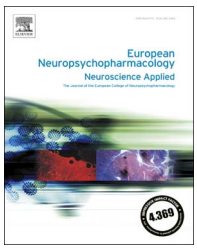
European Neuropsychopharmacology (▮▮▮▮) ▮, ▮▮▮–▮▮▮

www.elsevier.com/locate/euroneuro



Chemogenetic activation of dopamine neurons in the ventral tegmental area, but not substantia nigra, induces hyperactivity in rats

Linde Boekhoudt, Azar Omrani, Mieneke C.M. Luijendijk, Inge G. Wolterink-Donselaar, Ellen C. Wijbrans, Geoffrey van der Plasse, Roger A.H. Adan*

Brain Center Rudolf Magnus, Department of Translational Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands

Received 20 April 2016; received in revised form 27 July 2016; accepted 1 September 2016

KEYWORDS
Dopamine;
DREADD;
Chemogenetics;
Ventral tegmental area;
Substantia nigra;
Locomotor activity

Abstract
Hyperactivity is a core symptom in various psychiatric disorders, including attention-deficit/hyperactivity disorder, schizophrenia, bipolar disorders, and anorexia nervosa. Although hyperactivity has been linked to dopaminergic signalling, the causal relationship between midbrain dopamine neuronal activity and locomotor hyperactivity remains unknown. In this study, we test whether increased dopamine neuronal activity is sufficient to induce locomotor hyperactivity. To do so, we used designer receptors exclusively activated by designer drugs (DREADD) to chemogenetically enhance neuronal activity in two main midbrain dopamine neuron populations, i.e. the ventral tegmental area (VTA) and substantia nigra pars compacta (SN), in TH:Cre rats. We found that activation of VTA dopamine neurons induced a pronounced and long-lasting hyperactive phenotype, whilst SN dopamine neuron activation only modestly increased home cage locomotion. Furthermore, this hyperactive phenotype was replicated by selective activation of the neuronal pathway from VTA to the nucleus accumbens (NAC). These results show a clear functional difference between neuronal subpopulations in the VTA and SN with regards to inducing locomotor hyperactivity, and suggest that the dopaminergic pathway from VTA to NAC may be a promising target for the treatment of hyperactivity disorders.

*Correspondence to : Brain Center Rudolf Magnus, University Medical Center Utrecht,Dept Translational Neuroscience,Str 5.227 Universiteitsweg 100 3584CG Utrecht,The Netherlands.
E-mail address: r.a.h.adan@umcutrecht.nl (R.A.H. Adan).

http://dx.doi.org/10.1016/j.euroneuro.2016.09.003
0924-977X/

Please cite this article as: Boekhoudt, L., et al., Chemogenetic activation of dopamine neurons in the ventral tegmental area, but not substantia.... European Neuropsychopharmacology (2016), http://dx.doi.org/10.1016/j.euroneuro.2016.09.003

1. Introduction

Hyperactivity is a core symptom of several psychiatric disorders, including attention-deficit/hyperactivity disorder (ADHD), schizophrenia, manic episodes in bipolar disorders, and anorexia nervosa (Angst et al., 2003; Beumont et al., 1994; Casper, 2006; Mehler-Wex et al., 2006; Perry et al., 2010). The neurobiological substrates underlying hyperactive behaviour are incompletely understood, which hampers the development of novel, more effective, treatments. Clinical imaging studies have shown alterations in dopamine signalling in individuals diagnosed with hyperactivity (Jucaite et al., 2005; Ludolph et al., 2008; Volkow et al., 2009), and preclinical studies have shown a strong link between dopamine signalling in the striatum and locomotor hyperactivity in rodents (Canales and Iversen, 1998; Carr and White, 1987; Delfs et al., 1990; Dickson et al., 1994; Gong et al., 1999; Kelly et al., 1975). However, both clinical and preclinical studies have mainly focused on dopamine signalling in the target areas of midbrain dopaminergic neurons (primarily the striatum), rather than direct manipulation of the dopamine neurons themselves. Hence, it remains unclear if there is a causal relationship between dopaminergic neuronal activity and locomotor hyperactivity, and, if so, which neuronal subpopulations are involved.

Within the midbrain, we distinguish dopamine neurons in the ventral tegmental area (VTA) and substantia nigra pars compacta (SN). These dopamine neuron populations send projections to ventral and dorsal parts of the striatum, respectively. Previous studies have shown that increasing midbrain dopamine neuronal activity (including both VTA and SN) induced locomotor hyperactivity in mice (Wang et al., 2013). Also, disinhibition of these neurons, by inhibiting midbrain GABAergic neuronal activity, increased locomotor activity (Vardy et al., 2015). However, these studies were not designed to distinguish between dopamine neuronal subpopulations in the VTA and SN, and thus their relative contribution to the hyperactive phenotype remains unknown.

Preclinical and clinical studies have shown evidence for a role of both mesolimbic and nigrostriatal dopaminergic pathways – emerging from VTA and SN, respectively – in locomotor activity. Imaging studies have shown alterations in dopaminergic signalling in ADHD subjects in both ventral and dorsal striatal subregions (Jucaite et al., 2005; Ludolph et al., 2008; Volkow et al., 2009), suggesting that dopamine neuronal activity in both VTA and SN might be affected. Initial pharmacological studies in rodents have shown that psychostimulant-induced hyperactivity mainly results from actions in the nucleus accumbens (NAC) (Canales and Iversen, 1998; Carr and White, 1987; Delfs et al., 1990; Dickson et al., 1994; Kelly et al., 1975), rather than in the dorsal striatum (Carr and White, 1987; Dickson et al., 1994; Kelly et al., 1975), suggesting a primary role for dopamine neurons in the VTA. Indeed, dopamine-deficient mice did not show psychostimulant-induced hyperactivity unless dopaminergic signalling in the NAC was restored (Heusner et al., 2003). However, selective rescue of nigrostriatal dopamine signalling, also enhanced locomotor behaviour in these mice, in the absence of a psychostimulant drug (Hnasko et al., 2006). Previously, we reported that chemogenetic activation of VTA neurons projecting to the NAC increased home cage locomotor activity in rats (Boender et al., 2014). In contrast, several studies using optogenetic activation of VTA dopamine neurons failed to observe effects on locomotor activity (Chaudhury et al., 2013; Gunaydin et al., 2014; Tye et al., 2013). Taken together, dopaminergic pathways emerging from both VTA and SN appear to be involved in regulating locomotor activity, but their respective roles in inducing hyperactivity remain to be resolved.

In this study, we sought to investigate whether chemogenetic activation of dopamine neurons in the VTA or SN induces locomotor hyperactivity. In order to directly manipulate neuronal activity of dopaminergic neurons, we used designer receptors exclusively activated by designer drugs (DREADD) in TH:Cre transgenic rats. Additionally, we targeted selective pathways to identify which midbrain neuronal subpopulations are crucially involved in inducing locomotor hyperactivity.

2. Experimental procedures

2.1. Subjects and surgical procedures

TH:Cre transgenic rats (Witten et al., 2011) were bred in-house, by crossing heterozygous TH:Cre$^{+/-}$ rats with wild type Long Evans mates. Cre-negative (Cre−) littermates served as control. All experiments were performed in accordance with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC), and were approved by the Animal Ethics Committee of Utrecht University.

2.1.1. Surgical procedures

All experimental animals were injected bilaterally with 1 μl of AAV5-DIO-hSyn-hM3Dq-mCherry ("Dq") (6.4-8.0*E12 virus molecules/ml; UNC Vector Core). Prior to surgery, rats were anaesthetised by intramuscular fentanyl/fluanisone (0.315 mg/kg fentanyl, 10 mg/kg fluanisone, Hypnorm, Janssen Pharmaceutica, Belgium), and xylocaine was sprayed on the skull to provide local anaesthesia (Lidocaine 100 mg/ml, AstraZeneca BV, the Netherlands). All rats received three daily peri-surgical injections of carprofen (5 mg/kg, s.c., Carporal, AST Farma BV, the Netherlands), starting at the day of surgery. In order to allow for sufficient DREADD expression, there was a minimum of two weeks between surgery and electrophysiological recordings, and at least four weeks in between surgery and behavioural testing.

2.1.2. Experiment 1: chemogenetic activation of dopamine neurons *in vitro*

In vitro electrophysiology experiments were performed in TH:Cre rats injected with virus at 3-4 weeks old, at AP −4.8; ML +1.0 (5° angle); DV −7.1 for VTA, and AP −4.8; ML +1.9; DV −6.7 for SN. All coordinates are in mm relative to Bregma.

2.1.3. Experiment 2: chemogenetic activation of VTA and SN dopamine neurons

Male TH:Cre rats (n=16; age 14 weeks at start of behavioural testing) and Cre− littermates (n=14) were injected into either the VTA (young rats, 156±20 g [mean±SD], at AP −5.2; ML +1.1 [5° angle]; DV −7.4), or SN (adult rats, 337±23 g, at AP −5.4; ML +2.2; DV −7.7).

2.1.4. Experiment 3: chemogenetic activation of midbrain neuronal projections towards NAC and DMS

To induce DREADD expression selectively in midbrain neurons projecting to either NAC or dorsomedial striatum (DMS), we used Cre-dependent DREADD combined with canine-adenovirus2 expressing Cre recombinase (CAV2Cre) (Boender et al., 2014; Hnasko

Please cite this article as: Boekhoudt, L., et al., Chemogenetic activation of dopamine neurons in the ventral tegmental area, but not substantia.... European Neuropsychopharmacology (2016), http://dx.doi.org/10.1016/j.euroneuro.2016.09.003