm SEM with n=number of neurons analyzed from N = 3-5 mice for each condition. A-D and G: Two-way ANOVA and Bonferroni post-test; F: T-test; *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. Scale bar in F = 30 microns, G= 10 microns.

Figure 6. Time lapse imaging and molecular dissociation of spine growth and stabilization

(A) Schematic representation of the experiment. D1-SPN were labelled through intra-NAc viral infection with viruses AAV-PPTA::Cre, AAV-FLEX-tdTomato ; spine growth and stabilization were analysed in 3D in time-lapse images from striatal slices. Green arrows show

new spines forming during the hour following the co-stimulation, the red arrow indicates when these new spines are not stabilized.

(B) Maximum intensity projection of two-photon image stacks from D1-SPN in NAc acute slices. Time-lapse observations reveal spine growth and stabilization of the new spines upon co-stimulation. Also shown are slices pre-treated with the inhibitor of MNK-1 (CGP 57380), protein synthesis (anisomycin) and transcription (actinomycin D).

(C) Cumulative curves of spine growth following co-stimulation. In the presence of the ERK inhibitor (U0126, 10 μ M), the spine growth is absent, remaining at levels identical to vehicle condition. Note that spine growth kinetics and amplitude induced by co-stimulation are not altered by CGP 57380 (10 μ M), anisomycin (25 μ M) or actinomycin D (25 μ M). Two-way ANOVA and Bonferroni post-test.

(D) Survival curve showing the maintenance of newly formed spines through time. Spines formed in the presence of CGP 57380 or anisomycin show a marked loss of stability. Actinomycin D does not alter spine maintenance in this time window. Log rank test.

n = 5-7 neurons from N = 3-4 mice, with 27-44 spines formed in each condition. *: p < 0.05, **: p < 0.01, ***: p < 0.001. Scale bar = 2 microns.

Figure 7. MNK-1 is activated after acute cocaine injection and is necessary for structural plasticity *in vivo*

(A) Immunolabeling for pMNK-1 in the shell of the NAc from mice perfused ten minutes after either saline or cocaine injection. Single planes of confocal image stacks from the shell part of the NAc. The density increase in pMNK-1 positive cells induced by a single administration of cocaine is abolished by ERK inhibitor SL327 injected *in vivo* prior to cocaine. Mean \pm SEM, N = 3 mice for each condition. One-way ANOVA and Bonferroni post-test.

(B) Analysis of the density in P-MNK-1 positive cells in the shell of the NAc reveals a rapid and transient phosphorylation following a single cocaine administration. Two-way ANOVA and Bonferroni post-test, Drug effect: *, Time effect: °.

(C) Immunostaining for pMNK-1 in D1-SPN labeled with tdTomato reveal dendritic localization of pMNK-1. Upper panel shows single planes from confocal stack with arrows indicating pMNK-1 spots in the dendrite. Lower panel shows 3D volume rendering of the co-localization of pMNK-1 in a dendrite.

(D) The MNK-1 inhibitor CGP 57380 was injected into the NAc prior cocaine administration.

(E) *In vivo* analysis of dendritic spines labeled with DiI following injection in the NAc of the inhibitor CGP 57380 prior a single administration of cocaine. 3D surface rendering of confocal images from different conditions. The inhibition of MNK-1 blocks the increase in spine density induced by cocaine. Histograms show mean \pm SEM with n = number of neurons analyzed from N = 3-5 mice for each condition, Two-way ANOVA and Bonferroni post-test.

Scale bar in A = 50 microns, C-E = 1 micron. *: $p < 0.05$, ** or °: $p < 0.01$, *** or °°: $p < 0.001$.

Figure 8. Proposed model for synaptogenesis in the adult striatum

Dopamine, which level is elevated under cocaine condition, facilitates NMDAR functions and triggers ERK activation and spinogenesis. Synapse formation occurs through clustered spine growth and contact with pre-existing presynaptic boutons. ERK pathway regulates distinct steps of spine formation through different signaling modules. Firstly, spine growth is controlled by ERK, probably through phosphorylation of pre-existing cytoplasmic targets. Secondly, stabilization of the spine is controlled by ERK-induced MNK-1 dependent protein synthesis in a transcription independent manner. Importantly, MNK-1 and protein synthesis

are not involved in spine growth, so spine growth and stabilization involve different signaling molecules downstream ERK.

ACCEPTED MANUSCRIPT















