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Intracerebral Dialysis Coupled to a Novel Activity Box—A Method to Monitor Dopamine Release During Behaviour

T. SHARP, T. LJUNGBERG, T. ZETTERSTRÖM AND U. UNGERSTEDT

Department of Pharmacology, Karolinska Institutet, P.O. Box 60 400, S-104 01 Stockholm, Sweden

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SHARP, T., T. LJUNGBERG, T. ZETTERSTRÖM AND U. UNGERSTEDT. *Intracerebral dialysis coupled to a novel activity box—A method to monitor dopamine release during behaviour.* PHARMACOL BIOCHEM BEHAV 24(6) 1755–1759, 1986.—This paper describes a method for monitoring drug-induced changes in brain dopamine (DA) release and metabolism in the awake rat simultaneous to measurements of behavioural activation. Intracerebral dialysis was combined with a novel activity box based on a circular running track and a simple method for perfusate collection. "Locomotor" and "general" activity were automatically monitored using a system of photobeams lining the track, and stereotyped behaviours were scored by direct observation. Striatal perfusates were analysed for endogenous DA and its main metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), using HPLC with electrochemical detection. This methodology was tested by comparing the behavioural effects of systemically administered amphetamine (2 mg/kg SC) and its central DA releasing action. Amphetamine caused a marked increase in striatal DA release (16-fold) which was followed over the time course by increased locomotion and stereotyped sniffing and head and forepaw movements. The limitations and wider applications of the methodology are discussed.

Amphetamine DA-release Intracerebral dialysis Locomotor activity Stereotyped behaviour

BEHAVIOURAL change induced by pharmacological manipulation of brain dopamine (DA) systems is commonly monitored automatically using one of a variety of activity measuring devices (see e.g., [11,20]). Alternatively, behaviour is directly observed and scored using semiquantitative rating scales [19]. These behavioural measurements are usually complemented by separate biochemical experiments to determine more fully the mechanism of drug action on central DA neurons. Clearly, it would be of great value to directly monitor DA release in brain regions of the awake animal concomittant with measurements of the ongoing behaviour.

Various techniques have been used to monitor DA release in the animal brain *in vivo*. These include perfusion methods in which perfusates are collected from brain tissue using, for example, push-pull cannulae [4,5] or cortical cups [2] and analysed for DA or its metabolites. DA metabolites can also be measured in perfusates or samples taken from the cerebrospinal fluid [7,8]. An alternative and more recent approach is *in vivo* voltammetry which involves the recording of catechols at the tip of an implanted microelectrode [1, 6, 21]. However, all of these techniques have been hampered by the low levels of extracellular DA in the brain. This problem can be circumvented by, for example, monitoring the effux of preloaded, radiolabelled DA or detecting the comparatively high extracellular levels of DA metabolites but neither of these types of measurements may truly reflect changes in release of endogenous DA which is crucial when making comparisons with behaviour.

Intracerebral dialysis is a novel brain perfusion method which, in combination with sensitive HPLC assays, allows the continuous measurement of extracellular levels of DA and its metabolites in rat brain [9, 24, 25, 27]. The dialysis method has been previously used with simple methodology to quantify certain motor behaviours, catalepsy and rotational behaviour, concomittant with DA release measurements [28,29].

In the present paper we present further developments of the dialysis technique to enable a more detailed analysis of drug-induced behavioural activation in animals during measurement of brain DA release and metabolism. This has been achieved using both an improved method for collection of the perfusate and an activity measuring device designed to handle the particular problems that arise with freely moving animals connected to a perfusion system. The set-up was tested using amphetamine, a drug whose complex behavioural effects have long been associated with increased DA release in the brain (see [10,18] for review).

METHOD

Animals

Experiments were performed on male Sprague-Dawley rats (Alab, Stockholm) with a body weight of 280–320 g at the time of surgery. The animals arrived at least one week prior to the experiments and were housed individually in standard macrolon cages with food pellets and tap water ad lib under conditions of controlled humidity and temperature and on a

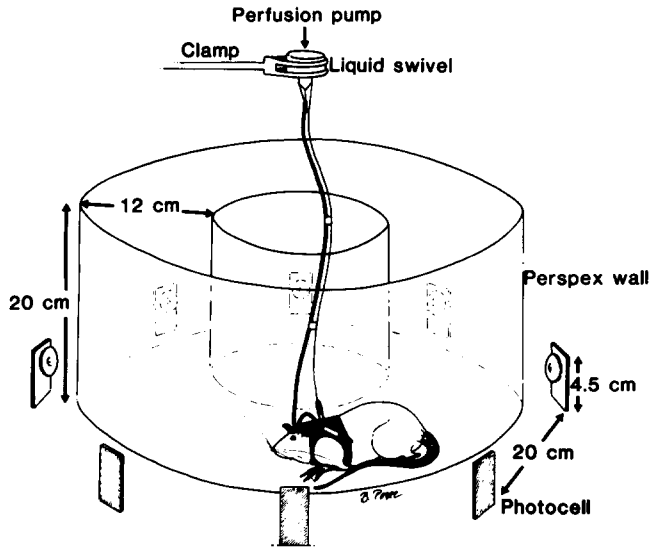

FIG. 1. Scaled drawing of the activity box used for behaviour/release experiments. The running track is enclosed within a sound-resistant, air-conditioned chamber (not shown). See text for details of construction.

12 hour light/dark cycle (lights on 6 a.m.). All animals were used only once.

Preparation and Implantation of the Dialysis Probe

Dialysis probes, consisting of short (2 mm) loops of cellulose dialysis membrane (DOW, 300 μm diameter, m.w. cut off 5000) were prepared as previously described [21,27]. Briefly, a 3 mm length of dialysis membrane glued between two stainless steel cannulae was wetted by connecting the inlet cannula to a microinfusion system and folded into a loop. The cannulae were clamped in a stereotaxic holder and a fine tungsten wire was temporarily inserted into the outlet cannula and used to extend the loop to a point during implantation.

For implantation the rats were anaesthetized with a 1.5% halothane/air mixture and mounted in a David Kopf stereotaxic frame. Dialysis probes were slowly implanted into the right striatum using the coordinates; rostral +2.5 mm, lateral +2.5 mm, ventral −6.5 mm from bregma and dura surface according to Pellegrino and Cushman [17]. The probe was secured using skull screws and dental cement, the latter also serving to seal the opened skin. Following surgery (typically 25–30 min) the ends of the loop were temporarily sealed and animals were returned to their home cage with free access to food and water.

Activity Box

The activity box design was based around a circular running track which was contained within a sound-resistant, air-conditioned chamber. The track (12 cm across) was constructed from an outer (44 cm diameter × 20 cm high) and inner (20 cm diameter × 20 cm high) ring of Plexiglas (4 mm thick) based on a hard PVC plastic base (see Fig. 1). The Plexiglas rings were held in place by supports positioned at intervals on the inside of the inner rings and the outside of the outer ring. This arrangement allowed the rings to be easily removed for cleaning.

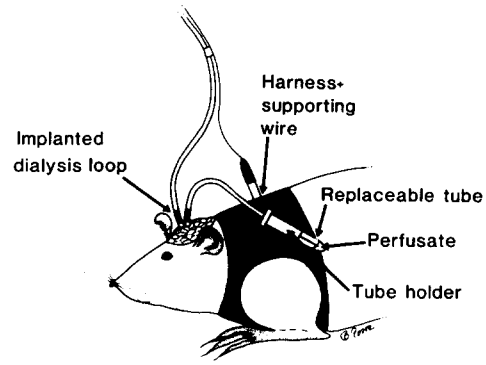

FIG. 2. Illustration of the animal harness which serves both to secure the animal within the confines of the running track and as a holder for replaceable perfusate collection tubes.

The outer chamber was made from 1 cm chip-board (75×65×60 cm) and had a hinged door on one side to allow easy access to the running track. A Plexiglas window was positioned in the door to allow direct observation of the animal. Holes were cut into the top of the chamber for the inlet perfusion tubing and for the air conditioning system. The running track was dimly lit by two 24 V, 2.5 watts, bulbs mounted overhead.

Activity Measurement and Scoring Procedure

Four horizontal photobeams, each placed 4.5 cm above the floor, symmetrically covered the running track (see Fig. 1). "General activity" was defined as each photobeam interruption. "Locomotor" counts were obtained when 2 of the beams, set at 90°, were consecutively broken. This corresponded to a distance of 25 cm travelled by the animal. The "general activity" and "locomotion" counts were displayed on a digital readout counter.

The intensity of two types of amphetamine-induced behaviour, sniffing and repetetive head and forelimb movements, were scored separately by direct observation according to the following 0–3 scales:

Sniffing: 0=as controls, 1=interrupted whisker and snout movements, 2=continuous whisker and snout movements with snout aimed at cage bottom, 3=intense continuous whisker and snout movements.

Head and forepaw movements: 0=as controls, 1=bursts of repetitive up and down head movements and shuffling of the forepaws, 2=continuous repetitive head and forepaw movements, 3=continuous repetitive head and forepaw movements of high intensity.

Scores were assigned following 1 min observation at 20 min intervals (i.e., the 1 min period preceding the change of the collecting tubes, see below).

Dialysis Procedure

The animals were connected to a microinfusion system placed outside the behavioural chamber via a liquid swivel (Carnegie Medicin AB, Solna, Stockholm) held above the track and polyethylene tubing (PE-20). Implanted dialysis probes were continually perfused (2 μl/min) with Ringer solution using an accurate slow infusion pump (CMA/100, Carnegie Medicin AB, Solna, Stockholm). Animals were secured in a specially prepared harness (Fig. 2) connected by a thin and flexible wire to the swivel. The harness, which was

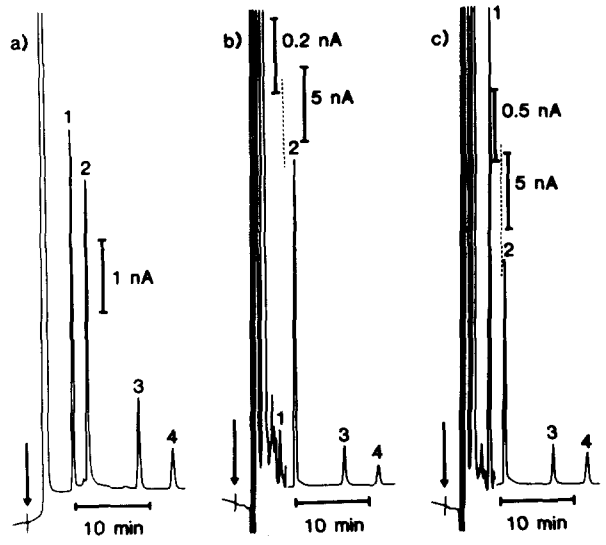

FIG. 3. HPLC separation of DA and monoamine metabolites in a 5 pmole standard solution (a), and in perfusate collected from striatum of an awake rat before (b) and 20–40 min after (c) 2 mg/kg amphetamine. Peak 1=DA, 2=DOPAC, 3=5-HIAA, and 4=HVA. Chromatographic conditions were as outlined in the text. Output current from the detector is indicated by the vertical bars. The pen recorder was set on 1 V full scale deflection. Sensitivity changes are indicated by the vertical dotted line.

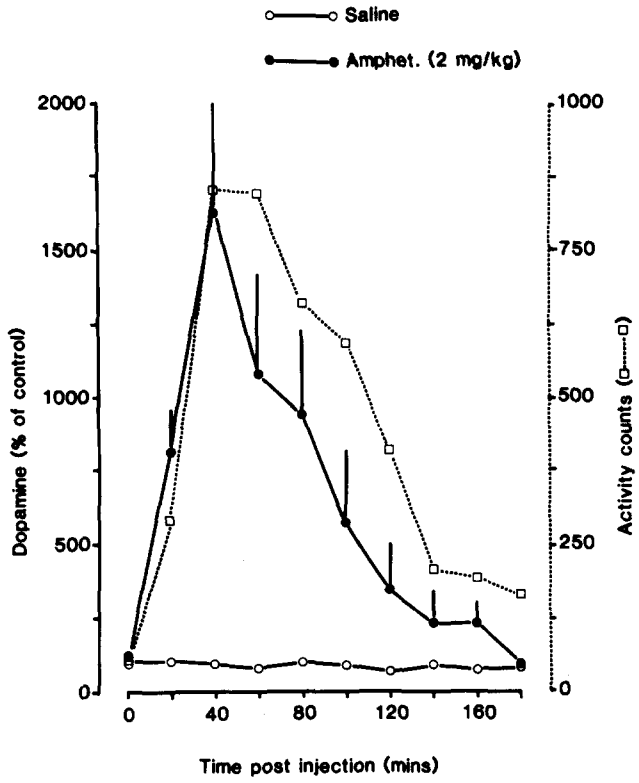

FIG. 4. Time course change of DA in striatal perfusates collected from the awake rat compared with "general" activity counts following amphetamine 2 mg/kg (n=6) or saline vehicle (n=5). Median general activity counts for saline treated controls were 60 per 20 min of the post injection period (n=5).

made out of light-weight nylon material, also served as a holder for the replaceable perfusate collecting tubes. Perfusates were collected every 20 min in miniature Eppendorf tubes (400 μl) containing 10 μl 1 M perchloric acid.

Monoamine Assay

Perfusate samples were assayed for DA, its main metabolites DOPAC and HVA, and the serotonin metabolite 5-HIAA, in a single run using HPLC with electrochemical detection. No sample preparation was necessary. The monoamines were separated on a Spherisorb 5 ODS column (250×4.6 mm) using 0.15 M sodium phosphate mobile phase containing 0.5 mM sodium octane sulphonic acid, 0.1 mM EDTA and 12% methanol (final pH 3.8), and detected by either a carbon paste or glassy carbon working electrode set at +0.65 V. Further details of this assay are described elsewhere [22]. Using a 1.2 ml/min flow-rate samples were analysed within 20 min (see Fig. 3) and could be run immediately following collection.

Experimental Protocol

The rats were handled and familiarized with the behavioural chamber on four separate occasions before implantation of the dialysis probe. Each habituation period was of 1 hour duration, with the final two sessions including the harness. Dialysis experiments were carried out on the day following surgery (i.e., 18–24 hours after loop implantation). Usually 4–5 control samples were taken before administration of amphetamine (2 mg/kg SC) or saline vehicle and then measurements were continued for 3 hours.

Drugs

d-Amphetamine sulphate (Sigma) was dissolved in saline and injected (2 mg/kg SC) in a volume of 1 ml/kg body weight. The dose refers to the base. Control animals were injected with saline only.

Data Analysis

The DA, DOPAC and HVA content in perfusates are expressed as a % of the final 2–3 stable baseline values before drug or vehicle injection and presented as means with S.E.M. (Fig. 4). General activity (Fig. 4) and locomotion counts (Fig. 5) are presented as group medians while sniffing and stereotypy ratings are presented as group total scores (Fig. 5).

RESULTS AND DISCUSSION

The present paper describes the application of intracerebral dialysis in combination with a specially designed activity box to monitor DA release and metabolism in the brain of the freely moving rat. The main feature of the activity box is a circular running track with a centrally positioned liquid swivel. This construction has the advantage over an open field design in that the animal is able to move freely within the confines of the track without stretching or becoming entangled with the perfusion tubing. Furthermore, an accurate measure of the distance moved by the animal is simply provided by a system of horizontal photobeams aimed across the track. This method of detection of locomotor activity is principally the same as for the "holeboard-

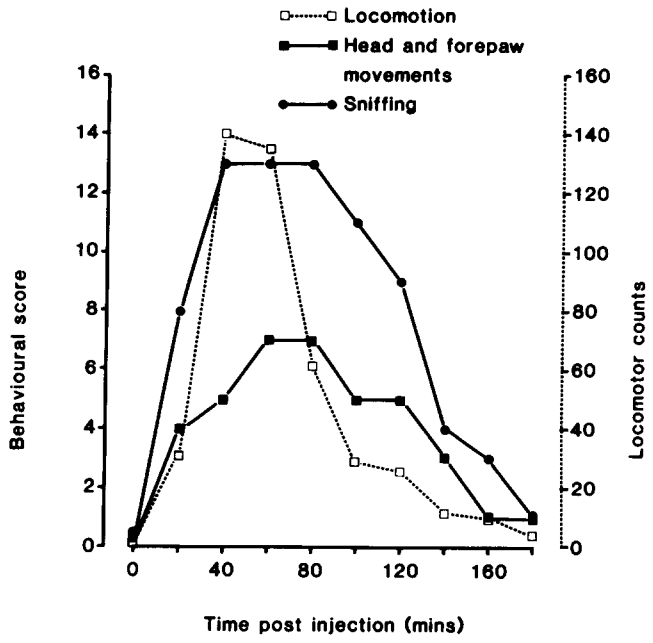

FIG. 5. Time course change in "locomotor" counts, repetitive head and forepaw movements and sniffing after amphetamine (2 mg/kg). This data was obtained from rats implanted with dialysis probes whose striatal DA release measurements are shown in Fig. 4. Median locomotor counts for the saline treated controls were 1 per 20 min of the post injection period.

apparatus" that we have been using in our laboratory for several years and which has been shown to provide a valid measure of locomotion [12–16, 23]. The automated activity recordings are complemented by direct observation and scoring of behaviour using semiquantitative rating scales. These scales are based on the idea that the intensity of different components of stereotyped behaviour should be determined separately rather than by the more common approach which scores the intensity of the general pattern of stereotyped behaviour (see discussion in [19]).

The simple method of collecting brain perfusates over 20 min periods in tubes mounted on the animal securing harness involves little disturbance to the animal. Perfusate tubes can be changed within seconds by an experienced hand without touching the animal directly. The constant interruption of the animal involved in more frequent sampling may necessitate sample collection outside the activity box although this approach would involve a large dead volume in the system and require a dual channel liquid swivel which is technically difficult to make.

A reversed-phase ion-pair HPLC system coupled with an electrochemical detector was used to analyse the monoamine content of striatal perfusates. This has previously been used to detect DA and its main metabolites DOPAC and HVA in dialysates collected from brain regions of anaesthetized rats [22]. In awake rats basal levels of DA, DOPAC and HVA in striatal perfusates were respectively 0.18±0.03 (11) (mean±SEM, (N)), 38.56±6.16 (11) and 27.06±4.12 (11) pmoles/20 min perfusate. These measurements obtained 18–24 hr following dialysis probe implantation compare well with those obtained in awake acutely operated rats but the amount of monoamine recovered declines significantly 48 hr post implantation, and 5 days after surgery DA responds poorly to amphetamine stimulation (Zetterström *et al.*, unpublished data). This latter finding is probably due to an increased gliosis around the dialysis probe which prevents molecular diffusion through the dialysis membrane. The problem of gliosis is likely to confront all methods using implanted probes to measure extracellular DA in brain regions over an extended period although not those techniques detecting monoamines in the CSF.

Whilst dialysis experiments on awake rats are restricted to a relatively short period of time after surgery, with sufficient habituation to the experimental environment brain perfused animals show spontaneous behaviour and are able to perform complicated tasks. This is evident from observations of spontaneous and drug-induced activity in rats in this study together with our finding that water deprived rats are able to perform various types of operant responses to obtain water reward whilst undergoing brain dialysis (unpublished observations).

After amphetamine (2 mg/kg SC) there was a marked increase in dopamine in striatal dialysates (Fig. 4, see also [27,29]). This effect was maximal (+1629%, n=6) during the collection period 20–40 min post drug and reduced to control levels within 3 hr. The time course change in DA release was followed by an increase in "general activity" as indicated by increased photobeam interruptions (Fig. 4). These time course measurements are further evidence in support of the generally held hypothesis that the behavioural effects of amphetamine are due to increased DA release in the brain. The present data complements our previous study showing that increased striatal DA release is closely associated with amphetamine-induced circling in unilaterally 6OHDA-lesioned rats [29].

Individual components of behaviour, increased locomotor activity (photobeam interruptions), sniffing (direct observation) and repetitive head and forepaw movements (direct observation) showed a similar duration to, but did not closely fit, the DA release profile (Fig. 5). In particular, repetitive head and forepaw movements as well as sniffing showed delayed responses relative to the peak release of striatal DA. Whilst this observation could at least partly reflect an artifact of the various methods of measurement, it might indicate that striatal DA release triggers certain of these behaviours, for example stereotypy, but does not maintain them.

The dose of amphetamine, 2 mg/kg, used in this study was chosen to provide a repertoire of behaviours for analysis and to induce a robust behavioural stimulation which would test the durability of the equipment. We have subsequently tested the effect of a lower dose of amphetamine (0.5 mg/kg) and the system was sufficently sensitive to detect a four-fold increase in release of DA in striatum as well as a stimulation of locomotor activity and stereotyped behaviour.

In contrast to DA, the DA metabolites DOPAC and HVA declined after amphetamine (DOPAC −71%, HVA −47% 100 min post drug, time course data not shown) in accordance with previous whole tissue and brain dialysis studies [26,27]. This result emphasizes the need to monitor DA release as well as metabolism when studying the relationship between drug-induced behaviour and dopaminergic neurotransmission *in vivo*. This point is furthered by our recent study showing that changes in DA release poorly correlate with alterations in DA metabolism after neuroleptic drugs [28]. However, continuous monitoring of extracellular levels

of monoamine metabolites offers a valuable means to study drug action in the brain, particularly when detected simultaneously to DA itself, but such measurements are probably more useful in behavioural studies under non-drug conditions when metabolite levels might give a better index of changes in dopaminergic activity [3].

In summary, this paper presents a novel activity box which allows drug-induced locomotion and stereotyped behaviours to be monitored during intracerebral dialysis in awake rats. This apparatus complements that outlined in our previous dialysis study in which DA release and rotational behaviour were monitored simultaneously in awake rats using a "rotometer" equipped with a liquid swivel [29]. In view of the versatility of the dialysis technique (see [25]) the present methodology could well be applied to experiments in which changes in amino acid, peptide or purine neurotransmitter substances, in addition to DA, need to be followed during drug-induced behavioural change.

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