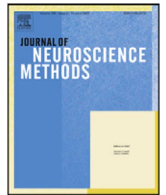
Journal of Neuroscience Methods 214 (2013) 149–155

Contents lists available at SciVerse ScienceDirect

Journal of Neuroscience Methods

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



Basic Neuroscience

Terminal effects of optogenetic stimulation on dopamine dynamics in rat striatum

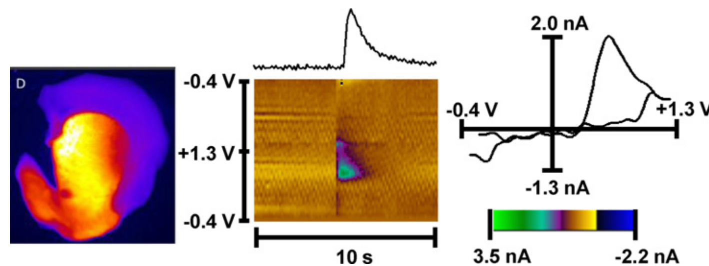
Caroline E. Bass[a], Valentina P. Grinevich[b], Alexandra D. Kulikova[b], Keith D. Bonin[c], Evgeny A. Budygin[b,*]

[a] Department of Pharmacology and Toxicology, State University of New York at Buffalo, Buffalo, NY 14214, USA
[b] Department of Neurobiology and Anatomy, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA
[c] Department of Physics, Wake Forest University, Winston-Salem, NC 27109, USA

HIGHLIGHTS

- Dopamine release from SN terminals in the striatum was studied with optogenetics.
- The amplitude of release can be controlled by different stimulation parameters.
- Optically-induced dopamine is stable after repeated stimulation (5 min interval).
- Optical stimulation can often result in a stimulating artifact.
- An artifact is distinguishable from dopamine by the cyclic voltammogram.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 November 2012
Received in revised form 26 January 2013
Accepted 28 January 2013

Keywords:
Voltammetry
Optogenetics
Dopamine
Brain slices
Adenoassociated virus
Channelrhodopsin-2

ABSTRACT

In this study, the first in-depth analysis of optically induced dopamine release using fast-scan cyclic voltammetry on striatal slices from rat brain was performed. An adeno-associated virus that expresses Channelrhodopsin-2 was injected in the substantia nigra. Tissue was collected and sectioned into 400 μm-thick coronal slices 4 weeks later. Blue laser light (473 nm) was delivered through a fiber optic inserted into slice tissue. Experiments revealed some difference between maximal amplitudes measured from optically and electrically evoked dopamine effluxes. Specifically, there was an increase in the amplitude of dopamine release induced by electrical stimulation in comparison with light stimulations. However, we found that dopamine release is more sensitive to changes in the pulse width in the case of optical stimulation. Light-stimulated dopamine was increased as the stimulation pulse widened. There was no difference with repeated stimulations at five minute intervals between stimulation sources and dopamine signal was stable during recording sessions, while one minute intervals resulted in a decline in the amplitude from both sources. Optical stimulation can also produce an artifact that is distinguishable from dopamine by the cyclic voltammogram. These results confirm that optical stimulation of dopamine is a sound approach for future pharmacological studies in slices.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Dopamine transmission in the striatum is critically involved in motor control, attention, learning, perception and processing of rewarding and aversive stimuli (Anstrom et al., 2009; Berridge, 2007; Budygin et al., 2012; Day et al., 2007; Grillner et al., 2005;

* Corresponding author. Tel.: +1 336 716 4072.
E-mail address: ebudygin@wakehealth.edu (E.A. Budygin).

0165-0270/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneumeth.2013.01.024

Redgrave et al., 2008; Rice et al., 2011). However, several other neurotransmitter systems impact these behaviors, and it is often difficult to dissect the role of dopamine in these functions. For many years the role of dopamine transmission in different physiological and pathological processes was studied using electrical or chemical/pharmacological stimulation. Recently, optogenetics has provided the field of neuroscience a new way to precisely target stimulation to select neuronal populations (Cao et al., 2011; LaLumiere, 2011), including dopamine neurons (Adamantidis et al., 2011; Bass et al., 2010; Stuber et al., 2010; Tecuapetla et al., 2010; Tsai et al., 2009; Witten et al., 2011). Recent findings demonstrate the precision by which dopamine can be released through optical stimulation of dopaminergic cell bodies in the midbrain (Adamantidis et al., 2011; Bass et al., 2010). In addition, it was observed that short-lasting optical stimulation of striatal dopamine release occurred without concomitant pH changes (Bass et al., 2010), which are a common phenomenon with chemical and electrical stimulation approaches (Venton et al., 2003), and which could interfere with receptor and neuronal functioning (Vyklicky et al., 1990). Therefore, optogenetics allows for a far more precise exploration of the role of dopamine transmission in different brain functions.

Several research groups began to combine optogenetics with fast-scan cyclic voltammetry (FSCV) to examine optically induced dopamine dynamics (Adamantidis et al., 2011; Bass et al., 2010; Stuber et al., 2010; Tecuapetla et al., 2010; Tsai et al., 2009; Witten et al., 2011). Indeed, FSCV detects dopamine *in vitro* and *in vivo* with subsecond temporal and micrometer spatial resolution that allows us to evaluate dopamine release and uptake changes in real time. Recently, by combining the tight spatial and temporal resolution of both optogenetics and FSCV we have performed the first in-depth analysis of substantia nigra (SN) optical stimulation on dopamine dynamics in the striatum of anesthetized rats (Bass et al., 2010). These experiments have demonstrated that the optical control over dopamine transmission at the level of cell bodies is highly reproducible, flexible and can be targeted to very precise, discrete subregions of the striatum *in vivo* based on placement of the opsin and light source in the midbrain. However, an important advantage of optogenetics is that light stimulation can be applied anywhere along the length of the neuron, including both the cell body in the midbrain and the terminals within the striatum. While our first study characterized striatal dopamine dynamics induced by light stimulation of the cell body region (SN) *in vivo*, the present experiments *in vitro* were designed to explore striatal dopamine release induced by optical stimulation of only the terminals.

As we did in our first study *in vivo*, we have used a generalized non-restricted promoter infused directly into the SN (Bass et al., 2010). It is important to note that this promoter should drive opsin expression in all neuronal subtypes in this region. However, in slice preparations only projections, which are sent from the SN, will be targeted for optical stimulation of dopamine release. This is in sharp contrast to the traditional electrical stimulation, which evokes neurotransmitter release from all terminals neighboring to the electrode, regardless of where the projection originates. Therefore, this optogenetic approach combined with FSCV allowed us to characterize dopamine efflux from exciting only SN terminals (dopaminergic and otherwise) in the absence of many other striatal terminals. While our results demonstrate that electrically and optically induced dopamine release is similar, there are some notable differences.

2. Experimental

2.1. Adeno-associated virus (AAV) packaging

The EF1α-ChR2-EYFP AAV plasmid was a kind gift from K. Deisseroth and consists of AAV2 inverted terminal repeats flanking the transgene cassette, which contains the EF1α promoter followed by a ChR2-EYFP fusion gene, woodchuck postregulatory element (WPRE) and human growth hormone polyA signal sequence, respectively. Packaging of the EF1α-ChR2-AAV10 was carried out according to a standard triple transfection protocol to create helper virus-free pseudotyped AAV2/10 virus (Xiao et al., 1998). An AAV2/10 rep/cap plasmid provided the AAV2 replicase and AAV10 capsid genes (Gao et al., 2002), while adenoviral helper functions were supplied by pHelper (Stratagene, La Jolla, CA). Briefly, AAV-293 cells (Stratagene, La Jolla, CA) were transfected with 10 μg of pHelper, and 1.15 pmol each of AAV2/10 and AAV vector plasmids *via* calcium phosphate precipitation. The cells were harvested 72 h later and the pellets resuspended in DMEM, freeze-thawed three times and centrifuged multiple times to produce a clarified viral lysate.

2.2. Stereotaxic virus injection

Male Sprague-Dawley rats (300–350 g; Charles River, Raleigh, NC) were anesthetized with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine hydrochloride (20 mg/kg, i.p.) and placed in a stereotaxic frame. The scalp was shaved, swabbed with iodine and a central incision made to expose the skull. Two small holes were drilled and 2 skull screws were placed in to secure a cement cap. A third hole was drilled above the right SN (from bregma: anterior-posterior, −5.6 mm; lateral, 2 mm) and 2 μl of EF1α-ChR2-AAV10 was slowly injected into SN (dorsal-ventral, −7.6 mm) over 3 min *via* a Hamilton syringe. The tissue was allowed to rest for 2 min before it was slowly retracted. The exposed scull was coated with dental cement secured by skull screws and upon drying the animals were returned to their home cages for recovery. All protocols were approved by the Institutional Care and Use Committee at Wake Forest School of Medicine. All experiments conformed to international guidelines on the ethical use of animals.

2.3. Fast-scan cyclic voltammetry (FSCV) in brain slices

Rats were sacrificed within several minutes by decapitation and the brains rapidly removed and cooled in ice-cold, pre-oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (aCSF). The tissue was then sectioned into 400 μm-thick coronal slices containing striatum. Slices were kept in a reservoir of oxygenated aCSF with HEPES buffer at room temperature for 30 min. The aCSF consisted of (in mM): NaCl (126), KCl (2.5), NaH_2PO_4 (1.2), $CaCl_2$ (2.4), $MgCl_2$ (1.2), $NaHCO_3$ (25), glucose (11), HEPES (20) and was pH adjusted to 7.4. One hour before each experiment, a brain slice was transferred to a submersion recording chamber, perfused at 1 ml/min with oxygenated aCSF, and allowed to equilibrate. The buffer was continuously bubbled with 5% CO_2 and O_2. Recordings were made in the dorsal part of the striatum. A cylindrical carbon fiber microelectrode (7 μm diameter, ∼100 μm long) was inserted into the slice and bipolar stimulating electrodes or fiber optic (Plastics One, Roanoke, VA, USA) were placed on the surface of the brain slice ∼200 μm away. Extracellular dopamine was monitored at the carbon fiber microelectrode every 100 ms using FSCV by applying a triangular waveform (−0.4 to +1.3 *vs.* Ag/AgCl, 300 V/s). Data were digitized (National Instruments, Austin, TX, USA) and stored on a computer. Dopamine release was evoked by electrical (4, 10, 20 and 40 ms/phase, monophasic, 300 μA,) or light (4, 10, 20 and 40 ms/phase, ≈5 mW) pulses.

2.4. Visual identification of ChR2 containing terminals

Typically, local electrical stimulation along the dorsal-ventral axis of a striatal slice will result in robust dopamine release at each point, including the ventral striatum. However, as we have

Download English Version:

https://daneshyari.com/en/article/4335160

Download Persian Version:

https://daneshyari.com/article/4335160

Daneshyari.com