Pharmacological Research 113 (2016) 81–91

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Perspective

The hallucinogen D-lysergic diethylamide (LSD) decreases dopamine firing activity through 5-HT_{1A}, D_2 and $TAAR_1$ receptors

Danilo De Gregorio[a,b], Luca Posa[a], Rafael Ochoa-Sanchez[a], Ryan McLaughlin[a], Sabatino Maione[b], Stefano Comai[a], Gabriella Gobbi (MD PhD)[a,*]

[a] *Neurobiological Psychiatry Unit, Department of Psychiatry, McGill University and McGill University Health Center, Montreal, QC, Canada*
[b] *Department of Experimental Medicine, Division of Pharmacology, Second University of Naples, Naples, Italy*

ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form 12 June 2016
Accepted 16 August 2016
Available online 17 August 2016

Chemical compounds studied in this article:
LSD (PubChem CID: 5761)
p-chlorophenylalanine (PubChem CID: 39972)
Haloperidol (PubChem CID: 3559)
MDL 100,907 (PubChem CID:60858)
EPPTB (PubChem CID: 25175634)
WAY 100,635 (PubChem CID: 5684)
Apomorphine (PubChem CID: 6005)
8-OH-DPAT (PubChem CID:1220)

Keywords:
LSD
In-vivo electrophysiology
Dopamine
Serotonin
$TAAR_1$ receptor

ABSTRACT

D-lysergic diethylamide (LSD) is a hallucinogenic drug that interacts with the serotonin (5-HT) system binding to 5-HT_1 and 5-HT_2 receptors. Little is known about its potential interactions with the dopamine (DA) neurons of the ventral tegmental area (VTA). Using *in-vivo* electrophysiology in male adult rats, we evaluated the effects of cumulative doses of LSD on VTA DA neuronal activity, compared these effects to those produced on 5-HT neurons in the dorsal raphe nucleus (DRN), and attempted to identify the mechanism of action mediating the effects of LSD on VTA DA neurons. LSD, at low doses (5–20 μg/kg, i.v.) induced a significant decrease of DRN 5-HT firing activity through 5-HT_{2A} and D_2 receptors. At these low doses, LSD did not alter VTA DA neuronal activity. On the contrary, at higher doses (30–120 μg/kg, i.v.), LSD dose-dependently decreased VTA DA firing activity. The depletion of 5-HT with *p*-chlorophenylalanine did not modulate the effects of LSD on DA firing activity. The inhibitory effects of LSD on VTA DA firing activity were prevented by the D_2 receptor antagonist haloperidol (50 μg/kg, i.v.) and by the 5-HT_{1A} receptor antagonist WAY-100,635 (500 μg/kg, i.v.). Notably, pretreatment with the trace amine-associate receptor 1 ($TAAR_1$) antagonist EPPTB (5 mg/kg, i.v.) blocked the inhibitory effect of LSD on VTA DA neurons. These results suggest that LSD at high doses strongly affects DA mesolimbic neuronal activity in a 5-HT independent manner and with a pleiotropic mechanism of action involving 5-$HT_{1A,}$ D_2 and $TAAR_1$ receptors.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Synthesized in 1938 by A. Hoffmann [1], D-lysergic diethylamide (LSD) is a hallucinogen drug with potent psychotropic effects, described as "mystical experiences" [2] including alterations of the state of consciousness, euphoria, enhanced capacity for introspection, and altered psychological functioning [3–5]. In particular, LSD may produce psychotic-like symptoms such as visual, tactile, acoustic hallucinations, change in body perception, synaesthesia, thought disorders, time distortions, etc. For these reasons, LSD-induced psychosis is considered a pharmacological model to better understand the pathogenesis of psychosis and schizophrenia. Common wisdom has associated the psychotropic properties of LSD to its effects at the level of the serotonergic (5-HT) system acting as a 5-HT_{2A} receptor partial agonist [6–8] and a 5-HT_{1A} receptor agonist/partial agonist [9,10], binding the 5-HT_{2A} receptor with an EC_{50} value of 7.2 nM [11]. *In-vivo* electrophysiological studies in rats have shown that LSD decreases the activity of 5-HT neurons in the dorsal raphe nucleus (DRN) [12] and increases the firing rate of cortical neurons in the somatosensory cortex [13]. However, several *in-vitro* studies have reported affinity not only for the 5-HT but also for dopamine (DA) receptors [14], in particular affinity for D_2 receptors in pig brain [15] and human cloned D_2 receptor [16], and like others psychostimulants, it induces the incorporation of $[^{35}S]$GTP-γ-S into G_i protein-coupled to D_2 receptors in homogenates of rat brain striatum [17]. Little is known about the *in-vivo* effects of LSD in the mesolimbic DA neurons of the ventral tegmental area (VTA), which is the main source of DA neurons in the brain and is also

* Corresponding author at: Neurobiological Psychiatry Unit, Room 220, Irving Ludmer Psychiatry Research and Training Building, 1033 Pine Avenue West, McGill University, Montreal, PQ, H3A 1A1, Canada.
E-mail address: gabriella.gobbi@mcgill.ca (G. Gobbi).

http://dx.doi.org/10.1016/j.phrs.2016.08.022
1043-6618/© 2016 Elsevier Ltd. All rights reserved.

implicated in the pathogenesis of psychosis [18], as well as the mechanism of action of antipsychotics [19].

Intriguingly, *in-vitro* studies have shown that LSD has high affinity for the G protein-coupled trace-amine associated receptor 1 ($TAAR_1$) [20], but its *in-vivo* interaction with $TAAR_1$ receptors has not yet been explored. Since TAAR 1 is expressed in the VTA, and mRNA and protein levels of D_2 receptors are over-expressed in the striatum of $TAAR_1$ knock-out mice [21], a close interaction between the DA system and $TAAR_1$ can be speculated.

Based on this previous evidence, we have hypothesized that LSD may act with a pleiotropic mechanism of action, involving not only 5-HT but also DA systems. The main goals of this study were thus 1) to better characterize the *in-vivo* contribution of the DA system to the effects of LSD, 2) to compare the effects of LSD on VTA DA neurons *vs.* those on DRN 5-HT neurons [22,23], and 3) to test the involvement of $5\text{-}HT_{1A}$, D_2 and $TAAR_1$ receptors in mediating the effects of LSD on VTA DA neurons.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats (Charles River, Saint-Constant, Quebec, Canada) weighing 300–330 g were housed under standard laboratory conditions with a 12 h light-dark cycle (lights on at 07:00 h) with *ad libitum* access to food and water. All experimental procedures were conducted from 9:00 a.m. to 3:00 p.m. and were in accordance with the guidelines set by the Canadian Institute of Health Research for animal care and scientific use and the Animal Care Committee of McGill University.

2.2. In-vivo *electrophysiological recording preparation*

Rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.) in their housing room and then transported in light-free boxes to the procedural room. Rats were placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) and a hole was drilled through the skull. Body temperature of the animals was measured using a rectal thermometer (Yellow Springs Instrument Co., Yellow Springs, OH, USA) and was maintained at 35–36.5 °C using an IR heating lamp (Philips, Infrared Heat). To maintain a full anesthetic state during the experiments, supplemental doses of chloral hydrate (100 mg/kg, i.p.) were periodically administered. Anesthesia was confirmed by the absence of nociceptive reflex reaction to a tail or a paw pinch and of an eye blink response to pressure. Extracellular single-unit recordings were performed using single-barreled glass micropipettes pulled from 2 mm Stoelting (Wood Dale, IL) capillary glass on a Narashige (Tokyo, Japan) PE-21 pipette puller and preloaded with fiberglass strands to promote capillary filling with 2% Pontamine Sky Blue dye in 2 M NaCl for DRN 5-HT recordings and sodium acetate 0.5 M for VTA DA recordings. The micropipette tips were broken down to diameters of 1–3 μm to reach an electrode impedance of 2–6 MΩ. Single-unit activity was recorded as large-amplitude action potentials captured by a software window discriminator, amplified by an AC Differential MDA-3 amplifier (BAK electronics, INC.), post-amplified and band-pass filtered by a Realistic 10 band frequency equalizer, digitized by a CED 1401 interface system (Cambridge Electronic Design, Cambridge, UK), processed online, and analyzed off-line using Spike2 software version 5.20 for Windows PC. The spontaneous single-spike activity of neurons was recorded for at least 2 min; the first 30 s immediately after detecting the neuron was not considered to eliminate mechanical artifacts due to electrode displacement. Once the recordings were terminated, pontamine sky blue dye was injected iontophoretically by passing a constant positive current of 20 μA for 5 min through the recording pipette to mark the recording site. Then rats were decapitated and their brains extracted and placed in a freezer at −20 Celsius (°C). Subsequent localization of the labeled site was made by cutting 20 μm-thick brain sections using a microtome (Leica CM 3050 S) and the electrode placement was identified with a microscope (Olympus U-TVO.5 × C-3).

2.3. Recording of DRN 5-HT neurons

In-vivo single-unit extracellular recordings of DRN 5-HT neurons were performed as previously described [24]. The electrode was advanced slowly into the DRN, guided by coordinates from the rat brain atlas of Paxinos and Watson (2007): 1.2 mm anterior to interaural zero on the midline, 5.0–6.5 mm from the dura mater. Under physiological conditions, spontaneously active 5-HT neurons exhibit characteristic electrophysiological properties distinguishable from non-5-HT neurons. These 5-HT neurons exhibit a slow (0.1–4 Hz) and a prominently regular firing rate (coefficient of variation, C.O.V., ranges from 0.12 to 0.87) and a broad biphasic (positive–negative) or triphasic waveform (0.8–3.5 ms; 1.4 ms first positive and negative deflections) [24–26]. Although, these criteria may vary in response to pharmacological or environmental manipulations [27], some spike features (i.e., waveform, shape and spike duration) have been shown to be stable across conditions, and are therefore reliable indicators for 5-HT neurons [28].

2.4. Recording of VTA DA neurons

The electrodes were descended into the VTA using a hydraulic micropositioner (David Kopf Instruments, Tujunga, CA, USA) according to the stereotaxic coordinates described in the rat brain atlas of Paxinos and Watson (2007): A–P: 3.4–4.1 mm from the interaural line; lateral: 0.6–1.1 mm from the midline; ventral: 7.5–8.8 mm from the brain surface [29]. Putative DA neurons were identified based on well-established electrophysiological properties: a wide action potential (>2.5 ms), biphasic or triphasic waveform and slow firing rate (0.5–10 Hz) [30,31]. Neuronal activity was measured by calculating the mean firing rate frequency, expressed as the number of spikes per second or Hz. Additionally, the burst activity of DA neurons was analyzed using a script for the Spike 2 software (available on line at www.ced.co.uk). Based on criteria previously described, a burst was defined as a train of at least two spikes with an initial interspike interval (ISI) ≤ 80 ms and a maximum ISI of 160 ms, within a regular low-frequency firing pattern and decreased amplitude from the first to the last spike within the burst [30,31]. The parameters analyzed with the Spike 2 script included the number of bursts found and the percentage of spikes fired in burst calculated for 200 s. All these parameters were expressed as percentage of vehicle injection [32,33].

2.5. Drugs

R-(−)-apomorphine hydrochloride hemihydrate (Apo), chloral hydrate (Sigma-Aldrich, Oakville, Canada), D-lysergic diethylamide (LSD) (Sigma-Aldrich, London, UK), 8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT), (Sigma-Aldrich, Oakville, Canada), WAY 100,635 maleate (WAY) (Tocris Bioscience, Missouri, USA), and *p*-chlorophenylalanine (PCPA) (Sigma-Aldrick, Oakville, Canada) were dissolved in a 0.9% NaCl vehicle (VEH) solution. (*R*)-(+)-α-(2,3-Dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperinemethanol (MDL 100 907) (Tocris Bioscience, Missouri, USA) was dissolved dropwise in acetic acid then titrated with distilled water. Haloperidol (Halo) (Sigma-Aldrich, Oakville, ON, Canada) was dissolved in a solution of 0.039% of 2-hydroxypropyl-γ-cyclodextrin in 0.9% NaCl. *N*-(3-*E*thoxyphenyl)-4-(1-pyrrolidinyl)-3-(trifluoromethyl)benzamide

(EPPTB) (Tocris Bioscience, Missouri, USA) was dissolved in a vehicle of 60% polyethylene glycol 400 (Sigma-Aldrich, Oakville, Canada) and 40% NaCl solution (0.9%). Apo, LSD, WAY, Halo and EPPTB were freshly prepared the day of the experiment. Drugs were injected intravenously (i.v.) using a 24G x 3/4" catheter (Terumo Medical Corporation, Elkton, MD, USA) inserted into the lateral vein of the tail. The maximum volume used for a single i.v. injection was 0.1 mL.

Cumulative doses of LSD (5, 10, 20, 30, 60, 90, 120 and 150 μg/kg, i.v.), Apo (25, 50, 100 μg/kg, i.v. [32]) Halo (50, 100, 150 and 200 μg/kg, i.v.) and 8-OH-DPAT (5, 10 μg/kg, i.v [34]) were tested. Once a stable DRN 5-HT or VTA DA neuron was found, its basal firing activity (CTRL) was recorded for at least five minutes. PCPA (350 mg/kg, i.p., [35]) was administered 48-h and 24-h before VTA DA recordings. Rats were i.v. injected with VEH and then every five minutes with sequential doses of one of the four drugs (LSD, Apo, Halo or 8-OH-DPAT) or with a singular injection of MDL 100 907 (200 μg/kg, i.v. [36]), WAY (500 μg/kg, i.v. [37]) or EPPTB (5 mg/kg, i.v.) until all doses and drug were tested according to the experimental plan. The dose of EPPTB was chosen following its pharmacokinetic parameters (at the dose of 2.5 mg/kg i.v., clearence 87 mL/min/kg, $t_{1/2}$ = 1.9 h, brain/plasma = 0.5 [38]). Only one neuron per rat was tested.

2.6. Statistical analysis

Data were analyzed using SigmaPlot 13 (Systat Software, Inc.). Neuronal responses to cumulative administration of drugs were calculated as percentage of change from VEH injection (100%), were reported as mean (% of VEH) ± standard error of the mean (SEM), and were computed using Student *t*-test or one-way ANOVA or two-way ANOVA for repeated measures followed by Bonferroni post hoc comparisons where appropriate. Statistical values of $P \leq 0.05$ were considered significant.

2.7. ED_{50} calculation

A log transformation of each LSD dose was computed. ED_{50} values were then determined by non-linear regression analysis using GraphPad Prism version 5.04 (GraphPad Software) following the method by Ford et al. [39].

3. Results

3.1. Low doses of LSD inhibit DRN 5-HT but not VTA DA firing activity in a dose-dependent manner

Based on previous findings [40]. and our experience testing LSD activity upon DRN 5-HT neurons, we first confirmed the effects of 5–20 μg/kg LSD in four DRN 5-HT neurons. Second, we investigated whether these doses were active in four VTA DA neurons. Fig. 1A and B report the example of an integrated histogram of spontaneous firing rate of a DRN 5-HT and a VTA DA neuron, respectively, following i.v. injection of cumulative doses of LSD. LSD (5–20 μg/kg) produced a dose-dependent decrease of DRN 5-HT cell firing frequency ($F(3, 8) = 28.090$, $P < 0.001$; Fig. 1D) as previously reported [40]. In particular, compared to VEH injection, 10 μg/kg LSD significantly decreased DRN 5-HT activity ($P = 0.007$), and 20 μg/kg LSD completely shut down 5-HT firing activity ($P < 0.001$). The ED50 value was 13.13 μg/kg (Fig. 3E). No significant effects were observed with 5 μg/kg LSD. Around 20% of DRN 5-HT neurons in rats usually display burst firing activity [27,41–43]; accordingly, we found only 1 out of 4 DRN 5-HT neurons discharging in bursts. The changes in 5-HT burst-firing parameters occurring in this neuron are reported in Table 2. On the contrary, 5–20 μg/kg LSD did not affect either spontaneous ($F(3, 9) = 0.93$, $P = 0.463$; Fig. 1E) or burst VTA DA firing activities (data not shown). After the injection of 20 μg/kg LSD, we tested the previously demonstrated inhibitory effects of cumulative doses of Apo (25–100 μg/kg, i.v.) on VTA DA neurons [32], and we found that at the dose of 50 μg/kg, the DA neural activity was completely shut down (Fig. 1B). Fig. 1D reports an example of the histological control of a recording site in the DRN. We have defined this range of LSD doses (5–20 μg/kg) affecting 5-HT but not DA neurotransmission as "low doses".

3.2. The D_2 antagonist haloperidol and the 5-HT_{2A} antagonist MDL 100 907 prevent the inhibitory effects of low doses of LSD on DRN 5-HT firing activity

Fig. 2A reports the integrated histogram of spontaneous firing rate of a DRN 5-HT neuron following the injection of Halo (50 μg/kg, i.v.) prior to cumulative low doses of LSD. Since no effects were observed until the injection of 20 μg/kg LSD, 30 μg/kg LSD was also injected. Since the maximal dose of 30 μg/kg LSD was also blocked by Halo, cumulative doses of the 5-HT_{1A} agonist 8-OH-DPAT (5–10 μg/kg, i.v. [34]) were injected, inducing a total decrease of 5-HT firing activity. These findings suggest that the blockade of D_2 receptors prevents the inhibitory effects of low doses of LSD on DRN 5-HT firing independently from 5-HT_{1A} receptors. As shown in Fig. 2B, Halo pre-treatment prevented the inhibitory effects of cumulative low doses of LSD on 5-HT firing activity (interaction: $F(5,30) = 11.06$, $P < 0.001$; Halo pre-treatment: $F(1,6) = 17.55$, $P = 0.006$; LSD treatment: $F(5,5) = 20.31$, $P < 0.001$). Bonferroni post-hoc comparisons revealed a different effect of LSD on Halo pre-treated and non pre-treated neurons ($P < 0.001$). In particular, while we found a dose-response decrease of DRN 5-HT firing activity after 10, 20, 30 μg/kg LSD compared to vehicle ($P < 0.001$), the pre-treatment with Halo blocked such effect (Fig. 2B). Halo (50 μg/kg) alone did not affect 5-HT firing activity (Fig. 2C). Pre-treatment with Halo also prevented the inhibitory effect of low dose LSD administration on DRN 5-HT burst-firing activity (Table 2; number of bursts per 200 s: $F(5,12) = 0.77$, $P = 0.58$; % of spikes in bursts: $F(5,12) = 1.08$, $P = 0.41$). Fig. 2D reports the integrated histogram of spontaneous firing rate of a DRN 5-HT neuron following the injection of MDL 100 907 (200 μg/kg, i.v.) prior to cumulative low doses of LSD. As shown in Fig. 2E, MDL 100 907 blocked the effect of cumulative low doses of LSD (interaction: $F(5,30) = 31.20$, $P < 0.001$; MDL 100 907 pre-treatment: $F(1,5) = 66.63$, $P < 0.001$; LSD treatment: $F(5,30) = 30.95$, $P < 0.001$). Bonferroni post-hoc comparisons revealed a different effect of LSD on MDL 100 907 pre-treated and non pre-treated neurons ($P < 0.001$), namely, a decrease of DRN 5-HT firing activity with 10, 20, 30 μg/kg LSD ($P = 0.007$, $P < 0.001$, $P < 0.001$, respectively) in non-pretreated neurons, and no effects of LSD in MDL 100 907 pre-treated neurons. MDL 100 907 (200 μg/kg) alone did not affect 5-HT firing activity (Fig. 2F). The injection of cumulative doses of 8-OH-DPAT (5–10 μg/kg, i.v.) after 30 μg/kg LSD silenced DRN 5-HT neurons (Fig. 1D) suggesting that the blockade of 5-HT_{2A} receptors prevents the inhibitory effects of low doses of LSD independently from 5-HT_{1A} receptors. One out of four DRN 5-HT neurons pre-treated with MDL 100 907 was discharging in bursts. No changes in DRN 5-HT burst-firing activity of this neuron were observed after either MDL 100 907 (200 μg/kg) or low doses of LSD (Table 2).

3.3. High doses of LSD inhibit VTA DA firing activity in a dose-dependent manner

Since 5–20 μg/kg LSD did not alter VTA DA neural activity, we then examined whether increasing the dose of LSD (30–120 μg/kg) any effect on VTA DA firing activity could be elicited. The acute effect of cumulative doses of LSD (30–120 μg/kg) was therefore tested in 6 VTA DA neurons. Fig. 3A reports the example of an inte-

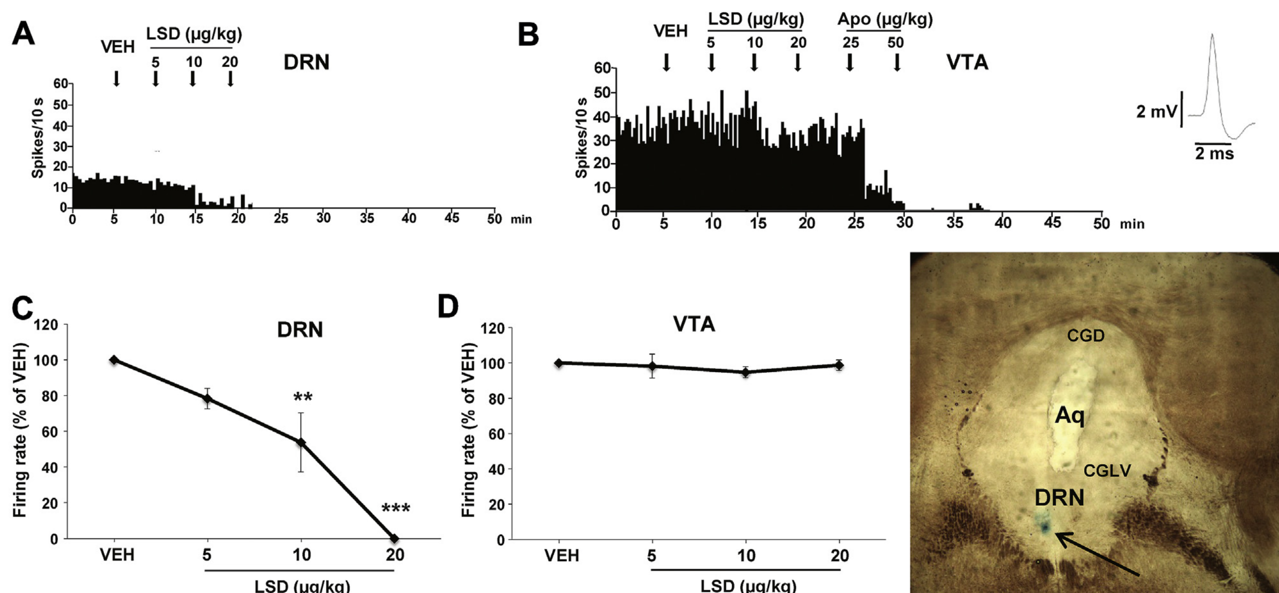

Fig. 1. Effects of intravenous LSD administration on the firing rate of dorsal raphe nucleus (DRN) serotonin (5-HT) neurons and of ventral tegmental area (VTA) dopamine (DA) neurons. (A) Representative integrated firing rate histograms showing the acute response of 5-HT neurons to LSD. Arrows indicate sequential injections of increasing doses of LSD (5 + 5 + 10 μg/kg). (B) Representative integrated firing rate histograms showing the acute response of DA neurons to LSD and apomorphine (Apo). Arrows indicate sequential injections of increasing doses of LSD (5 + 5 + 10 μg/kg) and Apo (25 + 25 μg/kg). The cumulative doses are indicated on top of each arrow. (C) The figure shows the typical spike waveform of a 5-HT neuron. The figure shows also the typical spike waveform of 5-HT neuron. (C) LSD administration induced a strong dose-dependent inhibition in the firing rate of DRN 5-HT neurons. Each point of the line represents mean± SEM expressed as percentage of firing rate after injection of vehicle (VEH). (D) LSD administration at low did not affect the firing rate of VTA DA neurons. Each point of the line represents mean± SEM expressed as percentage of firing rate after injection of vehicle (VEH). One-way ANOVA: ***$P < 0.001$ and **$P < 0.01$ *vs*. VEH. The figure show also representative photomicrograph of the recording site in DRN. Central Grey Dorsal (CGD); Central Grey Lateral Ventral (CGLV); Aqueduct (Aq). The black arrow indicates the site of the electrode recording labeled with pontamine sky blue dye.

Table 1
Burst-firing activity of VTA DA neurons following cumulative high doses of LSD (30–150 μg/kg, i.v.) in rats receiving vehicle (VEH), treated with PCPA, or pre-treated with haloperidol (Halo, 50 μg/kg, i.v.), WAY (500 μg/kg, i.v.) or EPPTB (5 mg/kg, i.v.). Data (mean ± SEM) are reported as% of change *vs*. VEH injection (100%).

	Pre-treatment	LSD					ANOVA
		30 μg/kg	60 μg/kg	90 μg/kg	120 μg/kg	150 μg/kg	
Controls	**VEH**						
# of bursts (200 s)	100	82.6 ± 9.0	45.0 ± 9.2***	18.8 ± 8.5***	2.4 ± 1.6***	0***	$F(5,18) = 35.0$, $p < 0.001$
% spikes in bursts	100	103.5 ± 12.3	69.4 ± 14.7	50.4 ± 18.1	7.8 ± 6.2**	0***	$F(5,18) = 7.6$, $p < 0.001$
PCPA treated rats	**VEH**						
# of bursts (200 s)	100	47.3 ± 12.8***	2.9 ± 2.5***	0***			$F(3,7) = 45.2$, $p < 0.001$
% spikes in bursts	100	67.1 ± 16.1	14.9 ± 10.3**	0***			$F(3,7) = 19.6$, $p < 0.001$
	Halo (50 μg/kg, i.v.)						
# of bursts (200 s)	117.6 ± 5.4*	113.4 ± 3.6	111.0 ± 2.9	108.8 ± 2.4	105.3 ± 4.5	109.0 ± 5.1	$F(6,24) = 3.2$, $p = 0.018$
% spikes in bursts	118.0 ± 4.1	120.8 ± 12.9	117.6 ± 19.0	116.0 ± 12.7	118.1 ± 13.9	112.3 ± 17.5	$F(6,24) = 0.4$, $p = 0.830$
	WAY (500 μg/kg, i.v.)						
# of bursts (200 s)	92.8 ± 2.8	89.3 ± 4.6	85.4 ± 7.4	84.9 ± 2.4	83.1 ± 3.7	91.5 ± 7.0	$F(6,24) = 1.9$, $p = 0.116$
% spikes in bursts	106.6 ± 3.9	110.9 ± 12.1	103.9 ± 8.6	107.3 ± 12.4	109.9 ± 13.3	91.6 ± 8.8	$F(6,24) = 1.7$, $p = 0.162$
	EPPTB (5 mg/kg)						
# of bursts (200 s)	249.2 ± 40.0	189.8 ± 58.6	198.9 ± 57.2	166.2 ± 51.0	207.4 ± 70.1	208.5 ± 44.4	$F(6,30) = 2.1$, $p = 0.086$
% spikes in bursts	136.9 ± 47.8	139.1 ± 30.1	117.5 ± 40.0	117.1 ± 39.2	99.3 ± 33.4	148.9 ± 47.3	$F(6,30) = 0.9$, $p = 0.534$

One-way ANOVA for repeated measures plus Bonferroni post-hoc comparisons.
* p<0.05.
** p<0.01.
*** p<0.001 *vs*. Vehicle.

grated histogram of spontaneous firing rate of a VTA DA neuron following injection of 30–120 μg/kg LSD. This range of LSD doses (30–120 μg/kg) active on VTA DA neurons has been defined as "high doses" in contrast to the "low doses" range (5–20 μg/kg) that affects 5-HT but not DA neurotransmission. Fig. 2A shows that cumulative high doses of LSD significantly decreased and silenced VTA DA firing activity, while subsequent cumulative injections of the selective D_2 antagonist Halo (50–150 μg/kg, i.v.) were able to reinstate DA firing activity. As illustrated in Fig. 3C, cumulative high doses of LSD induced a dose-dependent decrease in VTA DA neural activity ($F(4, 20) = 29.833$, $P < 0.001$). Compared to VEH, the decrease in firing rate was significant after administration of 60 μg/kg and 90 μg/kg LSD ($P < 0.001$), and importantly, 120 μg/kg LSD completely shut down VTA DA activity ($P < 0.001$). 30 μg/kg LSD did not significantly modify DA firing rate. The decrease produced by 120 μg/kg LSD was also significantly higher compared to that induced by 30 and 60 μg/kg LSD ($P < 0.001$ and $P = 0.003$, respectively). The ED_{50} value was 71.80 μg/kg (Fig. 3E). Linear regression analysis comparing the dose-response curve of the effect of LSD upon DRN 5-HT and VTA DA neurons revealed that the slopes of the two lines were significantly different ($F(2,7) = 39.34$, $P < 0.0001$; Fig. 3E). Therefore, LSD acts with higher potency toward 5-HT than toward DA neurons. A main effect of high doses of LSD was also detected when analyzing the effects of LSD on VTA DA burst-firing activity ((Table 1; number

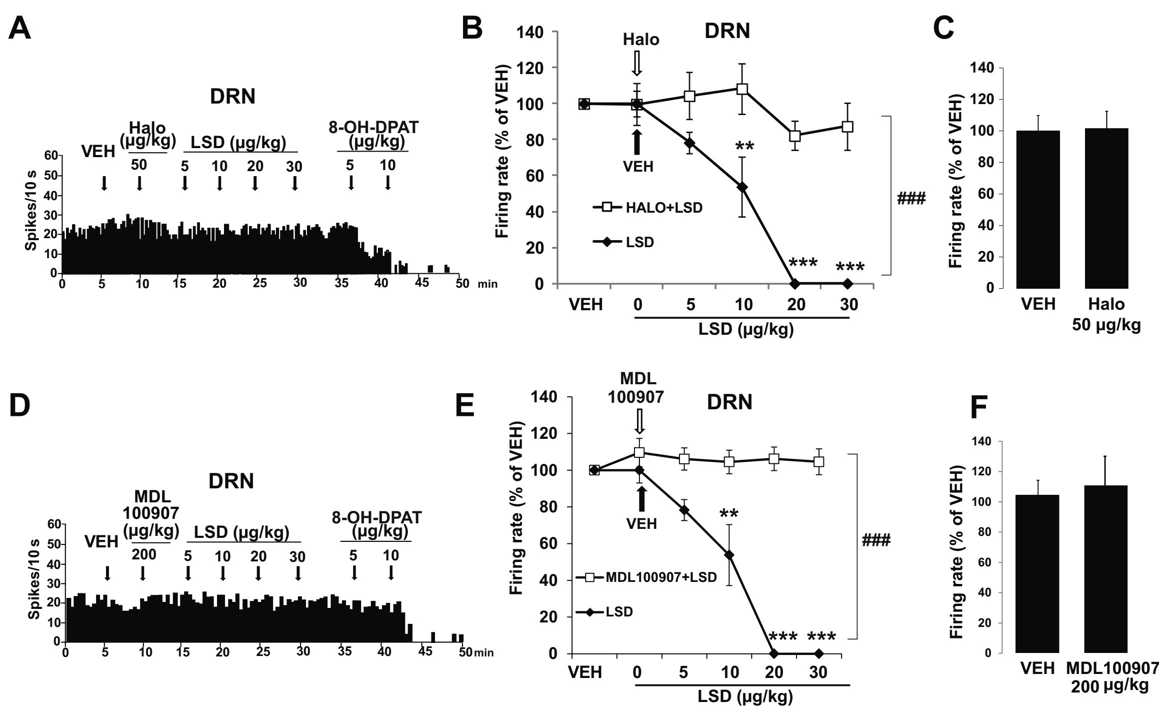

Fig. 2. Haloperidol (Halo) and MDL 100 907 prevent the inhibitory effects of LSD on DRN 5-HT firing activity. (A) Representative integrated firing rate histograms showing the acute response of 5-HT neurons to Halo, LSD and 8-OH-DPAT. Arrows indicate sequence of single injections of Halo (50 µg/kg) and of increasing doses of LSD (5 + 10 + 20 + 30 µg/kg) and 8-OH-DPAT (5 + 10 µg/kg). The cumulative doses are indicated on top of each arrow. (B) Pre-treatment with Halo prevented the inhibitory effects of LSD on 5-HT firing frequency. White and black arrows indicate the injection of Halo and vehicle (VEH), respectively, before the cumulative doses of LSD. Each point of the line represents mean ± SEM expressed as percentage of firing rate after injection of VEH. (C) The single injection of Halo does not change DRN 5-HT firing; bars represent mean ±SEM expressed as percentage of basal firing rate after injection of VEH. (D) Representative integrated firing rate histograms showing the acute response of 5-HT neurons to MDL 100 907, LSD and 8-OH-DPAT. Arrows indicate sequence of single injections of MDL 100 907 (200 µg/kg) and of increasing doses of LSD (5 + 10 + 20 + 30 µg/kg) and 8-OH-DPAT (5 + 10 µg/kg). The cumulative doses are indicated on top of each arrow. (E) Pre-treatment with MDL 100 907 prevented the inhibitory effects of LSD on 5-HT firing frequency. White and black arrows indicate the injection of MDL 100 907 and VEH, respectively, before the cumulative dose of LSD. Each point of the line represents mean ± SEM expressed as percentage of firing rate after injection of VEH. (F) The single injection of MDL 100 907 does not change DRN 5-HT firing; bars represent mean ± SEM expressed as percentage of basal firing rate after injection of VEH. Two-way ANOVA followed by Bonferroni post hoc comparisons and *t*-test were used: $^{**}P < 0.01$, and $^{***}P < 0.001$ *vs.* VEH; ### $P < 0.001$ vs Halo or MDL 100 907 pretreated group.

Table 2
Burst-firing activity of DRN 5-HT neurons following cumulative low doses of LSD (5–30 µg/kg, i.v.) in rats pre-treated with vehicle (VEH), haloperidol (Halo, 50 µg/kg, i.v.) or MDL 100 907 (200 µg/kg, i.v.). Data (mean ± SEM) are reported as% of change *vs.* vehicle (VEH) injection (100%).

	Pre-treatment	LSD				ANOVA
		5 µg/kg	10 µg/kg	20 µg/kg	30 µg/kg	
Controls	**VEH**					
# of bursts (200 s)	100	25	0	0	0	*N = 1, not performed*
% spikes in bursts	100	45.0	0	0	0	*N = 1, not performed*
	Halo 50 µg/kg, i.v.)					
# of bursts (200 s)	112.4 ± 21.2	101.7 ± 18.3	88.46 ± 14.3	91.4 ± 8.1	78.2 ± 5.7	N = 3, $F(5,12) = 0.77$, $p = 0.58$
% spikes in bursts	75.4 ± 12.7	100.0 ± 16.02	78.6 ± 9.1	93.9 ± 6.1	91.5 ± 9.3	N = 3, $F(5,12) = 1.0$, $p = 0.41$
	MDL 100 907 (200 µg/kg, i.v.)					
# of bursts (200 s)	80	80	100	100	80	*N = 1, not performed*
% spikes in bursts	107.32	90.02	92.81	94.86	87.07	*N = 1, not performed*

One-way ANOVA for repeated measures plus Bonferroni post-hoc comparisons.

of bursts per 200 s: $F(5,18) = 35.0$, $P < 0.001$; % of spikes in bursts: $F(5,18) = 7.6$, $P < 0.001$)). An example of the histological control of a recording site in the VTA is shown in Fig. 3C.

3.4. 5-HT depletion does not affect the inhibitory influence of high doses of LSD on VTA DA activity

Guiard et al. [44] previously demonstrated that the selective lesion of DRN 5-HT neurons produced by 5,7-dihydroxytryptamine (5,7-DHT) enhanced the firing activity of VTA DA neurons by 36%, thereby indicating an inhibitory influence of the 5-HT input upon DA neurons. On the other hand, the selective lesion of DA neurons elicited by 6-hydroxydopamine (6-OHDA) decreased the spontaneous firing activity of DRN 5-HT neurons by 60%, thus revealing the excitatory effect of the DA input upon 5-HT neurons. Given this reciprocal interaction between DRN 5-HT and VTA DA neurons and the effects of LSD on both neurotransmissions (see above), we examined a possible involvement of 5-HT in the effects of high doses of LSD on VTA DA neurons. We thus performed a 5-HT depletion using PCPA (350 mg/kg, i.p., [34]) injected 48-h and 24-h before testing cumulative high doses of LSD (30–120 µg/kg LSD). Using this protocol [34], 5-HT depletion induced by PCPA at the level of the VTA is more than 89% [45]. Fig. 4A reports an example of an integrated histogram of the spontaneous firing rate of a VTA DA neuron in a rat pretreated with PCPA and injected with cumulative high doses of LSD. Cumulative high doses of LSD equally

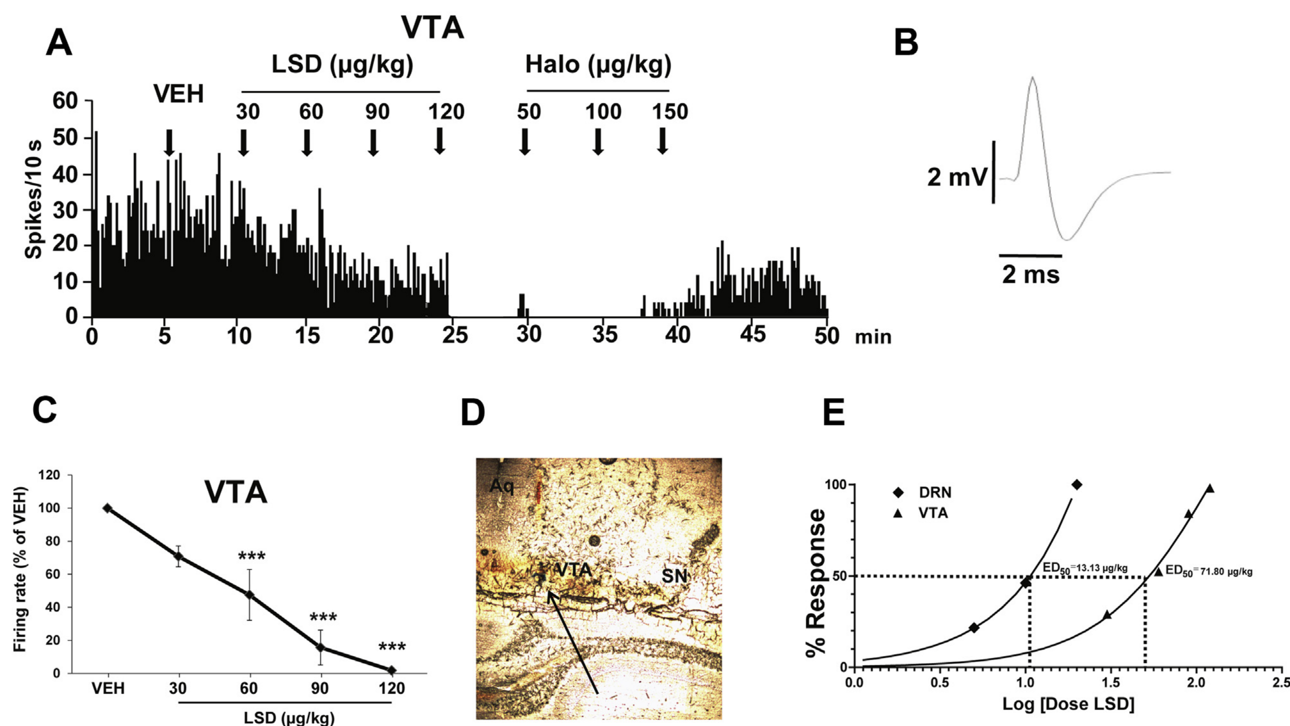

Fig. 3. Inhibitory effects of intravenous LSD administration on the firing rate of ventral tegmental area (VTA) dopamine (DA) neurons. (A) Representative integrated firing rate histograms showing the acute response of DA neurons to LSD and haloperidol (Halo). Arrows indicate sequential injections of increasing doses of LSD (30 + 30 + 30 + 30 μg/kg) and halo (50 + 50 + 50 μg/kg). (B) The figure shows the typical spike waveform of DA neurons. (C) High dose LSD administration induced a strong dose-dependent inhibition in the firing rate of VTA DA neurons. Each point of the line represents mean± SEM expressed as percentage of firing rate after injection of vehicle (VEH). One-way AOVA ***$P < 0.001$ *vs*. VEH. (D) Representative photomicrograph of the recording site in VTA: Aqueduct (Aq); SubstantiaNigra (SN). The black arrow indicates the site of the electrode recording labeled with pontamine sky blue dye. (E) LSD treatment on VTA DA neurons induced a shift to the right of the curve indicating the median effective dose (ED_{50}), as compared to DRN 5-HT neurons.

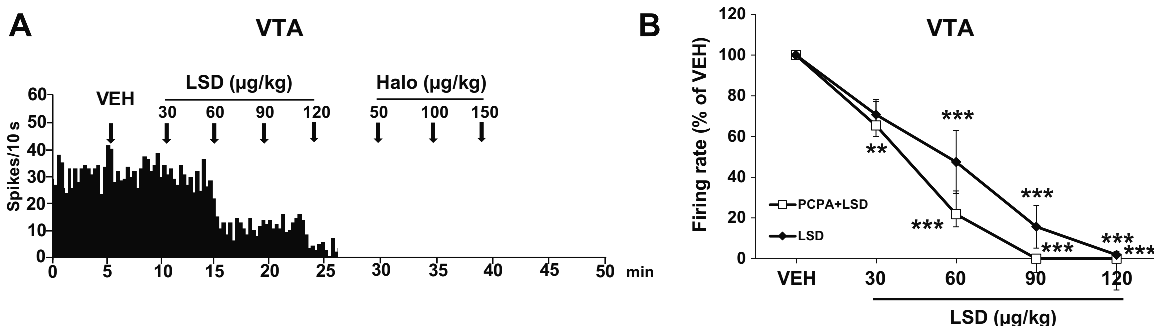

Fig. 4. Inhibitory effects of intravenous LSD administration on the firing rate and on the burst-firing activity of ventral tegmental area (VTA) dopamine (DA) neurons in PCPA-pretreated rats. (A) Representative integrated firing rate histograms showing the acute response of DA neurons to LSD. Arrows indicate sequential injections of increasing doses of LSD (30 + 30 + 30+ 30 μg/kg) and haloperidol (Halo) (50 + 50 + 50 μg/kg). (B) LSD administration induced a strong dose-dependent inhibition in the firing rate of VTA DA neurons in PCPA-pretreated rats. Each point of the line represents mean ± SEM expressed as percentage of firing rate after injection of vehicle (VEH). Two-way ANOVA followed by Bonferroni post hoc comparisons: ***$P < 0.001$ and **$P < 0.01$ *vs*. VEH.

decreased VTA DA neural activity in both PCPA pretreated and non-pretreated rats (no interaction ($F(4,31) = 1.08$, $P = 0.38$), no PCPA pre-treatment ($F(1,8) = 1.051$, $P = 0.335$), and an effect of LSD treatment ($F(4,31) = 54.32$, $P < 0.001$)). In particular, compared to VEH, we observed a decrease of VTA DA firing activity following 60, 90 and 120 μg/kg LSD (Fig. 4B, $P < 0.001$). No effect of 30 μg/kg LSD was found ($P = 0.20$). ED50 value for the PCPA-LSD dose-response curve was 59.24 μg/kg, and was not different than the ED50 of the dose-response curve of LSD in non-PCPA pre-treated animals (71.80 μg/kg). VTA DA burst-firing activity was decreased by cumulative high doses of LSD also in 5-HT depleted animals (Table 1; number bursts per 200 s: $F(3,7) = 45.2$, $P < 0.001$; % of spikes in bursts: $F(3,7) = 19.6$, $P < 0.001$). While LSD(30 μg/kg) did not significantly affect VTA DA firing rate in both 5-HT depleted and control animals, it decreased the # of bursts (200 s) ($P < 0.001$) in 5-HT depleted rats but not in controls, suggesting that 5-HT is very likely involved in the effects of LSD upon VTA DA burst-firing activity. Of note, unlike in the normal condition (Fig. 3A), the injection of Halo (50–150 μg/kg) after cumulative high doses of LSD in PCPA pre-treated animals did not restore VTA DA firing (Fig. 4A), suggesting that Halo may need an intact 5-HT system for its action on DA firing activity.

3.5. The D_2 antagonist haloperidol prevents the inhibitory effects of high doses of LSD on VTA DA firing activity

Fig. 5A reports the integrated histogram of spontaneous firing rate of a VTA DA neuron following the injection of Halo (50 μg/kg,

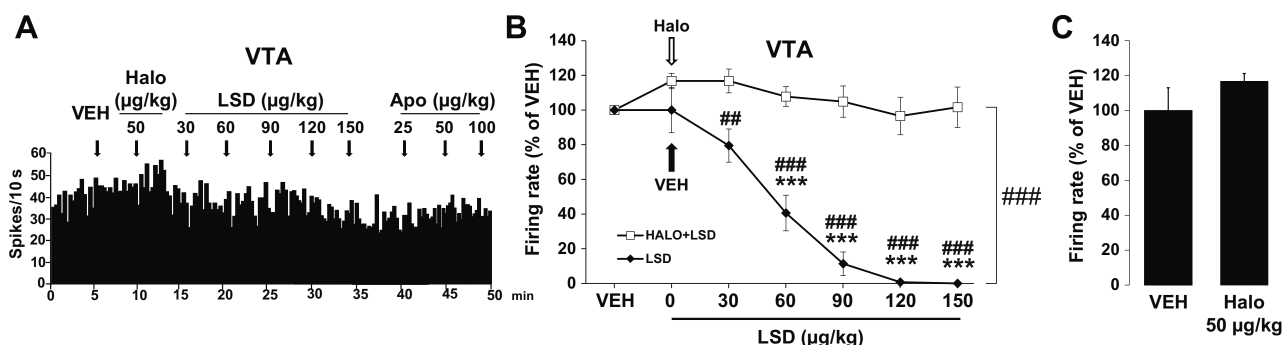

Fig. 5. Haloperidol (Halo) prevents the inhibitory effects of LSD on VTA DA firing activity. (A) Representative integrated firing rate histograms showing the acute response of DA neurons to Halo, LSD and apomorphine (Apo). Arrows indicate sequence of single injections of Halo (50 μg/kg) and of increasing doses of LSD (30 + 30 + 30 + 30 + 30 μg/kg) and Apo (25 + 25 + 25 μg/kg). The cumulative doses are indicated on top of each arrow. (B) Pre-treatment with Halo prevented the inhibitory effects of LSD on DA cell firing frequency. White and black arrows indicate the injection of halo and vehicle (VEH), respectively, before the cumulative dose of LSD. Each point of the line represents mean ± SEM expressed as percentage of firing rate after injection of VEH. (C) The single injection of Halo slightly increased the VTA DA firing; bars represent mean ±SEM expressed as percentage of basal firing rate after injection of VEH. Two-way ANOVA followed by Bonferroni post hoc comparisons and *t*-test were used: ***$P<0.001$, and ** $P<0.01$ *vs*. VEH; ### $P<0.001$ and ## $P<0.01$ vs Halo pretreated group.

i.v.) before that of cumulative high doses of LSD. Since no effects were observed until the injection of 120 μg/kg LSD, we increased the dose up to 150 μg/kg LSD. After the injection of 150 μg/kg LSD (which did not change firing activity), we tested the inhibitory effects of cumulative doses of Apo (25–100 μg/kg, i.v.) on VTA DA neurons [32], and we found that VTA DA neurons did not respond to Apo, thus confirming that haloperidol blocked LSD effects via DA D_2 receptors (Fig. 5A). As shown in Fig. 4B, Halo pre-treatment prevented the inhibitory effects of cumulative high doses of LSD on DA cell firing frequency (interaction: $F(6,46)=13.93$, $P<0.001$; Halo pre-treatment: $F(1,9)=54.03$, $P<0.001$; LSD treatment: $F(6,6)=13.93$, $P<0.001$). Bonferroni post-hoc comparisons revealed a different effect of LSD on Halo pre-treated and non pre-treated neurons ($P<0.001$). Indeed, while we found a decrease of VTA DA firing activity with 60, 90, 120 and 150 μg/kg LSD compared to vehicle in non pre-treated neurons ($P<0.001$), no effects of high doses of LSD were present in Halo pre-treated neurons (Fig. 5B). Interestingly, while 50 μg/kg Halo, prior LSD, induced only a slight but not significant increase of VTA DA firing activity(Fig. 5C), it did significantly increase the number of bursts per 200 s compared to vehicle (Table 1; $P=0.01$). On the other hand, Halo pretreatment prevented the ability of high doses of LSD to modify VTA DA burst-firing activity ((Table 1; number of bursts per 200 s: $F(6,24)=3.2$, $P=0.018$); % of spikes in bursts: $F(6,24)=0.4$, $P=0.830$).

3.6. *The 5-HT_{1A} receptor antagonist WAY-100,635 prevents the inhibitory effects of high doses of LSD on VTA DA firing activity*

Fig. 6A reports the integrated histogram of the spontaneous firing rate of a VTA DA neuron following the injection of WAY-100,635 (500 μg/kg, i.v.) prior to cumulative high doses of LSD (30–150 μg/kg LSD). VTA DA firing activity was not altered by LSD in WAY-100,635-treated rats. Similar to the previous experiment with Halo pre-treatment, after the injection of 150 μg/kg LSD, we tested the inhibitory effects of cumulative doses of Apo (25–100 μg/kg, i.v.). Unlike Halo pre-treatment, we found that Apo silenced VTA DA neurons. This finding suggests that the blockade of 5-HT_{1A} receptors prevents the inhibitory effects of high doses of LSD on VTA DA neurons independently of D_2 receptors. WAY-100,635 (500 μg/kg) alone did not affect DA neural activity (Fig. 6C). As shown in Fig. 6B, cumulative high doses of LSD did not affect VTA DA neural activity in neurons pre-treated with WAY-100,635. On the contrary, compared to vehicle, it significantly reduced DA firing at the doses of 30 ($P=0.011$) and 60–150 ($P<0.001$) μg/kg in non pre-treated neurons (interaction: $F(5,40)=15.98$, $P<0.001$; WAY-100,635 pre-treatment: $F(1,8)=58.23$, $P<0.001$; LSD treatment: $F(5,40)=25.05$, $P<0.001$). No variation after high doses of LSD was observed on VTA burst-firing activity after pre-treatment with WAY-100,635 (Table 1; number of bursts per 200 s: $F(6,24)=1.9$, $P=0.116$; % of spikes in bursts:$F(6,24)=1.7$, $P=0.162$).

3.7. *The $TAAR_1$ antagonist EPPTB prevents the inhibitory effects of LSD on VTA DA firing activity*

Fig. 7A reports the integrated histogram of the spontaneous firing rate of a VTA DA neuron following the injection of EPPTB (5 mg/kg, i.v.) prior to cumulative high doses administration of LSD. LSD did not affect VTA DA firing activity in the presence of EPPTB. The subsequent injection of cumulative doses of Apo did not alter DA neural activity suggesting that $TAAR_1$ antagonism also involves D_2 receptors. Interestingly, the single injection of 5 mg/kg EPPTB induced a significant increase of VTA DA firing activity ($P=0.002$, n = 7, Fig. 7C). The inhibitory effects of cumulative high doses of LSD were completely prevented by EPPTB pre-treatment (Fig. 7B; interaction: $F(6,53)=8.39$, $P<0.001$; EPPTB pretreatment: $F(1,10)=15.26$, $P=0.003$;LSD treatment: $F(6,53)=7.31$, $P<0.001$). Bonferroni post-hoc analysis revealed that compared to vehicle, none of the doses of LSD significantly altered firing activity of VTA DA neurons pretreated with EPPTB, whereas at the doses of 60–150 μg/kg it reduced VTA DA neural activity in non pre-treated neurons ($P<0.001$). Although there was a trend towards an increase of the number of bursts per 200 s induced by EPPTB 5 mg/kg, overall VTA DA burst-firing activity resulted unchanged when cumulative high doses of LSD were injected after EPPTB pre-treatment (Table 1; number of spikes in bursts per 200 s: $F(6,30)=2.1$, $p=0.086$; % of spikes in bursts: $F(6,30)=0.9$, $P=0.534$).

4. Discussion

In this study, we have demonstrated that the hallucinogenic drug LSD strongly influences dopaminergic neural activity of the VTA. In particular, at the doses inhibiting DRN 5-HT firing activity, LSD does not affect VTA DA neurons, but at higher doses, decreases VTA DA neural activity. Using selective antagonists, we have also demonstrated that the decrease in VTA DA neural activity induced by high doses of LSD occurs through a complex and multi-receptorial mechanism including activation of D_2, 5-HT_{1A}, and $TAAR_1$ receptors. Noteworthy, this is the first *in-vivo* evidence highlighting the involvement of $TAAR_1$ receptors in the action of LSD. In addition, we highlight that low doses of LSD also act on

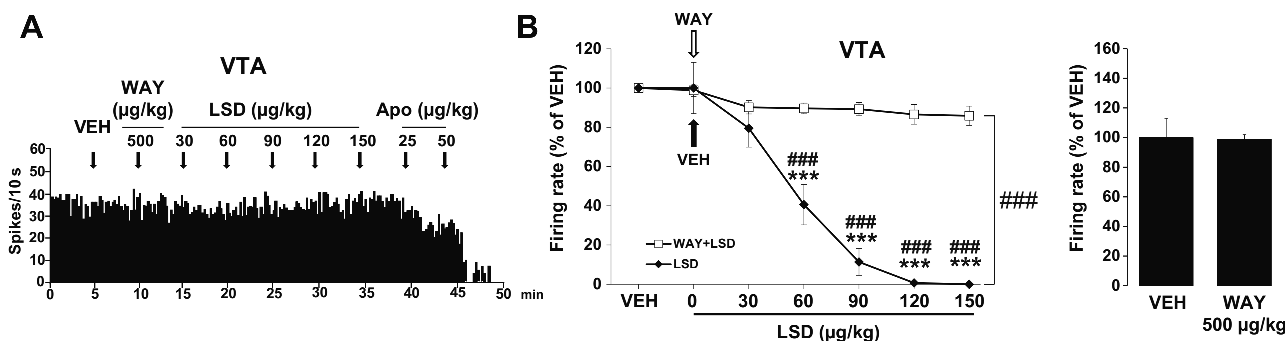

Fig. 6. WAY-100,635 (WAY) prevents the inhibitory effects of LSD on VTA DA firing activity. (A) Representative integrated firing rate histograms showing the acute response of DA neurons to WAY, LSD and apomorphine (Apo). Arrows indicate sequence of single injections of WAY (500 µg/kg) and of increasing doses of LSD (30 + 30 + 30 + 30 + 30 µg/kg) and apo (25 + 25 µg/kg). (B) Pre-treatment with WAY prevented the inhibitory effects of LSD upon DA cell firing frequency. White and black arrows indicate the injection of WAY and vehicle (VEH), respectively, before that of the cumulative doses of LSD. Each point of the line represents mean ± SEM expressed as percentage of firing rate after injection of VEH. (C) The single injection of WAY does not affect VTA DA firing; bars represent mean ± SEM expressed as percentage of basal firing rate after injection of VEH. Two-way ANOVA followed by Bonferroni post hoc comparisons and *t*-test were used: $^{***}P < 0.001$ *vs.* VEH; ### $P < 0.001$vs WAY pretreated group.

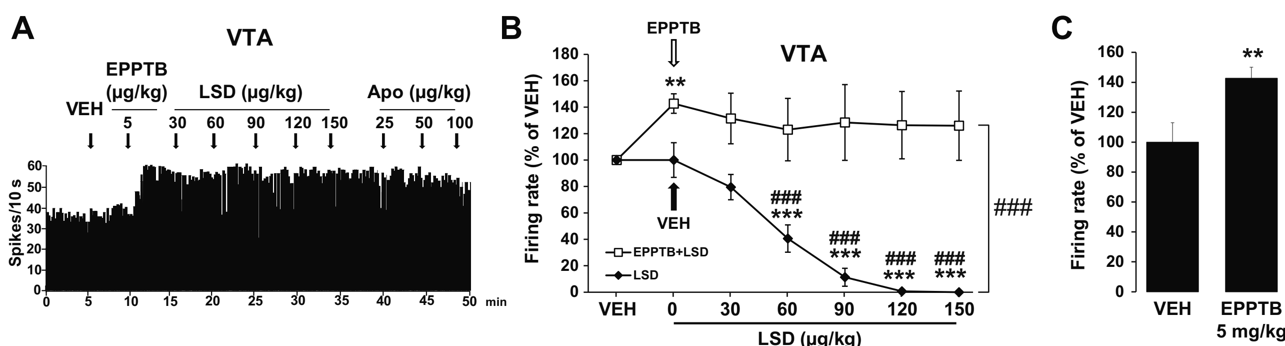

Fig. 7. EPPTB prevents the inhibitory effects of LSD on VTA DA firing activity. (A) Representative integrated firing rate histograms showing the acute response of DA neurons to EPPTB, LSD and apomorphine (Apo). Arrows indicate sequence of single injections of EPPTB (5 mg/kg) and of increasing doses of LSD (30 + 30 + 30 + 30 + 30 µg/kg) and Apo (25 + 25 + 50), the cumulative doses are indicated on top of each arrow. (B) Pre-treatment with EPPTB prevented the inhibitory effects of LSD on DA cell firing frequency. White and black arrows indicate the injection of EPPTB and vehicle (VEH), respectively, before that of cumulative doses of LSD. Each point of the line represents mean ± SEM expressed as percentage of firing rate after injection of VEH. (C) The single injection of EPPTB significantly increased the firing rate of VTA DA neurons; bars represent mean ± SEM expressed as percentage of basal firing rate after injection of VEH. Two-way ANOVA followed by Bonferroni post hoc comparisons and *t*-test were used: $^{***}P < 0.001$ and $^{**}P < 0.01$ *vs.* VEH; ### $P < 0.001$, ## $P < 0.01$ and # $P < 0.05$ vs EPPTB pretreated group.

DRN 5-HT neurons with a multi-receptorial mechanism including activation of 5-HT_{2A} and D_2 receptors. As previously reported, LSD acts on the serotonergic system by decreasing the firing rate of 5-HT neurons in the DRN [37]. In agreement, we found that LSD at low doses (5–20 µg/kg) ceased DRN 5-HT firing activity, but was ineffective at modulating DA neurons in VTA at this dose. Besides the well-known 5-HT_{1A} receptor-mediated effects of LSD [10], in keeping with previous studies describing an involvement of 5-HT_{2A} receptors on the effects produced by LSD [46] and in the inhibition of 5-HT neurons [36,47], we found that the decrease of 5-HT firing activity following LSD administration was blocked by the 5-HT_{2A} antagonist MDL 100 907.

Considering the ineffectiveness of low doses of LSD on DA neurons, we performed electrophysiological recordings of VTA DA neurons with higher doses of LSD (30–120 µg/kg), revealing that cumulative injections of high doses of LSD significantly decreased VTA DA neural activity. Intriguingly, the experiments show that the D_2 antagonist haloperidol prevented the decrease in VTA DA firing rate following cumulative injections of high doses of LSD, and also reinstated the basal DA firing activity when injected after the drug.

In addition, haloperidol also blocked the inhibitory effect of LSD on DRN 5-HT neurons, an effect likely due to the presence of D_2 receptors within the DRN [48].

Collectively, in keeping with previous studies [49–51], these experiments may suggest that D_2 receptor is involved in the mechanism of action of LSD. In particular, in vitro experiments demonstrated that LSD has a k_i of 2 nM for the human cloned dopamine D_2 receptor [50] and that it inhibited prolactin secretion by the pituitary cells in a concentration-dependent manner [48], and this effect was antagonized by the D_2 selective antagonist spiperone, but not by the D_1 selective antagonist SKF83566.

However, behavioral pharmacology studies are warranted to better understand the possible D_2 receptor- mediated mechanism of LSD.

Our results are partially in keeping with a previous study by White and Wang [52] who found that cumulative doses of LSD (1–500 µg/kg) decreased A10 VTA DA cell firing activity in 54% of the tested cells, and increased or had no effect on the remaining neurons. This discrepancy in percentage is likely due to the fact that today, compared to 30 years ago, we use computerized systems and software allowing us to recognize with high precision neuronal length, shape, duration, amplitude and bursts of DA neuron signals vs non-DA neurons.

Given the established role of 5-HT_{1A} receptors on the mechanism of action of LSD on 5-HT firing activity, we tested whether pre-treatment with the5-HT_{1A} antagonist WAY 100,635 could affect the activity of high doses of LSD on VTA DA neurons. We found that blockade of 5-HT_{1A} receptors prevented the inhibitory effects of high doses of LSD. Of note, this is the first *in-vivo* electrophysiological demonstration of the influence of 5-HT_{1A} receptors on the effect of LSD on VTA DA neuronal activity. Given the presence of 5-HT_{1A} receptors in the VTA [53], one can hypothesize that

LSD acts directly on 5-HT_{1A} receptors within VTA without any 5-HT mediated effect; indeed, 5-HT depletion with PCPA did not affect LSD responses on the firing activity of VTA DA neurons. On the other hand, we found that the depletion of 5-HT with PCPA sensitized the LSD-induced burst response, since a low dose of LSD (30 μg/kg) significantly reduced the number of bursts in 5-HT depleted animals compared to controls, shifting the curve to the left. Intriguingly, Halo did not reverse the effects of LSD in 5-HT depleted animals. Altogether, this data suggested that the lack of 5-HT enhances vulnerability to the hallucinogenic effects of LSD and make these animals resistant to the neuroleptic Halo, thus suggesting that 5-HT depletion can be a factor that influences neuroleptic-resistant psychosis [54]. This is in agreement with a previous work in which it has been demonstrated that the cataleptic effect of haloperidol is reduced after lesions of midbrain raphe serotonergic neurons or depletion of serotonin stores by PCPA [55]. On the other hand, Balsara et al. [56] have demonstrated that Quipazine, a 5-HT agonist, and clomipramine, a selective 5 HT neuronal uptake blocker, potentiated the cataleptic effect of Halo. However, more studies are needed to understand the neurobiological basis of neuroleptic-resistant psychosis and the role-played by 5-HT dysfunction. The dose-dependent electrophysiological effects of LSD on 5-HT and DA neurotransmission are in keeping with previous work [57] showing that the discriminative stimulus effects of LSD in rats occur in two temporal phases, with initial activation of 5-HT_{2A} receptors and a mediation of D_2 receptors in a second phase. Importantly the biphasic effects of LSD have also been reported in humans; while a “psychedelic experience” with “meaningfulness and portentousness” is experienced in the early phase, a later phase is described as “a clearly paranoiac state, with ideas of references and paranoia”[58]. Consequently, in light of these results one can hypothesize that LSD produces a mood elevation and creativity mediated by its initial serotonergic effect at low doses followed by a psychotic-like status at more elevated doses. Importantly, these psychotic-like symptoms are treated in emergency settings with the D_2 receptor antagonist haloperidol or chlorpromazine [59], further supporting the involvement of D_2 receptors in the effects of high doses of LSD. For the first time, the activity of a $TAAR_1$ receptor antagonist was explored *in-vivo,* and we found that the $TAAR_1$ receptor is involved in the effect of LSD on DA neurons. Discovered in 2001, TAAR1 is an aminergic G protein-coupled receptor [20,60,61] that is an important modulator of the dopaminergic and serotoninergic system and potentially of the glutamatergic system [62,63]. It binds the so-called trace-amines, a subgroup of biogenic amines, such as beta-phenylethylamine, *p*-tyramine or tryptamine [64] and also endogenous hallucinogens [65] and exogenous hallucinogens such as LSD [20]. Recent studies found that it could be linked to psychosis [66,67]. $TAAR_1$ knockout mice, don’t have a specific phenotype, but compared to wildtype controls, they have an increased firing activity of VTA DA neurons [68], and are more sensitive to a challenge of amphetamine, releasing more DA and 5-HT [68,69]. In addition, mRNA and protein levels of DA D_2 receptors are over-expressed in the striatum of $TAAR_1$ knockout mice [21]. Importantly, the $TAAR_1$ agonist RO 5256390 and more potently the $TAAR_1$ receptor partial agonist RO 5263397 can mediate cocaine-induced hyperlocomotion and improve performance on an object retrieval task, but only the partial agonist is effective at decreasing immobility in the forced swim test and increasing wakefulness [66]. Furthermore, using a visual whole-cell current clamp technique in brain slices, the $TAAR_1$ partial agonist RO5263397, but not the agonist RO5256390, augmented the firing frequency of DA VTA neurons[66], similar to other classes of antipsychotics [70]. The full agonist RO5166017 inhibits the firing frequency of DA neurons in vitro [61].

In our experiment, we pre-treated rats with the $TAAR_1$ antagonist EPPTB prior to the injection of LSD. EPPTB significantly increased the firing rate of VTA DA neurons, and blocked the inhibitory effects of LSD, suggesting that the effects of LSD over the DA system are also mediated by $TAAR_1$ receptor and further confirm the ability of $TAAR_1$ antagonism to counteract the inhibitory effects of hallucinogenic drugs on DA firing activity [71]. As a consequence, a potential role for $TAAR_1$ antagonists in the management of psychotic-like effects of LSD deserves to be investigated [18].However, more studies are needed to understand the differential pharmacological role of $TAAR_1$ antagonists and partial agonists in the treatment of pharmacological and non-pharmacological-induced psychosis.

The complex mechanism underlying the modulation of VTA DA activity by LSD was also confirmed by Apo. Pre-treatment with WAY 100,635 prevented the responsiveness of VTA DA neurons to the decreasing effect of LSD but not to those of Apo (Fig. 5A), which occur through D_2 receptors [72]. On the contrary, pre-treatment with EPPTB completely blocked not only the inhibiting effects of LSD but also those of Apo (Fig. 6A), suggesting a close relationship between $TAAR_1$ and D_2 receptors. In agreement with this finding, Sukhanov et al. [73] recently reported an involvement of $TAAR_1$ receptors in Apo-induced climbing in the forced swim test in mice. Further studies are needed to determine the exact mechanism underlying the interaction between $TAAR_1$ and D_2 receptors.

5. Conclusion

In conclusion, our results show that LSD acts with a pleiotropic mechanism of action and at low doses affects the 5-HT system interacting also with 5-HT_{2A} and D_2 receptors, while at higher doses it affects the DA system via 5-$HT_{1A,}$ D_2 and $TAAR_1$ receptors. Interestingly, this biphasic dose-dependent mechanism of action is paralleled by a similar biphasic psychotropic effect in humans. A combination of 5-$HT_{1A,}$ D_2 and $TAAR_1$ receptor antagonism could thus represent a novel avenue for drug-induced psychosis.

Authors’ contribution

DDG: performed electrophysiological experiments on DA neurons, analyzed data and wrote the manuscript; LP: assisted in DA electrophysiological experiments; ROS and RJM: performed electrophysiological experiments on 5-HT neurons, SM: read manuscript and gave feed-backs, SC: Analyzed data, wrote the manuscript, GG: conceived the hypothesis and experiments, supervised experiments, interpreted results and wrote the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgements

GG received a salary award and RJM a post-doc fellowship from the Fonds de la recherché du Quebec en Santé (FRQS), DDG received a PhD fellowship from the Ministero dell’Istruzione, dell’Università e della Ricerca (MIUR, Italy), ROS received a PhD fellowship from Consejo Nacional de Ciencia y Tecnología (CONACYT, Mexico).

References

[1] J.A. Hoffman, L.S.D. flashbacks, Arch. Gen. Psychiatry 41 (6) (1984) 631–632.
[2] W.N. Pahnke, W.A. Richards, Implications of LSD and experimental mysticism, J. Relig. Health 5 (3) (1966) 175–208.
[3] C. Savage, Lysergic acid diethylamide; a clinical-psychological study, Am. J. Psychiatry 108 (12) (1952) 896–900.
[4] H. Matthew, Lysergic acid diethylamide intoxication, Br. Med. J. 1 (5588) (1968) 380.

[5] T. Passie, J.H. Halpern, D.O. Stichtenoth, H.M. Emrich, A. Hintzen, The pharmacology of lysergic acid diethylamide: a review, CNS Neurosci. Ther. 4 (4) (2008) 295–314.
[6] E. Sanders-Bush, K.D. Burris, K. Knoth, Lysergic acid diethylamide and 2,5-dimethoxy-4-methylamphetamine are partial agonists at serotonin receptors linked to phosphoinositide hydrolysis, J. Pharmacol. Exp. Ther. 246 (3) (1988) 924–928.
[7] C.D. Nichols, E. Sanders-Bush, Molecular genetic responses to lysergic acid diethylamide include transcriptional activation of MAP kinase phosphatase-1, C/EBP-β and ILAD-1, a novel gene with homology to arrestins, J. Neurochem. 90 (3) (2004) 576–584.
[8] A.L. Halberstadt, M.A. Geyer, Effects of the hallucinogen 2,5-dimethoxy-4-iodophenethylamine (2C-I) and superpotent N-benzyl derivatives on the head twitch response, Neuropharmacology 77 (2014) 200–207.
[9] A.B. Norman, G. Battaglia, A.L. Morrow, I. Creese, [3H]WB4101 labels S1 serotonin receptors in rat cerebral cortex, Eur. J. Pharmacol. 106 (2) (1984) 461–462.
[10] C. Reissig, J. Eckler, R. Rabin, J. Winter, The 5-HT1A receptor and the stimulus effects of LSD in the rat, Psychopharmacology (Berl.) 182 (2) (2005) 197–204.
[11] C.T. Egan, K. Herrick-Davis, K. Miller, R.A. Glennon, M. Teitler, Agonist activity of LSD and lisuride at cloned 5HT2A and 5HT2C receptors, Psychopharmacology (Berl.) 136 (4) (1998) 409–414.
[12] M.E. Trulson, B.L. Jacobs, Raphe unit activity in freely moving cats: correlation with level of behavioral arousal, Brain Res. 163 (1) (1979) 135–150.
[13] G.J. Marek, G.K. Aghajanian, LSD and the phenethylamine hallucinogen DOI are potent partial agonists at 5-HT2A receptors on interneurons in rat piriform cortex, J. Pharmacol. Exp. Ther. 278 (3) (1996) 1373–1382.
[14] D.R. Burt, S.J. Enna, I. Creese, S.H. Snyder, Dopamine receptor binding in the corpus striatum of mammalian brain, Proc. Natl. Acad. Sci. U. S. A. 72 (11) (1975) 4655–4659.
[15] L. Minuzzi, G.G. Nomikos, M.R. Wade, S.B. Jensen, A.K. Olsen, P. Cumming, Interaction between LSD and dopamine D2/3 binding sites in pig brain, Synapse (New York, N.Y.) 56 (4) (2005) 198–204.
[16] P. Seeman, F. Ko, T. Tallerico, Dopamine receptor contribution to the action of PCP, LSD and ketamine psychotomimetics, Mol. Psychiatry 10 (9) (2005) 877–883.
[17] P. Seeman, H.C. Guan, H. Hirbec, Dopamine D2High receptors stimulated by phencyclidines, lysergic acid diethylamide salvinorin A, and modafinil, Synapse (New York, N.Y.) 63 (8) (2009) 698–704.
[18] G.K. Murray, P.R. Corlett, L. Clark, M. Pessiglione, A.D. Blackwell, G. Honey, P.B. Jones, E.T. Bullmore, T.W. Robbins, P.C. Fletcher, Substantia nigra/ventral tegmental reward prediction error disruption in psychosis, Mol. Psychiatry 13 (3) (2008) 267–276, 239.
[19] A.A. Grace, B.S. Bunney, H. Moore, C.L. Todd, Dopamine-cell depolarization block as a model for the therapeutic actions of antipsychotic drugs, Trends Neurosci. 20 (1) (1997) 31–37.
[20] J.R. Bunzow, M.S. Sonders, S. Arttamangkul, L.M. Harrison, G. Zhang, D.I. Quigley, T. Darland, K.L. Suchland, S. Pasumamula, J.L. Kennedy, Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor, Mol. Pharmacol. 60 (6) (2001) 1181–1188.
[21] S. Espinoza, V. Ghisi, M. Emanuele, D. Leo, I. Sukhanov, T.D. Sotnikova, E. Chieregatti, R.R. Gainetdinov, Postsynaptic D2 dopamine receptor supersensitivity in the striatum of mice lacking TAAR1, Neuropharmacology 93 (2015) 308–313.
[22] G. Aghajanian, LSD and CNS transmission, Annu. Rev. Pharmacol. 2 (1) (1972) 157–168.
[23] J. Baraban, G. Aghajanian, Suppression of firing activity of 5-HT neurons in the dorsal raphe by alpha-adrenoceptor antagonists, Neuropharmacology 19 (4) (1980) 355–363.
[24] F.R. Bambico, N. Katz, G. Debonnel, G. Gobbi, Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex, J. Neurosci. 27 (43) (2007) 11700–11711.
[25] K.A. Allers, T. Sharp, Neurochemical and anatomical identification of fast- and slow-firing neurones in the rat dorsal raphe nucleus using juxtacellular labelling methods in vivo, Neuroscience 122 (1) (2003) 193–204.
[26] C.P. Vandermaelen, G.K. Aghajanian, Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices, Brain Res. 289 (1–2) (1983) 109–119.
[27] B. Labonte, F.R. Bambico, G. Gobbi, Potentiation of excitatory serotonergic responses by MK-801 in the medial prefrontal cortex, Naunyn. Schmiedebergs Arch. Pharmacol. 380 (5) (2009) 383–397.
[28] N. Urbain, K. Creamer, G. Debonnel, Electrophysiological diversity of the dorsal raphe cells across the sleep–wake cycle of the rat, J. Physiol. 573 (3) (2006) 679–695.
[29] G. Gobbi, A.L. Muntoni, G.L. Gessa, M. Diana, Clonidine fails to modify dopaminergic neuronal activity during morphine withdrawal, Psychopharmacology (Berl.) 158 (1) (2001) 1–6.
[30] A.A. Grace, B.S. Bunney, Intracellular and extracellular electrophysiology of nigral dopaminergic neurons–1 Identification and characterization, Neuroscience 10 (2) (1983) 301–315.
[31] M.A. Ungless, A.A. Grace, Are you or aren't you? Challenges associated with physiologically identifying dopamine neurons, Trends Neurosci. 35 (7) (2012) 422–430.
[32] S. Dominguez-Lopez, R.D. Howell, M.G. Lopez-Canul, M. Leyton, G. Gobbi, Electrophysiological characterization of dopamine neuronal activity in the ventral tegmental area across the light-dark cycle, Synapse 68 (10) (2014) 454–467.
[33] B. Labonte, F.R. Bambico, G. Gobbi, Potentiation of excitatory serotonergic responses by MK-801 in the medial prefrontal cortex, Naunyn. Schmiedebergs Arch. Pharmacol. 380 (5) (2009) 383–397.
[34] R. Ghanbari, M. El Mansari, M. Shahid, P. Blier, Electrophysiological characterization of the effects of asenapine at 5-HT 1A, 5-HT 2A α 2-adrenergic and D 2 receptors in the rat brain, Eur. Neuropsychopharmacol. 19 (3) (2009) 177–187.
[35] P. Celada, M.V. Puig, R. Martín-Ruiz, J.M. Casanovas, F. Artigas, Control of the serotonergic system by the medial prefrontal cortex: potential role in the etiology of PTSD and depressive disorders, Neurotox. Res. 4 (5–6) (2002) 409–419.
[36] L. Boothman, K. Allers, K. Rasmussen, T. Sharp, Evidence that central 5-HT2A and 5-HT2B/C receptors regulate 5-HT cell firing in the dorsal raphe nucleus of the anaesthetised rat, Br. J. Pharmacol. 139 (5) (2003) 998–1004.
[37] N. Haddjeri, C. Ortemann, C. de Montigny, P. Blier, Effect of sustained administration of the 5-HT1A receptor agonist flesinoxan on rat 5-HT neurotransmission, Eur. Neuropsychopharmacol. 9 (5) (1999) 427–440.
[38] H. Stalder, M.C. Hoener, R.D. Norcross, Selective antagonists of mouse trace amine-associated receptor 1 (mTAAR1): discovery of EPPTB (RO5212773), Bioorg. Med. Chem. Lett. 21 (4) (2011) 1227–1231.
[39] A. Ford, A. Castonguay, M. Cottet, J.W. Little, Z. Chen, A.M. Symons-Liguori, T. Doyle, T.M. Egan, T.W. Vanderah, Y. De Konnick, Engagement of the GABA to KCC2 signaling pathway contributes to the analgesic effects of A3AR agonists in neuropathic pain, J. Neurosci. 35 (15) (2015) 6057–6067.
[40] P. Blier, C. de Montigny, Modification of 5-HT neuron properties by sustained administration of the 5-HT1A agonist gepirone: electrophysiological studies in the rat brain, Synapse (New York, N.Y.) 1 (5) (1987) 470–480.
[41] S.E. Gartside, E. Hajos-Korcsok, E. Bagdy, L.G. Harsing Jr., T. Sharp, M. Hajos, Neurochemical and electrophysiological studies on the functional significance of burst firing in serotonergic neurons, Neuroscience 98 (2) (2000) 295–300.
[42] G. Gobbi, F. Bambico, R. Mangieri, M. Bortolato, P. Campolongo, M. Solinas, T. Cassano, M. Morgese, G. Debonnel, A. Duranti, Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis, Proc. Natl. Acad. Sci. U. S. A. 102 (51) (2005) 18620–18625.
[43] F.R. Bambico, P.R. Hattan, J.-P. Garant, G. Gobbi, Effect of delta-9-tetrahydrocannabinol on behavioral despair and on pre-and postsynaptic serotonergic transmission, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 38 (1) (2012) 88–96.
[44] B.P. Guiard, M. El Mansari, Z. Merali, P. Blier, Functional interactions between dopamine, serotonin and norepinephrine neurons: an in-vivo electrophysiological study in rats with monoaminergic lesions, Int. J. Neuropsychopharmacol. 11 (5) (2008) 625–639.
[45] N.H. Chen, M.E. Reith, Monoamine interactions measured by microdialysis in the ventral tegmental area of rats treated systemically with (±)-8-hydroxy-2-(Di-n-propylamino) tetralin, J. Neurochem. 64 (4) (1995) 1585–1597.
[46] K. Rasmussen, G.K. Aghajanian, Effect of hallucinogens on spontaneous and sensory-evoked locus coeruleus unit activity in the rat: reversal by selective 5-HT 2 antagonists, Brain Res. 385 (2) (1986) 395–400.
[47] G. Quesseveur, C. Repérant, D. David, A. Gardier, C. Sanchez, B. Guiard, 5-HT2A receptor inactivation potentiates the acute antidepressant-like activity of escitalopram: involvement of the noradrenergic system, Exp. Brain Res. 226 (2) (2013) 285–295.
[48] D.M. Weiner, A.I. Levey, R.K. Sunahara, H.B. Niznik, B.F. O'Dowd, P. Seeman, M. Brann, D1 and D2 dopamine receptor mRNA in rat brain, Proc. Natl. Acad. Sci. U. S. A. 88 (5) (1991) 1859–1863.
[49] S. Giacomelli, M. Palmery, L. Romanelli, C.Y. Cheng, B. Silvestrini, Lysergic acid diethylamide (LSD) is a partial agonist of D 2 dopaminergic receptors and it potentiates dopamine-mediated prolactin secretion in lactotrophs in vitro, Life Sci. 63 (3) (1998) 215–222.
[50] P. Seeman, F. Ko, T. Tallerico, Dopamine receptor contribution to the action of PCP, LSD and ketamine psychotomimetics, Mol. Psychiatry 10 (9) (2005) 877–883.
[51] D. Marona-Lewicka, D.E. Nichols, Further evidence that the delayed temporal dopaminergic effects of LSD are mediated by a mechanism different than the first temporal phase of action, Pharmacol. Biochem. Behav. 87 (4) (2007) 453–461.
[52] F.J. White, R.Y. Wang, Comparison of the effects of LSD and lisuride on A10 dopamine neurons in the rat, Neuropharmacology 22 (6) (1983) 669–676.
[53] M.D. Doherty, V.M. Pickel, Targeting of serotonin 1A receptors to dopaminergic neurons within the parabrachial subdivision of the ventral tegmental area in rat brain, J. Comp. Neurol. 433 (3) (2001) 390–400.
[54] H.Y. Meltzer, The role of serotonin in antipsychotic drug action, Neuropsychopharmacology 21 (1999) 106S–115S.
[55] W. Kostowski, W. Gumułka, A. Członkowski, Reduced cataleptogenic effects of some neuroleptics in rats with lesioned midbrain raphe and treated with p-chlorophenylalanine, Brain Res. 48 (1972) 443–446.

[56] J. Balsara, J. Jadhav, A. Chandorkar, Effect of drugs influencing central serotonergic mechanisms on haloperidol-induced catalepsy, Psychopharmacology (Berl.) 62 (1) (1979) 67–69.

[57] D. Marona-Lewicka, R.A. Thisted, D.E. Nichols, Distinct temporal phases in the behavioral pharmacology of LSD: dopamine D2 receptor-mediated effects in the rat and implications for psychosis, Psychopharmacology (Berl.) 80 (3) (2005) 427–435.

[58] D.X. Freedman, Hallucinogenic drug research—if so, so what?: symposium summary and commentary, Pharmacol. Biochem. Behav. 24 (2) (1986) 407–415.

[59] A.J. Giannini, C. Nageotte, R.H. Loiselle, D.A. Malone, W.A. Price, Comparison of chlorpromazine, haloperidol and pimozide in the treatment of phencyclidine psychosis: DA-2 receptor specificity, J. Toxicol. Clin. Toxicol. 22 (6) (1984) 573–579.

[60] B. Borowsky, N. Adham, K.A. Jones, R. Raddatz, R. Artymyshyn, K.L. Ogozalek, M.M. Durkin, P.P. Lakhlani, J.A. Bonini, S. Pathirana, N. Boyle, X. Pu, E. Kouranova, H. Lichtblau, F.Y. Ochoa, T.A. Branchek, C. Gerald, Trace amines: identification of a family of mammalian G protein-coupled receptors, Proc. Natl. Acad. Sci. U. S. A. 98 (16) (2001) 8966–8971.

[61] D.B. Wainscott, S.P. Little, T. Yin, Y. Tu, V.P. Rocco, J.X. He, D.L. Nelson, Pharmacologic characterization of the cloned human trace amine-associated receptor1 (TAAR1) and evidence for species differences with the rat TAAR1, J. Pharmacol. Exp. Ther. 320 (1) (2007) 475–485.

[62] F.G. Revel, J.L. Moreau, R.R. Gainetdinov, A. Bradaia, T.D. Sotnikova, R. Mory, S. Durkin, K.G. Zbinden, R. Norcross, C.A. Meyer, V. Metzler, S. Chaboz, L. Ozmen, G. Trube, B. Pouzet, B. Bettler, M.G. Caron, J.G. Wettstein, M.C. Hoener, TAAR1 activation modulates monoaminergic neurotransmission, preventing hyperdopaminergic and hypoglutamatergic activity, Proc. Natl. Acad. Sci. U. S. A. 108 (20) (2011) 8485–8490.

[63] G.M. Miller, The emerging role of trace amine-associated receptor 1 in the functional regulation of monoamine transporters and dopaminergic activity, J. Neurochem. 116 (2) (2011) 164–176.

[64] D. Narang, S. Tomlinson, A. Holt, D.D. Mousseau, G.B. Baker, Trace amines and their relevance to psychiatry and neurology: a brief overview, Bull. Clin. Psychopharmacol. 21 (2011) 73–79.

[65] J. Wallach, Endogenous hallucinogens as ligands of the trace amine receptors: a possible role in sensory perception, Med. Hypotheses 72 (1) (2009) 91–94.

[66] F.G. Revel, J.L. Moreau, B. Pouzet, R. Mory, A. Bradaia, D. Buchy, V. Metzler, S. Chaboz, K. Groebke Zbinden, G. Galley, R.D. Norcross, D. Tuerck, A. Bruns, S.R. Morairty, T.S. Kilduff, T.L. Wallace, C. Risterucci, J.G. Wettstein, M.C. Hoener, A new perspective for schizophrenia: TAAR1 agonists reveal antipsychotic- and antidepressant-like activity, improve cognition and control body weight, Mol. Psychiatry 18 (5) (2013) 543–556.

[67] T.D. Sotnikova, M.G. Caron, R.R. Gainetdinov, Trace amine-associated receptors as emerging therapeutic targets, Mol. Pharmacol. 76 (2) (2009) 229–235.

[68] L. Lindemann, C.A. Meyer, K. Jeanneau, A. Bradaia, L. Ozmen, H. Bluethmann, B. Bettler, J.G. Wettstein, E. Borroni, J.L. Moreau, M.C. Hoener, Trace amine-associated receptor 1 modulates dopaminergic activity, J. Pharmacol. Exp. Ther. 324 (3) (2008) 948–956.

[69] T.D. Wolinsky, C.J. Swanson, K.E. Smith, H. Zhong, B. Borowsky, P. Seeman, T. Branchek, C.P. Gerald, The Trace Amine 1 receptor knockout mouse: an animal model with relevance to schizophrenia, Genes Brain Behav. 6 (7) (2007) 628–639.

[70] A.A. Grace, B.S. Bunney, H. Moore, C.L. Todd, Dopamine-cell depolarization block as a model for the therapeutic actions of antipsychotic drugs, Trends Neurosci. 20 (1) (1997) 31–37.

[71] S. Espinoza, A. Salahpour, B. Masri, T.D. Sotnikova, M. Messa, L.S. Barak, M.G. Caron, R.R. Gainetdinov, Functional interaction between trace amine-associated receptor 1 and dopamine D2 receptor, Mol. Pharmacol. 80 (3) (2011) 416–425.

[72] N.E. Anden, A. Rubenson, K. Fuxe, T. Hokfelt, Evidence for dopamine receptor stimulation by apomorphine, J. Pharm. Pharmacol. 19 (9) (1967) 627–629.

[73] I. Sukhanov, S. Espinoza, D.S. Yakovlev, M.C. Hoener, T.D. Sotnikova, R.R. Gainetdinov, TAAR1-dependent effects of apomorphine in mice, Int. J. Neuropsychopharmacol. 17 (10) (2014) 1683–1693.