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Preclinical molecular imaging of glutamatergic and dopaminergic neuroreceptor kinetics in obsessive compulsive disorder

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ABSTRACT

Background: Molecular neuroimaging was applied in the quinpirole rat model for compulsive checking in OCD to visualize the D2- and mGluR5-receptor occupancy with Raclopride and ABP-688 microPET/CT.
Methods: Animals (n = 48) were exposed to either saline (CTRL; 1 mL/kg) or quinpirole (QP; dopamine D2-agonist, 0.5 mg/kg) in a single injection (RAC and ABP acute groups) or twice-weekly during 7 weeks (chronic group). Animals underwent PET/CT after the 1st injection (acute) or before initial exposure and following the 10th injection in week 5 (chronic). For the latter, each injection was paired with an open field test and video tracking.
Results: The QP animals displayed a strong increase in visiting frequency (checking) in the chronic group (+ 699.29%) compared to the control animals. Acute administration of the drug caused significant ($p < 0.01$) decreases in D2R occupancy in the CP (− 42.03% ± 4.01%). Chronical exposure resulted in significantly stronger decreases in the CP (− 52.29% ± 3.79%). Furthermore significant increases in mGluR5 occupancy were found in the CP (10.36% ± 4.09%), anterior cingulate cortex (13.26% ± 4.01%), amygdala (24.36% ± 6.86%), entorhinal cortex (18.49% ± 5.14%) and nucleus accumbens (13.8% ± 4.87%) of the chronic group, not present after acute exposure.
Conclusions: Compared to acute exposure, sensitisation to QP as a model for OCD differs both on a dopaminergic and glutamateric level, indicating involvement of processes such as receptor internalization and changes in extracellular availability of both neurotransmitters.

1. Introduction

Obsessive-compulsive disorder (OCD) is a chronic, incapacitating, early onset psychiatric disorder that is characterized by obsessions and compulsions, alternating in a cyclic manner (Ahmari et al., 2013; Cicek et al., 2013). These obsessions are recurrent, intrusive, distressing, unwanted and meaningless in nature. To reduce this obsession-induced stress, patients often display repetitive, stereotyped, ritualistic and time-consuming behavior that in the end reinforces the obsession, resulting in a vicious cycle and a reduced quality of life for patients and their close relatives (Bobes et al., 2001). The implications of this disease urged the World Health Organization (WHO) in 2001 to rank it in the top 10 leading causes of disability (Khouzam, 1999).

Although the underlying pathophysiology of OCD is still poorly understood, research strongly points towards the disturbance of the cortico-striato-thalamico-cortical (CSTC) circuitry (Ahmari et al., 2013; Lipsman et al., 2013; Milad and Rauch, 2012). It is hypothesized that an altered local neurotransmission lies at the basis of a behavioral dysinhibition (Fineberg et al., 2011), which is not yet fully characterized. More specifically, when dopamine (DA) from nigral projections is released in the striatum, a direct pathway is activated resulting in a disinhibition of the thalamus and stronger projections returning to the cortex. Alternatively, when DA is absent in the striatum, an indirect pathway is activated, thereby inhibiting the thalamus resulting in less signalling to the cortex (Milad and Rauch, 2012). The intrinsic role of DA in this pathway hinted towards the manifestation of OCD as a DA-disorder rather then a serotonergic (5-HT) disorder, as was originally implied (Graybiel and Rauch, 2000). In the current consensus, a role for 5-HT is still present, although the inefficacy of selective serotonin reuptake inhibitors (SSRI's) and the long delay before effects are noticeable, doubted serotonine's status as the major trigger of the disease (Westenberg et al., 2007). Furthermore, the intricate connection between 5-HT and DA (Haleem et al., 2014), the amelioration of OCD symptoms after the addition of DA antagonists to the current treatment

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and the aggravation of symptoms after adding DA agonists strongly support the role of DA in the disorder (Graybiel and Rauch, 2000; Westenberg et al., 2007). However, since approximately one in three patients do not have a meaningful benefit after optimization of treatment with the current DA and 5-HT options (Pittenger, 2015a,b), it became obvious that the research frame should be expanded beyond the current hypothesis.

More recently another neurotransmitter, glutamate, has become the focus of OCD-related research (Wu et al., 2013). Modulation of the *N*-methyl-D-aspartate receptors (NMDAR) appeared to significantly reduce the compulsive behavior, not only in a preclinical study (Albelda and Joel, 2012) but also in humans (Ghaleiha et al., 2013), strongly hinting towards a potential role for glutamate. The direct involvement of glutamate and its receptors in the CSTC-circuit could mean that glutamate is directly implicated in the manifestation of the disease. Furthermore, the lowering of compulsive behavior in mice by administration of the mGluR5 antagonist 2-methyl-6-phenylethyl-pyrididine (MPEP) (Mehta et al., 2011) and the positive influence of a memantine augmentation to the SSRI treatment in moderate to severe OCD patients (Ghaleiha et al., 2013) supported this finding, opening up an entirely new line of glutamate-modelling treatment options (Pittenger, 2015a; Pittenger, 2015b). A recent PET imaging study investigating the occupancy of the metabotropic glutamate-5-receptor (mGluR5) revealed a significant positive correlation between the receptor's occupancy and the presence of obsessions (Akkus et al., 2014).

Clinical OCD research is often hindered by the lack of naïve patients on previous medication regimens or followed therapies as well as by the intrinsic differences in the individual manifestation of OCD itself. As such, preclinical studies provide an excellent tool to investigate the basic underlying pathophysiological mechanisms of OCD. A commonly used model in preclinical research is the quinpirole (QP) sensitization rat model for OCD (Szechtman et al., 1998). In this model, originally developed by Szechtman and colleagues in 1998, animals are chronically injected with the D2 agonist QP. After each injection, animals are introduced to a 30 min lasting open field test (OFT) on a 160 × 160 cm table with 4 carefully placed objects. In contrast to animals receiving saline injections, the QP-treated animals display an increase in locomotion and visit 1 or 2 objects excessively often (Szechtman et al., 1998). It can be hypothesized that the chronic administration of a D2-agonist causes a desensitisation of the D2-receptors thereby inhibiting the indirect and inhibitory D2-pathway, ultimately resulting in compulsive-like behavior. Later studies showed that certain environmental-dependent behavioral patterns are also present around these objects, strongly resembling the human condition (de Haas et al., 2011; Szechtman et al., 2001, 1993). This led to a variety of experiments, mainly focusing on behavioral research for pharmacological validation of certain drugs or treatments (De Carolis et al., 2011; Winter et al., 2008; Zadicario et al., 2007). The miniaturisation of molecular imaging techniques, such as positron emission tomography (PET), now allows for picomolar sensitive characterization of neuroreceptor kinetics in rodents.

In a previous study from our group (Servaes et al., 2016) we applied this molecular brain imaging technique in the aforementioned quinpirole rat model for compulsive disorder in order to measure the animal's neuronal activity through visualizing brain glucose metabolism with 18F–fluorodeoxyglucose (18F-FDG). Next to strong increases in visiting frequency and total distance travelled, we found a significant decreased metabolism in both the caudate putamen and the hippocampus. This change in brain metabolism was hypothesized to be a likely effect of D2 desensitisation caused by the constant exposure to the D2 agonist, quinpirole, resulting in a dysregulation of the neural feedback loop (Servaes et al., 2016). This hypothesis would benefit from a more in-depth look at the cause of this dysregulation by investigating the brain neurochemistry on a molecular level. Therefore we have now measured the DA D2 receptor occupancy by means of [^{11}C]-Raclopride (RAC) imaging. RAC is a selective antagonist for the D2 receptor and by labeling this molecule with the radioactive isotope ^{11}C, it is possible to visualize the occupancy of the D2 receptors in vivo with PET, expressed in a region specific binding potential (BPnd) (Ikoma et al., 2009). Furthermore, the glutamatergic system is investigated using [^{11}C]-ABP-688 (ABP) to visualize the metabotropic glutamate receptor subtype 5 (mGluR5) receptor. When labeled with radioactive ^{11}C, ABP, being a non-competitive highly selective antagonist for the mGluR5, allows us to visualize the distribution of this receptor by assessing the BPnd (Wyss et al., 2007) and how this changes with chronic exposure to the D2 agonist, quinpirole.

2. Material & methods

2.1. Animals

The present study was carried out in accordance with the European Communities Council Directive of November 24th, 1986 (86/609/EEC) for care of laboratory animals and after approval of the local ethical committee (University of Antwerp under number 2014–18). All efforts were made to minimize suffering and to reduce the number of animals. Forty-eight (n = 48) naïve male Sprague Dawley rats (Harlan, the Netherlands, 285–550 g during the experiment) were housed in a temperature- and humidity-controlled vivarium in IVC-cages with a 12-hour light-dark cycle. All experiments were performed during daytime. Food and water were available ad libitum. All applicable institutional and national guidelines for the care and use of animals were followed.

2.2. Experimental setup

Prior to the start of the experiments, all animals (n = 48) were handled during 2 min for 5 consecutive days. In order to distinguish the effect of acute drug administration to the chronic exposure, animals were divided into an acute and a chronic group. Furthermore, within these groups, animals were separated by being either a control condition (SAL) or an exposure condition (QP).

2.2.1. Acute group

Animals of the acute group received a subcutaneous injection of either QP (0.5 mg/kg dissolved in saline to achieve an injection volume of 1 mL/kg) or saline (1 mL/kg). Fifteen minutes after this injection, animals were injected with either RAC (n = 8; RAC Acute SAL and RAC Acute QP), to assess the receptor occupation of the DA D2 receptor, or with ABP (n = 8; ABP Acute SAL and ABP Acute QP) to assess the mGluR5 receptor distribution. Both tracers were administered under tracer dose conditions (< 3 nmol/kg for ABP; < 0.5 nmol/kg for RAC) to ensure that receptor occupancy was below 10%, thereby avoiding infringement of the physiological conditions. A dynamic acquisition protocol was started at the moment the tracer was injected (at a rate of 1 mL per minute), and consisted of a 60 min PET-scan followed by a 10 min CT-scan. In order to increase throughput, animals were scanned in a head-to-head position on the bed.

2.2.2. Chronic group

In order to measure how chronic QP administration affected the animals, the basal mGluR5 receptor distribution and the receptor occupation of the DA D2 receptor were assessed prior to model-setup. The animals underwent two baseline scans with ABP and RAC respectively in a similar manner as described above. In order to provide ample time for recovery and tracer washout, each scan was separated by a minimum of 2 days. Following baseline scans, animals in the QP condition (n = 8; Chronic QP) received twice a week a subcutaneous injection of QP (0.5 mg/kg dissolved in saline to achieve an injection volume of 1 mL/kg) for a total of 14 injections. Animals in the CTRL condition (n = 8; Chronic SAL) were similarly injected each time with saline (1 mL/kg). Fifteen minutes after each injection, animals underwent an Open Field Test (OFT) whereby the animal was video