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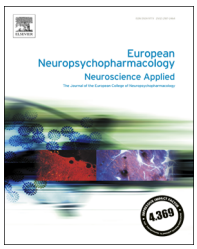

Correlating behaviour and gene expression endpoints in the dopaminergic system after modafinil administration in mouse

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Abstract
The mechanisms of action of modafinil continue to be poorly characterised and its potential for abuse in preclinical models remains controverted. The aim of this study was to further elucidate the mechanism of action of modafinil, through a potential behavioural and molecular association in the mouse. A conditioned place preference (CPP) paradigm was implemented to investigate the rewarding properties of modafinil. Whole genome expression and qRT-PCR analysis were performed on the ventral tegmental area (VTA), nucleus accumbens (NAC) and prefrontal cortex (PFC) of modafinil-treated and control animals. Modafinil administration (65 mg/kg) induced an increase in locomotor activity, an increase in the change of preference for the drug paired side after a conditioning period as well as changes to gene expression profiles in the VTA (120 genes), NAC (23 genes) and PFC (19 genes). A molecular signature consisting of twelve up-regulated genes was identified as common to the three brain regions. Multiple linear correlation analysis showed a strong correlation ($R^2 > 0.70$) between the behavioural and molecular endpoints in the three brain regions. We show that modafinil had a concomitant effect on CPP, locomotor activity, and up-regulation of interferon-γ (IFN-γ) regulated genes (Gbp2, Gbp3, Gbp10, Cd274, Igtp), while correlating the latter set of genes with behaviour changes evaluated through the CPP. A potential association can be proposed

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based on the dysregulation of p47 family genes and Gbp family of IFN-γ induced GTPases. In conclusion, these findings suggest a link between the behavioural and molecular events in the context of modafinil administration.

1. Introduction

Modafinil (2-diphenyl-methyl-sulphinyl-2-acetamide) is marketed to treat narcolepsy, used as a cognitive enhancer and in the treatment of substance-use disorders (Brady and Gray et al., 2011). The structure, sites of action, neurochemical and behavioural effects of modafinil differ to classical psychostimulants such as cocaine and amphetamine. There is increasing evidence that dopamine (DA) and the mesocortico-limbic pathways are involved in modafinil stimulatory effects (Nguyen and Tian et al., 2011). Modafinil has been demonstrated to bind directly to the DA transporter (DAT) and equilibrative nucleoside transporter (ENT) (Wisor and Eriksson 2005; Madras et al., 2006). However, *in-vitro* studies have shown that modafinil only exhibits a low affinity for the DAT, lower than that of other psychostimulants (Zolkowska et al., 2009; Schmitt and Reith, 2011, Loland et al., 2012). It has also been demonstrated that modafinil does not increase waking in DAT-deficient mice (Wisor, Nishino et al., 2001). *In-vivo* studies suggest that modafinil acts upon noradrenergic transmission to promote waking as adrenergic stimulation has been shown to be attenuated by α1 or β-adrenergic antagonists (Wisor and Eriksson, 2005). Additionally modafinil inhibits both catecholamine transporters at clinical doses and may enhance the levels of glutamate, promote serotonin release, activation of orexinergic neurons and promote gamma-aminobutyric acid (GABA) release (Ferraro et al., 1999, Scammell et al., 2000, Ferraro et al., 2002).

Psychostimulants are known to provoke addiction, however whether modafinil produces drug abuse liability in preclinical models is controverted. Contradictory data has shown that modafinil may be able to produce place preference at high doses (64–300 mg/kg) in mice (Nguyen et al., 2011; Wuo-Silva et al., 2011), consistent with its action upon the dopaminergic pathways; may have very low, if any, liability (Mitler et al., 2000; Roth et al., 2007); or that modafinil could act as a reinforcer to drug-experienced animals (Gold and Balster 1996, Deroche-Gamonet, Darnaudery et al. 2002). In contrast, modafinil has been shown to have little or no abuse liability in clinical studies. (Hart et al., 2008; Vosburg et al., 2010; Dackis et al., 2012, O'Brien, 2012). Due to the complex nature of addiction in humans, animal models cannot fully reproduce the development of drug use into addiction. Conditioned place preference (CPP) has been shown to successfully model drug-induced reward and has been widely used to assess the addictive properties of an extensive number of drugs of abuse, such as cocaine, opiates, and amphetamine (Mucha et al., 1982). Although drugs of abuse have various mechanisms of action, the common underlying relationship between brain region targeting and dysregulation of neuronal circuitry determines their potential for abuse and development of addiction.

There is a growing interest in correlating behavioural phenotypes with molecular events which occur in various brain regions. In the context of cocaine administration, studies have shown that the increased response to reward and increased motivation for cocaine is due to the amplified induction of DeltaFosB (Nestler, 2008). Cocaine administration also induces CREB activity, reducing sensitivity to drug-related reward and mediating the negative symptoms of drug withdrawal (McClung and Nestler, 2003). Studies evaluating if the effects that occur at a molecular level, after modafinil administration, account for alterations at a pharmacological and behavioural levels are scarce.

The aim of this study was two-fold. On the one hand to evaluate the behavioural response to modafinil in the mouse through the measurement of locomotor activity and the use of CPP and, on the other hand, to conduct molecular profiling, at the mRNA level, to identify expression regulation in the ventral tegmental area (VTA), nucleus accumbens (NAC) and prefrontal cortex (PFC) that could be associated with the behavioural endpoints. We show that modafinil increases locomotor activity, induces a preference for the drug-administrated place after a conditioning period of 8 days, and a concomitant up-regulation of Interferon gamma (IFN-γ) regulated genes – T cell specific GTPase 1 (Tgtp1), Interferon inducible GTPase 1 (Iigp1), Interferon gamma inducible protein 47 (Ifi47), Interferon gamma induced GTPase (Igtp), Guanylate binding protein 2 (Gbp2), Guanylate binding protein 3 (Gbp3), Guanylate binding protein 7 (Gbp7), Guanylate binding protein 10 (Gbp10), Immunity-related GTPase family M member 2 (Irgm2), Cd274, Lymphocyte antigen 6 complex locus A (Ly6a), Xanthine dehydrogenase (Xdh) – in the VTA, NAC, and PFC. Furthermore, correlation analysis suggests a link between the behavioural and molecular events in the context of modafinil administration.

2. Experimental procedures

2.1. Animals

All procedures described in this study have been approved by the ethical committee for animal experimentation of UCB Biopharma SPRL and were in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purpose and with the Belgian legislation on the use of laboratory animals. Male C57Bl/6J mice (Janvier, France) weighing 20–26 g were used. Animals were housed in groups of 3–5 per cage under standard laboratory conditions with access to food and water ad libitum. The housing facility was kept on a 12 h light/dark cycle, with lights on at 06:00 A.M. All procedures were performed in the light phase of the cycle.