Neuropharmacology 70 (2013) 43–50

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

Differential contribution of mesoaccumbens and mesohabenular dopamine to intracranial self-stimulation

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ARTICLE INFO

Article history:
Received 11 August 2012
Received in revised form
16 December 2012
Accepted 9 January 2013

Keywords:
D-**Amphetamine**
Dopamine
Nucleus accumbens
Shell
Core
Lateral habenula
Intracranial self-stimulation
Reward

ABSTRACT

The contribution of mesoaccumbens dopamine transmission to intracranial self-stimulation is well-established. However, although the nucleus accumbens comprises two main subregions, the shell and the core, little is known of the contribution of each to this behaviour. Our first aim was to study the effects of D-**amphetamine** infusions into the shell and core in order to understand their relative importance to reward and operant responding. Our second aim was to examine the contribution of a lesser studied group of dopamine neurons, those within the mesohabenular pathway, to intracranial self-stimulation. Male Sprague–Dawley rats were implanted with bilateral cannulae in the nucleus accumbens shell, core or in the lateral habenula and a monopolar stimulation electrode in the posterior mesencephalon, a brain site that is sensitive to changes in dopamine transmission. Using curve-shift scaling, we measured the reward- and performance-enhancing effects of intra-accumbens (1–20 μg) and intra-habenular (10–40 μg) infusions of D-**amphetamine** or vehicle. Within the nucleus accumbens, the use of multiple doses and long test sessions allowed us to observe an interaction between drug effect and infusion site. We show, for the first time, differences in the minimal doses necessary to enhance rewarding effectiveness and operant responding, in the magnitude of these enhancements as well as in their duration. Conversely, regardless of dose, intra-habenular D-**amphetamine** did not alter rewarding effectiveness or operant rate, highlighting the differential contribution of mesoaccumbens and mesohabenular dopamine pathways to intracranial self-stimulation.

1. Introduction

The contribution of mesolimbic dopamine (DA) transmission to goal-directed behaviour has been the focus of many years of study. This is especially true of the mesoaccumbens pathway. This pathway is important for mediating goal-directed approach or escape behaviour in a way that is sensitive to adaptive modifications via conditioned environmental cues (see Ikemoto and Panksepp (1999) for review). Dopamine in the mesoaccumbens pathway is also important for the acute rewarding properties of drugs of abuse as well as of electrical brain stimulation, although few other DA terminal sites have received as much research attention. With respect to brain stimulation reward, infusions of DA agonists into the nucleus accumbens enhance the rewarding effectiveness of the stimulation (Carlezon and Wise, 1996a; Carr et al., 2009; Colle and Wise, 1988; Hayes et al., 2009; Ranaldi and Beninger, 1994a, 1994b) while infusions of DA antagonists attenuate it (Nakajima and Patterson, 1997; Stellar et al., 1983; Stellar and Corbett, 1989). Drug infusions into the more dorsal caudate putamen or prefrontal cortex are relatively ineffective (Colle and Wise, 1988; Ranaldi and Beninger, 1994a, 1994b; Stellar and Corbett, 1989). Other sites, like the amygdala, have provided inconsistent findings (Stellar and Corbett, 1989; Waraczynski et al., 2010).

On account of neuroanatomy, neurochemistry and function, the nucleus accumbens can be divided into two broad compartments, a ventromedial shell and a dorsolateral core. On the basis of their anatomical connections, the shell and the core have traditionally been designated as preferentially limbic and motoric in function, respectively (Heimer et al., 1991; Zahm and Brog, 1992). For example, direct infusion of psychostimulants into the nucleus accumbens shell is rewarding (Carlezon et al., 1995; Carlezon and Wise, 1996b; Pontieri et al., 1995; Shin et al., 2008) while lesions of the core attenuate the locomotor activating properties of such drugs (Boye et al., 2001; Sellings et al., 2006, 2008; Sellings and Clarke, 2003). Intracranial self-stimulation (ICSS) relies on the rewarding effectiveness of the stimulation as well as on the animal's motoric capacity to emit the operant response. Although early ICSS studies often employed the rate of responding as an index of rewarding

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0028-3908/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuropharm.2013.01.005

effectiveness, the shortcomings of such measures are now well established (Hodos and Valenstein, 1962) and currently-available scaling techniques allow dissociation between reward and performance (Edmonds and Gallistel, 1974; Miliaressis et al., 1986). Rate of responding remains, however, a valid indicator of the overall capacity of the animal to perform the operant task, which is particularly useful in pharmacological experiments. Despite this, we know little of the role played by the shell and the core of the nucleus accumbens in ICSS. The first aim of this study was to infuse different doses of D-**amphetamine** into each subregion in order to characterize the relative contribution of increased DA transmission within these to rewarding effectiveness and operant responding.

The second aim of this study was to examine the contribution of another group of DA neurons, those comprising the mesohabenular pathway, to ICSS reward and performance. Mesohabenular DA neurons originate from the rostral portion of the ventral tegmental area, ascend via the fasciculus retroflexus and terminate in the lateral habenula (LHb) (Gruber et al., 2007; Herkenham and Nauta, 1977; Phillipson and Griffith, 1980). Within the ventral tegmental area, their cell bodies are located in paranigral, interfascicular, and parabrachial pigmented nuclei (Gruber et al., 2007; Phillipson and Griffith, 1980), sites that also give rise to DA afferents of the nucleus accumbens, particularly those that innervate the shell (Ikemoto, 2007). Very little is known about the contribution of mesohabenular DA transmission to goal-directed behaviour in general and to reward function in particular.

The notion that mesohabenular DA may be reward-relevant is supported by at least three observations. First, the LHb plays an important role in reward error prediction (Bromberg-Martin and Hikosaka, 2011) and DA signalling at this site may be pertinent. Second, mesohabenular and mesoaccumbens DA neurons share nuclei of origin (Gruber et al., 2007; Ikemoto, 2007; Phillipson and Griffith, 1980) suggesting common function. Third, studies of metabolic activity, as measured with (^{14}C)-2-deoxyglucose autoradiography, have consistently shown that pro-dopaminergic stimuli such as rewarding electrical brain stimulation or systemic administration of DA agonists reduce 2-deoxyglucose uptake in the LHb whereas anti-dopaminergic treatments such as reward-attenuating doses of DA antagonists reliably and significantly increase it (Gallistel et al., 1985; Gomita and Gallistel, 1982; McCulloch et al., 1980; Wechsler et al., 1979). These changes are consistent with the known effects of DA agonists and antagonists on the firing activity of midbrain DA neurons (Bunney et al., 1973). However, since the 2-deoxyglucose technique does not allow identification of the substrate mediating the observed changes in metabolic activity, changes in 2-deoxyglucose uptake may reflect modified afferent input from diverse brain sites. Here, we targeted DA terminals specifically by infusing D-**amphetamine** directly into the LHb.

2. Materials and methods

2.1. Subjects

Subjects were male Sprague–Dawley rats (Charles River, St Constant, Quebec), housed individually in a temperature (21 °C) and humidity (50%) controlled animal colony with a 12 h light/dark cycle (lights on at 6:30 a.m.). Rats had unrestricted access to food and water and were allowed to habituate to the animal colony for at least five days prior to surgery. All procedures followed Canadian Council on Animal Care guidelines and were approved by the Institutional Animal Care Committee.

2.2. Surgery

Rats (300–400 g) were anesthetized with a mixture of oxygen (0.6 L/min) and isoflurane (4%) and mounted onto a stereotaxic apparatus. During surgery, the level of anesthesia was reduced to 2–3%. Using aseptic techniques, bilateral holes were drilled over the nucleus accumbens or habenula, and a single hole over the posterior mesencephalon. Bilateral guide cannulae (26 ga, Plastics One, Roanoke, VA) were implanted into the LHb (flat skull coordinates: AP: −3.4, ML: ±0.6, DV: −5.0 mm), nucleus accumbens shell (AP: +1.7, ML: ±0.8, DV: −7.7 mm) or nucleus accumbens core (AP: +1.7, ML ±1.5, DV: −6.9 mm), and a stimulation electrode was implanted in the posterior mesencephalon (AP: −7.8, ML: 0, DV: −7.0 to −7.2 mm). All coordinates were obtained from the Paxinos and Watson atlas of the rat brain (Paxinos and Watson, 1997). All dorso-ventral (DV) coordinates are in reference to the surface of the skull. Stimulation electrodes were made from stainless steel wire (0.27 mm dia.) and were insulated with Epoxylite except for the rounded tip. A bare stainless steel wire, connected at one end to a male amphenol pin and wrapped around four to five miniature screws that were threaded into the cranium, served as the anode. Acrylic dental cement was used to chronically secure the electrode-cannula assembly to the skull. Prior to the end of surgery, all rats received an injection of the non-steroidal anti-inflammatory analgesic Ketoprofen (5 mg/kg, sc) and received a second injection the following day.

2.3. Intracranial self-stimulation

Operant conditioning chambers (28 cm wide × 29.4 cm deep × 68.6 cm high) were constructed from PVC (back and side walls) and Plexiglas (front wall). Each chamber was equipped with a lever located on the left wall, 3.4 cm above the metal grid floor. Operant conditioning chambers were encased in sound-attenuating boxes (48.6 cm wide × 50.7 cm deep × 95.4 cm high) made from melamine with a Plexiglas window allowing constant viewing of the rat. Depressions of the lever triggered a constant-current generator (PHM-152/2, Med Associates Inc, St Albans, VT) to deliver a single 400-ms train of rectangular cathodal pulses of 0.1 ms in duration, delivered on a FI-1s schedule. Current intensity was monitored on an oscilloscope by reading the voltage drop across a 1 kΩ resistor in series with the electrode.

Following one week of post-surgical recovery, rats were trained to emit the operant response by the method of successive approximations. Ten to 15 min of training were generally sufficient to establish consistent responding. Rats were immediately allowed to self-administer the stimulation for 1 h, at parameters set to support vigorous responding. On the following day, rats were allowed to self-administer the same stimulation parameters, but only during 45 s trials that were separated by 30 s inter-trial intervals and preceded by five trains of non-contingent stimulation delivered at 1 Hz. All parameters of the non-contingent stimulation were identical to those available during the 45 s trial. Beginning on the third day, the pulse frequency was systematically reduced by approximately 0.1 $\log_{10}$ units across trials, starting with a frequency that supported maximal responding and ending with one sufficiently low to induce extinction. The plot of the rate of responding as a function of pulse frequency comprised a single response–frequency curve; each curve took 15 min to complete and 6–8 were determined daily. Reward threshold was defined as the pulse frequency required for responding at a half-maximal rate and was derived from a regression line fit to the rising portion of individual response–frequency curves. In order to standardize the rewarding effectiveness of the stimulation across all rats prior to the start of drug testing, current intensities were adjusted to yield reward thresholds of approximately 50 Hz.

2.4. Drug tests

Rats were trained daily until reward thresholds varied by less than 0.1 $\log_{10}$ units within daily sessions and across a minimum of three days. Each drug test began with four threshold determinations (four response–frequency curves), the first of which was excluded from data analysis. Immediately after the end of the fourth curve, rats were removed from the operant chambers and brought to an adjacent room for drug infusion. The dust cap and obturator were first removed from the cannula assembly and replaced with an injector (31 ga) connected to two Hamilton microsyringes via polyethylene tubing. The injectors protruded beyond the tip of the guide cannula by 1 mm. Infusions were made with a dual syringe infusion pump (Pump 11 Plus, Harvard Apparatus, Holliston, MA) over 1 min, followed by an additional minute prior to retraction of the injector in order to allow drug diffusion away from the injector tip. Obturators and dust caps were then reinstalled and the rats were brought back to their respective operant conditioning chamber for six additional threshold determinations which required approximately 90 min of additional testing. At the end of the test session, rats were returned to their home cages.

Rats within each of the three groups (shell, core, LHb) received a total of three doses of D-**amphetamine** plus vehicle (sterile 0.9% saline) in a semi-counterbalanced sequence. In order to restrict drug diffusion to the target site, the first three doses in each group were delivered in a volume of 0.25 μl. In each of the three sites, the first three doses were delivered in a fully counterbalanced sequence. For the fourth and last infusion, we doubled the volume by increasing the infusion time of the highest dose to 2 min. Doses were as follows: shell and core: 0, 1 and 10 μg/0.25 μl plus 20 μg/0.5 μl; LHb: 0, 10 and 20 μg/0.25 μl plus 40 μg/0.5 μl. Pilot infusions of 1 μg/0.25 μl into the LHb of a separate group of rats did not produce any effect so we shifted up the range of doses administered to this site in an attempt to obtain an effect. All drug infusions were separated by 3–4 days.

2.5. Histology

At the end of the study, rats were anaesthetized with urethane (1.2 g/kg, ip) and a lesion was created at the tip of the stimulation electrode by passing direct anodal current (100 μA for 15 s). Rats were then perfused with 0.9% saline followed by a 10%