

PET study of the [¹¹C]raclopride binding in the striatum of the awake cat: effects of anaesthetics and role of cerebral blood flow

Wadad Hassoun¹, Marion Le Cavorsin², Nathalie Ginovart¹, Luc Zimmer¹, Veronique Gualda¹, Frederic Bonnefoi¹, Vincent Leviel²

¹ CERMEP, Lyon, France

² CNRS UMR5123, Bat R.Dubois, 8 rue Dubois; 43 bd du 11 nov. 1918, 69622 Villeurbanne, France

Published online: 17 September 2002

© Springer-Verlag 2002

Abstract. Cats were trained to stay in a containment box, without developing any signs of behavioural stress, while their head was maintained in a position that allowed positron emission tomography (PET) experiments to be performed. The binding potential for [¹¹C]raclopride (BP_{raclo}), a radioligand with good specificity for dopamine (DA) receptors of the D₂ type, was measured in the striatum and in three experimental situations: awake, anaesthetised with ketamine (50 mg kg⁻¹ h⁻¹; i.m.) and anaesthetised with halothane (1.5%). Non-specific binding was evaluated in the cerebellum. In the striatum of both sides, the BP_{raclo} was unmodified by ketamine anaesthesia when compared with awake animals. In contrast, a large increase in BP_{raclo} was observed under halothane anaesthesia. The non-specific binding of [¹¹C]raclopride, evaluated in the cerebellum, was also unchanged under ketamine anaesthesia but greatly increased under halothane anaesthesia. To evaluate whether changes in the cerebral blood flow (CBF) resulting from the different experimental situations could be at the root of these discrepancies, injections of [¹⁵O]H₂O were performed; measurements revealed a drastically increased CBF under halothane anaesthesia and a slight enhancement under ketamine anaesthesia, when compared with the waking state. These results are the first to be obtained on this topic in awake cats, and show that the BP_{raclo} is greatly dependent on alterations in the CBF.

Keywords: Positron emission tomography – D₂ receptors – Animal – Anaesthesia

Eur J Nucl Med (2003) 30:141–148

DOI 10.1007/s00259-002-0904-4

Vincent Leviel (✉)

CNRS UMR5123, Bat R.Dubois, 8 rue Dubois;
43 bd du 11 nov. 1918, 69622 Villeurbanne, France
e-mail: leviel@univ-lyon1.fr

Tel.: +33-472445840, Fax: +33-472431172

Introduction

Imaging techniques such as positron emission tomography (PET) or single-photon emission tomography (SPET) have been used over the past decade to evaluate acute fluctuations of neurotransmitter concentrations in the extracellular space [1, 2, 3]. This constitutes an important technological advance, allowing a non-invasive approach to assessment of the release of these substances, and permitting longitudinal studies in animals and humans. The method is based on competition for binding to receptors between the endogenous transmitter and an exogenous radioligand [4, 5]. Indeed, the binding potential (BP) of a radioligand is sensitive to the extracellular concentration of the corresponding neurotransmitter, the release of which may thus be quantified through the corresponding displacement of the pre-loaded radioligand [6, 7]. Such quantification has already been modelled using data obtained from human studies [8]. The use of this technique has also been confirmed in animals, by means of simultaneous evaluation of extracellular neurotransmitter concentrations with invasive techniques such as microdialysis [9, 10, 11].

Two main elements of this method are, however, subject to rapid changes that can produce bias in the observations: first, the affinity of the receptors and, second, the disposability of the radioligand delivered by the cerebral circulation. These alterations may be produced by the experimental conditions themselves, such as anaesthesia, waking states, stress etc. The BP of [¹¹C]raclopride (BP_{raclo}), a radioligand antagonist of dopamine (DA) receptors of the D₂ type, exemplifies this technical problem. In animals, a moderate reduction in BP_{raclo} was observed after amphetamine administration (2 mg kg⁻¹) under ketamine anaesthesia [12, 13] but amphetamine administration had no effect on BP_{raclo} under halothane anaesthesia [14]. However, amphetamine is known to produce a dramatic release of DA in these conditions [15, 16]. Conversely, in the urethane-anaesthetised rat,

complete displacement of the radioligand [^{11}C]raclopride was obtained by amphetamine [17]. It was therefore suggested, in the light of biochemical studies [18, 19], that this effect could be due to an anaesthesia-induced reduction in the affinity for DA receptors, as confirmed by an increase in the dissociation constant for DA receptors, measured under halothane anaesthesia [14].

Radioligand disposability must also be carefully considered in studies of neurotransmitter release using PET or other imaging methods. The disposability of the radioligand is a consequence of both the extracerebral metabolism and the amount of molecules delivered by the cerebral blood flow (CBF). When radioligands were administered at very low concentrations and under non-pharmacological conditions, alterations produced by changes in CBF were initially considered negligible, but recent experiments have suggested that this problem needs to be carefully addressed [7], and prompted the present study. To this end, two different conditions of anaesthesia (1.5% halothane, 50 mg kg $^{-1}$ h $^{-1}$ ketamine) were considered in cats, these conditions being chosen for their known ability to alter BP $_{\text{raclo}}$ [10, 14]. The values found for the BP $_{\text{raclo}}$ were compared with those found in awake animals and correlations between these changes and the CBF measured with the use of [^{15}O]H $_2\text{O}$ were sought. An original experimental set-up was developed to allow PET scans on awake cats for comparison with anaesthetic situations and during test-retest experiments performed on the same day. Possible CBF changes were measured, taking advantage of the possible use of the [^{15}O]H $_2\text{O}$ just before the evaluation of the BP $_{\text{raclo}}$.

Materials and methods

PET system. PET studies were performed on a Siemens ECAT Exact HR $^+$ used in three-dimensional mode. The system covers an axial distance of 15.5 cm [20]. The transaxial resolution of the reconstructed images is about 4.1 mm full-width at half-maximum (FWHM) in the centre. Transmission scans were acquired with three rotating $^{68}\text{Ge}/^{68}\text{Ga}$ sources and used to correct the emission scans for the attenuation of 511-keV photon rays through tissue and head support. The [^{11}C]raclopride was synthesised as previously described by methylation of the desmethyl precursor using [^{11}C]methyl iodide [21]. [^{11}C]raclopride (2–2.2 mCi) was injected as a bolus for 10 s, immediately followed by a flush with 2 ml saline.

Animals. Animal studies were performed by licensed investigators in accordance with French (87–848, Ministère de l'Agriculture et de la Forêt) and European Economic Community (86–60, EEC) guidelines for care of laboratory animals and were approved by the regional ethical animal use committee. In this study, two European male cats weighing about 4 kg were obtained from Iffa-Credo, France.

Surgery. A single surgical procedure was performed under general anaesthesia, induced with halothane (4%). As soon as deep anaesthesia was obtained, endotracheal intubation was performed and anaesthesia was maintained by constant insufflation of 2.5% halo-

thane in air. Carbon dioxide concentration in expired gases, heart rhythm and body temperature were continuously controlled during the surgical procedure [22]. A U-shaped piece of Plexiglas was stereotaxically fixed to the skull with acrylic cement and screws. This head-holder permitted painless restraint of the animal's head and ensured a reliable position of the head during PET measurement. During the week after surgery, the animals were treated daily with 50 mg kg $^{-1}$ sodium amoxicillin, an antibiotic.

Experimental set-up and animal training. After recovery, each cat was trained to lie down in a hammock, inside a box with a Plexiglas cover. It took about 2 months of daily training, based on kindness and alimentary motivation, to ensure that the animal remained quiet and in a waking state for the duration of the scanning procedure (1–2 h). The head movements of the animal were progressively restrained by attaching the head-holder to the cover of the box according to a previously described method [23]. The box was placed in the tomograph in such a way as to ensure that the head was in the centre of the field of view.

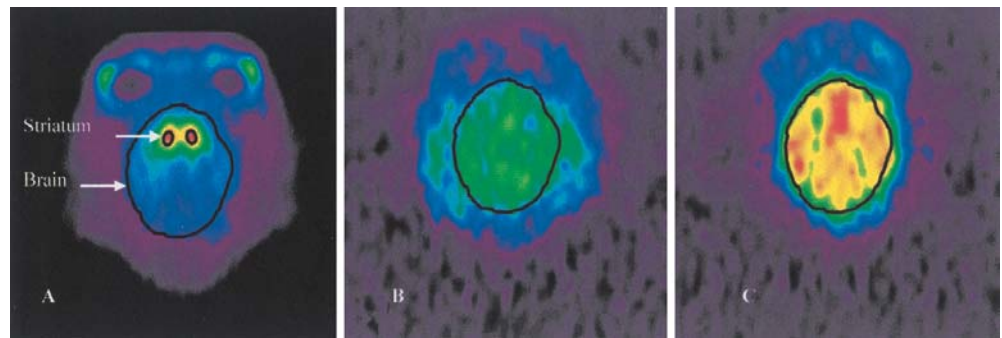
Anaesthetic procedures. Two anaesthesia protocols were employed. Ketamine (Panpharma, France) was administered intramuscularly as a single bolus 15 min before the beginning of the scan, yielding a dose of 50 mg kg $^{-1}$ h $^{-1}$. Halothane (Fluothane, Belamont) was 1.5% mixed in medical air supplied to the animal through a respiratory mask. The non-noxious restraint of the head of the animal allowed use of a low halothane concentration.

Scan test procedures. A catheter was first inserted in the cephalic vein for radiotracer injection. To measure the BP $_{\text{raclo}}$, each animal was first submitted to three simple scan tests (at least 1 week apart) under each experimental condition (awake, ketamine and halothane). A bolus injection of 1.5–2 mCi (55.5–74 MBq) of [^{11}C]raclopride was made via the catheter. Radioactivity was then measured in a series of sequential time frames of increasing duration from 30 s to 10 min. The total time for the measurement of the radioactivity in the brain was 67 min.

Test-retest experiments. Each animal was also submitted to three complex test-retest protocols entailing four radioligand injections. In the awake animal, a catheter was inserted in the cephalic vein for radiotracer injections. (1) A bolus injection of 1.5 mCi (55.5 MBq) of [^{15}O]H $_2\text{O}$ was made via the catheter and the radioactivity was measured in a series of six sequential time frames lasting for 10 s and two frames lasting for 20 s. (2) Fifteen minutes later, a bolus injection of 1.5–2 mCi (55.5–74 MBq) of [^{11}C]raclopride was made according to the same protocol as for the simple test. (3, 4) Three hours later, on the same day, the animal was anaesthetised according to one of the described procedures, and the two aforementioned protocols (1 and 2) were carried out again.

Data analysis. The position of the cat in the PET system was such that the reconstructed images followed the frontal plane. Regions of interest (ROIs) for the right and left caudate nuclei and the cerebellum were drawn on horizontally reconstructed PET images (Fig. 1). The striatum was defined on three successive slices according to a cat brain atlas [24]. The regional radioactivity concentration (Ci ml $^{-1}$) was determined for each frame, corrected for decay and plotted versus time. ROIs of the same surface as used in the striatum were defined in the cerebellum, a region with a low density of D $_2$ receptors, and were used to estimate free radioligand concentration and non-specific binding in the brain (F). Specific ligand binding (B) was defined as the difference between the total

Fig. 1. Horizontal reconstruction of the cat brain, presenting the in vivo distribution of [^{11}C]raclopride (A) and [^{15}O]H $_2$ O (B, C). Images B and C are normalised for the injected radioactivity. Two yellow spots with a red centre correspond to the striata in A. The brain is clearly detectable on C (yellow spot) but can hardly be differentiated from surrounding tissues on B



regional radioactivity and that of the cerebellum. The time curves for B [$B(t)$] and F [$F(t)$] were integrated from 40 min to 67 min. The binding potential (BP) was calculated using the simplified reference tissue model developed by Lammertsma et al. [25]. The BP_{raclo} was measured in the two striata for each experiment. The ROIs used to calculate BP_{raclo} were re-used to measure the regional time-activity curves in the striatum and the cerebellum after [^{15}O]H $_2$ O injection. The values from the two cats and the structures of both sides were pooled for statistical analysis. The means of the groups were compared using the two-tailed Student's t test.

Results

Daily training lasting about 2 months was necessary to ensure that the animals remained in a state of quiet wakefulness for the time required for PET scanning. After that, the animals accepted constraint for about 2 h without developing any sign of stress. A typical image of the [^{11}C]raclopride binding is presented in Fig. 1a. The two striata can easily be defined. Images obtained after injection of [^{15}O]H $_2$ O were more difficult to interpret, and no particular brain structures could be differentiated. In Fig. 1b, obtained in the awake cat, it can be seen that the amount of accumulated [^{15}O]H $_2$ O is higher in the brain than in the surrounding tissues (green vs blue). By comparison, in the animal anaesthetised with halothane (Fig. 1c), the difference between the brain and the surrounding tissues is amplified (yellow vs red).

Time-activity curves

Time-activity curves are presented in Fig. 2. The curves for the striatum and cerebellum are seen to be very different. After the bolus injection, the peak of maximum radioactivity was reached in 1.3 ± 0.2 min in the cerebellum and in 6 ± 0.2 min in the striatum. Wash-out was faster in the cerebellum. In both the cerebellum and the striatum, the maximum accumulation of radioactivity was considerably increased under halothane anaesthesia when compared with the results in the awake animals. No significant difference in the peak of radioactivity could be detected between the awake state and ketamine anaesthesia in either the cerebellum or the striatum.

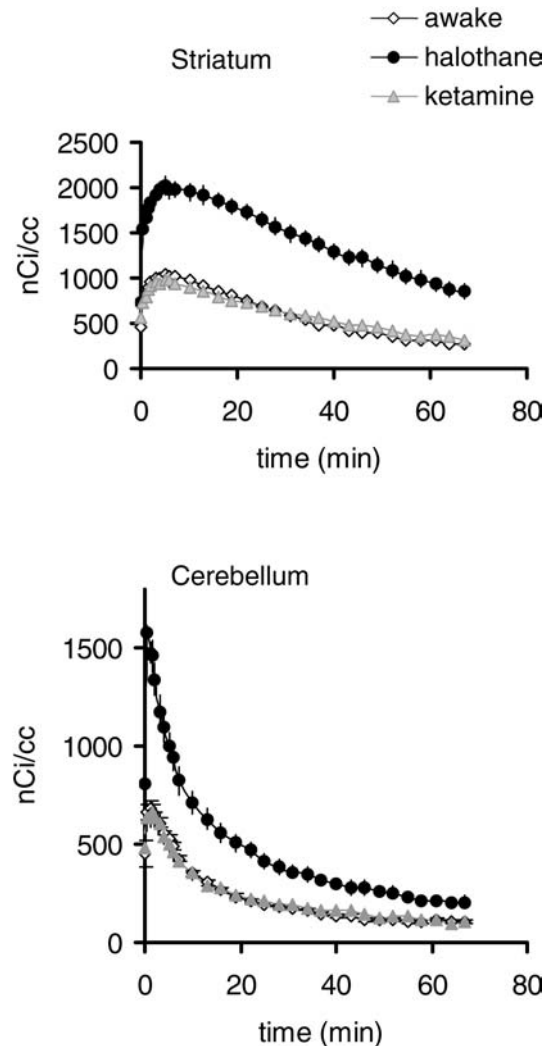


Fig. 2. Regional time-activity curves (nCi/ml) obtained in the cat striatum (top panel) and cerebellum (bottom panel) after i.v. injection of [^{11}C]raclopride. Three experimental situations are compared: awake animal with non-noxious restraint of the head (\diamond), 1.5% halothane (\bullet) and $50 \text{ mg kg}^{-1} \text{ h}^{-1}$ ketamine (\blacktriangle). Data are the mean (\pm SEM) of the measurements obtained bilaterally in two cats

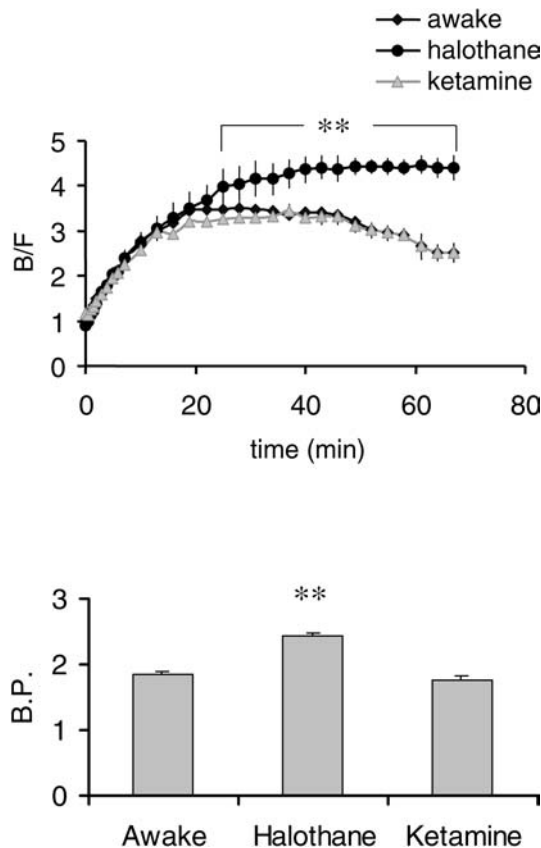


Fig. 3. Curve $B/F=f(t)$ and corresponding calculated values of BP_{raclo} obtained in awake animals with non-noxious head restraint (◆), 1.5% halothane (●) and $50 \text{ mg kg}^{-1} \text{ h}^{-1}$ ketamine (▲). Data are the mean (\pm SEM) of the measurements obtained bilaterally in two cats. Statistical differences were evaluated using the two-tailed Student's t test: $**P<0.05$)

BP_{raclo} in the striatum

The ROIs (striatum) included both the caudate nucleus and the putamen on horizontally reconstructed sections (Fig. 1). Time-activity curves of the [^{11}C]raclopride binding were obtained in each striatum in awake cats and gave a mean BP_{raclo} value of 1.848 ± 0.048 (Fig. 3, Table 1). The mean discrepancy between the right and the left structure in the same animal in a given experiment was 2.5%, and it never exceeded 6.5%. In experiments conducted under halothane anaesthesia, the mean BP_{raclo} was 2.455 ± 0.028 , and under ketamine it was 1.906 ± 0.074 . A constant discrepancy in the BP_{raclo} between the two animals was observed (10.6%).

Test-retest situations

The results of the test-retest protocols are presented in Fig. 4 and Table 1. No difference in the rate of accumulation of [^{15}O]H $_2$ O in either the striatum or the cerebellum was ever observed in awake or anaesthetised ani-

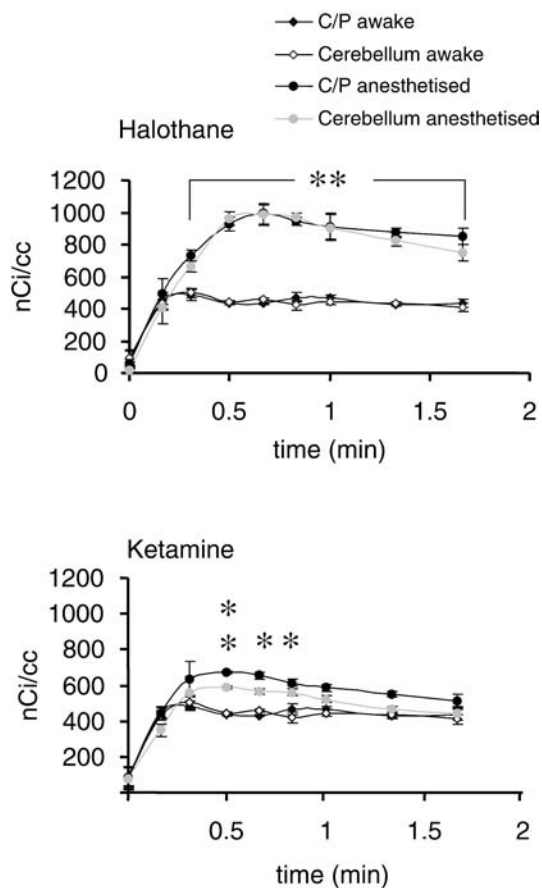


Fig. 4. Regional time-activity curves (nCi/ml) obtained under 1.5% halothane (top panel) and $50 \text{ mg kg}^{-1} \text{ h}^{-1}$ ketamine (bottom panel) after i.v. injection of [^{15}O]H $_2$ O. Curves obtained in awake animals are used as a control. Data are the mean (\pm SEM) of the measurements obtained bilaterally in two cats. Statistical differences were evaluated using the two-tailed Student's t test: $*P<0.5$, $**P<0.05$)

mals (Fig. 4). The average accumulation rate of [^{15}O]H $_2$ O was $460 \pm 14 \text{ nCi cc}^{-1} \text{ min}^{-1}$ in the striatum of the awake animals, $927 \pm 18 \text{ nCi cc}^{-1} \text{ min}^{-1}$ under halothane anaesthesia and $683 \pm 19 \text{ nCi cc}^{-1} \text{ min}^{-1}$ under ketamine anaesthesia. The BP_{raclo} measured during the test (awake) was the same as that found in simple test experiments. An increase was detected when the retest was done under halothane (2.430 ± 0.047), while no change was detected under ketamine (1.824 ± 0.102). No difference could be detected between simple test experiments and the re-test of the test-retest protocols.

Discussion

Only a few studies have involved the use of PET scans on the cat [22, 26, 27]. This animal nevertheless constitutes an interesting "halfway house" between rodents and primates. In addition, a large set of neurophysiological concepts have been established on the cat, and its

Table 1. Absolute and mean (\pm SEM) values of BP_{raclo} and $[^{15}\text{O}]\text{H}_2\text{O}$ accumulation (in $\text{nCi cc}^{-1} \text{min}^{-1}$) in the striatum of awake cats and in halothane- or ketamine-anaesthetised animals during 21 simple-test experiments and six test-retest experiments (awake/anaesthetised) performed on the same day^a

Cat	Awake	Halothane (1.5%)	Ketamine (50 mg $\text{kg}^{-1} \text{h}^{-1}$)			
<i>Simple test experiments</i>						
	$BP_{\text{raclo}} \times 10^{-3}$	$BP_{\text{raclo}} \times 10^{-3}$	$BP_{\text{raclo}} \times 10^{-3}$			
C1	1.703; 1.733					
C1	2.089; 2.057					
C1	2.152; 2.274					
C1	2.157; 2.097					
C1	1.868; 1.885					
C2	1.565; 1.565					
C2	1.845; 1.789					
C2	1.658; 1.699					
C2	1.747; 1.730					
C2	1.617; 1.731					
C1		2.462; 2.529				
C1		2.450; 2.417				
C1		2.550; 2.310				
C2		2.285; 2.336				
C2		2.548; 2.518				
C2		2.538; 2.527				
C1			2.184; 2.154			
C1			2.059; 2.077			
C2			1.519; 1.523			
C2			1.841; 1.925			
C2			1.856; 1.926			
Mean	1.848 \pm 0.048	2.455 \pm 0.028**	1.906 \pm 0.074 NS			
<i>Test-retest experiments</i>						
	$[^{15}\text{O}]\text{H}_2\text{O}$ ($\text{nCi cc}^{-1} \text{min}^{-1}$)	$BP_{\text{raclo}} \times 10^{-3}$	$[^{15}\text{O}]\text{H}_2\text{O}$ ($\text{nCi cc}^{-1} \text{min}^{-1}$)	$BP_{\text{raclo}} \times 10^{-3}$	$[^{15}\text{O}]\text{H}_2\text{O}$ ($\text{nCi cc}^{-1} \text{min}^{-1}$)	$BP_{\text{raclo}} \times 10^{-3}$
C1	444; 435	1.640; 1.703	998; 967	2.420; 2.505		
C1	467; 476	2.052; 2.074	879; 903	2.450; 2.610		
C2	455; 414	1.665; 1.675	920; 898	2.282; 2.346		
Mean	448 \pm 10	1.801 \pm 0.083	927 \pm 18***	2.43 \pm 0.047		
C1	502; 554	1.700; 1.730			635; 619	2.059; 2.077
C2	390; 540	1.548; 1.989			712; 722	1.519; 1.523
C2	450; 398	1.854; 1.799			685; 730	1.841; 1.925
Mean	472 \pm 28	1.770 \pm 0.061			683 \pm 19***	1.824 \pm 0.102**

For each experiment, the values obtained from left and right striatum are given. The mean corresponds to the average of both sides in all the experiments. In the test-retest experiments, each $[^{11}\text{C}]\text{raclopride}$ injection was preceded by an injection of $[^{15}\text{O}]\text{H}_2\text{O}$ to evaluate CBF

Statistical analysis was performed using the two-tailed Student's *t* test: * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$

brain is of sufficient size to allow the use of human-oriented PET cameras. Our investigations were undertaken with a view to substantiating the use of animal experiments for validation of human PET protocols. Unlike PET studies in humans, those performed in animals require anaesthesia in the majority of experimental situa-

tions. Great care must therefore be taken in defining the consequences of changes in the waking state for the binding of each radioligand. One of the main purposes of this study was to develop an original protocol in awake animals.

The BP_{raclo} has often been used as a tool to evaluate DA release in the striatum of animals and humans [7, 10]. Its sensitivity to alterations in the waking state and CBF was therefore estimated in this study. Measurements of BP_{raclo} obtained under two different anaesthesia settings were compared with those obtained in awake animals. The rate of [^{15}O]H $_2$ O accumulation was also measured in the same settings in an attempt to correlate BP_{raclo} with CBF.

Previous works in healthy human subjects have shown a high inter-individual variability in [^{11}C]raclopride binding [28]. Only two cats were used in the present study, and a 15% discrepancy was found between the mean values of their BP_{raclo} measured in the waking state. In contrast, a good intra-subject stability has been reported in the human [29] and confirmed in the animal [14]. This was confirmed by our finding that the discrepancy between the left and right structures was only 2.8% and the SEM of the BP_{raclo} values was low within the experimental groups. The greatest uncertainty thus concerns inter-individual comparisons.

The high BP_{raclo} found under halothane anaesthesia confirmed previous observations in monkeys [30] and cats [14]. In a previous work, also involving the cat, it was shown that the affinity of the DA receptors is lower under halothane anaesthesia than under ketamine anaesthesia [14]. This could, on its own, explain the increase in BP_{raclo} observed under halothane since [^{11}C]raclopride affinity remained unchanged. In our study, another effect of the volatile anaesthetic was observed that could also explain the increased BP_{raclo} under halothane. Indeed, two observations suggest an increased CBF. First, in halothane-anaesthetised cats, the peak of the maximum radioactivity in the cerebellum after the i.v. injection of [^{11}C]raclopride was considerably increased. Given the absence of specific receptors for this ligand in the cerebellum [31], the total amount of radioactivity is closely related to the amount of tracer delivered by CBF. Second, the rate of [^{15}O]H $_2$ O accumulation in the striatum and the cerebellum was greater under halothane anaesthesia when compared with the awake animal, showing that there was also a general increase in the CBF. This is not surprising since halothane has previously been claimed to be a CBF enhancer [32, 33, 34, 35]. Increased delivery of the tracer could thus be expected in the striatum, favouring the [^{11}C]raclopride binding.

Other influences of halothane on the DA system may be mentioned. Halothane is known to alter striatal extracellular DA. Could this action explain our results? Acting at the ligand-gated ionic channel, halothane depresses the Ca^{2+} -dependent release of DA [36, 37]. However, the basal DA release (independent of Ca^{2+}) is increased under halothane anaesthesia [38, 39]. Halothane and isoflurane enhance the drug-induced DA uptake blockade [16, 40] and halothane is even considered a DA uptake inhibitor on its own [41]. Thus, this substance probably increases extracellular DA by reducing DA uptake or ac-

tivating the carrier-dependent basal release. It could also affect the retro-inhibition of extracellular DA on DA release through its previously mentioned action on the pre-synaptic D_2 receptors. Whatever the mode of action of this substance, the consequence should be an increased extracellular amine concentration and thus a reduction in the [^{11}C]raclopride binding. It is unlikely that the increased [^{11}C]raclopride binding observed in our study would be relevant to the action of halothane on the extracellular DA concentration. If the effects of halothane on extracellular DA cannot be held responsible for the increase observed in BP_{raclo} , activated CBF is probably the main cause of this increase.

In our experiments, in the striatum BP_{raclo} was similar under ketamine and in the awake animals. The peak of the maximum radioactivity in the cerebellum was also comparable in the two situations. This suggests that ketamine had no effect on the CBF. However, a slight, but statistically significant, increase in the rate of accumulation of [^{15}O]H $_2$ O in the striatum and the cerebellum was observed. This is probably a sign of a higher sensitivity of the rate of accumulation of [^{15}O]H $_2$ O to CBF changes. No difference in [^{11}C]raclopride binding was found in the striatum under ketamine anaesthesia in spite of an apparent slight increase in the CBF. It may be that the CBF increase is too minor to produce a detectable effect, or that it is counteracted by a local action of this substance on the extracellular DA. The effect of ketamine on the extracellular DA is a matter of some controversy [42, 43, 44, 45], but it is well documented that DA release is under the control of NMDA receptors, on which ketamine exerts an antagonistic action. Thus the absence of an effect of ketamine on BP_{raclo} in the striatum could be due to both a slight increase in the CBF, favouring radioligand binding, and a simultaneous increase in the extracellular DA with which it is competing, as has previously been suggested [46, 47].

Recent works [7, 10, 14, 46, 48] have led to questioning of the existence of a simple relationship between extracellular DA and BP_{raclo} . This study confirmed the limitations of such a concept by evidencing a high sensitivity of BP_{raclo} to anaesthesia and probably CBF. This could explain the conflicting results obtained when using this parameter to evaluate in vivo DA release.

Acknowledgements. We want thank Astra Zeneca for furnishing standard solutions and precursor of raclopride.

References

1. Tedroff J, Pedersen M, Aquilonius SM, Hartvig P, Jacobsson G, Langström B. Levodopa-induced changes in synaptic dopamine in patients with Parkinson's disease as measured by [^{11}C]raclopride displacement and PET. *Neurology* 1996; 46:1430–1436.
2. Laruelle M, Iyer RN, Al-Tikriti MS, Zea-Ponce Y, Malisson R, Zoghbi SS, Baldwin RM, Kung HF, Charney DS, Hoffer PB

- Innis RB. Microdialysis and SPECT measurements of amphetamine-induced dopamine release in nonhuman primates. *Synapse* 1997; 25:1–14.
3. Koeppe MJ, Gunn RN, Lawrence AD, Cunningham VJ, Dagher A, Jones T, Brooks DJ, Bench CJ, Grasby PM. Evidence for striatal dopamine release during a video game. *Nature* 1998; 393:266–268.
 4. Ginovart N, Farde L, Halldin C, Swahn CG. Effect of reserpine-induced depletion of synaptic dopamine on [¹¹C]raclopride binding to D2-dopamine receptors in the monkey brain. *Synapse* 1997; 25:321–325.
 5. Watabe H, Endres CJ, Breier A, Schmall B, Eckelman WC, Carson RE. Measurement of dopamine release with continuous infusion of [¹¹C]raclopride: optimization and signal-to-noise considerations. *J Nucl Med* 2000; 41:522–530.
 6. Fisher RE, Morris ED, Alpert NM, Fischman AJ. In vivo imaging of neuromodulatory synaptic transmission using PET: a review of relevant neurophysiology. *Hum Brain Mapping* 1995; 3:24–34.
 7. Laruelle M. Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. *J Cereb Blood Flow Metab* 2000; 20:423–451.
 8. Friston KJ, Malizia AL, Wilson S, Cunningham VJ, Jones T, Nutt DJ. Analysis of dynamic radioligand displacement or “activation” studies. *J Cereb Blood Flow Metab* 1997; 17:80–93.
 9. Dewey SL, Smith GS, Logan J, Alexoff D, Ding YS, King P, Pappas N, Brodie JD, Ashby CR Jr. Serotonergic modulation of striatal dopamine measured with positron emission tomography (PET) and in vivo microdialysis. *J Neurosci* 1995; 15:821–829.
 10. Tsukada H, Harada N, Nishiyama S, Ohba H, Sato K, Fukumoto D, Kakiuchi T. Ketamine decreased striatal [¹¹C]raclopride binding with no alterations in static dopamine concentrations in the striatal extracellular fluid in the monkey brain: multi-parametric PET studies combined with microdialysis analysis. *Synapse* 2000; 37:95–103.
 11. Zimmer L, Mauger G, LeBars D, Bonmarchand G, Luxen A, Pujol JF. Effect of endogenous serotonin on the binding of the 5-HT_{1a} PET ligand ¹⁸F-MPPF in the rat hippocampus: Kinetic β measurements combined with microdialysis. *J. of Neurochemistry* 2002; 80:278–286.
 12. Dewey SL, Smith GS, Logan J, Brodie JD, Fowler JS, Wolf AP. Striatal binding of the PET ligand ¹¹C-raclopride is altered by drugs that modify synaptic dopamine levels. *Synapse* 1993; 13:350–356.
 13. Hartvig P, Torstenson R, Tedroff J, Watanabe Y, Fasth KJ, Bjurling P, Langstrom B. Amphetamine effects on dopamine release and synthesis rate studied in the rhesus monkey brain by positron emission tomography. *J Neural Transm* 1997; 104:329–339.
 14. Ginovart N, Hassoun W, Veyre L, Leviel V. Effects of amphetamine and evoked dopamine release on [¹¹C]raclopride binding in anesthetized cats. *Neuropsychopharmacology* 2002; 27: 72–84.
 15. Zetterstrom T, Sharp T, Ungerstedt U. Further evaluation of the mechanism by which amphetamine reduces striatal dopamine metabolism: a brain dialysis study. *Eur J Pharmacol* 1986; 132:1–9.
 16. Fink-Jensen A, Ingwersen SH, Nielsen PG, Hansen L, Nielsen EB, Hansen AJ. Halothane anesthesia enhances the effect of dopamine uptake inhibition on interstitial levels of striatal dopamine. *Naunyn-Schmiedeberg Arch Pharmacol* 1994; 350: 239–244.
 17. Zimmer L, Hassoun W, Pain F, Bonnefoi F, Laniece P, Mastripolito R, Pinot L, Pujol JF, Leviel V. SIC, an intracerebral β -radiosensitive probe for radiopharmacology investigations in small laboratory animals: binding studies with [¹¹C]raclopride. *J Nucl Med* 2002; 43:227–233.
 18. Wreggett KA, Seeman P. Agonist high and low affinity states of the D2 dopamine receptor in calf brain. Partial conversion by guanine nucleotide. *Mol Pharmacol* 1984; 25:10–17.
 19. Martin DC, Dennison RL, Introna RP, Aronstam RS. Influence of halothane on the interaction of serotonin₁ and adenosine₁ receptors with G protein in rat brain membranes. *Biochem Pharmacol* 1991; 42:1313–1316.
 20. Brix G, Zaers J, Adam LE, Bellemann ME, Ostertag H, Trojan H, Haberkorn U, Doll J, Oberdorfer F, Lorenz WJ. Performance evaluation of a whole-body PET scanner using the NE-MA protocol. National Electrical Manufacturers Association. *J Nucl Med* 1997; 38:1614–1623.
 21. Halldin C. Radioligands for dopamine receptor PET studies: benzamides and ligands for dopamine D-1 receptors. In: Baron JC, Comar D, Farde L, Martinot JL, Mazoyer B, eds. *Brain dopaminergic systems: imaging with positron tomography*. Dordrecht: Kluwer Academic; 1991:23–38.
 22. Ginovart N, Hassoun W, Le Bars D, Weissmann D, Leviel V. In vivo characterization of *p*-[¹⁸F]MPPF, a fluoro analog of WAY-100635 for visualization of 5HT_{1A} receptors. *Synapse* 2000; 35:192–200.
 23. Goffart L, Pelisson D. Orienting gaze shifts during muscimol inactivation of caudal fastigial nucleus in the cat. I. Gaze dysmetria. *J Neurophysiol* 1998; 79:1942–1958.
 24. Jasper HH, Ajmone-Marsan C. In: *A stereotaxic atlas of the diencephalon of the cat*. The National Research Council of Canada. Ottawa, Canada, 1954.
 25. Lammertsma AA, Bench CJ, Hume SP, Osman S, Gunn K, Brooks DJ, Frackowiak RSJ. Comparison of methods for analysis of clinical [¹¹C]raclopride studies. *J Cereb Blood Flow Metab* 1996; 16:42–52.
 26. Sakiyama Y, Sato A, Senda M, Ishiwata K, Toyama H, Schmidt RF. Positron emission tomography reveals changes in global and regional cerebral blood flow during noxious stimulation of normal and inflamed elbow joints in anesthetized cats. *Exp Brain Res* 1998; 118:439–446.
 27. Hashizume K, Tamakawa H, Hashimoto N, Miyake Y. Single-step synthesis of [¹⁸F]haloperidol from the chloro-precursor and its applications in PET imaging of a cat’s brain. *Appl Radiat Isot* 1997; 48:1179–1185.
 28. Farde L, Hall H, Pauli S, Halldin C. Variability in D2-dopamine receptor density and affinity: a PET study with [¹¹C]raclopride in man. *Synapse* 1995; 20:200–208.
 29. Schlosser R, Brodie JD, Dewey SL, Alexoff D, Wang CJ, Fowler JS, Volkow N, Logan J, Wolf AP. Long-term stability of neurotransmitter activity investigated with [¹¹C]raclopride PET. *Synapse* 1998; 28:66–70.
 30. Kobayashi K, Onoue O, Watanabe Y, Onoe H, Langström B. Difference in response of D2 receptor binding between ¹¹C-N-methylspiperone and ¹¹C-raclopride against anesthetics in rhesus monkey brain. *J Neural Transm* 1995; 100:147–151.
 31. Ishiwata K, Hayakawa N, Ogi N, Oda K, Toyama H, Endo K, Tanaka, Senda M. Comparison of three PET dopamine D2-like receptor ligands, [¹¹C]raclopride, [¹¹C]nemonapride and [¹¹C]N-methylspiperone, in rats. *Ann Nucl Med* 1999; 13:161–167.
 32. Paut O, Bissonnette B. Effect of halothane on the cerebral circulation in young children: a hysteresis phenomenon. *Anaesthesia* 2001; 56:360–365.

33. Brussel T, Fitch W, Brodner G, Arendt I, Van Aken H. Effects of halothane in low concentrations on cerebral blood flow, cerebral metabolism, and cerebrovascular autoregulation in the baboon. *Anesth Analg* 1991; 73:758–764.
34. Hansen TD, Warner DS, Todd MM, Vust LJ. Effects of nitrous oxide and volatile anaesthetics on cerebral blood flow. *Br J Anaesth* 1989; 63:290–295.
35. Madsen JB, Cold GE, Hansen ES, Bardrum B. Cerebral blood flow, cerebral metabolic rate of oxygen and relative CO₂-reactivity during craniotomy for supratentorial cerebral tumours in halothane anaesthesia. A dose-response study. *Acta Anaesthesiol Scand* 1987; 31:454–457.
36. Kress HG, Muller J, Eisert A, Gilge U, Tas PW, Koschel K. Effects of volatile anesthetics on cytoplasmic Ca²⁺ signaling and transmitter release in a neural cell line. *Anesthesiology* 1991; 74:309–319.
37. Salord F, Keita H, Lecharny JB, Henzel D, Desmots JM, Mantz J. Halothane and isoflurane differentially affect the regulation of dopamine and gamma-aminobutyric acid release mediated by presynaptic acetylcholine receptors in the rat striatum. *Anesthesiology* 1997; 86:632–641.
38. Mantz J, Varlet C, Lecharny JB, Henzel D, Lenot P, Desmots JM. Effects of volatile anesthetics, thiopental, and ketamine on spontaneous and depolarization-evoked dopamine release from striatal synaptosomes in the rat. *Anesthesiology* 1994; 80:352–363.
39. Stahle L, Collin AK, Ungerstedt U. Effects of halothane anaesthesia on extracellular levels of dopamine, dihydroxyphenylacetic acid, homovanillic acid and 5-hydroxy indolacetic acid in rat striatum: a microdialysis study. *Naunyn-Schmiedeberg Arch Pharmacol* 1990; 342:136–140.
40. Tsukada H, Nishiyama S, Kakiuchi T, Ohba H, Sato K, Harada N, Nakanishi S. Isoflurane anesthesia enhances the inhibitory effects of cocaine and GBR12909 on dopamine transporter: PET studies in combination with microdialysis in the monkey brain. *Brain Res* 1999; 849:85–96.
41. el Maghrabi EA, Eckenhoff RG. Inhibition of dopamine transport in rat brain synaptosomes by volatile anesthetics. *Anesthesiology* 1993; 78:750–756.
42. Bacopoulos NG, Redmond DE, Roth RH. Serotonin and dopamine metabolites in brain regions and cerebrospinal fluid of a primate species: effects of ketamine and fluphenazine. *J Neurochem* 1979; 32:1215–1218.
43. Koshikawa N, Tomiyama K, Omiya K, Kobayashi M. Ketamine anesthesia has no effect on striatal dopamine metabolism in rats. *Brain Res* 1988; 444:394–396.
44. Mc Cowin TJ, Mueller RA, Breese GR. Effects of anesthetics and electrical stimulation on nigrostriatal dopaminergic neurons. *J Pharm Exp Ther* 1983; 224:489–493.
45. Mereu G, Fanni B, Gessa GL. General anesthetics prevent dopamine neuron stimulation by neuroleptics. In: Usdin E, Carlsson A, Dahlström A, Engel J, eds. *Catecholamines. Part B. Neuropharmacology and central nervous system-theoretical aspects*. New York: Alan Liss; 1984:353–358.
46. Breier A, Adler CM, Weisenfeld N, Su TP, Elman I, Picken L, Malhotra AK, Pickar D. Effects of NMDA antagonism on striatal dopamine release in healthy subjects: Application of a novel PET approach. *Synapse* 1998; 29:142–147.
47. Verma A, Moghaddam B. NMDA receptor antagonists impair prefrontal cortex function as assessed via spatial delayed alternation performance in rats: modulation by dopamine. *J Neurosci* 1996; 16:373–379.
48. Laruelle M, Abi-Dargham A, van Dyck CH, Rosenblatt W, Zea-Ponce Y, Zoghbi SS, Baldwin RM, Charney DS, Hoffer PB, Kung HF, Innis RB. SPECT imaging of striatal dopamine release after amphetamine challenge. *J Nucl Med* 1998; 36:1182–1190.