A microdialysis and behavioural investigation of modafinil in freely-moving rats
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INTRODUCTION
Modafinil (Provigil®) is an unusual stimulant that is widely used to treat narcolepsy in USA and Europe. It has an enigmatic mechanism of action (see reviews by Minzenberg & Carter, 2008; Heal et al, 2012). Modafinil has stimulant-like effects in humans and animals and its pharmacology is different from the catecholaminergic stimulants d-amphetamine and methylphenidate. Although modafinil has low micromolar affinity for DAT (Zolowska et al, 2006; Madsen et al, 2006) and no affinity for NET or SERT (Madsen et al, 2006; Minzenberg & Carter, 2008), positron emission tomography (PET) experiments have paradoxically revealed that therapeutic doses of modafinil occupy a substantial proportion of striatal DAT sites in the brains of humans (Volkow et al, 2009). Modafinil also decreased d-CTx binding (Volkow et al, 2009), indicating occupancy of DAT sites resulted in increased synaptic concentrations of dopamine.

METHODS
Experiments were carried out in male Sprague-Dawley rats (250-350g; Charles River). Two concentric microdialysis probes were stereotaxically implanted under isoflurane anesthesia into A) the PFC (2 mm tip, coordinates: AP: +3.2 mm; L: +/- 2.5 mm relative to bregma; V: -4.0 mm relative to the skull surface) and B) the striatum (4 mm tip, coordinates: AP: +0.2 mm; L: +/- 3.0 mm; V: -7.8 mm) according to the atlas of Paxinos and Watson (2007). Following surgery, rats were individually housed in the Culex Bambino (BASi, West Lafayette IN) dialysis bowls and allowed to recover for at least 16 hr with ad libitum food and water. Probes were continuously perfused with artificial cerebrospinal fluid (ACSF) (sodium 150 mM; potassium 3 mM; magnesium 0.8 mM; calcium 1.4 mM; phosphate 1.0 mM; chloride 155 mM) at a flow rate = 1.2 µl/min. On the day of the experiment, dialysate samples were collected from freely-moving rats into vials containing 5.0 µl of 0.1 M perchloric acid at 15 min intervals from 60 min before until 5 hr after drug administration (4 basal samples, 20 post-drug samples). Measurement of noradrenaline, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT and 5-hydroxyindolacetic acid (5-HIAA) in dialysis samples was by reverse-phase, ion-pair HPLC coupled with electrochemical detection using an ALEXYS monoamine analyzer (Antec, Leyden).

Data are the AUC (% baseline) (n=5-6) in the extracellular concentrations of NA, DA and 5-HT that were statistically significant at the time point indicated. Results were analysed by ANCOVA with each dose of drug compared to vehicle-treated control group by Williams’ test. Significant differences are denoted by *p<0.05.

RESULTS
In the PFC, modafinil (300 and 600 mg/kg po) significantly increased the extracellular concentrations of noradrenaline (Figure 1A) and dopamine (Figure 1B). These effects were most pronounced in the first 2.0 hr after administration and supported by AUC analysis (Figure 1C). This stimulant was without effect at the lowest dose of 100 mg/kg. Modafinil (100 - 600 mg/kg po) did not alter 5-HT efflux in the PFC (Figure 1C).

• In the striatum, modafinil (300 and 600 mg/kg po) increased dopamine efflux (Figure 2A,B). The increases were rapid in onset and lasted for approximately 1 hr and were approximately equal in magnitude at both doses (Figure 2A,B). Modafinil was without effect at the lowest dose of 100 mg/kg (Figures 2A, B).

• Modafinil (100 - 600 mg/kg po) did not alter striatal 5-HT efflux (Figure 2B).

• Modafinil (100 - 600 mg/kg po) produced moderate dose-dependent increases in the locomotor activity of the rats (Figure 3). However, the locomotor activity evoked by modafinil was not significantly correlated with the increase in extracellular dopamine in the striatum (r² = 0.024 at 300 mg/kg; r² = 0.002 at 600 mg/kg).

CONCLUSIONS
• The results revealed that the lowest dose of 100 mg/kg of modafinil was at the threshold of pharmacological effect. Higher doses of modafinil increased the extracellular concentrations of noradrenaline and dopamine in PFC and dopamine in the striatum.

• Although the magnitude of modafinil’s effect on extraneuronal catecholamine concentrations in the PFC is similar to that observed with lidocamphetamine or methylphenidate (Rowley et al, 2012), the increase in striatal dopamine efflux was much smaller.

• Modafinil has a much greater ability to enhance catecholaminergic function in the PFC than dopaminergic neurotransmission in the striatum, and in addition, that another as yet unknown neurochemical mediator may be the primary driver of modafinil’s stimulant actions help to explain why modafinil has an unusual pharmacological profile.