NeuroImage **16,** 1015–1027 (2002)
doi:10.1006/nimg.2002.1121

In Vivo Detection of Striatal Dopamine Release during Reward: A PET Study with [^{11}C]Raclopride and a Single Dynamic Scan Approach

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Received December 23, 2001

A new simple method is proposed to detect, using PET and [^{11}C]raclopride, changes in striatal extracellular dopamine concentration during a rewarded effortful task. This approach aimed to increase the sensitivity in detection of these effects. It requires a single-dynamic PET study and combines the classic kinetic compartmental model with the general linear model of SPM to provide statistical inference on changes in [^{11}C]raclopride time–activity curve due to endogenous dopamine release during two short periods of activation. Kinetic simulations predicted that 100% dopamine increase during two 5-min periods starting at 30 and 60 min after the injection can be detected. Moreover the effects of dopamine release on the [^{11}C]raclopride time-activity-curve are different from those induced by CBF increase. These simulated curves were used to construct the statistical linear model and to test voxel-by-voxel in healthy subjects the hypothesis that dopamine is released in the ventral striatum during periods of unexpected monetary gains, but not during periods of unexpected monetary loss. The experimental results are in line with the expected results although the amplitude of the effects due to dopamine release is moderate. The advantages and the limits of this method as well as the relevance of the results for dopamine involvement in reward processing are discussed. © 2002 Elsevier Science (USA)

Key Words: **positron emission tomography; endogenous dopamine release; dopamine receptors; reward; raclopride; statistical parametric mapping; kinetic model; activation studies.**

INTRODUCTION

Mesolimbic dopaminergic neurotransmission may be involved in the processing of the reward information (Schultz *et al.,* 1997; Kalivas and Nakamura, 1999; Spanagel and Weiss, 1999). The results of recent studies performed by Schultz *et al.* (1997) in alert monkey showed that dopamine neurones of the ventral tegmental area (VTA), which project to the nucleus accumbens and frontal cortex, respond to primary positive rewards as a function of their predictability. They are activated by primary unpredicted rewards or earliest conditioned stimuli predictive of reward. Inversely, their activity is reduced when a fully predicted reward fails to occur. The same neurones are activated in a course of a learning task when the errors are frequent and reward is unpredictable, and their activity is progressively reduced with the improvement of the performance and the predictability of reward (Hollerman and Schultz, 1998). Based on these data it has been suggested that dopamine neurones code errors in the prediction of both the occurrence and the time of reward, and thus may play an important role in learning. While a large body of functional neuroimaging methods (i.e., PET-CBF and fMRI) have been applied to map, in humans, the mesocortical limbic networks implicated in monetary and drug-associated rewards (Elliot *et al.,* 2000; Breiter *et al.,* 2001; Knutson *et al.,* 2001), the direct investigation of the role of striatal dopamine in mediating the responses to reinforcing stimuli remains almost unexplored. New insights into this issue could be provided by the application of PET-SPECT/radioligand techniques to the study of changes in endogenous dopamine release during rewarded behavioural activation. The theoretical (Morris *et al.,* 1995) and the experimental (Koepp *et al.,* 1998) feasibility of PET detection of endogenous dopamine changes during cognitive activation have been reported. The method proposed used [^{11}C]raclopride as radioligand and required two PET studies, with and without activation. Thus some limitations have to be considered like the

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1053-8119/02 $35.00

variability of physiological and experimental conditions between the studies and the necessity to inject a double radioactive dose to healthy subjects. A second critical point is the small amplitude of PET signal changes induced by cognitive activation, an issue that has been recently addressed by the development of more powerful statistical method for the dual scan approach (Aston *et al.*, 2000).

The goals of the present study were: (1) to develop a method that allows to increase the sensitivity in detection of endogenous dopamine release during activation and that requires a single PET study with [^{11}C]raclopride; (2) to test the hypothesis that dopamine is released in the ventral striatum during positive unpredicted monetary rewards but not during loss of an expected positive monetary reward.

To achieve these aims we propose a single-scan design approach in which two periods of short activation are carried out at some points during the scan (i.e., displacement study). The basic idea is to use the classic radioligand compartmental model to predict, by means of computer simulations, the expected activation changes induced by dopamine release on the striatal [^{11}C]raclopride time-activity curve (TAC) and to construct, using these simulated curves, the statistical linear model. Then the effects of interest (i.e., dopamine release) are tested on a voxel-by-voxel basis using SPM. The theory of this approach has been in part described (Friston *et al.*, 1997) but never applied to cognitive activations. The theoretical advantages of this method, as compared to the dual scan approach, should be an increase in the statistical power while reducing the interscans variability. To increase the differences in the shape of the uptake curve during the two periods of activation we developed a paradigm that should provide different dopamine changes: a period of unexpected monetary gain, theoretically associated to strong dopamine release and [^{11}C]raclopride displacement, and a period of unexpected monetary loss theoretically associated to unchanged and/or reduction of dopamine release and unchanged [^{11}C]raclopride binding.

In this study we present the theoretical feasibility of this new approach and the preliminary results of its applications to experimental data obtained in healthy volunteers engaged in a monetary rewarded effortful task.

MATERIALS AND METHODS

Experimental Design

The experimental paradigm engaged subjects in an effortful task while manipulating the monetary reward earned. Subjects started with a sum of 1000 FF, which they were told that they could either lose, or keep, or even double or triple by earning or losing a small amount of money on each trial of a response-time task. Our goal was to image dopamine release during two testing period of 5 min each, separated by a half-hour: one period during which subjects systematically performed the task correctly and earned an important monetary reward (R+ period), and another during which subjects systematically performed badly at the same task and lost an important sum of money (R− period). This was achieved by modifying the proportion of trials in which subjects were told that their responses were too slow (see below). To further increase the contrast between the R+ and R− blocks, and because theory predicts that dopamine release indexed violations in subjects' expectations of reward (Schultz *et al.*, 1997), the gains or losses always went against any expectations that subjects could have developed based on the preceding blocks. This was achieved by using a short training period performed just before PET scanning. Subjects received a short training period without monetary rewards (2 blocks of 30 trials each), and were then presented with a training period of 90 trials with monetary gains or losses, after which their money was restored to its initial value of 1000 FF. When the subjects gained during this training period, they were presented during the PET scanning, first with the R− block (quick loss, violating their expectation of a gain based on the training period), and then with the R+ block (quick gain, violating their expectation of a loss based on the previous experimental block). Similarly, when the subject started with a losing streak during the training period, they went on with the R+ and then R− blocks. If dopamine indexes positive reward above and beyond the subject's expectation (Schultz *et al.*, 1997), then there should be strong dopamine release on R+ blocks, but none on R− blocks. If, however, dopamine merely indexes attention, stress, or effort during a cognitive task (Feenestra, 2000; Pruessner *et al.*, 2000), then there should be dopamine release on both R+ and R− blocks.

Behavioral Task

The behavioral task was a previously studied number comparison task (Dehaene *et al.*, 1998; Dehaene, 1996; Pinel *et al.*, 1999). Briefly, arabic digits (1, 4, 6, or 9; 8 mm width, 15 mm height) or spelled-out French number words (UN, QUATRE, SIX, or NEUF; 16–50 mm width 15 mm height) were presented for 200 ms at the center of a computer screen, about 50 cm from the subjects' eyes. Subjects were requested to decide whether each digit was larger or smaller than 5. They responded as quickly as possible by depressing a left button with their right index if the number was smaller than five or a right button with their right middle finger if the number was larger than five.

Three basic changes were made to this simple paradigm to turn it into a task requiring conscious, effortful

processing and behavioural control based on external monetary reward. First, speed pressure was added by presenting one trial every 2 s for a period of 5 min, for a total of 150 trials per R+ or R− block. Second, another distracting number, which was similar but uncorrelated with the first, was presented for 50 ms just prior to the target number, with a stimulus onset asynchrony of only 117 ms. Contrary to previous studies in which this preceding number was masked and used as an unconscious prime (Dehaene *et al.*, 1998), here it was clearly visible and served to enhance the difficulty of the task. Subjects were told to try to ignore this first flashed number and to focus their attention on the second number which was presented for a longer duration.

A third change was that, after each trial, subjects received a verbal and monetary reward. When subjects responded incorrectly, the word "FAUX!" (false) appeared in yellow at fixation and a random amount between 10 and 30 FF was withdrawn. When subjects responded correctly, but slower than a response deadline, the words "PLUS VITE!" (faster) appeared in yellow and a similar sum was withdrawn. Otherwise, the word "CORRECT" appeared in green and a sum between 10 and 20 FF was gained. If this was the third correct response in a row, the word "SUPER" appeared and the gain was between 15 and 25 FF. The total gain was displayed on a visible meter with a scale ranging between 0 and 2000 FF. Subjects could thus constantly visualize their current gains or losses, and furthermore, every minute during testing, the numerical value in French Francs also appeared at fixation. To control the subject's gain, the response deadline was updated as follows. Initially, it was set at the subject's mean RT as measured during the second training block. Then on every trial its value was updated by (1) calculating the desired value of the subject's gains at this point in the experiment; (2) calculating how the current total gains deviated from this desired value; and (3) increasing the response deadline if the current gains were below the desired value, or decreasing it otherwise, by an amount proportional to the deviation in gains. For instance, in the first R+ block in which we wanted subjects to move from their initial 1000 FF to 2000 FF in five minutes, after 3 min of playing we expected the subject to have reached 1600 FF and hence relaxed the speed pressure if the subject was below this goal. This is essentially a cybernetic approach to controlling subject's gain. After some fine tuning of the updating equations (reported in appendix A), this manipulation was quite successful, the final gain approaching the desired value with a small standard deviation across subjects.

Kinetic Simulations

Kinetic simulations were performed to test the feasibility of a single-scan design in which two 5-min periods of activation are performed during the study separated by 30 min. Simulations were done using the nonequilibrium nonlinear compartmental model (Delforge *et al.*, 1993), with the addition of the kinetics of the endogenous neurotransmitter (Delforge *et al.*, 2001).

The kinetics of exogenous labeled and unlabeled ligand are described by four compartments (non-metabolised free ligand in plasma, free ligand in tissue, specifically and nonspecifically bound ligand) and seven parameters: k_1 and k_2 (the rate constants between the plasma and the free ligand compartment), k_{on}/V_R and k_{off} (the association and dissociation rate constants), k_5 and k_6 (the rate constants between the plasma and the nonspecific binding compartments) and B'_{max} (the receptor site concentration). The kinetics of the neurotransmitter are simplified and described by only two compartments, corresponding to the free (F^{en}) and the bound (B^{en}) endogenous neurotransmitter (Morris *et al.*, 1995; Delforge *et al.*, 2001). This is based on several assumptions. First, the mean concentration of the (F^{en}) is considered as a constant at the synaptic cleft throughout each state of the experiment. It means that the free endogenous dopamine concentration variations between rest and activation is modelled as a step function. Although the fluctuations over time of the neurotransmitter are much more complex, the time scale of these changes is so short (ms) with respect to that of PET acquisition (s–min) that an averaged value can be justified (Logan *et al.*, 1991; Fisher *et al.*, 1995). Second, the concentration of the free endogenous neurotransmitter is known and specified at any time during the experiment. Third, the kinetics of the endogenous neurotransmitter is thought to be much faster than the kinetics of the exogenous ligand.

The parameters of [^{11}C]raclopride were derived from the results obtained in a group of normal subjects (Gregoire *et al.*, 2000). The nonspecific binding rate constants (k_5 and k_6) were set to 0 in the striatum. The metabolite-corrected plasma activity curve obtained in the same control was used as input function. The typical parameters values were: k_1 = 0.0918 ml/min/g; k_2 = 0.4484/min; B_{max} = 43.90 pmol/ml; k_{on}/V_r = 0.0282 ml/(pmol/min); k_{off} = 0.1363/min; VF (vascular fraction) = 0.04 ml/ml. The baseline parameters values for endogenous dopamine were derived from the literature (Morris *et al.*, 1995; Laruelle *et al.*, 2000) as follows: free F^{en} = 100 pmol/ml; k^{en}_{on} = 0.25/min; k^{en}_{off} = 25/min.

One single bolus injection study was simulated. The [^{11}C]raclopride time-activity curve (TAC) was predicted for 90 min. The effects on the putamen TAC of two periods of activation lasting 5 min and starting at 30 and 60 min after the injection were simulated. We assumed that during the activation the endogenous dopamine release was increased by 100%. This estimation was provided by Fisher *et al.* (1995) on the basis of data from animals studies and recently supported by *in*

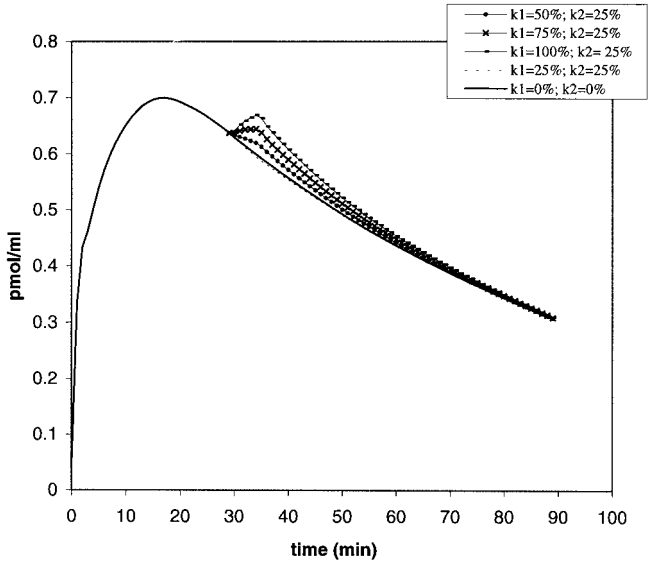

FIG. 1. Effects of variation of k_1 and k_2 on ^{11}C-raclopride curve in the putamen estimated by simulating 5-min period of activation performed at 30 min. Assuming similar 25% increases in both k_1 and k_2 lead to almost no change as compared to the curve obtained during rest condition (the solid line). However, the ^{11}C-raclopride TAC was increased with respect to the rest curve when k_1 was supposed to increase more than k_2.

vivo data obtained with microdialysis in human brain (amygdala) during cognitive activation (Fried *et al.*, 2001).

Since most activation paradigms can induce CBF changes, this effect could be a confounding factor in the detection of endogenous dopamine release. This is a critical point that has not been sufficiently addressed in the literature. The results of the few available reports are in part discordant, and there are no available data in the literature close to our experimental conditions. Using [^{11}C]flumazenil and $H_2{}^{15}O$, Holthoff *et al.* (1991) found a 20% increase in CBF and k_1 but no change in DV in the occipital cortex during a sustained visual stimulation. These results suggest that a similar variation was induced on k_1 and k_2 since the ratio (DV) was unaffected by the activation. On the other hand Logan *et al.* (1994), using [^{11}C]racopride in one healthy subject, showed that a CBF decrease induced by hyperventilation resulted in a reduction of k_1 (43%) and DV (18%), the largest variation occurring in k_1. Given these difficulties several possibilities were simulated: either a similar increase in both k_1 and k_2 by 25%; or a greater increase in k_1 than in k_2, obtained by increasing k_2 by 25% and k_1 by 50, 75, or 100%.

Results of simulations. Figure 1 shows the predicted effects of increased k_1 and k_2 on the putamen TAC during the activation performed at 30 min. Endogenous dopamine was supposed to be unchanged during activation. The curve reported represents the total radioactivity, i.e., the sum of the [^{11}C]raclopride concentration in the plasmatic, free, and bound compartments. If one assumes that blood flow increase induces the same rate of change in both k_1 and k_2, then there is essentially no net effect on the TAC. Only a very mild decrease of the TAC is seen. If, however, one assumes that blood flow increase induces a greater change on k_1 than in k_2, the model predicts an increase in the TAC. When different amounts of percent increase in k_1 are compared, the shapes of the predicted blood flow effects are largely unchanged, only their amplitude is affected. This indicates that, within the range of relatively small variations in k_1 and k_2, a single linear model can accomodate a variety of hypotheses about k_1 and k_2, the exact amplitude of k_1 and k_2 changes being unimportant. Thus, we arbitrarily modeled the effects of increased CBF on the TAC by an increase in k_1 by 50% and an increase in k_2 by 25%. Figure 2A shows the predicted effects of increased CBF on the putamen TAC during the two periods of activation performed at 30 and 60 min after the injection simulated as a 50% increase in k_1 and a 25% increase

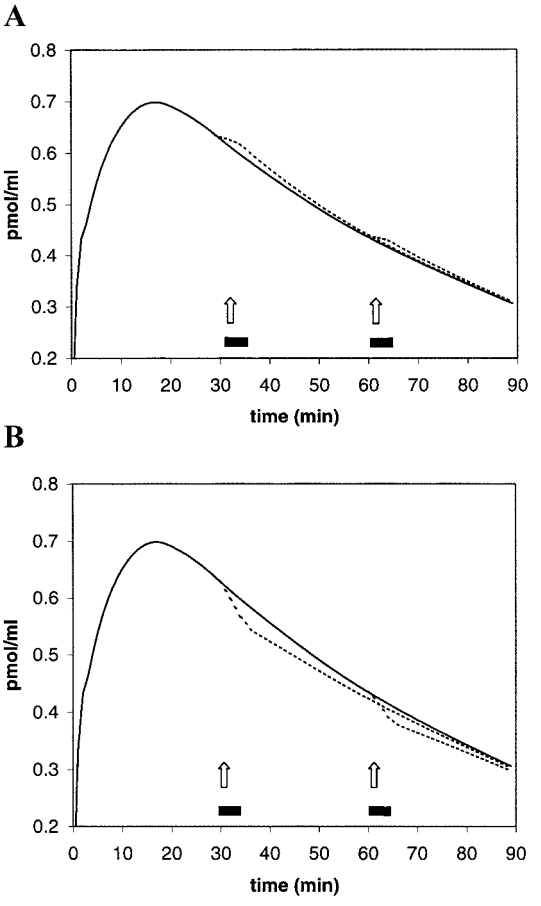

FIG. 2. Effects of CBF increase (A) and dopamine release (B) on the total ^{11}C-raclopride curves in the putamen estimated by simulating two 5-min periods of activation performed at 30 and 60 min after the tracer injection. The solid line represents the control condition and the broken line the activated condition.

in k_2 but no change in dopamine release. The results show that in these conditions the [^{11}C]raclopride radioactivity is increased during the activation. Further simulations showed that this variation has an opposite direction as compared to the effects of endogenous dopamine release. Figure 2B shows the predicted effects of increase in endogenous dopamine level from 100 to 200 pmol/ml on putamen TAC during the two periods of activation. The CBF was supposed to be unchanged. As expected the [^{11}C]radioactive concentration decreases during the activation periods. This effect results from the decrease of specific binding of the [^{11}C]raclopride due to increased endogenous dopamine binding to the same receptor during the activation. The "displacement" of [^{11}C]raclopride activity during the first activation was relatively fast and transient, the TAC appeared to return slowly to base-line values that were almost, but not completely, reached before the beginning of the second activation. The same aspect of the TAC was observed during the second activation.

PET Images Recording and Data Analysis

Subjects. Twelve normal male right-handed volunteers (mean age: 23 ± 2; range 20–27 years) took part to this study. Four subjects were scanned in the R+ R− conditions, four subjects in the R− R+ conditions, and four while resting throughout the 90 min scanning period. This study was approved by the ethical committee on clinical investigation (Comité consultatif de protection des Personnes dans la Recherche Biomédicale, Hôpital Bicêtre, Paris, France). Informed written consent was obtained for all subjects.

Scanning procedures. Dynamic PET scans were acquired in three-dimensional mode using the ECAT EXACT HR+ camera (Siemens Medical Systems, Germany). It consists of 32 rings of 576 crystals providing 63 simultaneous transaxial slices. The spatial resolution is 4.5 mm in the transverse direction and 4.1 mm in the axial direction, while the slice thickness is 2.425. The subject's head was carefully positioned in a headholder by means of a crossed laser-beam system. An individually thermoplastic mask insured the maintenance of head position. Correction for 511-keV gammaray attenuation by tissue was performed using ^{68}Ge-^{68}Ga-transmission scan. A total of 90 1-min sequential frames were acquired after a bolus injection of 8.6 ± 1.7 mCi (the Specific Radioactivity (SRA) was 401 ± 116 mCi/μmol at time of injection). Two 5-min blocks R+ and R−, which order was counterbalanced were presented at 30 and 60 min after injection.

T1 3-D images were obtained for the purpose of anatomical localisation using the 1.5-T Signa MRI system (Signa General Electric Medical Systems).

Data analysis. Data were analyzed using statistical parametric mapping (SPM99; Welcome Department of Cognitive Neurology, London, UK, implemented in Matlab). The images were realigned and spatially normalized to the standard Montreal Neurological Institute (MNI) brain space (Friston *et al.*, 1995a). The algorithms implemented in SPM were applied for realignment of dynamic emission scans. These algorithms use a least squares approach and a six-parameter (rigid body) spatial transformation and are designed to minimize the differences between the realigned image and the reference image. This approach assumes that the radioactivity distribution in the two images to be realigned is similar. However, the cerebral uptake of [^{11}C]raclopride varies over time. In the early frames the radioactivity distribution reflects the tracer delivery (CBF), while in the late frames it depends mostly on the D2 receptor distribution and the highest signal is observed within the striatum. Thus to reduce errors in realignment due to changes over time in the cerebral radioactivity distribution all frames were realigned using as reference the averaged image obtained from frames acquired between 25 and 28 min after the injection (i.e., immediately before the activation). The pattern of distribution of this "late image" is typical of receptor distribution and it is similar to that of frames obtained between 25 and 90 min after the injection. This approach should improve the realignment of late frames that have a more important contribution than the early images to the model used for statistical analysis (see statistical analysis methods session). The averaged amount of correction for translation applied by the realignment algorithm was 0.89 ± 0.67 in the x direction, 0.75 ± 0.55 in the y direction, and 2.11 ± 1.3 in the z direction. After realignment the frames were normalized using a linear and/or non linear transformation that best matched the late image to an [^{11}C]raclopride template. This should result in a more robust spatial normalization (Meyer *et al.*, 1999). The final pixel size of normalized images was 3 × 3 × 3 mm. The [^{11}C]raclopride template was created using the [^{11}C]raclopride images obtained in all subjects as follow. The early CBF-dependent averaged image (averaged 1–5 min frames) was first coregistered to the subject's MRI using a mutual information algorithm (Ashburner *et al.*, 1997a, 1997b). The MRI was then normalized to the MRI T1 template taken from the SPM library using a non linear transformation. This transformation was applied to both early (averaged 1- to 5-min frames) and late images (averaged 25- to 28-min frames). Finally an averaged image was calculated from the spatially normalized late images obtained in 11/12 subjects. This normalized averaged image was flipped and added to the unflipped image to obtain a symmetrical [^{11}C]raclopride template that conformed to the MNI anatomical space. The normalized images were then smoothed with an isotropic gaussian kernel (FWHM of 10 mm).

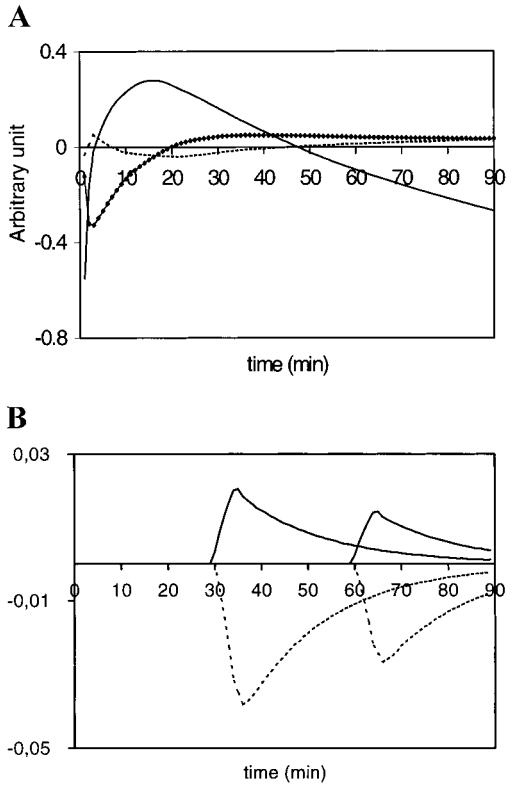

FIG. 3. Set of functions used to model the temporal behaviour of the measured activity. The first three functions (A) allowed for the variation in time during baseline (solid line, basic function 1; dotted line, basic function 2; broken line, basic function 3). The remaining four functions (B) were used to model the first and the second experimental condition, respectively, for both dopamine release (broken line) and blood flow increase (solid line).

Statistical model. The statistical model was constructed as follows. First, the expected variations of activity in several brain regions (striata, grey matter, and cerebellum) were modeled under the null hypothesis (no dopamine release) using two different sets of parameters taken from a previous study (see above simulations paragraph). This provided a series of functions that could predict the temporal behaviour across regions and subjects. To reduce redundancy in the model, a singular value decomposition of the expected signals was performed and only the first 3 components (accounting for more than 98% of the variance) were kept to model baseline (Fig. 3A). Second, variations due to endogenous dopamine release (100%) in the striata were modeled as described in the previous sections (simulations paragraph) for the first and the second experimental conditions (R+/R− and R−/R+). Third, we included predictors for possible variations induced by changes in blood flow ($k_1 = +50\%$; $k_2 = +25\%$). We included in the model the regressors for possible blood-flow effects in order to adopt the most inclusive strategy. As shown in the simulations, if k_1 and k_2 varied at the same rate, there would be no net effect on the TAC, or at most a very mild decrease, and the data would still be modeled efficiently by setting the regressor weights to zero or to a small negative value for these regressors. As it turned out, however, we observed significantly positive weights for the CBF regressors in many voxels, indicating that it was indeed useful to model the possible impact of CBF changes. For both dopamine and CBF changes, the corresponding baseline functions were subtracted. The resulting regressors (Fig. 3B) represent the expected perturbations induced by stimulation. Their predicted temporal profiles differ not only in the direction but also in the shape of the induced change. The CBF temporal profile shows an earlier peak and return to the base-line than the DA temporal profile. Those shape differences, although small, are sufficient to allow us to separately estimate these two effects by linear regression. All seven regressors were entered concurrently in a linear model that could approximate the temporal behaviour across regions, under dopamine release or/and blood flow increase or under baseline, hence allowing to test specifically for the endogenous dopamine release. The same model also allowed us to test the opposite effects, i.e., the increase in D2 availability due to a possible decrease in extracellular DA release and the decrease in CBF. Within the range of small changes, the impact of either type of change on the TAC is linear (as a first approximation) (for a full description of this approach, see Friston *et al.*, 1997). Thus, within the linear range, the same model can account for both increased or decrease CBF, as well as increased or decreased D2 receptor availability. Increases and decreases will simply be distinguished by the sign of the regressions weights. The parameters of the model were fitted using a weighted least square procedure taking into account that the noise variance increases with time due to radioactive decay.

Statistical analysis. We will refer to the terms "dopamine release" and "CBF increase" to describe respectively the effects induced on the [^{11}C]raclopride TAC by an increase in levels of extracellular dopamine (decrease of [^{11}C]raclopride total concentration) and in CBF (increase of [^{11}C]raclopride total concentration). The changes in dopamine release and CBF were tested in R+ and R− blocks voxel by voxel using a group analysis and a fixed effect model pooling together the dynamic data from the 12 subjects to increase statistical power. We also tested whether dopamine release and CBF increase were higher in R+ than R− blocks or in R− than R+ blocks. To assess the reliability of our model we also tested in subjects studied at rest ($n = 4$) the effects of dopamine changes and CBF changes during two 5-min blocks starting at 30 and 60 min after the injection of the tracer. Statistical inferences, corrected for the volume analyzed, were based on the theory of random Gaussian fields (Friston *et al.*,

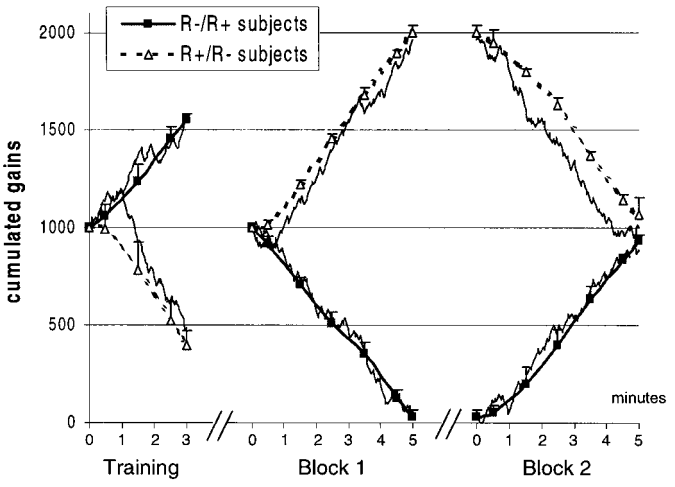

FIG. 4. Evolution of monetary gains in the two groups of subjects. The R+/R− lost money during the training period, gained money during PET block 1, and then lost money during PET block 2. The converse schedule held for the R−/R+ group. For each block, the initial value, final value, and minute-by-minute average of the subjects' gains are shown for each group (thick curves, means ± 1 Se) as well as for two representative subjects (thin lines).

1995b). The statistical analysis was performed only on the striatal volume, as defined using a thresholded mask obtained from the symmetrical normalized [^{11}C]raclopride template. Two types of statistical analyses were used. First, based on the literature, we defined two regions of interest in the left and right ventral striata, which were spheres of 6-mm radius centered on Talairach coordinates +/−18, +12, −6 mm (Elliot *et al.*, 2000). Voxels within that sphere were defined as significant if they passed $P < 0.05$ corrected for both voxel height and for cluster level, as calculated using the small volume correction (SVC) option of SPM99. Second, we performed an analysis of the entire striata without *a priori* hypothesis. In that case, regions were considered significant at threshold of $P < 0.001$ uncorrected for voxel height and $P < 0.05$ corrected for cluster extent.

RESULTS

Behavior

As shown in Fig. 4, the reward schedule was successful in imposing approximately fixed gains or losses per minute during R+ and R− blocks.

Analyses of variance were conducted on response times and error rates, with block type (R+ or R−) as a within-subject factor and group (R+/R− or R−/R+) as a between-subject factor. As expected, subjects responded faster on R− blocks than on R+ blocks (353 versus 394 ms; F (1,6) = 30.0, P = 0.0015), but this was at the expense of accuracy (27.2% versus 17.1% errors; F (1,6) = 59.3, $P < 0.001$). There was no difference between groups on response times (F (1,6) = 1.96), but the R+/R− group made more errors (26.8% vs 17.4%; F (1,6) = 7.92, P = 0.03).

PET

Our model allowed to detect significant DA release and CBF increase effects in the striatum. The results showed that the regressor weights for blood-flow effects differed significantly from zero, suggesting that blood flow changes did contribute to the observed TAC.

Results of SVC analysis. A significant effect due to dopamine release was found in the ventral striatum bilaterally during periods of monetary gain (R+). The peaks were located in the anterior part of the right ventral striatum (Fig. 5), close to the nucleus accumbens, and in the medial part of the left ventral striatum. During the periods of monetary loss (R−), no effect was found. This resulted in a greater dopamine release in R+ than in R− in the left ventral striatum (Table 1). No greater dopamine release was found in the ventral striatum when R− conditions were compared to R+ conditions. Significant CBF increase was found in both R+ and R− conditions. During the R+ condition the peaks were located in the lateral part of the ventral striatum bilaterally (Table 2; Figs. 6A and 6B). During R− periods significant activation was found only in the right ventral striatum (Table 2; Figs. 6C and 6D). No CBF increase was found in the direct comparison of R+ and R− conditions. No significant dopamine release and CBF increases were found in the ventral striatum when the subjects were studied during the rest condition.

No significant decrease in CBF and/or extracellular DA levels were found in the ventral striatum

Analysis on the entire striatum. At the thresholds used for this second statistical analysis no significant dopamine release was found during the R+ and R− conditions and no difference could be detected when R+ conditions were compared to R− conditions. The CBF increase survived to the thresholds established

TABLE 1

Voxels with Effects Due to Dopamine Release within the Small Volume of the Ventral Striatum (SVC Analysis)

Region	Left/right	MNI coordinates (x, y, z)			*Z* value
R+					
Ventral striatum	R	15	15	−9	2.80
Ventral striatum	L	−18	12	−12	2.92
R−	No significant voxels				
R+ vs R−					
Ventral striatum	L	−18	9	−9	2.55

Note. Height threshold = $P < 0.05$ corrected; extent threshold = $P < 0.05$ corrected.

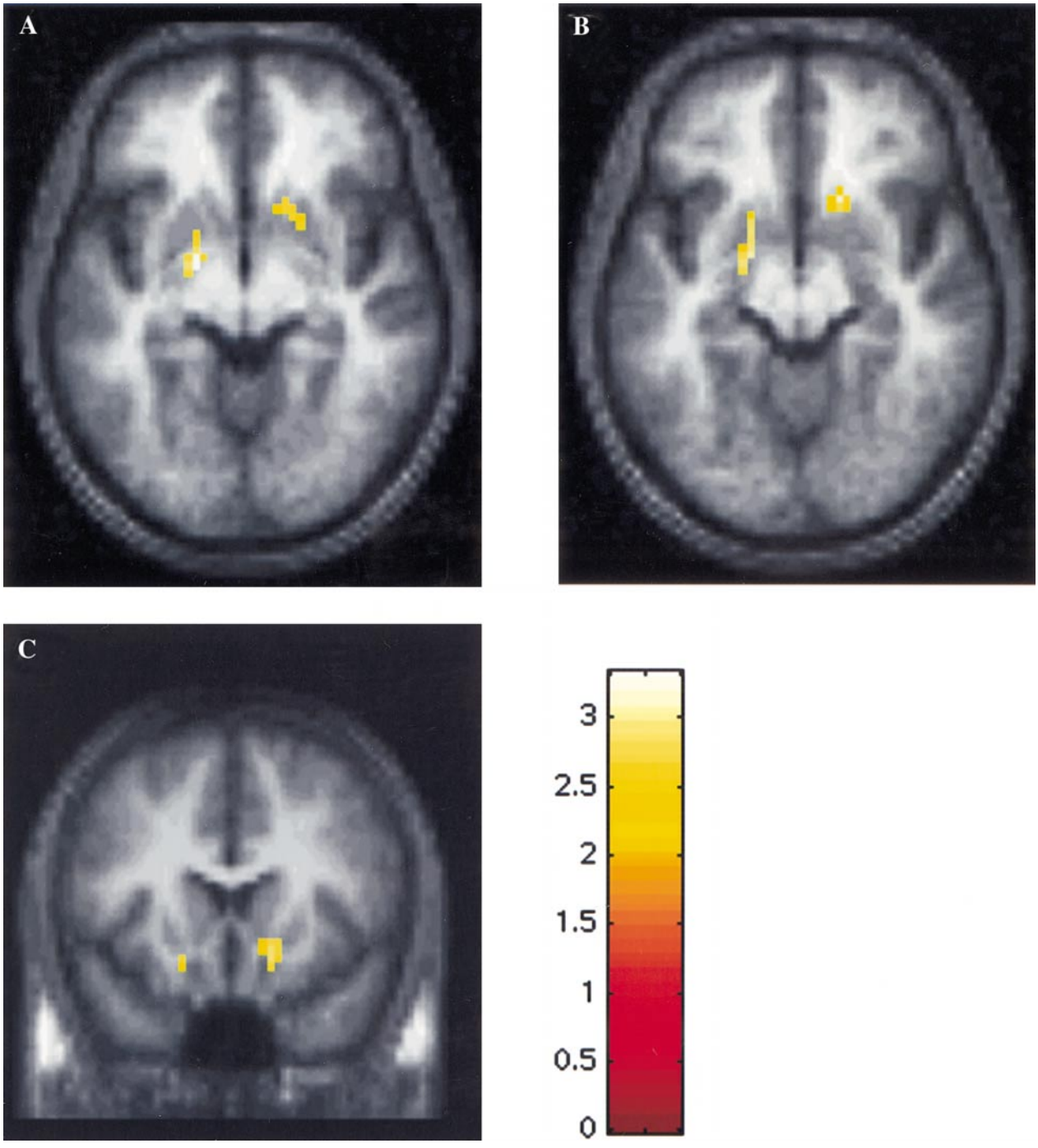

FIG. 5. Voxels displaying dopamine release in the striatum in response to R+ and rendered onto the averaged T1-MRI images obtained in the subjects and spatially normalized to the MNI space. For illustrative purpose the *t* statistic maps were thresholded at the significance level of $P < 0.01$, uncorrected for the peak height and $P < 0.1$ corrected for cluster size. These images show a bilateral activation in the ventral striatum. A ($z = -6$); B ($z = -9$); C ($y = 15$).

for this analysis (i.e., $P < 0.001$ uncorrected for the peak height and $P < 0.05$ corrected for the extent). This effect was globally more marked and more widespread during R+ than R− condition (Table 3). The foci of activation were located in the right and left ventral striatum but also in regions near the left caudate nucleus and pallidum. During R+ condition the activation in the right ventral striatum appeared somewhat spread to the inferior orbitofrontal cortex while in the left ventral striatum involved also the anterior cingulate cortex. This could be an effect resulting from the smoothing and/or an edge effect due to the striatal

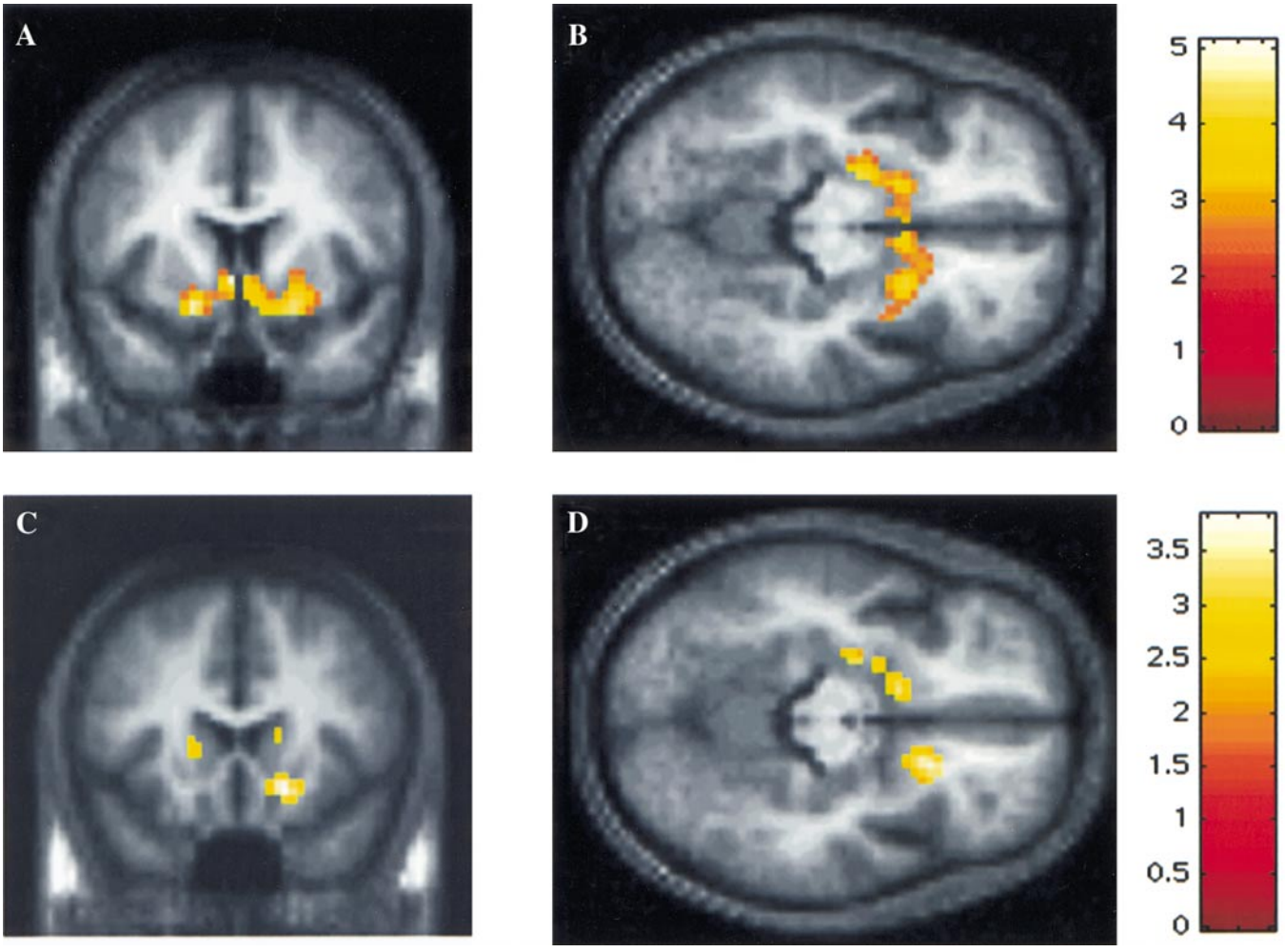

FIG. 6. Voxels displaying CBF increase in response to R+ (A, $y = 9$; B, $z = -9$) and R− (C, $y = 16$; D, $z = -12$) rendered onto the averaged T1-MRI image obtained in the subjects and spatially normalized to the MNI space. For illustrative purpose the t-statistic maps were thresholded at the significance level of $P < 0.01$, uncorrected for the peak height and of $P < 0.1$ corrected for cluster size. The CBF increase involved the ventral striatum bilaterally and other striatal areas (C).

mask used for statistical analysis. During R− condition only the right ventral striatum revealed significant CBF effects. No significant differences were found at this threshold when R+ blocks were compared to R− blocks, or in the inverse comparison.

At the same threshold, in the subjects studied at rest no significant dopamine release was observed while a significant CBF increase was found in a region located between the putamen and the caudate nucleus close to the anterior arm of internal capsule (−21 12 9; *x*, *y*, *z*).

No significant decrease in CBF and/or extracellular DA levels were found in the entire striatum analysis.

DISCUSSION

The results of the present study suggest that our method allows to detect changes in [^{11}C]raclopride binding in the ventral striatum compatible with an increase in dopamine release during a positively rewarded effortful task. These changes are moderate but consistent with data reported in animals and suggest that the mesolimbic dopaminergic system is implicated in reward-associated processing.

In a pioneering study with PET and [^{11}C]raclopride, Koepp *et al.* (1998) showed that dopamine release could be detected in human striatum while subjects were engaged in a financially rewarded video-game. They quantified the dopamine release using a dual scan approach, that requires two separate PET scanning sessions, and used a task that did not separate reward, attention and sensorimotor contributions. Our study proposes a new method for *in vivo* detection of dopamine release at rest and during two short activation conditions using a single PET study. Moreover it specifically addresses the issue of striatal dopamine involvement in the processing of reward information. In the dual scan approach (Koepp *et al.*, 1998), the increased release of endogenous dopamine was inferred

TABLE 2

Voxels with Effects Due to CBF Increase within the Small Volume of the Ventral Striatum (SVC Analysis)

Region	Left/right	MNI coordinates (x, y, z)			Z value
R+					
Ventral striatum	R	21	9	−9	4.01
Ventral striatum	L	−18	12	−12	3.91
R−					
Ventral striatum	R	18	12	−12	3.11
	R	15	15	−9	3.08
R+ vs R−	No significant voxels				

Note. Height threshold = $P < 0.05$ corrected; extent threshold = $P < 0.05$ corrected.

from the reduction of the binding potential (BP) values in the striatum during the activation as compared to those obtained in the control study. To maximize the effects the subjects were engaged in a sustained videogame task for 60 min (from 10 min before to 50 min after the bolus injection of the tracer). Despite this strong activation task, that engaged both the motor and the cognitive components of striatal dopamine, the averaged BP reduction was only 13% in the ventral striatum. Recently a statistical method has been proposed aimed to increase the sensitivity of this approach (Aston *et al.*, 2000). The main limitations of this method remain, however, the high injected dose and the variability of the input function and of the endogenous dopamine levels between the baseline and the activation studies (usually performed on two different days). Finally the sustained activation may be not adapted to some experimental paradigms. The single-scan design proposed in our approach reduces these limitations. It may also enhance the sensitivity in the detection of the effects induced by the activation by increasing the statistical power, due to a combination of the increase in the number of degrees of freedom (i.e., the number of frames), the within-subject design, and the voxel-based analysis. It is noteworthy that moderate dopamine release could be detected even during short periods of activation (5 min).

Our study allows us to better specify the relation between dopamine release and reward. Our results support the hypothesis that the ventral striatum, and particularly the nucleus accumbens, seems to be the critical structure in mediating the response to reward signals. This region receives dopaminergic projections from the midbrain VTA neurons. VTA neurons are thought to code for errors in the prediction of occurrence of rewards. Their activity is increased when positive rewards are received beyond previous expectation while it is depressed when an expected positive reward is omitted (Schultz *et al.*, 1997). The experimental paradigm used in this study was developed to probe similar phenomena in humans. It was designed to compare two conditions in which rewards are mostly positive (periods of monetary gain or R+) or mostly negative (periods of monetary loss or R−). Furthermore, training periods with outcomes opposite to those of the subsequent experimental block were used to prevent subjects from anticipating the periods of monetary gain and monetary loss. Thus R+ blocks were periods of unpredicted positive reward and R− blocks were periods with loss of an expected positive reward. We found an increase in dopamine release in the ventral striatum bilaterally during R+ blocks but none during R− blocks. These results are consistent with the hypothesis proposed by Schultz and suggest that ventral striatal dopamine release is associated with rewarding rather than stressful stimuli. The alternative hypothesis that dopamine release is associated with stress and/or attention (Feenestra, 2000; Pressner *et al.*, 2000) would have predicted a release of dopamine in the striatum during periods of R− where the subjects were loosing money and were, of anything, more stressed than in R+ blocks. However, this stress hypothesis was rejected in our data.

Our findings also suggest that the effects induced by dopamine release on the striatal [^{11}C]raclopride time-activity curves could be distinguished from those presumably due to an increase in CBF during the task. These CBF effects have been modeled and their changes during the activation were statistically tested. The results showed significant CBF increase during both the R+ and R− blocks. Interestingly, the spatial localization and magnitude of these effects did not perfectly parallel that of dopamine release. They were more marked and widespread within the striatum and they were associated to both R+ and R− activation

TABLE 3

Voxels with Effects Due to CBF Increase (Whole Striatum Analysis)

Region	Left/right	MNI coordinates (x, y, z)			Z value
R+					
Ventral striatum/OF	R	21	6	−15	5.06
	R	9	6	−15	4.00
Ventral striatum	L	−3	9	−3	4.79
	L	−18	9	−12	4.73
Ventral striatum/CG	L	−9	24	−6	4.16
Ventral striatum	L	−21	−9	−9	4.15
Caudate	L	−15	−9	18	3.85
R−					
Ventral striatum	R	18	18	−12	3.76
Pallidum	L	−18	−6	6	3.81

Note. Height threshold = $P < 0.001$ uncorrected; extent threshold = $P < 0.05$ corrected. OF, orbitofrontal cortex; CG, cingulate gyrus.

periods, though less marked in this latter. These results are in part in line with those reported by fMRI studies and showing that significant activation in the right ventral striatum was associated with positive financial rewards (Elliot *et al.*, 2000; Breiter *et al.*, 2001). We found CBF increase not only in the right but also in the left ventral striatum. The peak locations in right and left ventral striatum were however different. Moreover CBF increase was found in the right ventral striatum in both R+ and R−. This could be in agreement with the lack of a main effect of winning or loosing in this structure during financial rewards (Elliot *et al.*, 2000). Other striatal regions showed significant CBF increase. The peaks of activation were sometimes outside the structure (i.e., between the caudate and the putamen). It is difficult to establish whether these foci are true activation or residual artefacts after correction for movement. It is tempting to speculate that the CBF effects in regions near the left pallidum found in both R+ and R− could be a result of the motor response made with the index and middle finger of the right hand (Lehericy *et al.*, 1998).

The approach proposed in this study may suffer from some methodological limitations. The first potential criticism is that the occurrence of endogenous dopamine release and CBF increase during the time of activation is inferred from changes in the shape of the uptake curve predicted on the basis of simulations. Thus, the reliability of this method depends on the validity of the assumptions used for simulations and on how well the simulated curves describe the biological variables. The first critical point is the model applied to describe these changes. We used a simple competitive binding model and assumed that transient changes in extracellular DA could lead to transient changes in D2 receptor availability. Although it remains debated, this is the model which underlies all studies in the field and to date there are no available experimental data to confirm or reject its appropriateness.

The second critical point concerns our assumptions about the amount and temporal changes in extracellular DA levels during the activation and the rest conditions. We assumed that endogenous dopamine was increased by 100% during activation, an estimation derived from animals studies (Fisher *et al.*, 1995) and recently supported by human data (Fried *et al.*, 2001). This exact numerical value is not important, however, because the linear model can accomodate a large range of quantitative changes in dopamine release, and quasilinearity was checked on the range 0–1000%. We also assumed that the endogenous dopamine variations occurred only during the activation periods and in a step way. This temporal behaviour of endogenous dopamine release fits with the temporal profile of extracellular dopamine changes measured by microdialysis in the human amygdala during cognitive activation (Fried *et al.*, 2001). The simulations showed that these assumptions lead to a "relatively" transient decrease of striatal [^{11}C]raclopride radioactive concentration. In fact, after the peak the TAC slowly returned to baseline values within a period of about 30 min. It is noteworthy, however, that the activated curve, although converging to the baseline curve, did not completely reach the same value. These results are in line with simulated data from Morris *et al.* (1995) showing that 7-min activation resulted in a rise in [^{11}C]raclopride TAC with a return to near baseline curve within 40 min, that is 33 min after the end of activation. These data are, however, not completely in line with previous results of sustained cognitive activation (Koepp *et al.*, 1997) and amphetamine challenge (Laruelle, 2000), which both showed a persistent drop in [^{11}C]raclopride TAC long after the end of the challenge. It should be stressed, however, that sustained behavioral activation for an hour may possibly result in prolonged extracellular DA changes that extend beyond the period of stimulation proper. Likewise, the effects of amphetamine challenge are likely different from those induced by short periods of cognitive activation. It is noteworthy that at 30 min after amphetamine bolus injection, extracellular levels of DA, as measured with microdialysis in baboon, are still five fold higher than baseline values (250 nM versus 50 nM) (Endres *et al.*, 1997). Finally, it should be also stressed that both the amplitude and the temporal profile of dopamine increase could be variable among subjects and in part related to the cognitive processing involved in the task (Fried *et al.*, 2001). We found that the maximum of signal variation induced by a 100% dopamine increase during 5 minutes was around 8%. This amplitude is consistent with that reported by Morris *et al.* (1995). A period of activation of ten minutes and/or an increase in the number of subjects could further improve the detectability of dopamine release.

A third important methodological issue concerns the effects of CBF changes on [^{11}C]raclopride binding. This point is debated and only anecdotal reports have addressed this issue in the literature, as reported in the Methods section. It is generally proposed that a change in CBF should similarly affect both constants k_1 and k_2. On the other hand experimental data suggest that k_1 can be affected more than k_2 by CBF changes (Logan *et al.*, 1994). Our results seem more in line with the assumption than CBF increase during activation results in a greater increase in k_1 than k_2. If blood flow always induced the same rate of change in both k_1 and k_2 constants, then our simulations suggest that there should be little or no net effect on the TAC. In that case, there would be no need to include a regressor for blood-flow effects in the statistical model, and all changes in the TAC would be essentially imputable to changes in endogenous dopamine level. Our simulations, however, show that this is no longer true if k_1 varies differently from k_2. In this case the direction of

the effect is opposite to that of DA release and the shape in part different. Our results showed that the regressor weights for blood-flow effects differed significantly from zero, suggesting that blood flow changes did contribute to the observed TAC.

Another potential limitation of our approach relates to the validity of the statistical model in describing the biological effects. To be valid, the statistical model must fit closely with the measured PET signal. Therefore we checked the residuals of the model in the regions where the dopamine release and CBF increase were detected. No clear structure was observed in the residuals suggesting that the model provided a good fit.

Finally, another crucial issue relates to the effects of head motion during the study. We found that even small movements in different directions could result in false positive CBF increase and endogenous dopamine release effects. This is especially critical when the movement is correlated with the paradigm and the targeted structure is small and located in the inferior part of the striatum. Late after the injection, [^{11}C]raclopride uptake is maximal within the striatum and low in the surrounding regions. Thus a displacement of the subject could lead to a false detection of a change in raclopride uptake at the border of the striatum. To reduce these confounding artefactual effects we had to correct the dynamic [^{11}C]raclopride frames for movements. To realign our data we have used the sum of square differences (SSD) algorithm implemented in SPM. This choice was based on the observation that when we applied other algorithms, such as those that use the mutual information (Maes *et al.*, 1997), the results were less robust. In particular we observed that this latter approach was very sensitive to the noise in the late frames, resulting in realignment parameters that are not realistic. It should be stressed that the issue of realignment of this kind of data is still debated and there are no conclusive data in the literature on the robustness of the different methods actually available.

In conclusion, the single-scan approach proposed in this study seems capable of detecting dopamine release in the ventral striatum associated with positive unexpected reward. This is a new approach that should be applied to PET ligands that reach equilibrium conditions within a relatively short time and should theoretically allow to detect in a single study both CBF and neurotransmitters changes during activations. Further studies are required to better validate this method and the underlying assumptions and to improve the experimental design in order to increase the detectability of these changes.

APPENDIX A: RESPONSE DEADLINE ALGORITHM

This algorithm was used to modify, on each trial, the response deadline (RD), which is the minimal response time below which a trial is classified as too slow. Using this algorithm, one can tightly control the gains or losses of the subject, though only in a statistical sense because the gain or loss on any single trial remains strictly determined by whether the subject responds correctly and meets a preestablished deadline.

Assume that the subject starts with a certain amount of money SA (starting amount).

Experimenter desires this value to reach a certain target amount TA (which can be smaller or larger than SA) after N trials of testing. The algorithm requires the computation of MRT, the mean of the subject's response time. Initially, it is computed on an unrewarded training block. Later, MRT is updated every minute to the subject's mean response time during the preceding minute.

Then, on each trial n,

- Update the current amount of the subject's gains (CA) based on subject's performance on this trial.
- Compute the desired amount of the subject's gains (DA) at this point in this experiment: $DA = SA + (TA - SA)n/N$
- Compute the normalized deviation to the desired amount, $\Delta = CA - DA/SA$
- Then the response deadline for the next trial is $RD = MRT/1 + \alpha \cdot \Delta$

α is a parameter that determines how stiffly the algorithm responds to deviations from the desired gain curve. In the present experiments, we used $\alpha = 1.5$ throughout, except during the last 10 trials of each block where we used $\alpha = 3$ to ensure strong convergence to the desired amount DA.

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