Demonstration of the relationship between in vivo dopamine efflux, behaviour and drug pharmacokinetics

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INTRODUCTION
One of the major challenges in studying the actions of drugs is to define how their neurochemical effects translate into functional outcomes. This poster describes how we have attempted to achieve this objective by:
1. Performing carefully controlled in vivo microdialysis and behavioural experiments in parallel groups of rats
2. Employing the Culex Bambino (BASI) that permits automated microdialysis in freely-moving rats coupled with independently programmable blood sampling and behavioural monitoring (Raturn® system) in the same animals.

MATERIALS AND METHODS
All studies were performed on male Sprague-Dawley rats (250-300g). Surgery, microdialysis in freely-moving rats (n=11) and measurement of dopamine (DA) in the dialysate samples were performed as described in detail by Rowley et al (2000)1. Locomotor activity was measured using single rats (n=8) from a parallel group of animals with an automated photo-beam interruption system as described by Rowley et al (2000)1.

For experiments with the Culex Bambino system, animals were anaesthetised using isoflurane (5% to induce, 2% to maintain) in an O₂/N₂O (1 litre/min each) mixture. A concentric dialysis probe with a 2mm membrane tip was stereotaxically implanted either into the nucleus accumbens (coordinates AP: +2.2mm; L: -1.5mm relative to bregma; V: -8.0mm relative to the skull surface) or into the prefrontal cortex (PFC) (coordinates: AP: +3.2 mm; L: +/–2.5 mm; V: –4.0 mm). During the same surgery, a catheter (BASI, USA) was implanted into the jugular vein. Rats were implanted in the Culex Bambino for overnight recovery from surgery for at least 16 h with food and water available ad libitum. The dialysis probe was continuously perfused with aCSF (Harvard, UK) at a flow rate of 1.2 µl/min. The patency of the jugular vein catheter was maintained automatically by flushing through with periodic small pulses of sterile saline/heparin (10 U/ml). The next day, following automated collection of four basal dialysate and blood samples, rats were administered d-amphetamine (1.5 mg/kg ip, n=4) or olanzapine (1 mg/kg ip, n=3) and dialysates automatically collected every 20 min for a further 4 h. Blood samples (150 µl) for the olanzapine study were collected automatically every 20 min for the duration of the experiment. Dialysate samples were analysed for DA content by HPLC with EC detection. Blood samples were centrifuged to provide plasma samples which were analysed for olanzapine by HPLC-MS/MS and corticosterone by enzyme immunosassay (BASI UK).

REFERENCES

RESULTS
Experiment 1: d-Amphetamine profoundly increased DA efflux in the nucleus accumbens (Fig.1). Typical of releasing agents, its effects were rapid in onset and of relatively short duration. The locomotor response to d-amphetamine measured in a parallel group of rats showed a similar profile (Fig. 2). There was an excellent correlation between the magnitude of DA efflux and locomotor activity (Fig. 3).

Experiment 2: The Culex Bambino (Fig. 4) - the validation study shown in Fig. 5 revealed that d-amphetamine evoked exactly the same effect on nucleus accumbens DA efflux as reported by Rowley et al (2000)1. Although it appeared to be somewhat less sensitive than photo-beam interruption measurements, automated behavioural monitoring data obtained with the Raturn® system were similar (Fig. 6).

Experiment 3: The effects of olanzapine administration on the extracellular level of DA in PFC and plasma [olanzapine] in rat PFC and plasma [olanzapine] (Fig. 7). Automated blood sampling was a stress-free procedure as shown by the plasma corticosterone measurements (Fig. 8). When the data for the group of 3 rats were analysed, there was an excellent correlation between the change in DA efflux and plasma olanzapine concentrations (Fig. 9).

SUMMARY
- In vivo microdialysis is a valuable tool to study the actions of drugs on neurotransmitters in the CNS.
- The value of drug effects on extracellular concentrations of neurotransmitters is greatly enhanced if they can be effectively linked to a functional outcome, e.g. locomotor activity.
- The Culex Bambino provides the additional benefits of being able to combine microdialysis and behavioural monitoring in the same animal together with blood sampling to correlate neurotransmitter and behavioural changes with drug pharmacokinetics.