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Salin Adélie, Dugast Emilie, Lardeux Virginie, Solinas Marcello, Belujon Pauline. The amygdala-ventral pallidum pathway contributes to a hypodopaminergic state in the ventral tegmental area during protracted abstinence from chronic cocaine. *British Journal of Pharmacology*, 2023, 180 (14), pp.1819-1831. 10.1111/bph.16034 . hal-03971992

HAL Id: hal-03971992

<https://hal.science/hal-03971992>

Submitted on 15 Mar 2023

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RESEARCH ARTICLE

The amygdala–ventral pallidum pathway contributes to a hypodopaminergic state in the ventral tegmental area during protracted abstinence from chronic cocaine

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Funding information

Institute National de la Santé et de la Recherche Médicale; Centre National pour la Recherche Scientifique; University of Poitiers; CHU of Poitiers; SFR FED 4226; Agence Nationale de la Recherche, Grant/Award Number: ANR-15-CE37-0010

Abstract

Background and Purpose: Incubation of craving, the progressive increase in drug seeking over the first weeks of abstinence, is associated with temporal changes during abstinence in the activity of several structures involved in drug-seeking behaviour. Decreases of dopamine (DA) release and DA neuronal activity (hypodopaminergic state) have been reported in the ventral tegmental area (VTA) during cocaine abstinence, but the mechanisms underlying these neuroadaptations are not well understood. We investigated the potential involvement of a VTA inhibiting circuit (basolateral amygdala [BLA]–ventral pallidum [VP] pathway) in the hypodopaminergic state associated with abstinence from chronic cocaine.

Experimental Approach: In a model of cocaine self-administration, we performed in vivo electrophysiological recordings of DA VTA neurons and BLA neurons from anaesthetised rats during early and protracted abstinence and evaluated the involvement of the BLA–VP pathway using a pharmacological approach.

Key Results: We found significant decreases in VTA DA population activity and significant increases in BLA activity after protracted but not after short-term abstinence from chronic cocaine. The decrease in VTA DA activity was restored by pharmacological inhibition of the activity of either the BLA or the VP, suggesting that these regions exert a negative influence on DA activity.

Conclusion and Implications: Our study sheds new lights on neuroadaptations occurring during incubation of craving leading to relapse. In particular, we describe the involvement of the BLA–VP pathway in cocaine-induced decreases of DA activity in the VTA. This study adds important information about the specific brain network dysfunctions underlying hypodopaminergic activity during abstinence.

KEYWORDS

amygdala, cocaine, dopamine, dysphoria, incubation of craving, relapse, ventral pallidum

Abbreviations: BLA, basolateral amygdala; DA, dopamine; TTX, tetrodotoxin; VP, ventral pallidum; VTA, ventral tegmental area.

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1 | INTRODUCTION

Chronic **cocaine** produces persistent neuroadaptations in brain networks that underlie long-term risks of relapse and incubation of drug craving (van Huijstee & Mansvelter, 2015). Rewarding properties of psychostimulants such as cocaine are associated with hyperdopaminergic state (increase of **dopamine** [DA] release and increase in activity of DA neurons) (Wise & Hoffman, 1992) whereas acute withdrawal from acute and chronic psychostimulants leads to a hypodopaminergic state (decrease of DA release and decrease in DA neuronal activity), specifically in the mesolimbic system (Belujon et al., 2016; Salin et al., 2021; Shen et al., 2007), leading to hedonic deficits (Belujon & Grace, 2017; Wise, 2008). A hypodopaminergic state has been proposed to be a major mechanism involved in drug addiction (Melis et al., 2005). Reduction of DA activity is also observed after long abstinence (Diana et al., 1996; Salin et al., 2021); this reduction could be involved in dysphoria (Haake et al., 2019) and anhedonia (Hatzigiakoumis et al., 2011) described after protracted abstinence. In patients, a positive correlation has been shown between craving and anhedonia in detoxified users, suggesting a strong association between hedonic capability and craving (Janiri et al., 2005). Several studies in humans and animal models have also suggested that incubation in cocaine seeking and craving during abstinence may be associated with alterations in DA neurotransmission (Alonso et al., 2021; Calipari et al., 2015; Shin et al., 2016). In particular, a withdrawal-dependent decrease in cue-induced DA release has been shown in cocaine-seeking rats (Shin et al., 2016). Altogether, these studies support a putative role of a prolonged hypodopaminergic state during abstinence as a negative prognostic factor for relapse.

DA activity is regulated by different afferent circuits. In particular, the basolateral nucleus of the amygdala (BLA)–ventral pallidum (VP) pathway has been shown to inhibit VTA DA activity (Chang & Grace, 2014). The BLA is a crucial brain structure involved in emotional processes (Adhikari et al., 2015; Janak & Tye, 2015; LeDoux, 2000) and in reward-associated learning and memory (Murray, 2007; Wassum & Izquierdo, 2015). In drug addiction, the BLA is a major brain substrate for drug-associated cue memory and imaging studies in humans report increased activity in the amygdala in cocaine craving addicts exposed to drug-cues (Childress et al., 1999; Garavan et al., 2000; Maas et al., 1998). In rodents, increased activity of BLA neurons has been described during abstinence from chronic cocaine (Munshi et al., 2019) and exposure to drug-related environmental cues triggers drug-seeking behaviour which is alleviated by lesion of the BLA (Meil & See, 1997). The VP is a central site for limbic reward signals (Smith et al., 2009) and is involved in drug-seeking behaviour after long-term abstinence (Farrell et al., 2019), in particular its projections to the VTA (Mahler et al., 2014). Importantly, the BLA-dependent decrease in VTA activity found in animal models of psychiatric disorders such as major depressive disorders appears to be regulated by the VP (Chang & Grace, 2014). However, it is not known whether the same circuit plays a role in the DA hypoactivity that characterizes prolonged abstinence.

What is already known

- Protracted abstinence from cocaine self-administration produces hyperactivity of BLA and hypoactivity of VTA DA neurons.
- The BLA–ventral pallidum pathway is a potent inhibitory circuit of VTA DA activity.

What does this study add

- Hyperactivity of the BLA and hypoactivity of VTA DA neurons requires several weeks to develop.
- Increased activity of the BLA–VP pathway inhibits DA activity after long-term abstinence.

What is the clinical significance

- Hypodopaminergic activity after prolonged abstinence might underlie dysphoria leading to relapse.
- Imaging of the circuit regulating DA activity might give critical information on emotional symptoms.

In this work, we investigated the potential involvement of the BLA and the VP in VTA hypodopaminergic activity during withdrawal from chronic **cocaine**, using a model of cocaine self-administration, and in vivo electrophysiological recordings of DA VTA neurons and BLA putative projection neurons from anaesthetised rats during early and protracted abstinence.

2 | METHODS

2.1 | Animals

Rats were used in this study because the rat behavioural repertoire makes them a species of choice for the investigation of addiction-related processes (Parker et al., 2014). Eighty-three (cocaine $n=53$, no cocaine $n=30$) male Sprague–Dawley rats (Charles River Laboratories, Ecully, France) weighing 325–350 g upon arrival were housed in a temperature and humidity-controlled environment and maintained on a 12-h light/12-h dark cycle (on at 07:00 AM). Animals were housed two per cage from arrival to surgery and one per cage after surgery. When animals were isolated, enrichment (wood blocks) was added in the home cage. Experiments were performed during the light phase. Animals had ad libitum access to standard chow and water in the home cage. All experiments were

conducted in accordance with the European Union directives (2010/63/EU) for the care of laboratory animals and all experimental protocols were approved by the local ethics committee (COMETHEA, n°7147).

Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the *British Journal of Pharmacology* (Lilley et al., 2020; McGrath & Lilley, 2015).

2.2 | Cocaine self-administration and seeking

2.2.1 | Catheter implantation

Rats were prepared for cocaine self-administration by surgical catheterization of the right jugular vein. Briefly, rats were anaesthetised with isoflurane (isoflurane-Belamont, Centre Spécialités Pharmaceutiques, Cournon d'Auvergne, France) (5% induction, 2.5% maintenance) in O₂ and administered with the nonsteroidal anti-inflammatory ketoprofen (ketofen[®], Ceva, Brussels, Belgium, 2.5 mg·kg⁻¹, s.c.). Animals were placed on a heating pad from the induction of anaesthesia to the end of surgery. A handmade silastic catheter (external diameter: 0.94 mm; ref. 228-0670, HelixMark™, Fisher Scientific, Illkirsh, France) was inserted into the jugular vein, and the distal end was led to the back between the scapulae. Rats were allowed to recover for 7 days and flushed daily with 0.1-ml sterile saline (0.9%), gentamicin (20 mg·ml⁻¹), and heparin (100 UI·ml⁻¹) in sterile saline to help protect against infection and catheter occlusion.

2.2.2 | Apparatus

Experiments were conducted in operant-conditioning chambers, equipped with nose-pokes as *operanda*, a cue-light above the active nose-poke and a house light, controlled by Imetronic interfaces and software (Imetronic, Pessac, France).

2.2.3 | Cocaine self-administration procedure

Rats were allowed to self-administer cocaine (Cooper, Melun, France; 6 g·L⁻¹ in saline solution 0.9%; 0.75 mg·kg⁻¹ per infusion). Control rats received saline infusions according to a yoked procedure, which consists of the delivery of an injection of saline each time the paired “master” rat self-administered an injection of cocaine. Rats were randomly assigned to the yoked saline or cocaine group. One cohort consisted of 12 rats with at least four yoked saline rats in each cohort. Rats were placed in the self-administration chamber for 6 h·day⁻¹ for 10 sessions, using a fixed ratio 1 (FR1) schedule of reinforcement. The response to an active nose poke resulted in one intravenous (i.v.) cocaine infusion with the concomitant activation of the light that remained on for 5 s

and then pulsed for 5 s, followed by a 5-s time-out. Inactive nose-pokes were recorded but did not produce any consequences. After the last self-administration session, rats remained in their home cage for up to 45 days of forced abstinence. To avoid any effect of the relapse test on electrophysiological measures, animals were then either used for electrophysiological recordings or tested for cocaine-seeking behaviour.

2.2.4 | Cocaine-seeking behaviour

Abstinent cocaine rats were re-exposed to the operant chambers after 1 and 30 days of abstinence as previously described (Chauvet et al., 2012). Active nose-pokes triggered cue-light and syringe holder activation (similarly to self-administration sessions), but rats were not connected to tubing and did not receive any cocaine infusions. Cocaine-seeking behaviour was measured for 1 h where active and inactive responses were recorded. The same groups of rats were used for each time point.

2.3 | In vivo single-unit extracellular recordings

2.3.1 | Randomization

Recordings were randomized in the morning or afternoon between saline and cocaine rats.

2.3.2 | Local drug administration

A cannula was lowered into targeted regions at the following coordinates on the right hemisphere (antero-posterior [AP] relative to bregma, medio-lateral [ML] relative to midline, and dorso-ventral [DV] relative to brain surface): BLA: AP -2.8 mm, ML +4.8 mm, DV -6.8 mm; VP: AP -0.3 mm, ML +2.0 mm, DV -6.8 mm. Drug solutions were prepared with Dulbecco's PBS buffer: tetrodotoxin (TTX), 8 µg/0.5 µl/infusion (Tocris, Bio-Techne, Noyal Châtillon sur Seiche, France, CAS 4368-28-9), **kynurenic acid** (5 µg in 0.5 µl) (Sigma-Aldrich, Saint-Quentin-Fallavier, France, CAS 492-27-3). Kynurenic acid, a glutamate receptor antagonist, is known to block glutamatergic inputs onto VP (Chang & Grace, 2014). Drugs or vehicle were infused 1 min after cannula lowering, by slow manual infusion. The cannula was left in place for an additional 1 min and then removed before VTA DA neuron recordings.

For acute withdrawal, extracellular recordings were performed from 18 h up to 72 h after the last cocaine self-administration session. For protracted withdrawal, recordings were performed from 26 days to 45 days after the last session. Naïve rats were also used for recording of BLA (3 naïve rats) and DA (7 naïve rats) activity. Since there was no difference in BLA and DA activity between naïve rats and saline WD1–3 and saline WD26–45, data were pooled.

2.3.3 | Single-unit extracellular recordings

Rats were anaesthetised with isoflurane (5% induction, 2.5% maintenance) and placed in a stereotaxic frame. Body temperature was maintained at 37°C with a temperature-controlled heating pad. The scalp was exposed, and burr holes were drilled in the skull overlying the VTA, the BLA, or the VP. Extracellular recording electrodes were pulled from glass micropipettes (WPI; impedance 10–20 MΩ for BLA recordings, 6–8 MΩ for VTA recording). The tip of the glass microelectrode was broken to a diameter of 2 μm and filled with a 2% Chicago Sky Blue dye (Sigma-Aldrich, CAS 2610-05-01) solution in 2-M NaCl.

For DA neurons, the recording electrode was lowered using a microdrive through the right VTA in nine sequential vertical tracks of 0.2 mm (Figure S1). Spontaneously active cells encountered identified as DA neurons (online analysis) were recorded to determine population activity as previously described (Salin et al., 2021). Single-unit activity recorded from the VTA was amplified 10 times, filtered (low pass: 30 Hz; high pass: 16 kHz), and further amplified 50 times (Multiclamp 700b, Axon Instruments, Union City, CA). The signal was digitized at 16 kHz (Cambridge Electronics Design, Cambridge, UK, CED 1401) and acquired on a computer using Spike 2 7.0 software (Cambridge Electronics Design). Three parameters were analysed: the number of neurons/electrode track, firing rate, and the percentage of spikes in bursts.

For BLA recordings, the electrode was lowered through the right BLA at the following coordinates: AP –2.8 mm to –3.2 mm (from bregma), ML +4.6 mm to 5.2 mm (from midline) and DV –7.0 mm to –9.5 (from brain surface). Encountered cells in the targeted area meeting BLA putative pyramidal neuron criteria (action potential from peak to trough ≥0.5 ms; Bienvenu et al., 2012) and with a signal-to-noise ratio of 3:1 (or greater) were recorded for at least 5 min. Single-unit signals were amplified 10 times, filtered using a high-pass filter at 30 Hz and a low-pass filter at 10 kHz, and further amplified 50 times (Multiclamp 700b, Axon Instruments). The signal was digitized at 16 kHz (CED 1401) and acquired on a computer using Spike 2 7.0 software (Cambridge Electronics Design). Bursts were detected using characteristic parameters of pyramidal neurons (Vitrac et al., 2014) (maximum interval to start a burst: 40 ms, maximum interval to end a burst: 10 ms, minimum interval between burst: 20 ms, minimum duration of a burst: 5 ms, and minimum number of spikes in a burst: 2) and analysed using NeuroExplorer® (Nex Technologies, Colorado Springs, USA).

For all recorded cells, the following electrophysiological parameters were analysed: basal firing rate, bursting rate, and the percentage of spikes in bursts.

2.4 | Histology

Electrode placements were verified via electrophoretic ejection of Chicago Sky Blue dye (Sigma-Aldrich) at the recording site. Rats were killed with a lethal dose of pentobarbital (182.2 mg·kg⁻¹, IP), and brains were removed. The tissue was fixed in 8% paraformaldehyde for at least 48 h and transferred to a 25% sucrose solution

for cryoprotection. Once saturated, the brains were frozen and sliced coronally at 60 μm thick using a cryostat (Thermo Fischer Scientific™ Cryostat CryoStar™ NX70, Illkirch, France) and mounted onto gelatin-coated slides. Tissue was stained with a combination of neutral red and cresyl violet. Only rats with verified electrode placements for both recording and infusion sites were included in the data analysis.

2.5 | Statistical analysis

The manuscript complies with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology (Curtis et al., 2022). The statistical analysis was performed only where each group size was at least $n = 5$. Group mean values and statistical analysis used independent values and n is number of animals. The sample size and animal numbers were determined by power analysis of pre-existing data (Belujon et al., 2016; Belujon & Grace, 2014; Chang & Grace, 2014; Salin et al., 2021).

Data were analysed with the statistical program GraphPad Prism (Version 8.0.1; GraphPad Prism, San Diego, California, USA, RRID: SCR_002798).

All the results are expressed as the mean ± SEM. Results from individual neurons were used as replicates in calculating the mean value ± SEM per rat. A Grubb's test was used to identify statistical outlier animals. Two outliers were found in this study and were removed from the analyses. The level of probability (P), for determining differences between groups, was set at $P < 0.05$. We followed significant main effects and interactions ($P < 0.05$) with post hoc tests (Sidak's multicomparison test). Post-hoc tests were run only if F achieved $P < 0.05$ and there was no significant variance of inhomogeneity.

For active/inactive responses in yoked saline animals, a two-way repeated measure (RM) ANOVA was performed with session as the within-subject factor and number of nose-pokes as the between-subject factor. Since corresponding data for cocaine animals did not pass the normality test, a mixed-effects model was used. For incubation of cocaine craving, a paired Mann–Whitney test was performed.

Electrophysiological data were analysed using either a two-way ANOVA with time (WD1–3 and WD26–45) and drug (saline and cocaine) as factors followed by Sidak's post hoc when comparing more than two experimental conditions. When comparing two groups, first a Kolmogorov–Smirnov normality test was used. If data passed the normality test, an unpaired t test was used; otherwise, an unpaired Mann–Whitney test was used.

Complete statistical analyses and exact P values are reported in Table S1.

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, and are

permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander et al., 2022).

3 | RESULTS

3.1 | Cocaine self-administration and cocaine seeking behaviour after 1 and 30 days of withdrawal

All rats were allowed to self-administer cocaine for 10 sessions under an FR1 schedule of reinforcement. During the 10 sessions of self-administration, cocaine rats had more active than inactive nose-pokes (Figure 1b; $n = 53$), whereas there were no differences in yoked saline rats (Figure 1c; $n = 20$). The number of injections was stable across the self-administration sessions in cocaine rats (Figure 1d). As previously described (Chauvet et al., 2012; Grimm et al., 2001), cocaine

seeking (measured in a 1-h-extinction session) was significantly higher after 30 days of withdrawal compared with 1 day of withdrawal (Figure 1e; $n = 6$).

3.2 | Abstinence from cocaine intake induces changes in dopaminergic activity in the VTA after 30 days of withdrawal, but not 1 day of withdrawal

We have previously shown a significant persistent decrease in the dopaminergic population activity recorded in the VTA after 7 and 30 days of abstinence from extended cocaine intake (25 days) (Salin et al., 2021). Here, we investigated potential changes in DA VTA activity after a shorter 10-day period of cocaine self-administration, in the early phase of abstinence (withdrawal days 1–3), and after protracted abstinence (withdrawal days 26–45). An example of the location of the

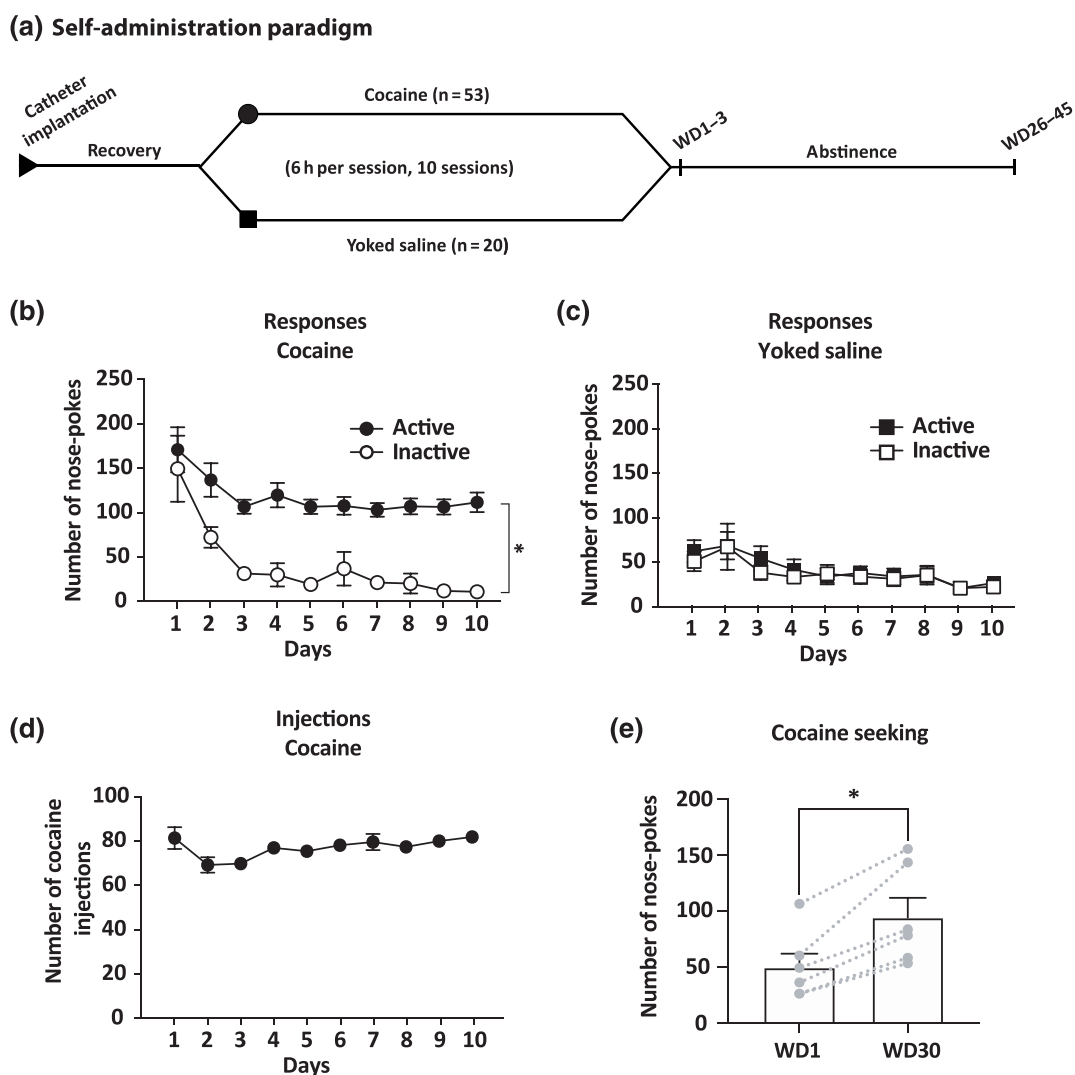


FIGURE 1 Self-administration and relapse test. Experimental design timeline (a). Number of active (black) and inactive (white) nose-pokes during the 10 sessions in cocaine rats (b) and in yoked saline rats (c). Number of cocaine injections ($0.75 \text{ mg}\cdot\text{kg}^{-1}$ per infusion) during the ten self-administration sessions (d). Number of active responses during cocaine-seeking over a 1-h-session, 24 h (WD1) and 30 days (WD30) after the last self-administration session (e). Data are mean \pm SEM; * $P < 0.05$; cocaine rats: $n = 53$, yoked saline rats: $n = 20$, cocaine seeking: $n = 6$.

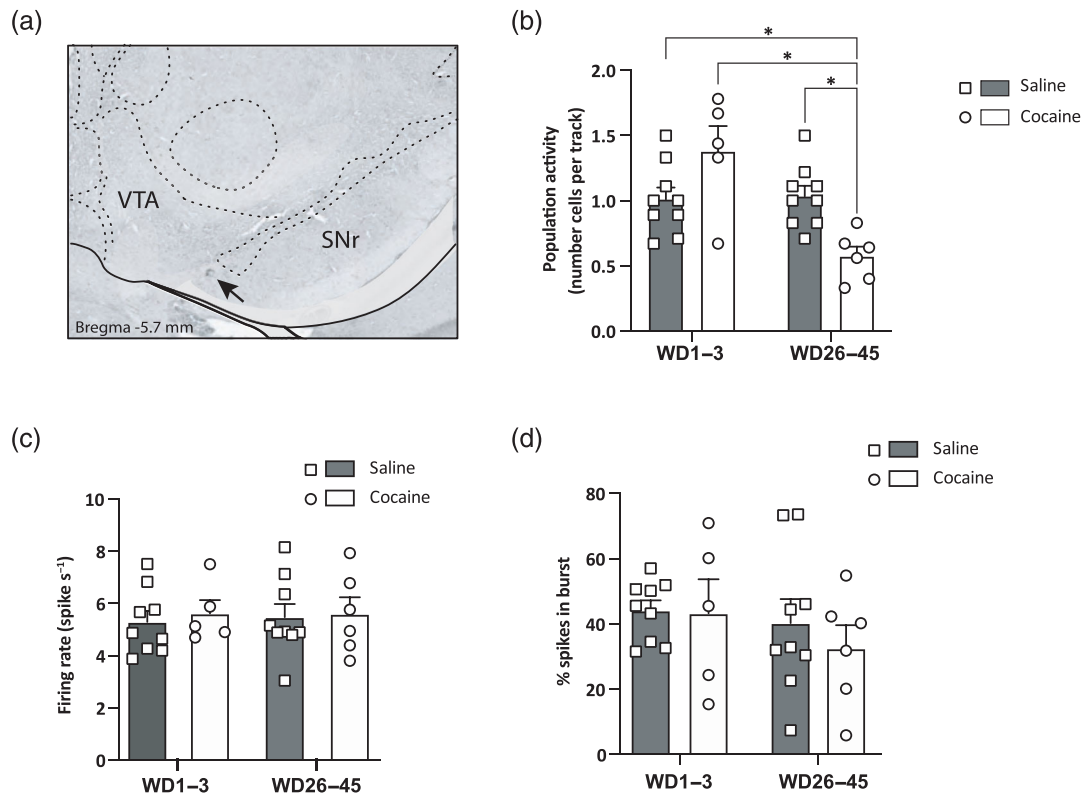


FIGURE 2 Dopaminergic activity in the ventral tegmental area after short- (24 h: WD1) and long- (30 days: WD30) term withdrawal from chronic cocaine and yoked saline. Histological slice showing a Chicago sky blue deposit dot in the VTA (a). Mean number of spontaneously active DA neurons recorded per electrode track (b), number of spikes per second (firing rate) (c), and percentage of action potential firing in bursts (d) in saline/naive and cocaine rats after 18–72 h (WD1–3) and saline/naive and cocaine rats after 26–45 days after the last self-administration session (WD26–45). Data are mean \pm SEM; * $P < 0.05$; saline/naive WD1–3: nine rats (five saline, four naive), 64 neurons; cocaine WD1–3: five rats, 61 neurons; saline/naive WD26–45: nine rats (six saline, three naive), 65 neurons; cocaine WD26–45: six rats, 30 neurons. Coronal brain sections adapted from Swanson (2018).

recording electrode at the end of a recording is presented in Figure 2a. Population activity (Figure 2b), firing rate (Figure 2c), and percentage of spikes firing in bursts (Figure 2d) were analysed for VTA DA neurons in saline/naive and cocaine rats after 1–3 and 26–45 days of abstinence. Exposure to cocaine induced a significant decrease in population activity of VTA DA neurons during long-term but not short-term abstinence, compared with saline/naive rats (Figure 2b). Post hoc analysis revealed a significant difference between saline/naive WD1–3 and cocaine WD26–45, saline/naive WD26–45 and cocaine WD26–45, and cocaine WD1–3 and cocaine WD26–45 rats. There was no change in the firing rate (Figure 2c), or in the percentage of spike firing in bursts (Figure 2d) of DA neurons between groups.

3.3 | Abstinence from cocaine intake induces changes in neuronal activity in the BLA after 26–45 days of withdrawal, but not 1–3 days of withdrawal

Previous studies have shown that chronic cocaine intake increases the spontaneous firing rate of BLA neurons after 15 days of abstinence (Munshi et al., 2019) and that the BLA is part of the regulating

circuit that decreases tonic DA (Chang & Grace, 2014). Here, we investigated the firing rate and the percentage of spikes firing in burst of BLA neurons in saline and cocaine rats after 1–3 and 26–45 days of abstinence (Figure 3). An example of the location of the recording electrode is presented in Figure 3a. An example of spike doublet and triplet (Rainnie et al., 1993) recorded from a putative projection neuron (Bienvenu et al., 2012; Washburn & Moises, 1992) in the BLA and an example of a spike shape (averaged over 5 min) are presented in Figure 3b. We found a significant drug effect for the firing rate (Figure 3c,d top). Post hoc analysis revealed a significant difference between saline WD26–45 and cocaine WD26–45. No significant difference was found for the percentage of spikes in burst (Figure 3c,d bottom).

3.4 | Attenuation of BLA activity restores DA neuron activity after long-term abstinence from cocaine

The DA system is regulated by an inhibitory circuit including the BLA (Belujon et al., 2016; Belujon & Grace, 2017), and amygdala-driven

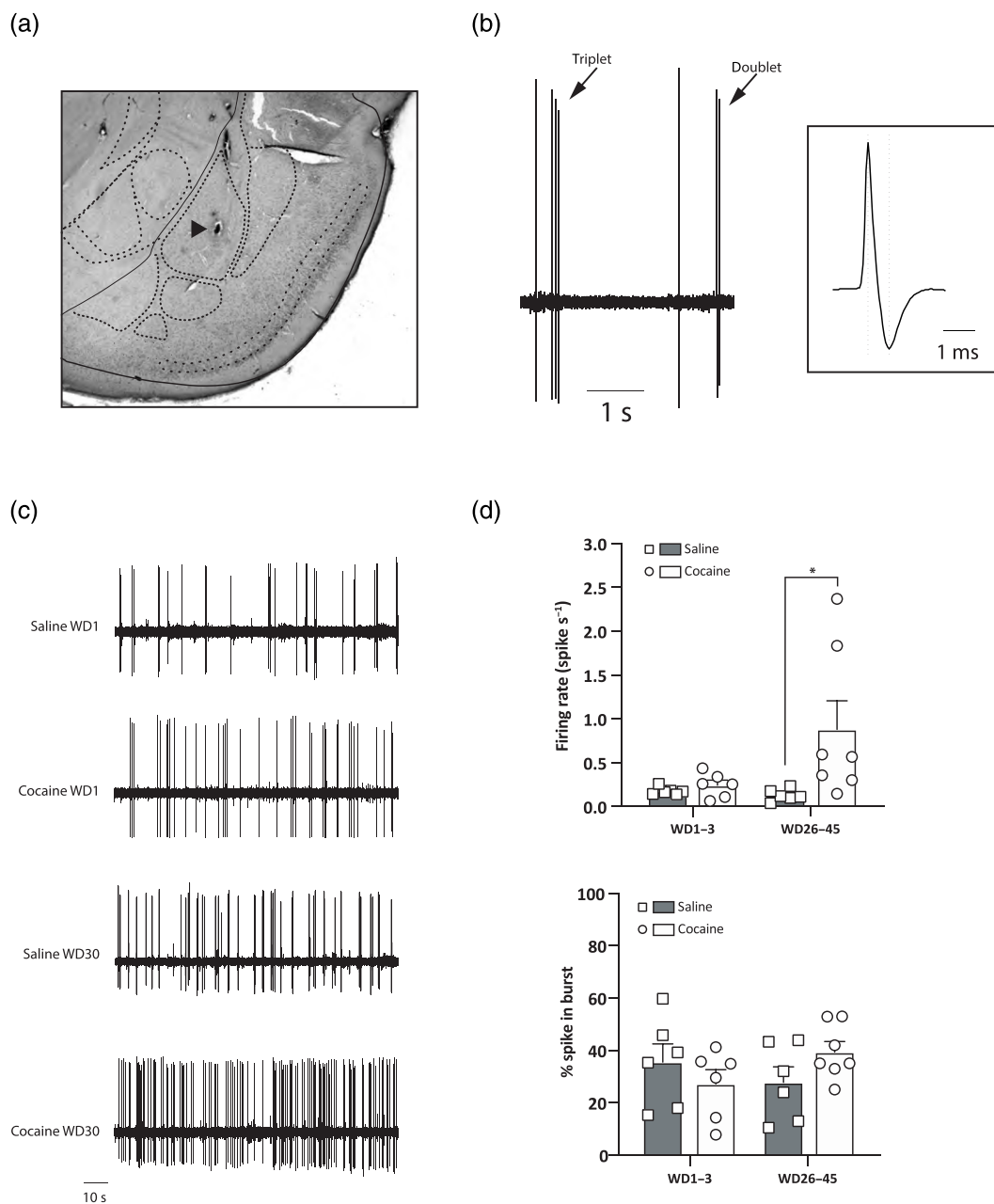


FIGURE 3 Activity of neurons recorded from the basolateral amygdala after short- (1-3 days: WD1-3) and long- (26-45 days: WD26-45) term withdrawal from chronic cocaine and yoked saline. Histological slice showing a Chicago sky blue deposit dot in the BLA (a). Representative electrophysiological trace of a BLA neurons showing the presence of a triplet and doublet of spikes (black arrows). Inset represents the shape of action potentials (averaged over 5 min), with the action potential duration measured from peak to trough (dashed line) (b). Two-minute representative electrophysiological trace of a BLA neurons recorded from (top to bottom): saline WD1-3, cocaine WD1-3, saline WD26-45, and cocaine WD26-45 rats (c). Number of action potentials per second (firing rate; top) and percentage of action potentials firing in burst (bottom)(d). * $P < 0.05$. Saline WD1-3: 6 rats (4 saline and 2 naive), 33 neurons; cocaine WD1-3: 30 neurons; saline WD26-45: 6 rats (5 saline, 1 naive), 32 neurons; cocaine WD26-45: 7 rats, 56 neurons. Coronal brain sections adapted from Swanson (2018).

attenuation of DA neuron activity has been described after withdrawal from acute amphetamine (Belujon et al., 2016). Thus, we then tested whether DA neuron population activity could be restored by attenuating BLA activity by local infusion of the sodium channel blocker TTX (or vehicle), immediately before VTA recordings. A

representative example of placement of the infusion cannula is presented in Figure 4a, and the location of the vehicle and TTX infusion cannula is presented in Figure 4b. As expected, cocaine rats infused with vehicle (VEH) after 26-45 days abstinence had fewer spontaneously active DA neurons, in comparison to after inactivation of BLA

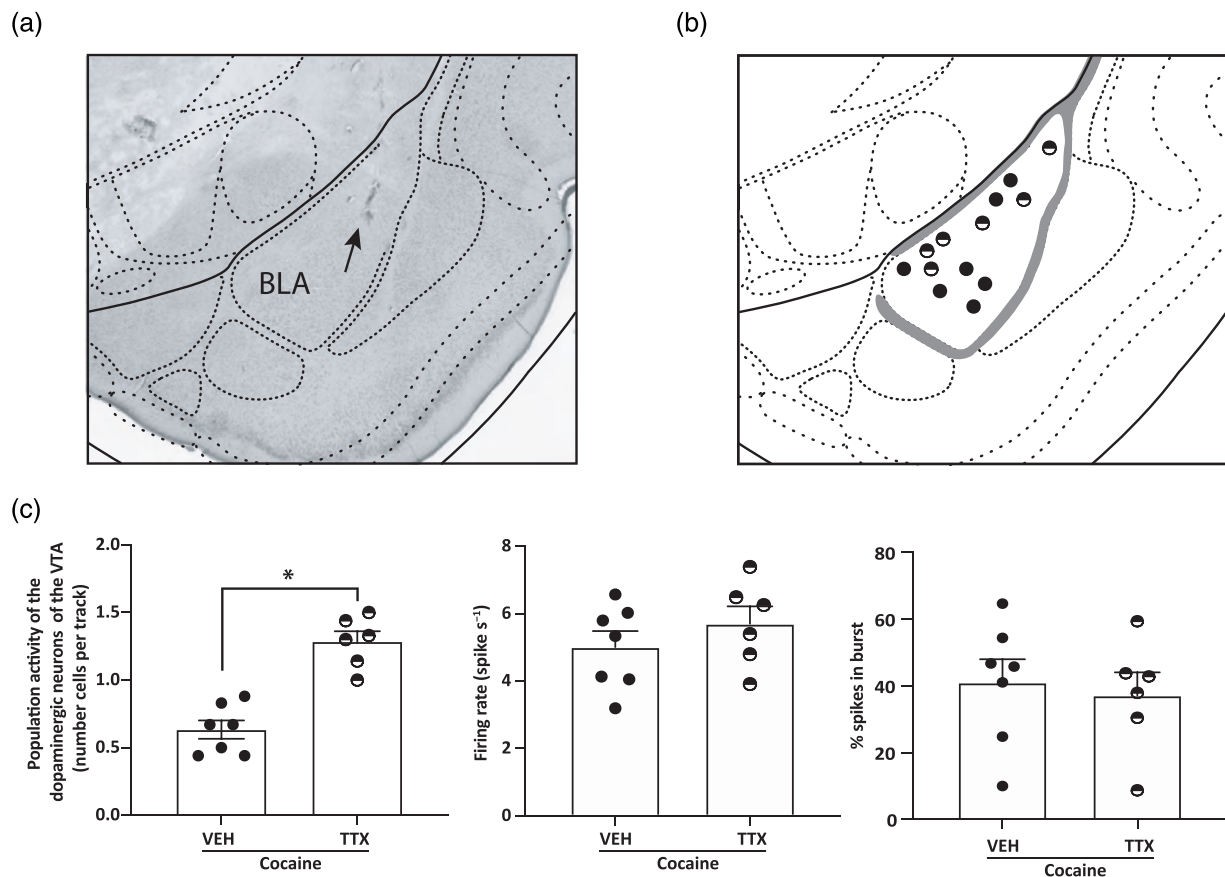


FIGURE 4 Effect of inactivating the BLA with TTX on VTA DA neuron activity. Histological slice showing track of the guide cannula in the BLA (a). Coronal brain section adapted from Swanson (2018) (vehicle, VEH: black dots; TTX: white and black dots) (b). Number of spontaneously active DA neurons recorded per electrode track (c, left), number of spikes per second (firing rate; c, middle), and percentage of action potential firing in bursts (c, right) in cocaine long term withdrawal (26–45 days: WD26–45) rats after vehicle (VEH) or TTX infusion in the BLA. Data are mean \pm SEM; * $P < 0.05$; cocaine WD26–45 VEH: seven rats, 32 neurons; cocaine WD26–45 TTX: six rats, 50 neurons. Coronal brain sections adapted from Swanson (2018).

activity with TTX (Figure 4c left). No differences in the mean firing rate (Figure 4c middle) or the percentage of spikes in a burst (Figure 4c right) were observed.

3.5 | Blockade of ventral pallidum glutamatergic inputs restored DA neuron activity after long-term-abstinence from chronic cocaine

The ventral pallidum (VP) attenuates VTA DA neuron population activity (Floresco et al., 2001) and receives glutamatergic inputs from the BLA (Maslowski-Cobuzzi & Napier, 1994), and blocking glutamatergic inputs in the VP using kynurenic acid has been shown to restore DA population activity in the CMS model of depression (Chang & Grace, 2014). We used **kynurenic acid**, a broad-spectrum **glutamate receptor** antagonist (Chang & Grace, 2014; Floresco et al., 2001), to block glutamatergic afferents from the BLA to the VP. A representative example of placement of the infusion cannula is presented in Figure 5a, and the location of the vehicle and kynurenic acid (KYN) infusion cannula is presented in Figure 5b. Cocaine rats infused with

vehicle (VEH) after 26–45 days abstinence had fewer spontaneously active DA neurons, in comparison to when VP glutamatergic inputs were blocked by kynurenic acid (Figure 5c left). No differences in the mean firing rate (Figure 5c middle) or the percentage of spikes in a burst (Figure 5c right) were observed.

4 | DISCUSSION

In this study, we found a significant decrease of VTA DA population activity after long term, but not short term, abstinence from chronic cocaine self-administration, a pattern paralleling the phenomenon of incubation of craving (Grimm et al., 2001). We also found a significant increase of BLA activity after long, but not short-term, abstinence. Finally, we show that the decrease in VTA DA activity depends on the BLA–VP pathway.

We have previously shown that extended exposure to cocaine (25 days) followed by abstinence (7 and 30 days) persistently decreases DA activity in the VTA (Salin et al., 2021). Here, we extend these findings using a shorter 10-day self-administration protocol,

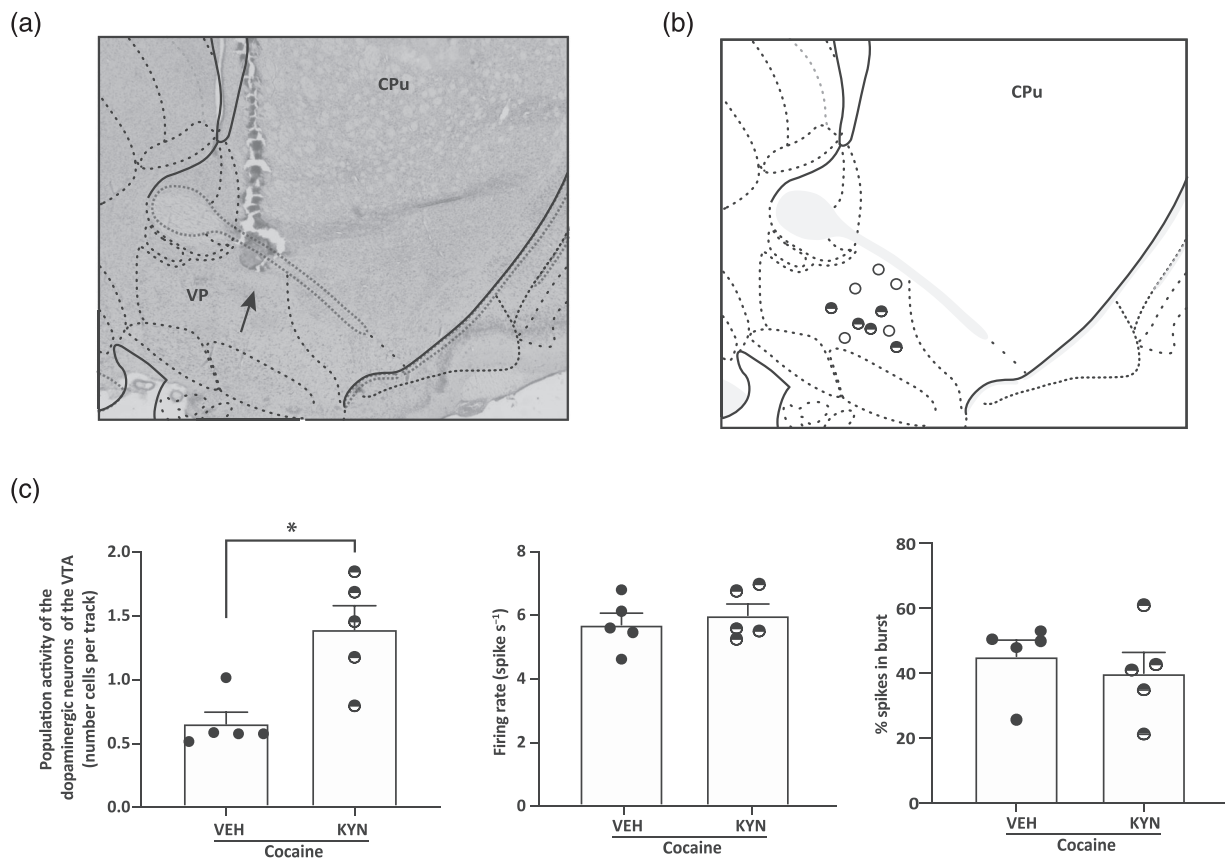


FIGURE 5 Effect of blocking glutamatergic afferents with kynurenic acid in the ventral pallidum on VTA DA neuron activity. Histological slice showing track of the guide cannula in the VP (a). Coronal brain section adapted from Swanson (2018) (vehicle, VEH: black dots; kynurenic acid, KYN: white and black dots) (b). Number of spontaneously active DA neurons recorded per electrode track (c, left), number of spikes per second (firing rate; c, middle), and percentage of action potential firing in bursts (c, right) in cocaine WD30 rats after VEH or KYN infusion in the VP. Data are mean \pm SEM; * $P < 0.05$; cocaine WD26–45 VEH: five rats, 27 neurons; cocaine WD26–45 KYN: five rats, 49 neurons. Coronal brain sections adapted from Swanson (2018).

showing a decrease in DA population activity after 26–45 days but not 1–3 days of abstinence. We found no changes of DA population activity during acute withdrawal, suggesting no changes in tonic DA release in VTA target structures (Floresco et al., 2003) at this time point. This is consistent with some studies using microdialysis that showed no changes in baseline DA during acute withdrawal (Calipari et al., 2014; Cameron et al., 2016), but it is inconsistent with other work (Hurd et al., 1989; Weiss et al., 1992) demonstrating reduced basal DA levels during acute withdrawal. There are multiple differences between studies that can account for these discrepancies, including the use of slice versus *in vivo* preparation, the type of self-administration paradigm (length of the self-administration period for example), and the time of microdialysis measurements relative to the last self-administration sessions (12 h, 24 h, or up to 72 h after the last self-administration session). In our study, DA neuron activity was recorded from 18 h up to 72 h after the last session, suggesting that potential decreases in population activity and subsequent decreased DA release might normalize rapidly following discontinuation of cocaine self-administration. Another explanation could be that tonic DA decreases independently of population activity (via

changes in presynaptic DA release or increased reuptake). Although there are discrepancies in changes of DA level or DA activity during acute withdrawal, a decrease in DA activity has been consistently described in the literature following long-term withdrawal (Salin et al., 2021; Shen et al., 2007). This decrease could participate in the dysphoria described after protracted cocaine abstinence (Haake et al., 2019).

Previous work has shown that activation of the BLA potently decreases the number of DA neurons firing (Chang & Grace, 2014). Increased activity in the BLA has been previously described after extended withdrawal from chronic cocaine (Munshi et al., 2019), which is consistent with the increased firing rate and percentage of spikes in bursts after long-term abstinence in our study. However, Munshi et al. did not study BLA activity after acute withdrawal. Here, we show no changes in the firing rate or the percentage of spikes firing in burst after 1–3 days of withdrawal between cocaine and saline rats. The increased firing rate after long term abstinence in the BLA could be attributed to multiple factors, such as changes in intrinsic excitability, in the expression of NMDA or AMPA receptors, or changes in different inputs to pyramidal neurons. Changes in

membrane properties have been described in several pathological conditions such as drug addiction (Kourrich et al., 2015). Numerous studies have described increased excitability of BLA neurons after chronic stress (Rau et al., 2015; Rosenkranz et al., 2010). Since chronic stress and drug of abuse share common substrates (Belujon & Grace, 2011; Munshi et al., 2019), the increased firing rate and bursting activity of BLA neurons after long abstinence could be due to increased neuronal excitability. Another factor that might contribute to increased firing is changes in the expression of NMDA or AMPA receptors in the BLA. Indeed, previous work has shown an increase in the expression of **GLUR1** and **GLUR2** subunits as well as a decrease in the expression of NR2B subunits in the BLA during cocaine abstinence (Lu et al., 2005). Interneurons in the BLA tightly regulate excitability of principal neurons (Ehrlich et al., 2009) by targeting their perisomatic region (Bienvenu et al., 2012) and blocking activity in principal neurons (Woodruff & Sah, 2007). Since activity of BLA principal neurons is regulated by local interneurons, dysregulation of interneuron activity in the BLA after chronic cocaine could increase spontaneous activity of principal neurons. Although increased activity of parvalbumin (PV) interneurons has been described in the central amygdala after chronic opiate withdrawal (Wang et al., 2016), there is a lack of information concerning PV interneurons in the BLA after psychostimulant withdrawal. Moreover, BLA activity can be potently modulated by the prefrontal cortex through inhibitory control via reciprocal connections (Rosenkranz & Grace, 2002). Changes in activity in the medial prefrontal cortex has been observed in abstinent cocaine abusers (Bolla et al., 2004), which could lead to pathological activation of the amygdala. Therefore, after protracted abstinence of chronic cocaine, hyperactivity of the BLA could underlie the reduced DA VTA population activity observed in the present study.

Chronic stress has been shown to increase BLA activity (Munshi et al., 2019) and decrease DA population activity in the VTA (Chang & Grace, 2014). Inactivation of the BLA in a model of chronic stress has also been shown to increase DA population activity (Chang & Grace, 2014). In our model, we tested the effect of BLA inactivation on DA population activity after long-term abstinence. In agreement with the stress literature (Chang & Grace, 2014), BLA inactivation increased dopaminergic activity in cocaine rats after long-term withdrawal. These results support the existence of common substrates between addiction and stress-related emotional disorders (Belujon & Grace, 2011; Polter & Kauer, 2014) and emphasize that normalization of BLA activity may be involved in reducing abstinence-related emotional symptoms.

The BLA does not project directly to the VTA but has strong projections to the VP (Maslowski-Cobuzzi & Napier, 1994), and the BLA-VP-VTA has been shown to exert potent actions on DA neuron activity states (Chang & Grace, 2014). In our model, blocking glutamatergic afferents in the VP after long-term abstinence restores DA activity suggesting that the BLA-VP pathway is involved in the decreased DA activity after protracted abstinence. Mahler et al. have shown that GABAergic neurons from the rostral part of the VP (RVP) projecting to the VTA, but not the caudal part, are activated during cue-induced reinstatement and that inactivation of the RVP-VTA pathway reduces

cocaine seeking behaviour after long-term abstinence (Mahler et al., 2014). In our study, glutamatergic afferents were blocked in the RVP, suggesting that increased activity of the BLA-RVP pathway leading to hypodopaminergic activity in the VTA after long-term withdrawal might play a critical role in cue-induced seeking. Another region such as the rostromedial tegmentum nucleus could be an additional relay structure in restoring DA activity since it receives direct projections from the BLA (Kaufling et al., 2009) and sends direct GABAergic inhibitory afferents to the VTA (Lecca et al., 2012). However, the rostromedial tegmentum nucleus activation inhibits DA neuron firing rate (Lecca et al., 2012), but firing rate of VTA neurons was not altered during withdrawal in our study. It should be noted that the current findings on the BLA-VP-VTA pathway after long-term abstinence only apply to male rats and future studies are needed to confirm these findings in female rats.

5 | CONCLUSION

Our study sheds new light on neuroadaptations occurring during incubation of craving, a phenomenon believed to play a role in persistent risks of relapse, which is crucial to improve novel therapeutic strategies. In particular, we demonstrate that after long-term withdrawal, the decrease of DA activity in the VTA is associated with hyperactivity of the BLA and involves a circuit comprising the BLA and the VP. Dysfunctions of this circuit could underlie dysphoria, which has been described during acute but also long-term withdrawal from cocaine. Treating/reversing hypodopaminergia in detoxified patients could be critical in terms of relapse prevention strategies, and imaging studies focusing on the interactions of regions in this specific network could be used as a marker of vulnerability to dysphoria, craving, and relapse in these patients.

AUTHOR CONTRIBUTIONS

Adélie Salin: Conceptualization (equal); data curation (lead); formal analysis (equal); investigation (equal); methodology (equal); writing—original draft (equal); writing—review and editing (equal). **Emilie Dugast:** Data curation (supporting); methodology (supporting); project administration (supporting); writing—review and editing (supporting). **Virginie Lardeux:** Data curation (supporting); methodology (supporting); writing—review and editing (supporting). **Marcello Solinas:** Conceptualization (equal); methodology (equal); validation (supporting); writing—review and editing (equal).

ACKNOWLEDGEMENTS

This work has benefited from the facilities and expertise of PREBIOS platform (University of Poitiers). This work was supported by the Institute National de la Santé et de la Recherche Médicale, the Centre National pour la Recherche Scientifique, the University of Poitiers, CHU of Poitiers, SFR FED 4226, and the Agence Nationale de la Recherche (ANR JC, ANR-15-CE37-0010 to P.B.). The authors thank Catherine Le Goff and Eric Balado for technical support.

CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflict of interest.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for [Design and Analysis](#), and [Animal Experimentation](#), and as recommended by funding agencies, publishers, and other organizations engaged with supporting research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

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REFERENCES

- Adhikari, A., Lerner, T. N., Finkelstein, J., Pak, S., Jennings, J. H., Davidson, T. J., Ferenczi, E., Gunaydin, L. A., Mirzabekov, J. J., Ye, L., Kim, S. Y., Lei, A., & Deisseroth, K. (2015). Basomedial amygdala mediates top-down control of anxiety and fear. *Nature*, *527*, 179–185. <https://doi.org/10.1038/nature15698>
- Alexander, S. P., Mathie, A., Peters, J. A., Veale, E. L., Striessnig, J., Kelly, E., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Aldrich, R. W., Attali, B., Baggetta, A. M., Becirovic, E., Biel, M., Bill, R. M., Catterall, W. A., ... Zhu, M. (2021). THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: Ion channels. *British Journal of Pharmacology*, *178*(S1), S157–S245. <https://doi.org/10.1111/bph.15539>
- Alonso, I. P., O'Connor, B. M., Bryant, K. G., Mandalaywala, R. K., & España, R. A. (2022). Incubation of cocaine craving coincides with changes in dopamine terminal neurotransmission. *Addiction Neuroscience*, *3*, 100029. <https://doi.org/10.1016/j.addicn.2022.100029>
- Belujon, P., & Grace, A. A. (2011). Hippocampus, amygdala, and stress: Interacting systems that affect susceptibility to addiction. *Annals of the New York Academy of Sciences*, *1216*, 114–121. <https://doi.org/10.1111/j.1749-6632.2010.05896.x>
- Belujon, P., & Grace, A. A. (2014). Restoring mood balance in depression: Ketamine reverses deficit in dopamine-dependent synaptic plasticity. *Biological Psychiatry*, *76*, 927–936. <https://doi.org/10.1016/j.biopsych.2014.04.014>
- Belujon, P., & Grace, A. A. (2017). Dopamine system dysregulation in major depressive disorders. *The International Journal of Neuropsychopharmacology*, *20*, 1036–1046. <https://doi.org/10.1093/ijnp/pyx056>
- Belujon, P., Jakobowski, N. L., Dollish, H. K., & Grace, A. A. (2016). Withdrawal from acute amphetamine induces an amygdala-driven attenuation of dopamine neuron activity: Reversal by ketamine. *Neuropsychopharmacology*, *41*, 619–627. <https://doi.org/10.1038/npp.2015.191>
- Biennu, T. C. M., Busti, D., Magill, P. J., Ferraguti, F., & Capogna, M. (2012). Cell-type-specific recruitment of amygdala interneurons to hippocampal theta rhythm and noxious stimuli in vivo. *Neuron*, *74*–20, 1059–1074.
- Bolla, K., Ernst, M., Kiehl, K., Mouratidis, M., Eldred, D., Contoreggi, C., Matochik, J., Kurian, V., Cadet, J., Kimes, A., Funderburk, F., & London, E. (2004). Prefrontal cortical dysfunction in abstinent cocaine abusers. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *16*, 456–464. <https://doi.org/10.1176/jnp.16.4.456>
- Calipari, E. S., Ferris, M. J., & Jones, S. R. (2014). Extended access of cocaine self-administration results in tolerance to the dopamine-elevating and locomotor-stimulating effects of cocaine. *Journal of Neurochemistry*, *128*, 224–232. <https://doi.org/10.1111/jnc.12452>
- Calipari, E. S., Siciliano, C. A., Zimmer, B. A., & Jones, S. R. (2015). Brief intermittent cocaine self-administration and abstinence sensitizes cocaine effects on the dopamine transporter and increases drug seeking. *Neuropsychopharmacology*, *40*, 728–735. <https://doi.org/10.1038/npp.2014.238>
- Cameron, C. M., Wightman, R. M., & Carelli, R. M. (2016). One month of cocaine abstinence potentiates rapid dopamine signaling in the nucleus accumbens core. *Neuropharmacology*, *111*, 223–230. <https://doi.org/10.1016/j.neuropharm.2016.09.006>
- Chang, C., & Grace, A. A. (2014). Amygdala-ventral pallidum pathway decreases dopamine activity after chronic mild stress in rats. *Biological Psychiatry*, *76*, 223–230. <https://doi.org/10.1016/j.biopsych.2013.09.020>
- Chauvet, C., Goldberg, S. R., Jaber, M., & Solinas, M. (2012). Effects of environmental enrichment on the incubation of cocaine craving. *Neuropharmacology*, *63*, 635–641. <https://doi.org/10.1016/j.neuropharm.2012.05.014>
- Childress, A. R., Mozley, P. D., McElgin, W., Fitzgerald, J., Reivich, M., & O'Brien, C. P. (1999). Limbic activation during cue-induced cocaine craving. *The American Journal of Psychiatry*, *156*, 11–18. <https://doi.org/10.1176/ajp.156.1.11>
- Curtis, M. J., Alexander, S. P. H., Cirino, G., George, C. H., Kendall, D. A., Insel, P. A., Izzo, A. A., Ji, Y., Panettieri, R. A., Patel, H. H., Sobey, C. G., Stanford, S. C., Stanley, P., Stefanska, B., Stephens, G. J., Teixeira, M. M., Vergnolle, N., & Ahluwalia, A. (2022). Planning experiments: Updated guidance on experimental design and analysis and their reporting III. *British Journal of Pharmacology*, *179*, 3907–3913. <https://doi.org/10.1111/bph.15868>
- Diana, M., Pistis, M., Muntoni, A., & Gessa, G. (1996). Mesolimbic dopaminergic reduction outlasts ethanol withdrawal syndrome: Evidence of protracted abstinence. *Neuroscience*, *71*, 411–415. [https://doi.org/10.1016/0306-4522\(95\)00482-3](https://doi.org/10.1016/0306-4522(95)00482-3)
- Ehrlich, I., Humeau, Y., Grenier, F., Ciocchi, S., Herry, C., & Lüthi, A. (2009). Amygdala inhibitory circuits and the control of fear memory. *Neuron*, *62*, 757–771. <https://doi.org/10.1016/j.neuron.2009.05.026>
- Farrell, M. R., Ruiz, C. M., Castillo, E., Faget, L., Khanbajian, C., Liu, S., Schoch, H., Rojas, G., Huerta, M. Y., Hnasko, T. S., & Mahler, S. V. (2019). Ventral pallidum is essential for cocaine relapse after voluntary abstinence in rats. *Neuropsychopharmacology*, *44*, 2174–2185. <https://doi.org/10.1038/s41386-019-0507-4>
- Floresco, S. B., Todd, C. L., & Grace, A. A. (2001). Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental area dopamine neurons. *The Journal of Neuroscience*, *21*, 4915–4922. <https://doi.org/10.1523/JNEUROSCI.21-13-04915.2001>
- Floresco, S. B., West, A. R., Ash, B., Moore, H., & Grace, A. A. (2003). Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nature Neuroscience*, *6*, 968–973. <https://doi.org/10.1038/nn1103>
- Garavan, H., Pankiewicz, J., Bloom, A., Cho, J.-K., Sperry, L., Ross, T. J., Salmeron, B. J., Risinger, R., Kelley, D., & Stein, E. A. (2000). Cue-induced cocaine craving: Neuroanatomical specificity for drug users and drug stimuli. *The American Journal of Psychiatry*, *157*, 1789–1798. <https://doi.org/10.1176/appi.ajp.157.11.1789>
- Grimm, J. W., Hope, B. T., Wise, R. A., & Shaham, Y. (2001). Neuroadaptation. Incubation of cocaine craving after withdrawal. *Nature*, *412*, 141–142. <https://doi.org/10.1038/35084134>
- Haake, R. M., West, E. A., Wang, X., & Carelli, R. M. (2019). Drug-induced dysphoria is enhanced following prolonged cocaine abstinence and dynamically tracked by nucleus accumbens neurons. *Addiction Biology*, *24*, 631–640. <https://doi.org/10.1111/adb.12627>

- Hatzigiakoumis, D. S., Martinotti, G., Giannantonio, M. D., & Janiri, L. (2011). Anhedonia and substance dependence: Clinical correlates and treatment options. *Frontiers in Psychiatry*, 2, 10.
- Hurd, Y. L., Weiss, F., Koob, G. F., And, N. E., & Ungerstedt, U. (1989). Cocaine reinforcement and extracellular dopamine overflow in rat nucleus accumbens: An in vivo microdialysis study. *Brain Research*, 498, 199–203. [https://doi.org/10.1016/0006-8993\(89\)90422-8](https://doi.org/10.1016/0006-8993(89)90422-8)
- Janak, P. H., & Tye, K. M. (2015). From circuits to behaviour in the amygdala. *Nature*, 517, 284–292. <https://doi.org/10.1038/nature14188>
- Janiri, L., Martinotti, G., Dario, T., Reina, D., Paparello, F., Pozzi, G., Addolorato, G., di Giannantonio, M., & de Risio, S. (2005). Anhedonia and substance-related symptoms in detoxified substance-dependent subjects: A correlation study. *Neuropsychobiology*, 52, 37–44. <https://doi.org/10.1159/000086176>
- Kaufling, J., Veinante, P., Pawlowski, S. A., Freund-Mercier, M.-J., & Barrot, M. (2009). Afferents to the GABAergic tail of the ventral tegmental area in the rat. *The Journal of Comparative Neurology*, 513, 597–621. <https://doi.org/10.1002/cne.21983>
- Kourrich, S., Calu, D. J., & Bonci, A. (2015). Intrinsic plasticity: An emerging player in addiction. *Nature Reviews. Neuroscience*, 16, 173–184. <https://doi.org/10.1038/nrn3877>
- Lecca, S., Melis, M., Luchicchi, A., Muntoni, A. L., & Pistis, M. (2012). Inhibitory inputs from rostromedial tegmental neurons regulate spontaneous activity of midbrain dopamine cells and their responses to drugs of abuse. *Neuropsychopharmacology*, 37, 1164–1176. <https://doi.org/10.1038/npp.2011.302>
- LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience*, 23, 155–184. <https://doi.org/10.1146/annurev.neuro.23.1.155>
- Lilley, E., Stanford, S. C., Kendall, D. E., Alexander, S. P. H., Cirino, G., Docherty, J. R., George, C. H., Insel, P. A., Izzo, A. A., Ji, Y., Panettieri, R. A., Sobey, C. G., Stefanska, B., Stephens, G., Teixeira, M., & Ahluwalia, A. (2020). ARRIVE 2.0 and the *British Journal of Pharmacology*: Updated guidance for 2020. *British Journal of Pharmacology*, 177, 3611–3616. <https://doi.org/10.1111/bph.15178>
- Lu, L., Dempsey, J., Shaham, Y., & Hope, B. T. (2005). Differential long-term neuroadaptations of glutamate receptors in the basolateral and central amygdala after withdrawal from cocaine self-administration in rats. *Journal of Neurochemistry*, 94, 161–168. <https://doi.org/10.1111/j.1471-4159.2005.03178.x>
- Maas, L. C., Lukas, S. E., Kaufman, M. J., Weiss, R. D., Daniels, S. L., Rogers, V. W., Kukes, T. J., & Renshaw, P. F. (1998). Functional magnetic resonance imaging of human brain activation during cue-induced cocaine craving. *The American Journal of Psychiatry*, 155, 124–126. <https://doi.org/10.1176/ajp.155.1.124>
- Mahler, S. V., Vazey, E. M., Beckley, J. T., Keistler, C. R., McGlinchey, E. M., Kaufling, J., Wilson, S. P., Deisseroth, K., Woodward, J. J., & Aston-Jones, G. (2014). Designer receptors show role for ventral pallidum input to ventral tegmental area in cocaine seeking. *Nature Neuroscience*, 17, 577–585. <https://doi.org/10.1038/nn.3664>
- Maslowski-Cobuzzi, R. J., & Napier, T. C. (1994). Activation of dopaminergic neurons modulates ventral pallidum responses evoked by amygdala stimulation. *Neuroscience*, 62, 1103–1119. [https://doi.org/10.1016/0306-4522\(94\)90347-6](https://doi.org/10.1016/0306-4522(94)90347-6)
- McGrath, J. C., & Lilley, E. (2015). Implementing guidelines on reporting research using animals (ARRIVE etc.): New requirements for publication in *BJP*. *British Journal of Pharmacology*, 172, 3189–3193. <https://doi.org/10.1111/bph.12955>
- Meil, W. M., & See, R. E. (1997). Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. *Behavioural Brain Research*, 87, 139–148. [https://doi.org/10.1016/S0166-4328\(96\)02270-X](https://doi.org/10.1016/S0166-4328(96)02270-X)
- Melis, M., Spiga, S., & Diana, M. (2005). The dopamine hypothesis of drug addiction: Hypodopaminergic state. In *International review of neurobiology* (pp. 101–154). Academic Press. [https://doi.org/10.1016/S0074-7742\(05\)63005-X](https://doi.org/10.1016/S0074-7742(05)63005-X)
- Munshi, S., Rosenkranz, J. A., Caccamise, A., Wolf, M. E., Corbett, C. M., & Loweth, J. A. (2019). Cocaine and chronic stress exposure produce an additive increase in neuronal activity in the basolateral amygdala. *Addiction Biology*, 26, e12848.
- Murray, E. A. (2007). The amygdala, reward and emotion. *Trends in Cognitive Sciences*, 11, 489–497. <https://doi.org/10.1016/j.tics.2007.08.013>
- Parker, C. C., Chen, H., Flagel, S. B., Geurts, A. M., Richards, J. B., Robinson, T. E., Solberg Woods, L. C., & Palmer, A. A. (2014). Rats are the smart choice: Rationale for a renewed focus on rats in behavioral genetics. *Neuropharmacology*, 76 Pt B, 250–258. <https://doi.org/10.1016/j.neuropharm.2013.05.047>
- Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Karp, N. A., Lasic, S. E., Lidster, K., MacCallum, C. J., Macleod, M., ... Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biology*, 18, e3000410. <https://doi.org/10.1371/journal.pbio.3000410>
- Polter, A. M., & Kauer, J. A. (2014). Stress and VTA synapses: Implications for addiction and depression. *The European Journal of Neuroscience*, 39, 1179–1188. <https://doi.org/10.1111/ejn.12490>
- Rainnie, D. G., Asprodini, E. K., & Shinnick-Gallagher, P. (1993). Intracellular recordings from morphologically identified neurons of the basolateral amygdala. *Journal of Neurophysiology*, 69, 1350–1362. <https://doi.org/10.1152/jn.1993.69.4.1350>
- Rau, A. R., Chappell, A. M., Butler, T. R., Ariwodola, O. J., & Weiner, J. L. (2015). Increased basolateral amygdala pyramidal cell excitability may contribute to the anxiogenic phenotype induced by chronic early-life stress. *The Journal of Neuroscience*, 35, 9730–9740. <https://doi.org/10.1523/JNEUROSCI.0384-15.2015>
- Rosenkranz, J. A., & Grace, A. A. (2002). Cellular mechanisms of infralimbic and prelimbic prefrontal cortical inhibition and dopaminergic modulation of basolateral amygdala neurons in vivo. *Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 22, 324–337. <https://doi.org/10.1523/JNEUROSCI.22-01-00324.2002>
- Rosenkranz, J. A., Venheim, E. R., & Padival, M. (2010). Chronic stress causes amygdala hyperexcitability in rodents. *Biological Psychiatry*, 67, 1128–1136. <https://doi.org/10.1016/j.biopsych.2010.02.008>
- Salin, A., Lardeux, V., Solinas, M., & Belujon, P. (2021). Protracted abstinence from extended cocaine self-administration is associated with hypodopaminergic activity in the VTA but not in the SNc. *The International Journal of Neuropsychopharmacology*, 24, 499–504. <https://doi.org/10.1093/ijnp/pyaa096>
- Shen, R.-Y., Choong, K.-C., & Thompson, A. C. (2007). Long-term reduction in ventral tegmental area dopamine neuron population activity following repeated stimulant or ethanol treatment. *Biological Psychiatry*, 61, 93–100. <https://doi.org/10.1016/j.biopsych.2006.03.018>
- Shin, C. B., Serchia, M. M., Shahin, J. R., Ruppert-Majer, M. A., Kippin, T. E., & Szumlanski, K. K. (2016). Incubation of cocaine-craving relates to glutamate over-flow within ventromedial prefrontal cortex. *Neuropharmacology*, 102, 103–110. <https://doi.org/10.1016/j.neuropharm.2015.10.038>
- Smith, K. S., Tindell, A. J., Aldridge, J. W., & Berridge, K. C. (2009). Ventral pallidum roles in reward and motivation. *Behavioural Brain Research*, 196, 155–167. <https://doi.org/10.1016/j.bbr.2008.09.038>
- Swanson, L. W. (2018). Brain maps 4.0—Structure of the rat brain: An open access atlas with global nervous system nomenclature ontology and flatmaps. *The Journal of Comparative Neurology*, 526, 935–943. <https://doi.org/10.1002/cne.24381>
- van Huijstee, A. N., & Mansvelder, H. D. (2015). Glutamatergic synaptic plasticity in the mesocorticolimbic system in addiction. *Frontiers in Cellular Neuroscience*, 8, 466. <https://doi.org/10.3389/fncel.2014.00466>

- Vitrac, C., Péron, S., Frappé, I., Fernagut, P.-O., Jaber, M., Gaillard, A., & Benoit-Marand, M. (2014). Dopamine control of pyramidal neuron activity in the primary motor cortex via D2 receptors. *Frontiers in Neural Circuits*, 8, 13. <https://doi.org/10.3389/fncir.2014.00013>
- Wang, L., Shen, M., Jiang, C., Ma, L., & Wang, F. (2016). Parvalbumin interneurons of central amygdala regulate the negative affective states and the expression of corticotrophin-releasing hormone during morphine withdrawal. *The International Journal of Neuropsychopharmacology*, 19, pyw060. <https://doi.org/10.1093/ijnp/pyw060>
- Washburn, M. S., & Moises, H. C. (1992). Electrophysiological and morphological properties of rat basolateral amygdaloid neurons in vitro. *Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 12, 4066–4079. <https://doi.org/10.1523/JNEUROSCI.12-10-04066.1992>
- Wassum, K. M., & Izquierdo, A. (2015). The basolateral amygdala in reward learning and addiction. *Neuroscience and Biobehavioral Reviews*, 57, 271–283. <https://doi.org/10.1016/j.neubiorev.2015.08.017>
- Weiss, F., Markou, A., Lorang, M. T., & Koob, G. F. (1992). Basal extracellular dopamine levels in the nucleus accumbens are decreased during cocaine withdrawal after unlimited-access self-administration. *Brain Research*, 593, 314–318. [https://doi.org/10.1016/0006-8993\(92\)91327-B](https://doi.org/10.1016/0006-8993(92)91327-B)
- Wise, R. A. (2008). Dopamine and reward: The anhedonia hypothesis 30 years on. *Neurotoxicity Research*, 14, 169–183. <https://doi.org/10.1007/BF03033808>
- Wise, R. A., & Hoffman, D. C. (1992). Localization of drug reward mechanisms by intracranial injections. *Synapse*, 10, 247–263. <https://doi.org/10.1002/syn.890100307>
- Woodruff, A. R., & Sah, P. (2007). Inhibition and synchronization of basal amygdala principal neuron spiking by parvalbumin-positive interneurons. *Journal of Neurophysiology*, 98, 2956–2961. <https://doi.org/10.1152/jn.00739.2007>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Salin, A., Dugast, E., Lardeux, V., Solinas, M., & Belujon, P. (2023). The amygdala–ventral pallidum pathway contributes to a hypodopaminergic state in the ventral tegmental area during protracted abstinence from chronic cocaine. *British Journal of Pharmacology*, 1–13. <https://doi.org/10.1111/bph.16034>