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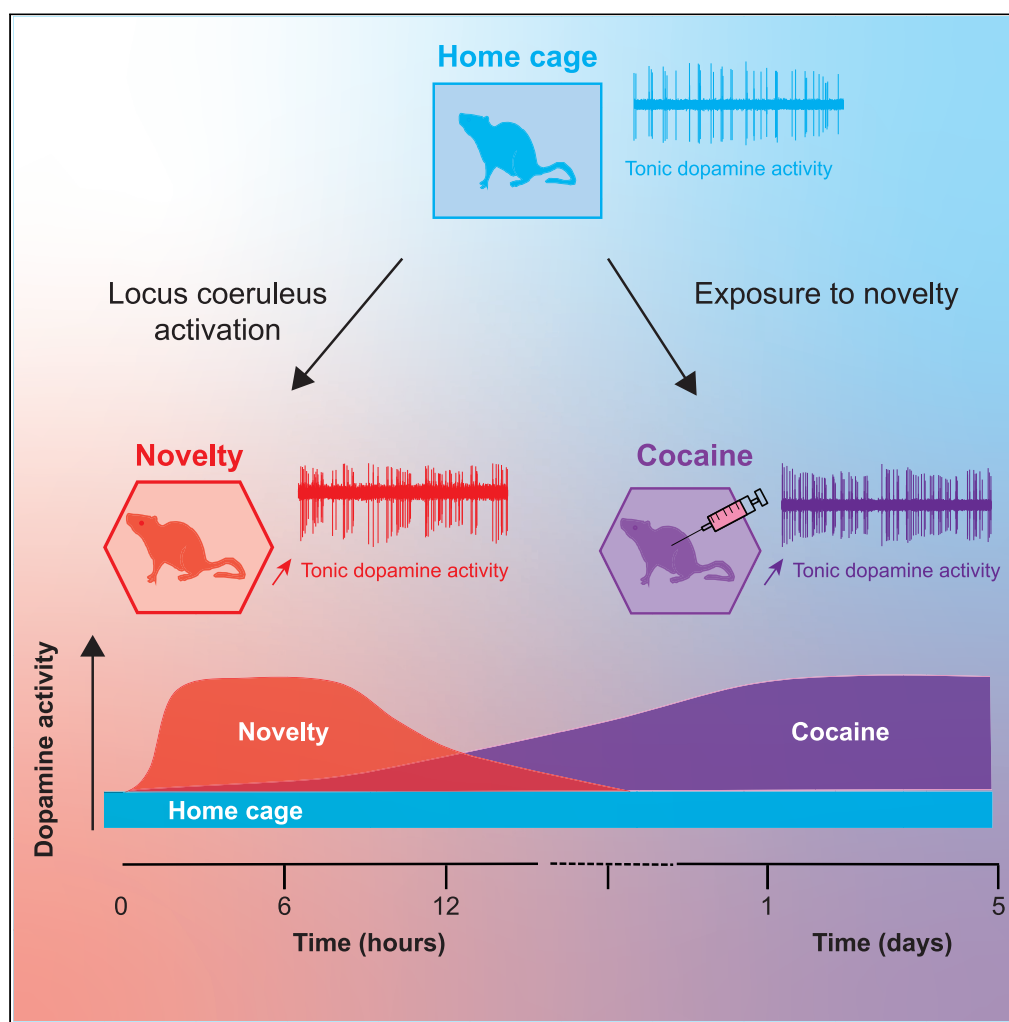
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## Article

## Locus coeruleus activation during environmental novelty gates cocaine-induced long-term hyperactivity of dopamine neurons



Giulia R. Fois, Karl Y. Bosque-Cordero, Rafael Vazquez-Torres, ..., Carlos A. Jimenez-Rivera, Stéphanie Caille, François Georges

francois.georges@u-bordeaux.fr

## Highlights

Exposure to a novel environment increases the tonic activity of dopamine neurons

Non-spatial contextual novelty had no excitatory effect on dopamine neurons

Locus coeruleus (LC) controls the impact of novelty on dopamine neurons

Novelty gates cocaine's effect on dopamine neurons through LC activation

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## Article

## Locus coeruleus activation during environmental novelty gates cocaine-induced long-term hyperactivity of dopamine neurons

Giulia R. Fois,<sup>1</sup> Karl Y. Bosque-Cordero,<sup>2</sup> Rafael Vazquez-Torres,<sup>3</sup> Cristina Miliano,<sup>4</sup> Xavier Nogues,<sup>5</sup> Carlos A. Jimenez-Rivera,<sup>3</sup> Stéphanie Caille,<sup>6</sup> and François Georges<sup>1,7,\*</sup>

## SUMMARY

**A key feature of the brain is the ability to handle novelty. Anything that is new will stimulate curiosity and trigger exploration. Novelty preference has been proposed to predict increased sensitivity to cocaine. Different brain circuits are activated by novelty, but three specific brain regions are critical for exploring a novel environment: the noradrenergic neurons originating from the locus coeruleus (LC), the dopaminergic neurons from the ventral tegmental area (VTA), and the hippocampus. However, how exploring a novel environment can interfere with the reward system and control cocaine impact on VTA dopamine neuron plasticity is unclear. Here, we first investigated the effects of exposure to a novel environment on the tonic electrophysiological properties of VTA dopamine neurons. Then, we explored how exposure to a novel environment controls cocaine-evoked plasticity in dopamine neurons. Our findings indicate that LC controls VTA dopamine neurons under physiological conditions but also after cocaine.**

## INTRODUCTION

Being exposed to a new environment may be perceived by the individual as an anxiogenic situation. However, in the absence of threats, the individual, driven by curiosity, will explore the novel environment in order to integrate salient events and discover new opportunities with a beneficial outcome. This behavior is preserved in different species as it is essential for survival (Berlyne, 1966; Henry et al., 2010). However, this exploratory behavior can persist even in the absence of stimuli that are beneficial or situations that are harmful to the individual (Henry et al., 1992). Exploratory behavior in the absence of salient stimuli suggests that animals are motivated by curiosity to explore their environment (Berlyne, 1966). In fact, curiosity has been conceptualized as a motivated behavior to obtain information, and this information becomes the reward (Kidd and Hayden, 2015). **Exploration of a novel environment in search of beneficial or instructive information is the first step in the physiological processes associated with reinforcement learning, motivation, or reward seeking.** One of the best-described roles of ventral tegmental area (VTA) dopamine neurons is their phasic activation in response to new, unexpected, and salient events (Takeuchi et al., 2016). Although its role has been understudied, it has recently been shown that the tonic activity of VTA dopamine neurons codes for reward values in a given situation (Wang et al., 2021). Moreover, we recently demonstrated that tonic VTA dopamine neuron activity is directly correlated with motivation to engage in a rewarding behavior (Muguruza et al., 2019). The importance of the tonic activity of the dopaminergic system for novelty seeking and exploration has also been assessed through pharmacological interruption of dopamine transmission in the striatum and inhibition of VTA dopamine neuron tonic activity, which results in a decrease of novelty seeking in the environment (Hooks and Kalivas, 1995). Moreover, it has been shown that curiosity drives activity in midbrain dopaminergic regions (Gruber et al., 2014; Lisman and Grace, 2005). In this context, the fact that curiosity causes animals to spontaneously engage in a wide range of exploratory behaviors without temporal coding led us to hypothesize that the behavioral exploration of a new context will affect the tonic activity of dopamine neurons.

It has been shown that increased locomotor activity in a novel environment may be predictive of increased sensitivity to psychostimulants (Bardo et al., 1996; Belin et al., 2011). It is well known that cocaine-evoked dopamine release is controlled by the tonic activity of VTA dopamine neurons (Sombers et al., 2009). Moreover, synaptic plasticity in VTA dopamine neurons induced by the first exposure to cocaine conditions the

<sup>1</sup>CNRS, IMN, UMR5293, Université de Bordeaux, 33000 Bordeaux, France

<sup>2</sup>Biology Department, University of Puerto Rico Rio Piedras Campus, San Juan, Puerto Rico

<sup>3</sup>Physiology Department, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico

<sup>4</sup>Department of Biomedical Sciences, Division of Neuropsychopharmacology, University of Cagliari, Cagliari, Italy

<sup>5</sup>Université de Bordeaux, 33000 Bordeaux, France

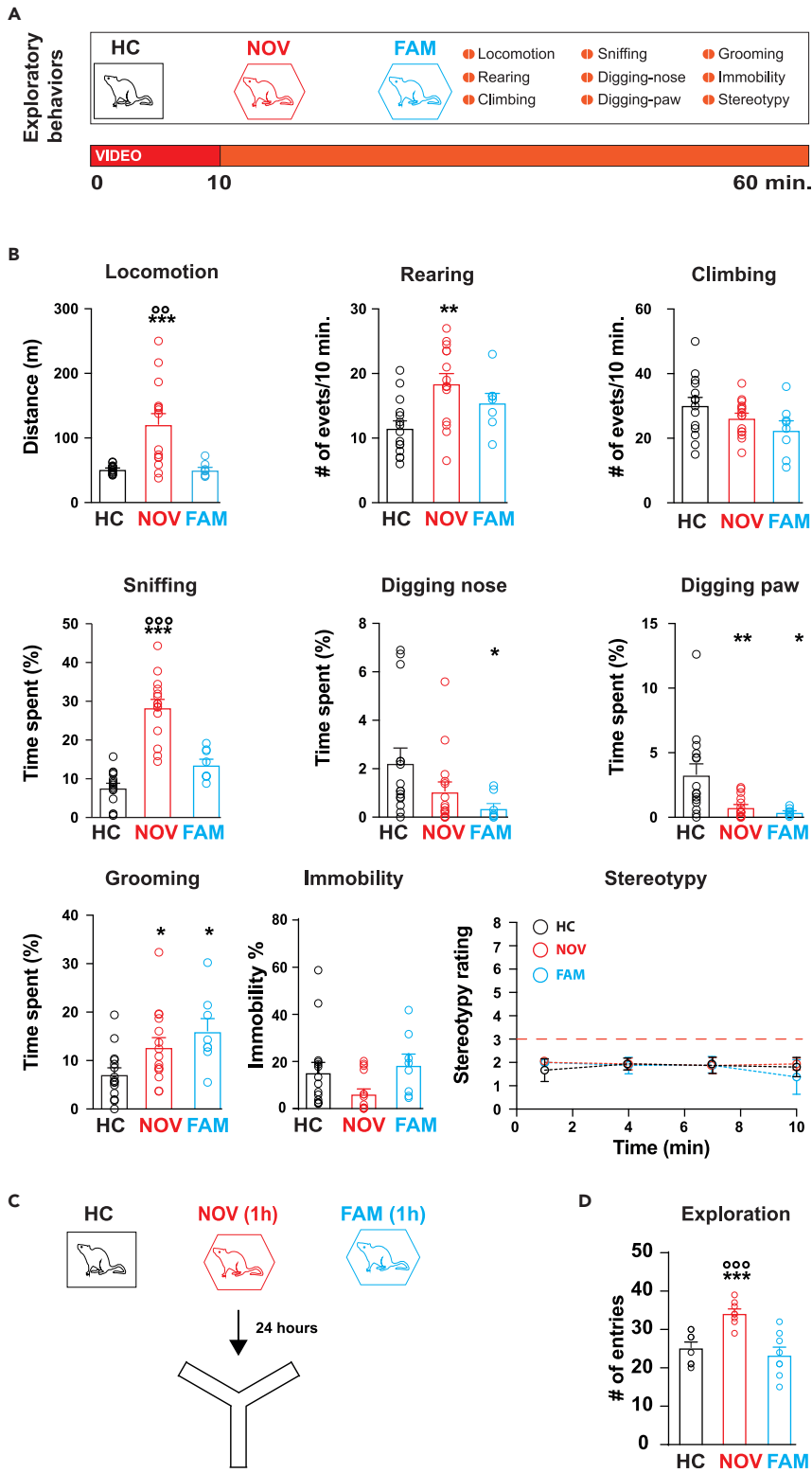
<sup>6</sup>CNRS, EPHE, INCIA, UMR5287, Université de Bordeaux, 33000 Bordeaux, France

<sup>7</sup>Lead contact

\*Correspondence: francois.georges@u-bordeaux.fr

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**Figure 1. Exposure to a novel environment drives explorative behavior and promotes the exploration of other novel environments the next day**

(A) Schematic of the experimental procedure.

(B) Quantification of explorative behavior: locomotor activity, rearing, climbing, sniffing, digging, grooming, immobility, and stereotypies.

**Figure 1. Continued**

(C) Schematic of the experimental procedure.

(D) Quantification of Y-maze test 24 h after exposure to a novel or a familiar environment.

HC, home cage; NOV, novel environment; FAM, familiar environment.

\*Significance compared with HC; <sup>○</sup>significance compared with FAM. Details of statistical analysis in Table S1.

initial phases of consumption (Saal et al., 2003; Ungless et al., 2001). Cocaine's impact on behavior can be strongly influenced by the environmental context in which the drug is experienced (Caprioli et al., 2007). However, it remains to be explored how exposure to a novel environment interferes with long-lasting changes induced by cocaine in the mesolimbic dopamine system.

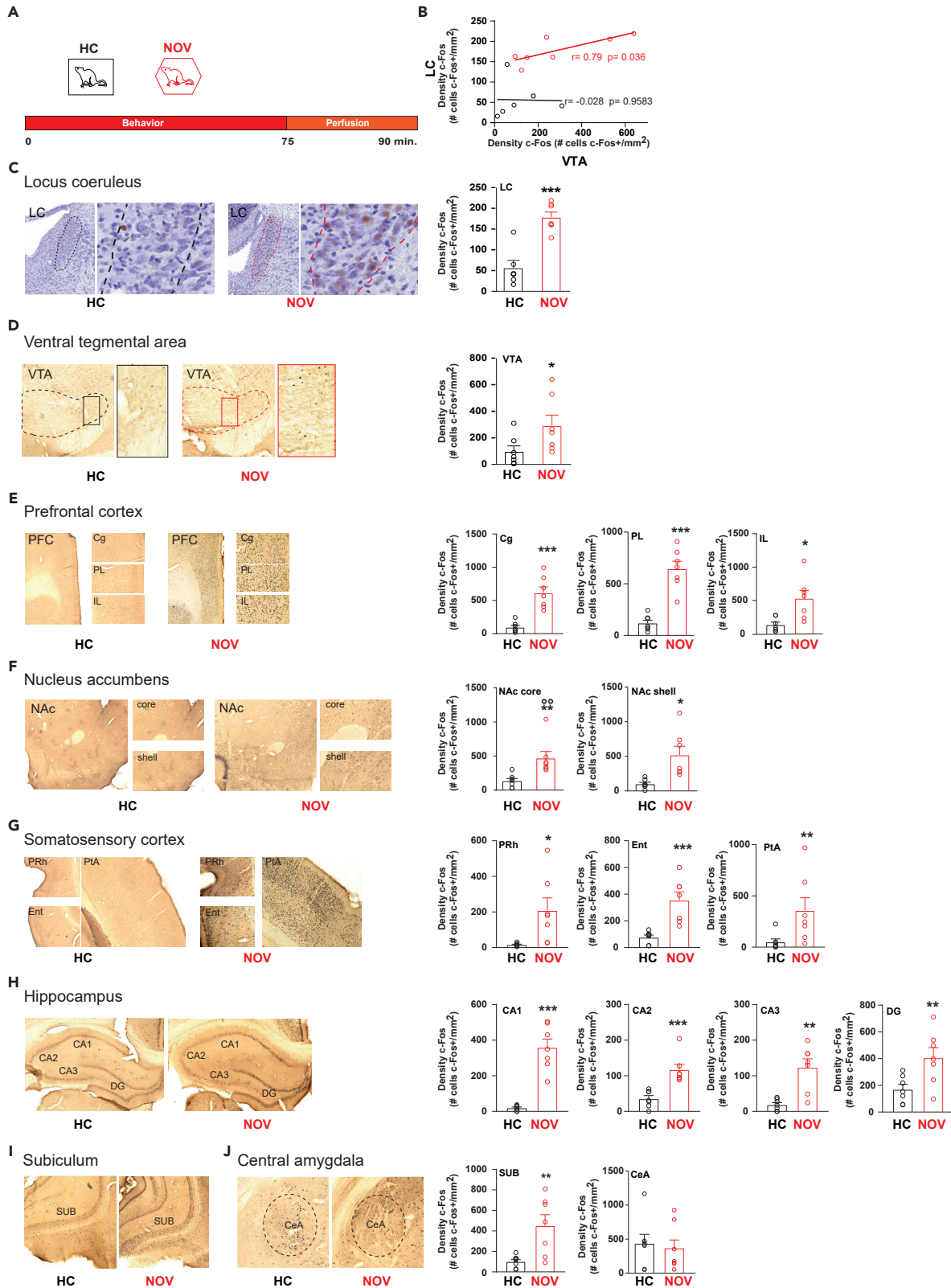
The locus coeruleus (LC) noradrenergic system is directly connected to the VTA dopamine system (Isingrini et al., 2016) and is as critical as the dopamine system for novelty detection (Takeuchi et al., 2016). Recent studies confirm that LC noradrenergic neurons increase their tonic activity in a novel environment (Lisman and Grace, 2005; Takeuchi et al., 2016), and like dopamine neurons, respond to unpredicted environmental stimuli that are salient through their novel and surprising features (Horvitz, 2000; Takeuchi et al., 2016). However, it is not clear whether novelty is biologically relevant for the integrative properties of VTA dopamine neurons in physiological conditions or after cocaine. We hypothesized that LC noradrenergic neurons control the impact of novelty and cocaine on VTA dopamine neurons. To test this, we used a combination of measures of exploratory behavior, chemogenetic manipulations in freely behaving animals, and *in vivo* and *ex vivo* recordings of VTA dopamine neurons. In this study, we report that exploration of a new environment causes a change in the tonic activity of the VTA dopamine neurons and that this change is controlled by the LC. We further show that exposure to a novel environment is required for the cocaine-induced plastic changes of VTA dopamine neurons, which is mediated by the LC. Based on our data, we propose that curiosity-driven exploratory behavior in rats has the power to change the excitability state of VTA dopamine neurons for several hours, acting as a booster, thus making them more sensitive to the integration of subsequent salient stimuli such as cocaine exposure.

**RESULTS****Explorative behavior in a novel environment**

Activation of VTA dopamine neurons is required for the acute expression of novelty-induced motor activity (Hooks and Kalivas, 1995), but the long-term influence of novelty on VTA dopamine neuron activity is unknown. First, three groups of rats were tested for their motor and explorative behaviors in their home cage (HC), in a novel environment (NOV), or in an environment made familiar by re-exposure for 1 h over five consecutive days (FAM) (Figure 1A). Rats exposed to a novel environment exhibited high locomotor activity (*n* values refer to the number of rats, unless otherwise stated; HC, *n* = 15; NOV, *n* = 15; FAM, *n* = 8;  $p < 0.0001$ ) and sustained explorative behavior characterized by an increase in rearing (HC, *n* = 15; NOV, *n* = 15; FAM, *n* = 8;  $p = 0.0023$ ), sniffing (HC, *n* = 15; NOV, *n* = 15; FAM, *n* = 8;  $p < 0.0001$ ), and grooming (HC, *n* = 15; NOV, *n* = 15; FAM, *n* = 8;  $p = 0.0105$ ) behaviors (Figure 1B). These behavioral changes decreased when the environment had become familiar (Figure 1B). Exploratory behavior did not include any stereotypy (HC, *n* = 15; NOV, *n* = 15; FAM, *n* = 8; ns; Figure 1B). Interestingly, we observed that a 1-h exposure to a novel environment promotes the exploration of other novel environments (exploration of a Y-maze) the next day (Figures 1C and 1D). This sensitization of explorative behavior was specific to the novelty episode of the previous day, as it decreased when rats were instead in a familiar environment (HC, *n* = 8; NOV, *n* = 8; FAM, *n* = 8;  $p = 0.0002$ ; Figures 1C and 1D).

**Impact of exposure to a novel environment on c-Fos activation in the brain**

The comparison process that is necessary to identify an environment as familiar or novel requires that sensory, attentional, motivational, and memory systems together form a putative novelty neuronal circuit (Johnston and Edwards, 2002). To map the brain regions responding to novelty, we examined the expression of the immediate-early gene c-Fos, 90 min after exposure to a novel environment (Figure 2). Control animals housed in their home cage displayed few c-Fos + nuclei in identified brain areas of the novelty neuronal circuit. However, the rats exposed to a novel environment exhibited elevated expression of c-Fos in the LC (HC, *n* = 6; NOV, *n* = 7;  $p < 0.0001$ ), VTA (HC, *n* = 7; NOV, *n* = 7;  $p = 0.0122$ ), prefrontal cortex (cingular cortex: HC, *n* = 7; NOV, *n* = 7;  $p = 0.001$ ; prelimbic cortex: HC, *n* = 7, NOV, *n* = 7;  $p = 0.0002$ ; infralimbic cortex: HC, *n* = 7, NOV, *n* = 7;  $p = 0.0167$ ), nucleus accumbens (core: HC, *n* = 7; NOV, *n* = 7;  $p = 0.0039$ ; shell: HC, *n* = 7; NOV, *n* = 7;  $p = 0.0035$ ), somatosensory associative cortices (parietal associate



**Figure 2. Impact of exposure to a novel environment on c-Fos activation in the brain**

(A) Experimental groups and kinetics of the experimental procedure used for quantification of c-Fos activation in rats in their home cage (HC) or exposed to a novel environment (NOV).

(B) Correlative Pearson analysis of c-FOS activation levels in ventral tegmental area (VTA) and LC in home cage animals (HC, black circles), and in animals exposed to a novel environment (NOV, red circles).

(C–J) The impact of exposure to a novel environment on c-Fos in the LC (C); in the VTA (D); in the prefrontal cortices ((E), cingulate cortex, Cg; prelimbic cortex, PL; infralimbic cortex, IL); in the nucleus accumbens ((F), nucleus accumbens core, NAc core; nucleus accumbens shell, NAc shell); in the somatosensory cortex ((G), parietal associate cortex, PtA; perihinal cortex, PRh; entorhinal cortex, Ent); in the hippocampus ((H), cornu ammonis: CA1, CA2, CA3; dentate gyrus, DG), in the subiculum ((I), SUB), and in the central amygdala ((J), CeA).

\*Significance compared with HC. Details of statistical analysis in [Table S2](#).

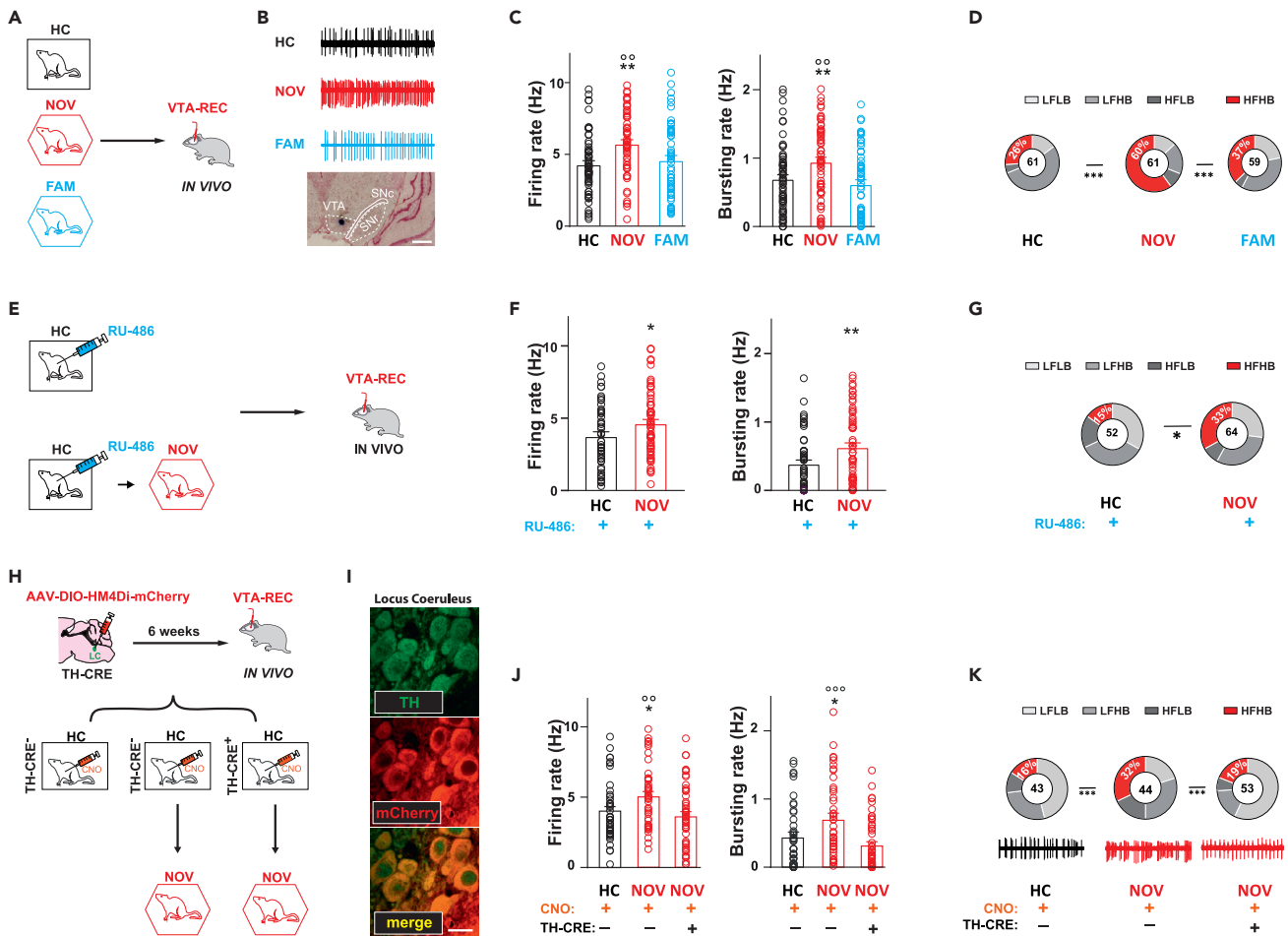
cortex: HC,  $n = 7$ ; NOV,  $n = 7$ ;  $p = 0.0173$ ; entorhinal cortex: HC,  $n = 7$ , NOV,  $n = 7$ ;  $p = 0.0005$ ; perihinal cortex: HC,  $n = 7$ ; NOV,  $n = 7$ ;  $p = 0.0175$ , and hippocampal formation (CA1: HC,  $n = 7$ ; NOV,  $n = 7$ ;  $p < 0.0001$ ; CA2: HC,  $n = 7$ ; NOV,  $n = 7$ ;  $p = 0.0003$ ; CA3: HC,  $n = 7$ , NOV,  $n = 7$ ;  $p = 0.0006$ ; dentate gyrus HC,  $n = 7$ , NOV,  $n = 7$ ;  $p = 0.0084$ ; subiculum HC,  $n = 7$ ; NOV,  $n = 7$ ;  $p = 0.0059$ ) ([Figures 2C–2I](#)). No change in c-Fos expression was observed in the central amygdala after exposure to a novel environment ([Figure 2J](#)). Furthermore, a Pearson correlative analysis demonstrated a correlation between c-Fos expression in the VTA and the LC in novelty-exposed group (HC,  $n$  (rats) = 6;  $r = 0.028$ ; ns; NOV,  $n$  (rats) = 7;  $r = 0.79$ ;  $p = 0.036$ ; [Figure 2B](#)).

**LC controls the impact of novelty on VTA dopamine neurons**

Phasic activation of VTA dopamine neurons is required for the acute expression of novelty-induced motor activity ([Hooks and Kalivas, 1995](#)), but the long-term influence of novelty on tonic VTA dopamine neuron activity is unknown. To investigate the neural circuits underlying the response to novelty, we subjected rats to a novel environment for 1 h and examined the consequences on the activity of VTA dopamine neurons ([Figures 3, S1–S3, and S5](#)). The firing rate, bursting activity, and modes of firing patterns ([Mameli-Engvall et al., 2006](#)) of VTA dopamine neurons were measured *in vivo* in rats exposed to a novel environment ([Figures 3A–3D](#)). During a period of 6 h after exposure to a novel environment, VTA dopamine neuron impulse activity was higher than in animals in their home cage or exposed to a familiar environment (HC,  $n = 10$ ;  $n$  (neurons) = 61; NOV,  $n = 5$ ;  $n$  (neurons) = 61; FAM,  $n = 7$ ,  $n$  (neurons) = 59;  $p = 0.0016$ ; [Figures 3A–3D](#)). Moreover, exposure to a novel environment increased the proportion of VTA dopamine neurons in the high frequency and high burst firing (HFHB) mode ( $p < 0.0001$ ; [Figure 3D](#)) ([Mameli-Engvall et al., 2006](#)). The relative distribution of the four main VTA dopamine neuron firing patterns was identical when rats were in their home cage or exposed to a familiar environment ([Figure 3D](#)). Beyond 6-h post-exposure to a novel environment, the activity of the VTA dopamine neurons was similar to that of rats in their home cage ([Figure S1 and Table S6](#)). To test whether these changes in neuronal activity induced by exposure to a novel environment could be owing to the change in housing conditions during the experimental procedure, rats were isolated for 1 h or 5 days (in their home cage by removing their littermates) and the impact on VTA dopamine neurons activity was measured ([Figure S2 and Table S6](#)). Firing activity and relative distribution of the four main modes of VTA dopaminergic firing patterns were similar in rats in their home cage and rats isolated for 1 h, suggesting no impact of a 1-h isolation period on VTA dopamine neuron activity. However, a 5-day isolation period triggered hypoactivity of the VTA dopamine neurons, probably owing to stressful housing conditions ([Manouze et al., 2019](#)).

Next, we tested whether the effect of novelty on VTA dopamine neurons was specifically driven by a change in a spatial context, or if any change in the context produces similar effects. The effect of non-spatial contextual novelty was measured in rats exposed to a male intruder in their home cage or a novel environment. In both conditions, in response to a novel intruder, there was an immediate decrease in bursting activity and the proportion of HFHB VTA dopamine neurons, changes that lasted 24 h ([Figure S3 and Table S6](#)), suggesting a specific effect of spatial exploration on the activation of VTA dopamine neurons during an episode of novelty. The fact that every electrophysiological parameter of the dopaminergic neurons decreases in the presence of the intruder suggests a stress effect probably owing to possible male-to-male aggressions ([Anstrom et al., 2009](#)).

Stress is known to trigger synaptic changes in VTA dopamine neurons ([Saal et al., 2003](#)). In rodents, stress is characterized by a rapid release of corticosterone, which acts on the glucocorticoid receptor (GR). To test whether GR activation plays a role in the effects of a novel environment on VTA dopamine neurons, we injected the GR antagonist RU-486 30 min before exposure to a novel environment ([Figures 3E–3G](#)). RU-486 did not prevent the increase of activity in VTA dopamine neurons (HC:  $n = 4$ ,  $n$  (neurons) = 52; NOV:  $n = 5$ ,



**Figure 3. LC controls the impact of novelty on VTA dopamine neurons**

(A) Schematic of the experimental procedure for *in vivo* recordings.

(B) Representative electrophysiological traces for each group and representative histological control of the VTA recording site.

(C) Bar graphs of the quantification of VTA dopamine neuron firing and bursting activity. \*Significance compared with HC; <sup>○</sup>significance compared with FAM.

(D) Donut representation of the proportion of dopaminergic neurons classified on the basis of their firing and bursting activity.

(E) Schematic of the experimental procedure for *in vivo* recordings after systemic injection of the GR antagonist RU-486 (40 mg/kg, i.p.).

(F) Bar graphs of the quantification of VTA dopamine neuron firing and bursting activity. \*Significance compared with HC-RU-486.

(G) Donut representation of the proportion of dopaminergic neurons classified on the basis of their firing and bursting activity.

(H) Schematic of the experimental procedure for viral transfection used for chemogenetic manipulations, clozapine N-oxide (CNO) injection (1 mg/kg i.p.) and *in vivo* recordings.

(I) Representative histological controls of mCherry-tagged viral transfections into LC noradrenergic neurons identified by tyrosine hydroxylase (TH) immunostaining. Scale bar: 20  $\mu$ m.

(J) Bar graphs of the quantification of VTA dopamine neuron firing and bursting activity. \*Significance compared with TH-CRE- HC; <sup>○</sup>significance compared with TH-CRE + NOV.

(K) Donut representation of the proportion of dopaminergic neurons classified on the basis of their firing and bursting activity. In (D, G, and K), four categories of neurons are defined: low-frequency and low-burst firing (LFLB), low-frequency and high-burst firing (LFHB), high-frequency and low-burst firing (HFLB), and high-frequency and high-burst firing (HFHB). HC, home cage; NOV, novel environment, FAM, familiar environment; VTA-REC, VTA recordings. Details of statistical analysis in Table S3.

$n$  (neurons) = 64; firing rate:  $p < 0.05$ , bursting rate:  $p = 0.0035$ ; Figure 3F), nor the change in the relative distribution of the four main modes of VTA dopamine firing patterns ( $p = 0.0181$ ; Figure 3G), suggesting that the involvement of stress-related effects mediated by the GR is unlikely.

The correlation between LC and VTA activation during exposure to a novel environment (Figure 2B), and the fact that LC noradrenergic neurons control VTA dopamine neurons (Isingrini et al., 2016) led us to



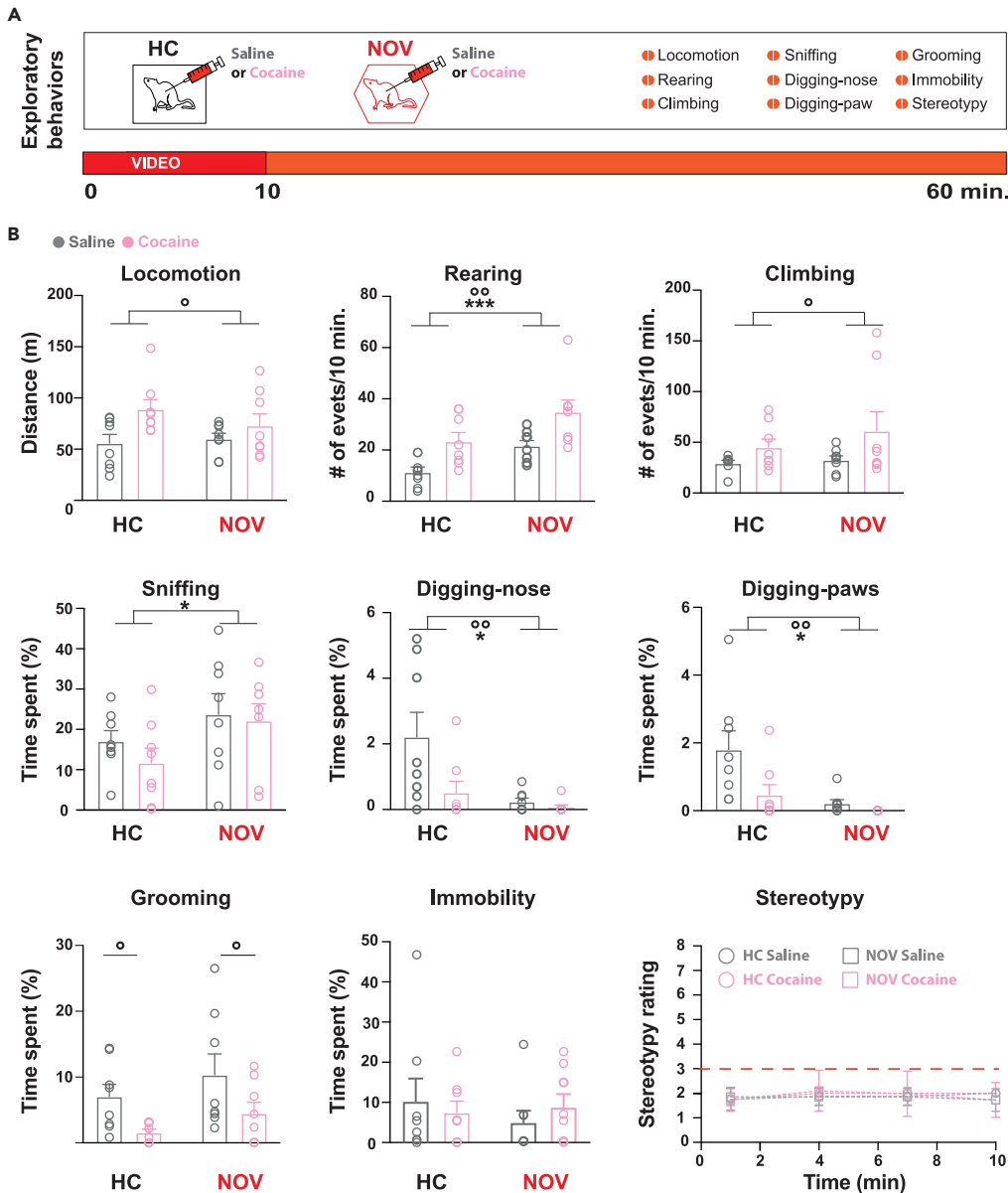
hypothesize that changes in the activity of LC noradrenergic neurons during exposure to a novel environment gate changes in the tonic activity of VTA dopaminergic neurons. To further determine whether LC noradrenergic neurons are specifically required for the novelty-induced activation of VTA dopamine neurons, we perturbed the activation of LC noradrenergic neurons while animals were exposed to a novel environment (Figures 3H–3K). To this end, we first inhibited LC noradrenergic neurons by expressing the inhibitory designer receptor exclusively activated by designer drug (DREADD) hM4Di via stereotaxic LC injections of an AAV5-DIO-hM4Di-mCherry in transgenic rats where the rat tyrosine hydroxylase (TH) promoter drives expression of Cre-recombinase in catecholaminergic cells (TH-CRE+), or the control genotype TH-CRE-. All groups received acute clozapine-N-oxide (CNO, the DREADD agonist) before exposure to a novel environment (Figure 3H–3K). Notably, we found that six weeks after AAV infection, there was a specific expression of mCherry in LC noradrenergic neurons (Figure 3I) and *in vivo* chemogenetic inhibition of neurons with CNO caused a reduction in LC noradrenergic neuronal tonic activity and excitability evoked by sensory stimulation (Figure S4 and Table S6). We found that a CNO injection (1 mg/kg i.p.) before exposure to a novel environment prevented the increase in firing activity parameters of VTA dopamine neurons in TH-CRE+ rats expressing hM4Di in LC noradrenergic neurons (TH CRE- HC,  $n = 3$ ,  $n$  (neurons) = 43; TH CRE- NOV,  $n = 3$ ,  $n$  (neurons) = 44; TH CRE+ NOV,  $n = 4$ ,  $n$  (neurons) = 53; firing rate:  $p = 0.0065$ ; bursting rate:  $p = 0.0003$ ) (Figures 3H–3K, S4, and S5 for control conditions, Table S6). Interestingly, we observed that in the TH CRE- NOV group, there is an increase in the HFHB neuronal subpopulation ( $p < 0.0001$ ) but it is blocked in the TH CRE+ NOV group, thus keeping the distribution of the four main firing modes of VTA dopamine neurons comparable with the TH CRE- HC group. These results indicate that the activation of LC noradrenergic neurons triggered by exposure to a novel environment is necessary for VTA dopamine neuron activation.

### Novelty gates cocaine's effect on VTA dopamine neurons through LC noradrenergic neuron activation

Acute behavioral effects of cocaine are owing to the complex combination of the molecular and neuronal targets affected by the drug but also the context in which cocaine is experienced (Caprioli et al., 2007). As it is well established that novelty potentiates cocaine-induced locomotor activity (Day et al., 2001), we evaluated the effect of cocaine experienced in a novel or familiar environment on exploratory behavior (Figure 4). The effectiveness of acute cocaine treatment on behavior was confirmed by the decrease in grooming observed in rats in their home cage and rats exposed to a novel environment (Figure 4B) (Carey et al., 2005). Only the novel environment triggered exploratory behavior, but this was not affected by cocaine treatment (sal-HC,  $n = 8$ ; coc-HC,  $n = 8$ , sal-NOV,  $n = 8$ ; coc-NOV,  $n = 8$ ; Figure 4B). Finally, because most rodents exhibit stereotypic behaviors following repeated exposure to psychostimulants, we measured stereotypies in our experimental conditions (Jaber et al., 1995; Robinson and Becker, 1986). As expected, an acute injection of cocaine under our experimental conditions did not induce any stereotypic behavior (Figure 4B). The plastic adaptation of VTA dopamine neurons after acute cocaine is thought to represent an indispensable initial step leading to addictive behavior (Chen et al., 2010; Creed and Luscher, 2013). However, the role of exposure to a novel environment as a necessary step for this synaptic adaptation remains unknown. Thus, we hypothesized that the impact of cocaine on VTA dopamine neurons would depend on the context in which the cocaine is experienced.

First, we confirmed that acute cocaine, 24 h after treatment, increased the AMPA-to-NMDA receptor-mediated EPSC ratio in VTA dopamine neurons (Saal et al., 2003; Ungless et al., 2001) (Figures 5A and 5B). Interestingly, the magnitude of this ratio is increased when rats receive cocaine in a novel environment (sal-HC,  $n = 8$ ,  $n$  (neurons) = 11; coc-HC,  $n = 6$ ,  $n$  (neurons) = 8, sal-NOV,  $n = 7$ ,  $n$  (neurons) = 7; coc-NOV,  $n = 7$ ,  $n$  (neurons) = 7;  $p < 0.05$ ; Figure 5B). Surprisingly, cocaine experienced in the home cage had no effect after 24 h on *in vivo* firing activity parameters in VTA dopamine neurons, but did trigger hyperactivity in all these parameters when experienced in a novel environment (Figures 5C and 5D) (Creed et al., 2016).

Next, we hypothesized that a temporal association of novelty-evoked hyperactivity with VTA dopamine neurons (Figure 2) and cocaine challenge would lead to long-lasting synaptic adaptations. We exposed rats to a novel environment for 1 h, then subjected them to an acute cocaine or saline injection in their HC, and recorded VTA dopamine activity the next day. This pre-exposure to a novel environment modified the impact of cocaine treatment received in the home cage by triggering, after 24 h, an increase in firing activity parameters of VTA dopamine neurons similar to that observed when cocaine was injected in a



**Figure 4. Behavioral consequences of acute cocaine exposure in the home cage or a novel environment**

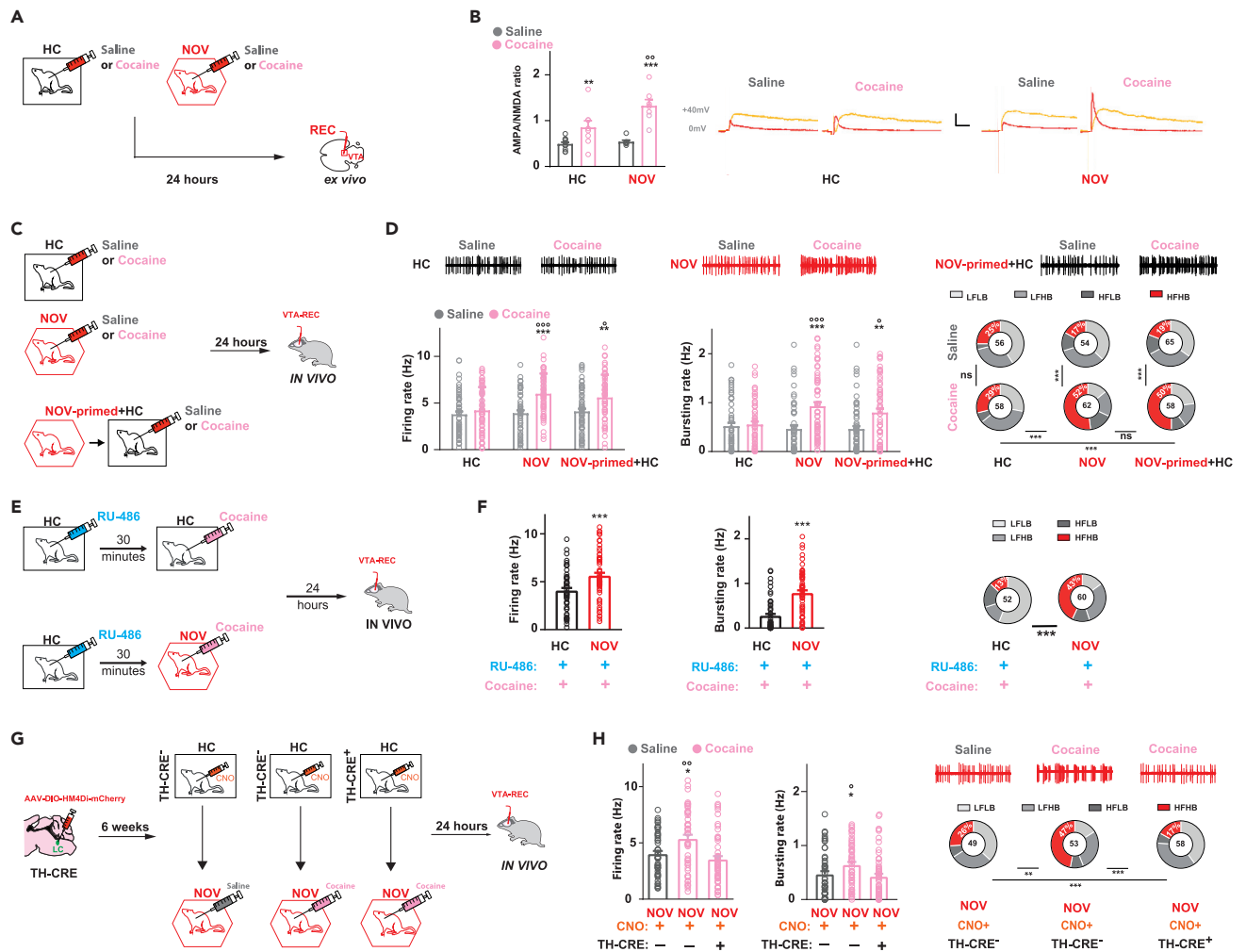
(A) Schematic of the experimental procedure.

(B) Quantification of explorative behavior: locomotor activity, rearing, climbing, sniffing, digging, grooming, immobility, and stereotypies.

HC, home cage; NOV, novel environment, FAM, familiar environment.

\*Group effect; °treatment effect. Details of statistical analysis in Table S4.

novel environment (sal-HC,  $n = 6$ ,  $n$  (neurons) = 56; coc-HC,  $n = 7$ ,  $n$  (neurons) = 58; sal-NOV,  $n = 5$ ,  $n$  (neurons) = 54; coc-NOV,  $n = 9$ ,  $n$  (neurons) = 62; NOV-primed + sal-HC,  $n = 5$ ,  $n$  (neurons) = 65; NOV-primed + coc-HC,  $n = 6$ ,  $n$  (neurons) = 58; firing rate:  $p = 0.0249$ ; bursting rate:  $p = 0.0102$ ; population neurons:  $p < 0.0001$ ; Figure 5D). To confirm that stress was not a confounding factor inherent to the novelty exposure procedure (Saal et al., 2003), we injected RU-486 before cocaine injection and confirmed that exposure to a novel environment indeed controls the long-term impact of cocaine on VTA dopamine neurons (HC-RU-486-coc,  $n = 4$ ,  $n$  (neurons) = 52; NOV-RU-486-coc,  $n = 4$ ,  $n$  (neurons) = 60; firing rate:  $p < 0.0001$ ; bursting rate:  $p < 0.0001$ ; population neurons:  $p < 0.0001$ ; Figures 5E and 5F).



**Figure 5. Novelty gates cocaine's effect on VTA dopamine neurons through LC noradrenergic neuron activation**

(A) Schematic of the experimental procedure for ex vivo recordings.

(B) Quantification of the AMPAR/NMDAR ratio and representative glutamatergic synaptic currents in VTA dopamine neurons after cocaine in the home cage (HC) or a novel environment (NOV). \*Significance compared with saline group; °significance compared with coc-HC.

(C) Schematic of the experimental procedure for in vivo recordings in HC, NOV, and rats exposed first to NOV and receiving their treatment in the HC (NOV-primed + HC).

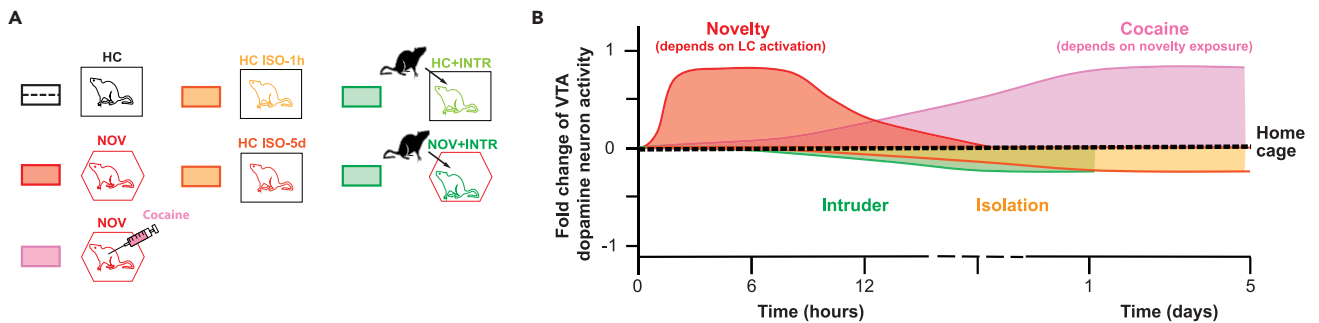
(D) Representative electrophysiological traces, bar graphs of the quantification of VTA dopamine neuron firing and bursting activity (\*significance compared with the saline group; °significance compared with coc-HC), and donut representation of the proportion of dopaminergic neurons classified on the basis of their firing and bursting activity.

(E) Schematic representation of the experimental procedure for in vivo recordings. The GR receptor antagonist RU-486 (40 mg/kg i.p.) was injected 30 min before cocaine injection in a novel environment.

(F) Bar graphs of the quantification of VTA dopamine neuron firing and bursting activity (\*significance compared with HC-RU-486-coc) and donut representation of the proportion of dopaminergic neurons classified on the basis of their firing and bursting activity. RU-486 did not prevent the increase of VTA dopamine neuron activity evoked by cocaine exposure in a novel environment nor in HC.

(G) Schematic of the experimental procedure for viral transfection used for chemogenetic manipulations, clozapine N-oxide (CNO) injection (1 mg/kg i.p.), and in vivo recordings.

(H) Representative electrophysiological traces, bar graphs of the quantification of VTA dopamine neuron firing and bursting activity (\*significance compared with TH-CRE- sal-NOV; °significance compared with TH-CRE + coc-NOV), and donut representation of the proportion of dopaminergic neurons classified on the basis of their firing and bursting activity. In (D, F, and H), four categories of neurons are defined: low-frequency and low-burst firing (LFLB), low-frequency and high-burst firing (LFHB), high-frequency and low-burst firing (HFLB), and high-frequency and high-burst firing (HFHB). HC, home cage; NOV, novel environment; VTA-REC, VTA recording. Details of statistical analysis in Table S5.



**Figure 6. Graphical highlights showing that LC noradrenergic neurons control VTA dopaminergic neurons under physiological conditions and after cocaine**

(A) Schematic of the experimental procedure. (B) Graphic representation of the variation of the activity of the VTA dopaminergic neurons according to the different experimental conditions. The red curve shows that exposure to a novel environment increases the tonic activity of VTA dopamine neurons and opens a time window of excitation for 6 h. The orange curve shows that 1 h or 5 days of isolation leads to a reduction in VTA dopamine neuron activity. The green curve shows that non-spatial contextual novelty (exposure to an intruder) has no excitatory effect on VTA dopamine neurons. The pink curve shows that cocaine experienced in a novel environment, but not in the home cage, triggers hyperactivity of VTA dopamine neurons, which lasts up to 5 days. This study demonstrates that LC noradrenergic neuron activation during novelty exposure gates cocaine-induced hyperactivity of VTA dopamine neurons. HC, home cage; NOV, novel environment, HC ISO-1 h, 1-h isolation in the home cage; HC ISO-5days, 5-day isolation in the home cage; HC + INTR, intruder in the home cage; NOV + INTR, intruder in the novel environment.

Thus, we demonstrated that (1) the engagement of LC noradrenergic neurons during exposure to a novel environment is necessary for VTA dopamine neuron activation (Figure 3) and (2) exposure to a novel environment gates the cocaine-induced *in vivo* effect on VTA dopamine neuron activity (Figure 5). Therefore, we hypothesized that the activation of LC noradrenergic neurons evoked by exposure to a novel environment permits the effects of cocaine on VTA dopamine neurons. We injected AAV-DIO-hM4Di into the LC of TH-CRE animals for selective expression of hM4Di in LC noradrenergic neurons. If CNO was injected 30 min before cocaine treatment in a novel environment, animals expressing hM4Di in LC noradrenergic neurons (TH-CRE + rats) showed a blockade of cocaine-evoked increase of firing and bursting activity in VTA dopamine neurons (TH CRE- sal-NOV,  $n = 3$ ,  $n$  (neurons) = 49; TH CRE- coc-NOV,  $n = 5$ ,  $n$  (neurons) = 53; TH CRE + coc-NOV,  $n = 5$ ,  $n$  (neurons) = 58; firing rate:  $p = 0.0002$ ; bursting rate:  $p = 0.0207$ ). They also showed a reversal of the cocaine-induced change in the relative distribution of the four main modes of VTA dopamine neuronal activity ( $p < 0.0001$ ; Figures 5G and 5H). In saline-treated animals in a novel environment, injecting CNO in TH-CRE + rats expressing hM4Di in LC noradrenergic neurons did not affect the electrophysiological parameters of VTA dopamine neuron at 24 h, in comparison with controls (Figure S6 and Table S6). These results indicate that the inhibition of LC noradrenergic neurons during exposure to a novel environment is sufficient to block cocaine-induced changes in VTA dopamine neuron activity.

## DISCUSSION

Here we demonstrate that LC noradrenergic neuron activation during exposure to a novel environment is necessary to trigger cocaine-induced changes in tonic electrophysiological properties of VTA dopamine neurons. We show that exposure to a novel environment provides a temporal window during which priming of VTA dopamine neuron excitability will occur. Coincidence in time between this “booster effect” and events challenging the dopamine system (including acute cocaine challenge) will lead to long-lasting synaptic adaptations (Figure 6). There are different categories of novelty, including spatial novelty and stimulus novelty (Schomaker and Meeter, 2015). Our experimental procedures were aimed at manipulating spatial novelty. Whereas stimulus novelty improves perception, spatial novelty increases motivation and promotes learning by involving the VTA-nucleus accumbens projections (Schomaker and Meeter, 2015; Segovia et al., 2010). Although the identity of VTA dopaminergic neurons on the basis of their projections was not defined in the present study, we can reasonably suggest that the effects reported here concern mainly the population of mesoaccumbal dopaminergic neurons. Indeed, this assumption is supported by the fact that acute cocaine challenge increases the AMPA-to-NMDA receptor ratio only in VTA dopamine neurons projecting to the nucleus accumbens (Lammel et al., 2011), and that the majority of our recordings were done in the part of the VTA that mostly contains neurons projecting to the nucleus accumbens (Ford et al., 2006).

Our study demonstrates that LC noradrenergic activation during a novelty episode promotes an increase in the tonic activity of VTA dopamine neurons. Further investigation is needed to clarify the distinct sub-circuit that supports the LC noradrenergic neuron control of dopaminergic neurons through direct (Isingrini et al., 2016) or indirect polysynaptic projections (Glangetas et al., 2015; Luo et al., 2011). As the hippocampal formation is intensely activated when rats are exposed to a novel environment (Figure 2) and exerts excitatory control over dopaminergic neurons (Glangetas et al., 2015; Luo et al., 2011), the hippocampus-VTA pathway could be a potential candidate to relay the control of VTA dopaminergic neurons by the LC.

Our study echoes the concept of “soft-supervision” (Nogues et al., 2012), where curiosity leads to the exploration of a novel environment, acting on the dopaminergic system by temporarily modifying its tonic firing activity in a non-specific manner, thus allowing subsequent stimuli (i.e., stimulus novelty or cocaine-exposure) (Schomaker and Meeter, 2015) to trigger long-lasting plastic changes (Wang et al., 2021). Overall, our study provides a mechanistic framework that explains why the rewarding effects of cocaine are enhanced when consumed in a novel environment (Caprioli et al., 2007). The discovery of this cooperative effect between the influence of the novel environment and the effects of cocaine resulting in hyperactivity of VTA dopamine neurons could lead to a state of “high novelty,” and may also help explain why people lose control over their cocaine consumption in an attempt to feel the same high as one experienced the first time in a novel environment (Bardo et al., 1996; Belin et al., 2011).

### Limitations of the study

In this study, we chose the option of a chemogenetic rather than optogenetic approach to manipulate LC noradrenergic neurons. This approach has the advantage of decreasing the excitability of the neurons in a time-extended manner (up to 1 h in our conditions). The use of an inhibitory approach by optogenetics could also have been an interesting option to evaluate the impact of a complete silencing of LC noradrenergic neurons on VTA dopamine neuron activity.

When examining the effect of novelty and cocaine on VTA dopamine neuron activity, only adult male rats were used in our study. This was done in order to complement a recent study from our laboratory performed on males, which explored the molecular mechanism by which cocaine experienced in a new environment changed the *in vivo* activity of dopaminergic neurons (Creed et al., 2016). However, previous studies reported sex-related differences in behavioral reactivity to a novel environment (Borbelyova et al., 2019) and also greater tonic activity of VTA dopamine neurons in females than in males (Calipari et al., 2017). To determine if our findings can be generalized to both sexes or if there is a sex-dependent response to novelty, these experiments would need to be repeated in both sexes.

### STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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### SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.104154>.

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## AUTHOR CONTRIBUTIONS

G.R.F., C.A. J-R., S.C., and F.G. conceptualized and designed the study. G.R.F. and C.M. performed *in vivo* electrophysiological experiments. K.Y. B-C., and R.V-T performed *ex vivo* electrophysiology experiments. G.R.F and X.N. analyzed behavioral and neural data. F.G. wrote the manuscript; all authors participated in proofreading and revision of the manuscript. F.G. supervised the entire study, provided resources, and acquired funding.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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## STAR★METHODS

### KEY RESOURCE TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
anti-TH primary antibody	Millipore	N/A
anti-cFos primary antibody	Cell Signaling	N/A
anti-RFP primary antibody	Life Technology	N/A
donkey anti-mouse secondary antibody	Invitrogen	N/A
donkey anti-rabbit secondary antibody	Life Technology	N/A
donkey anti-rabbit biotinylated secondary antibody	Millipore	N/A
<b>Bacterial and viral strains</b>		
AAV2.5-hSyn-DIO-hM4Di-mCherry	ADDGENE	44362
<b>Chemicals, peptides, and recombinant proteins</b>		
<b>Isoflurane</b>		
NDS	SIGMA	N/A
BSA	SIGMA	N/A
pontamine sky blue	SIGMA	N/A
CNO	SIGMA	N/A
RU-486	SIGMA	N/A
DMSO	SIGMA	N/A
Vectashield medium	Vector Laboratories	N/A
Dako Envision+TM Kit	Agilent	K4011
Dako DAB Kit	Agilent	K3468
Cocaine hydrochloride	Cooperation Pharmaceutique Française	N/A
<b>Experimental models: Organisms/strains</b>		
Sprague Dawley rats	Janvier-Labs	RjHan:SD
TH::CRE rats	SCA-Bordeaux	Long Evans-Tg(TH-Cre)3.1Deis
<b>Software and algorithms</b>		
Microsoft Excel	Microsoft	16.55
Prism	GraphPad Prism	9.2.0
NDP.view2	Hamamatsu Photonics KK.	2.8
Spike2	Cambridge Electronic Design; UK	V8.21
Image-J	JAVA	1.8.0-172
PClamp	Molecular Device	9
<b>Other</b>		
Stereotaxic frame	M2E	N/A
Data acquisition interface	Cambridge Electronic Design; UK	CED 1401
Stimulator	Digitimer	DS3
Confocal microscope	Leica	DM6 CFS TCS SP8
Nanozoomer	Hamamatsu	2.0
Electrode Puller	WPI	N/A
Amplifier	Axoclamp	2B
Vibratome	Leica	VT1000S
Amplifier	Axon instrument	Multiclamp 700B



## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, François Georges: [francois.georges@u-bordeaux.fr](mailto:francois.georges@u-bordeaux.fr).

### Materials availability

This study did not generate unique reagents.

### Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

### Animals

Male Sprague Dawley rats (218 in total; 275–300 g; 10 weeks old; Elevage Janvier, France) and male Long Evans-Tg(TH-Cre)<sup>3.1</sup>Deis rats (32 in total) were used. Rats were housed two to three per cage in standard cages (48.2 × 26.7 × 21 cm, surface 940 cm<sup>2</sup>), without any enrichment and under controlled conditions (22–23°C, 40% relative humidity, 12 h light/dark illumination cycle; lights on from 07:00–19:00). Rats were acclimatized to laboratory conditions 1 week before the experiment, with food and water *ad libitum*. All procedures were conducted following the European directive 2010-63-EU and with approval from the University of Bordeaux Animal Care and Use Committee (N-50120205-A).

## METHOD DETAILS

### Contextual paradigm

Before behavioral tests, all rats were handled daily for 5 days to reduce the stress reactivity induced by the presence of the experimenter. For the novelty group, rats were placed for 1 h in a clean cage with the same characteristics as the home cages. This new cage was placed in an experimental room without animals and with different characteristics. The specificity of novelty effects was assessed by exposing animals to a familiar environment. To make the environment familiar, animals were exposed to the same environment for 1 h per day for 5 consecutive days. Exposure to the new or familiar environment lasted 60 min and behavioral observations were video-taped during the first 10 min. Animals from the familiar group were exposed to the same “novel” context for 5 consecutive days, and behavior was quantified during the last session. For home cage animals, the cage was located in the animal facility, in the presence of other rat cages. The experimenter was not present in the room during the behavioral tests, and rats were observed through a camera from an adjacent room. All quantifications were performed offline by experimenters blinded to the group attribution. For the Y-maze test, animals were alone in the room and observed through a camera from an adjacent room. All quantifications were performed offline by experimenters blinded to the group attribution. In the isolation group, rats were isolated in their HC by removing their littermates for 1 hour or 5 days. In the intruder group, rats were housed in their HC and exposed, once, to an unknown male Sprague Dawley rat (275–300 g; 10 weeks old) for 1 h and observed online by the experimenter. Finally, intraperitoneally drug-treated rats were given either a single dose of cocaine (15 mg/kg in saline) or the same volume of saline as the experimental groups (Creed et al., 2016; Ungless et al., 2001).

### Stereotaxy

Stereotaxic surgeries for viral injections were performed under 1.0–1.5% isoflurane anesthesia (in 50% air/50% O<sub>2</sub>; 1 L/min) (Glangetas et al., 2015). After animal placement in the stereotaxic frame, flat-skull position was verified, and a hole was made above the LC at the following coordinates: –9.84 mm/bregma, ±1.4 mm/midline, 5.7 mm/brain surface (Paxinos and Watson, 1982). The sagittal suture provides a reasonably good marker for the midline. Viruses were subdivided into aliquots and stored at –80°C until use. AAV2.5-hSyn-DIO-hM4Di-mCherry (7 × 10<sup>12</sup> vg/mL; ADDGENE) virus was injected into the LC in TH-CRE rats (300 nL per hemisphere). The surgery was completed by suturing the skin over the craniotomy, and rats were kept under post-operative monitoring for 5 days. Chemogenetic experiments were performed 6 weeks after the initial surgery.

### **In vivo electrophysiology**

Stereotaxic surgeries for electrophysiology experiments were performed under 1.0–1.5% isoflurane (in 50% air/50% O<sub>2</sub>; 1 L/min) anesthesia. Recording pipettes or injection pipettes were inserted into the VTA (−5.3 mm/bregma, 0.7 mm/midline, 7.5–8.5 mm/brain surface), the LC (−9.84 mm/bregma, 1.4 mm/midline, 5.7 mm/brain surface (Paxinos and Watson, 1982)). A glass micropipette (tip diameter, 1–2 mm; 10–15 MΩ) filled with a 2% pontamine sky blue solution in 0.5 M sodium acetate was lowered into the VTA or the LC. Extracellular potentials were recorded with an Axoclamp-2B amplifier and filter (300 Hz/0.5 Hz). Spikes were collected online (CED 1401, SPIKE 2; Cambridge Electronic Design; UK). VTA dopamine neurons were identified according to well-established electrophysiological features: 1) an action potential width  $\geq 1.1$  ms (measured from the start of the action potential to the negative trough); 2) slow spontaneous firing rate (<12 Hz); 3) single and burst spontaneous firing patterns (characterized by spike-amplitude decrement); 4) inhibition of spontaneous activity by dopaminergic receptor agonists and subsequent reversal by dopaminergic receptor antagonists (data not shown). Neurochemical identification through single-cell labeling and immunohistochemistry (Jalabert et al., 2011), confirmed that the use of the electrophysiological criteria was extremely reliable for the online identification of dopamine neurons. Two parameters of VTA dopamine neuron impulse activity were computed over 200 s epochs after a 5 min stable baseline period: 1) the basal firing rate and 2) the bursting rate. The onset of a burst was defined as the occurrence of two spikes with an interspike interval <80 ms (Grace, 1988). Both the frequency and the bursting rate were used to classify VTA dopamine neurons in four subpopulations according to the previously described criteria (Mameli-Engvall et al., 2006): low frequency low bursting (LFLB), low frequency high bursting (LFHB), high frequency low bursting (HFLB) and high frequency high bursting (HFHB). LFLB is characterized by a firing rate lower than 5 Hz and a % of spikes in burst lower than 20%. LFHB is characterized by a firing rate lower than 5 Hz and a % of spikes in burst in the range of 20–60%. HFLB has high frequency (>5 Hz) and low bursting with a % of spike in burst <40%. Lastly, HFHB corresponds to high frequency (>5 Hz) and a % of spike in burst >40%.

Electrical footshock was used as a nociceptive stimulus to measure the sensory integrative properties of LC-NOR-neurons. The footshock was applied to the contralateral paw. The stimulus was applied through two electrodes (0.5 mm diameter). The one used for stimulation was placed subcutaneously in the digit, and the second one in the palm. The electrodes were linked to a stimulator (Digitimer) distributing a constant current with the following parameters for one pulse (5 mV; 1 ms duration; positive current). This stimulator was linked to a computer through a CED (CED 1401, SPIKE 2; Cambridge Electronic Design; UK) allowing a stimulation protocol corresponding to 100 pulses with a frequency of 0.5 Hz. At the end of each recording experiment, the recording pipette placement was marked with an iontophoretic deposit of pontamine sky blue dye (−20  $\mu$ A, 30 min). RU-486 was used at 40 mg/kg (i.p. in 2% DMSO) to antagonize the glucocorticoid receptors.

### **In vivo chemogenetics**

The hM4Di DREADD experiment started 6 weeks after surgery (described above). More than 6 weeks was allowed between virus injection and clozapine N-oxide (CNO) administration. CNO was dissolved in saline and was administered at a dose of 1 mg/mL/kg (i.p.) before exposing rats to a novel environment. For validation of chemogenetic experiments, CNO was locally infused into the LC (5  $\mu$ M, 60 nL), using double-barrel micropipettes.

### **Whole cell patch-clamp (AMPA / NMDA ratio)**

Animals were anesthetized with chloral hydrate (400 mg/kg) and decapitated. Brains were immediately removed and placed in cold (2–4°C) and oxygenated (95% O<sub>2</sub> and 5% CO<sub>2</sub> at pH 7.4) artificial cerebrospinal fluid (ACSF) containing 127 mM NaCl; 2.5 mM KCl; 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>; 25 mM NaHCO<sub>3</sub>; 2 mM CaCl<sub>2</sub>; 1 mM MgCl<sub>2</sub>; and 25 mM D-glucose. Horizontal VTA sections were made using a Vibratome (VT1000S, Leica, Germany). The slices were then transferred to an incubation chamber containing oxygenated ACSF at 32°C for 45 min to 1 h and subsequently placed in a recording chamber superfused with oxygenated ACSF at 2 mL/min. ACSF was supplemented with picrotoxin (100  $\mu$ M) to block GABA<sub>A</sub> receptor-mediated inhibitory postsynaptic currents. Putative dopamine neurons were identified by the presence of a large hyperpolarization-activated cation current (I<sub>h</sub> > 200 pA) in most of the recorded neurons, similar to a previously published method (Glangetas et al., 2015). I<sub>h</sub> was present in about 84% of VTA dopamine neurons, and VTA GABA cells rarely express this conductance (Margolis et al., 2006). Therefore, the contribution of non-dopaminergic neurons to the experimental recordings performed in this study is not likely to be significant. The

recorded neurons were located lateral to the retroflexus fasciculus and medial to the medial terminal nucleus of the accessory optic tract (Paxinos and Watson, 1982). Therefore, recordings of the substantia nigra compacta, identified as the regions rostral and caudal to the medial terminal nucleus, were avoided. Borosilicate glass patch pipettes were pulled to a final resistance of 3–6 M $\Omega$  and filled with 120 mM cesium methanesulfonate, 20 mM HEPES, 0.4 mM EGTA, 2.8 mM NaCl, 5 mM TEA-Cl, 2.5 mM MgATP and 0.25 mM NaGTP, pH 7.2–7.3 (270 to 285 mOsm). A bipolar stimulating electrode was placed 50–150  $\mu$ m rostral to the recording electrode to stimulate at 100 ms intervals. For the AMPA / NMDA ratios, cells were clamped at +40 mV after stabilization of the NMDAR inhibitor D-2-amino-5-phosphonovalerate (D-AP5; 50  $\mu$ M) perfused into the bath chamber. NMDAR responses were calculated by subtracting the average responses (15 traces) in the presence of D-AP5 (only mediated by AMPAR) from that recorded in its absence. Experiments with a series resistance change of more than 20% were discarded. Data were collected with PClamp 9 software through a Multiclamp 700B amplifier.

### Tissue processing and immunohistochemistry

Rats were perfused transcardially (4% paraformaldehyde solution). Sections were incubated (overnight at 4°C) with mouse anti-TH primary antibody (1/8000, Millipore), rabbit anti-cFos primary antibody (1/1000; Cell Signaling, France), or rabbit anti-RFP primary antibody (1/1000; Life Technology, France). For double staining, primary antibodies were incubated together. For fluorescence microscopy, after washing, sections were incubated overnight at 4°C with a donkey anti-mouse secondary antibody (labeling of TH, 1/1000, Invitrogen, Alexa 488), donkey anti-rabbit secondary antibody (labeling of cFOS, 1/1000, Life Technology, France, Alexa 568), donkey anti-rabbit secondary antibody (labeling of AAV2.5-hSyn-DIO-hM4D-mCherry, 1/1000, Life Technology, France, Alexa 568). Sections were washed and then mounted in Vectashield medium (Vector Laboratories), cover-slipped, and imaged on a laser scanning confocal microscope. The confocal microscope was a Leica DM6 CFS TCS SP8 (Leica Microsystems, Germany).

The acquisition of images was done with a resonant scanner (8,000 Hz–16,000 Hz; Hamamatsu Nanozoomer 2.0 HT). C-Fos immunohistochemistry was performed in serial tissue sections. After thorough rinsing with PBS, endogenous peroxidase was inhibited with peroxidase-blocking solution (Dako REAL, S2023) for 10 min. After again thorough rinsing with PBS, non-specific labeling was prevented by blocking antigenic sites in PBS containing 2% bovine serum albumin (BSA), 0.3% triton X-100 and 0.01% thimerosal, for 30 min. Sections were then incubated overnight at room temperature in primary antibody (monoclonal rabbit anti-C-Fos, clone 9E6, mAb#2250 - Cell Signaling Technology) diluted at 1/5000 in PBS containing 0.2% BSA, 0.3% triton X-100 and 0.01% thimerosal. The next day, sections were thoroughly rinsed with PBS and incubated with labeled polymer-horseradish peroxidase (HRP) anti-rabbit (Dako EnVision + TM Kit, K4011) for 30 min. After thorough rinsing with PBS, TH staining was then revealed with 3,3'-diaminobenzidine (DAB/Ni) (Dako DAB Kit, K3468) for 30 s and stopped with several PBS washes. Sections were then mounted on gelatin-coated slides, counterstained with Nissl stain, dehydrated, cleared in xylene and cover-slipped in permanent mounting media. cFos-immunoreactive cells were counted using Image-J.

### Behavior

#### Observations of exploration behavior

Exploratory behavior refers to a set of behavioral features that allow the animal to assess and obtain information about its environment (Berlyne, 1966; Henry et al., 1992). We decided to measure general exploration induced by exposure to a novel environment at light levels of 70 LUX, to have a safe environment with a minimal baseline level of anxiety (Glangetas et al., 2017). The following behaviors were monitored during the first 10 min in the experimental context: (1) Grooming: animals clean, scratch, or lick their body; (2) Sniffing: nose twitches and rapid vibrissae movements, including nose being very close to the floor while moving; (3) Locomotion: walks, runs, or turns; (4) Rearing: animal rises up on its hind legs and stretches the body; (5) Climbing: animal hangs with its paws in contact with a vertical surface; (6) Immobility: animal stands or sits with all four limbs touching the floor; (7) Digging: rats move litter with their paws or nose. Finally, because most rodents exhibit stereotypic behaviors following repeated exposure to psychostimulants, we measured stereotypies in our experimental conditions (Jaber et al., 1995; Robinson and Becker, 1986). Stereotypic behaviors were assessed for 1 min every 2 min, for a total observation period of 10 min. Scores were defined as follows: 0 = inactive; 1 = intermittent activity; 2 = continuous activity; 3 = intermittent stereotypy; 4 = continuous stereotypy over a wide area including stereotyped locomotor activity, sniffing and rearing; 5 = continuous stereotypy over a narrow area (mainly sniffing

and rearing); 6 = marked continuous stereotypy over a narrow area (mainly sniffing); 7 = intermittent licking or biting; and 8 = continuous licking or biting (Jaber et al., 1995).

### Y maze

Spontaneous alternation in a Y maze is a behavioral test measuring the willingness of rodents to explore new environments (Bertholet and Crusio, 1991). Rodents typically prefer to investigate a new arm of the maze rather than returning to the one that was previously visited. The apparatus was made of grey plastic, each arm was 50 cm long x 17 cm wide x 35 cm high, and each arm was 1 m above the floor. The animals were put into the maze and watched for 10 min. During this time, the number of entries into each arm was recorded. The number of explorations of each arm was recorded every minute. During the Y-maze test, each rat is observed in an adjacent room by a computer coupled to a camera. Behavioral analysis of the test was performed offline.

## QUANTIFICATION AND STATISTICAL ANALYSIS

The data were analyzed with independent or paired two-tailed *t*-tests, one-way or two-way analysis of variance (ANOVA), followed up by post hoc tests (Newman-Keuls). Kolmogorov-Smirnov tests or the Shapiro-Wilk criterion were used to determine the normality of the data and non-parametric Kruskal-Wallis statistics test were applied with Dunn's post hoc test. All bars and error bars represent the mean  $\pm$  the standard error of the mean (SEM), respectively, and significance was set at \* and  $^{\circ}p < 0.05$ , \*\* and  $^{\circ\circ}p < 0.001$  and \*\*\* and  $^{\circ\circ\circ}p < 0.0001$ . All statistics were performed on data using GraphPad Prism version 9.2.0 (GraphPad Software, La Jolla California USA). The electrophysiological activity of VTA dopamine neurons was analyzed offline using Spike 2. Chi square tests were used to compare the trends in the distribution of the population. Explorative behavior was analyzed offline manually. Behavioral observations were quantified during the first 10 min of exposure to the contextual paradigm. The percentage of time that rats spent grooming, sniffing, digging and immobility were measured. Rearing and climbing were reported as the number of events occurring in 10 min. Locomotor activity is expressed as distance, measured in cm and reported in meters on the graph. For c-Fos experiments, correlations between VTA and LC were made using the Pearson correlation test. Sections at the LC level were counterstained by the cresyl violet method to label the cytoplasm of the neurons. This counter-staining confirms the nuclear appearance of the c-fos labeling and was used to confirm its specificity. Paired or unpaired two-tailed *t*-tests were used. Results from all statistical analyses are shown in [Tables S1–S6](#).