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Measuring the neuromodulatory effects of drugs in man with positron emission tomography

K.J. Friston[a], P.M. Grasby[a,c], C.J. Bench[a,c], C.D. Frith[a], P.J. Cowen[b], P.F. Liddle[a], R.S.J. Frackowiak[a] and R. Dolan[a,c]

[a]*MRC Cyclotron Unit, Hammersmith Hospital, London (UK),* [b]*MRC Psychopharmacology Research Unit, Littlemore Hospital, Oxford (UK) and* [c]*Academic Department of Psychiatry, Royal Free Hospital Medical School, London (UK)*

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Cognitive activation in conjunction with pharmacological challenge was used to demonstrate neuromodulation in man. Using positron emission tomography (PET), measurements of regional cerebral blood flow were made during the performance of memory tasks, before and after the administration of apomorphine (dopamine agonist), buspirone (5-HT_{1A} partial agonist) or placebo. Drug effects on memory-induced increases in regional cerebral blood flow were assessed, on a voxel-by-voxel basis, using statistical parametric mapping. Increases of regional cerebral blood flow in response to the memory challenge were attenuated by apomorphine in the dorsolateral prefrontal cortex and augmented in the retrosplenial region of the posterior cingulate. Conversely, buspirone attenuated blood flow increases in the retrosplenial region. These interactions between drugs and a cognitive challenge can best be interpreted as neuromodulatory effects.

Monoaminergic neurotransmitters have been proposed to have a neuromodulatory role in behavioural regulation [1, 2, 4]. The measurement of a neuromodulatory effect requires the conjoint manipulation of neuromodulatory neurotransmission and an independent activation of another neural system to be modulated. We report experiments in which a memory activation in conjunction with pharmacological challenge was used to investigate neuromodulation in man.

Dopamine neurotransmission in the dorsolateral prefrontal cortex (DLPFC) is important in the execution of mnemonic tasks in primates [2, 18]. In addition, electrophysiological evidence supports a neuromodulatory role for dopamine at this site [11, 14, 16]. The hippocampal formation is also implicated in memory function [6, 12] and 5-HT_{1A} receptor-active drugs have been shown to have neuromodulatory effects in this region [3, 21]. Thus monoaminergic neuromodulatory effects have been demonstrated at two discrete brain areas implicated in the functional anatomy of memory. We therefore predicted that: (1) a memory task would activate the DLPFC and the hippocampal formation, (2) apomorphine (a dopamine agonist) would attenuate this activation in the DLPFC and (3) buspirone (a 5-HT_{1A} partial agonist) would attenuate activation in the hippocampal formation. We tested, and partially confirmed, these predictions using combined cognitive and pharmacological activation in conjunction wtih positron emission tomographic (PET) measurements of regional cerebral blood flow (rCBF) in man.

Twenty-four normal (right handed) male subjects were subjected to cognitive and pharmacological activation during 6 serial PET measurements of rCBF over a 75 min period. We used a CTI model 931-08/12 (Knoxville, TN, USA) scanner giving a (reconstructed) transaxial resolution of 8.5 mm [20]. To measure rCBF subjects inhaled $C^{15}O_2$, mixed with air, at an activity of 6 MBq/ml and a flow rate of 500 ml/min through a face mask for a period of 2 min [13]. Integrated counts per pixel for the 2 min build-up phase of radioactivity in the brain during $C^{15}O_2$ inhalation were used as an index of rCBF [5]. Permission to administer radioactive substances was obtained from the local ethical committee and was approved by the Advisory Committee on the Administration of Radioactive Substances (UK).

During scanning each subject performed 3 successive pairs of tasks consisting of a baseline task (subspan memory task during scans 1, 3 and 5) and an activation task (supraspan memory task during scans 2, 4 and 6).

Correspondence: P. Grasby, MRC Cyclotron Unit, Hammersmith Hospital, DuCane Road, London W12 OHS, UK.

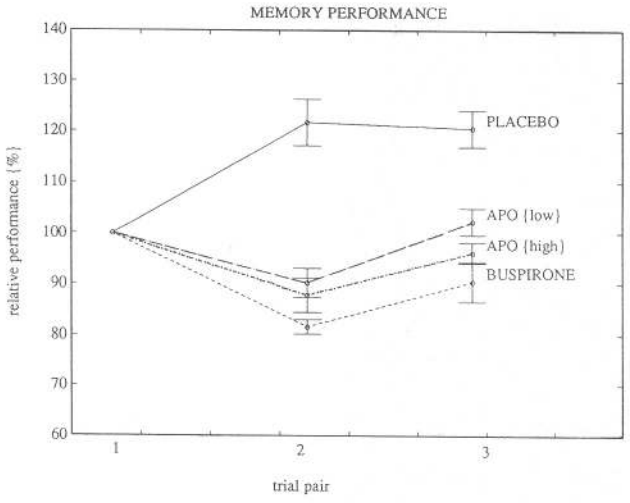

Fig. 1. Mean performance (with S.E.M.) for each drug group on the post-drug supraspan memory tasks (expressed as the percentage of words recalled during the first trial). 100% on ordinate = 26 ± 2 words recalled for pre-drug placebo, 25 ± 3 for pre-drug buspirone, 29 ± 3 for pre-drug apomorphine (5 μg/kg) and 34 ± 5 for pre-drug apomorphine (10 μg/kg) means ± S.D. Abscissa = trial 1 (pre-drug), trial 2 and 3 (first and second post-drug trials).

Each pair of scans were 10 min apart. In the subspan condition subjects repeated lists of words presented auditorally 5 at a time. In the supraspan task words were presented 15 at a time. Subjects were asked to immediately repeat the words they could remember after each presentation of a 5 or 15 word list. A total of 45 words were presented in both conditions (i.e. nine 5-word lists during subspan condition and three presentations of a 15-word list during supraspan condition). The difference in the demand placed on auditory-verbal memory by the sub and supraspan tasks served as the memory challenge. After the first task pair an active drug or placebo was administered. Six subjects received placebo (water s.c.), six apomorphine (5 μg/kg s.c.), six apomorphine (10 μg/kg s.c.) and six buspirone (30 mg orally).

Memory performance was recorded as the number of words recalled in the supraspan tasks. To examine the effects of drugs on memory, subjects performance following drug was expressed as a percentage of the words recalled in the pre-drug task (Fig. 1). The second and third supraspan tasks were then entered into an ANOVA (4{drug} × 2{trial}). There was a significant effect of drug (F=12.5, P<0.0001, df 3,40) but no time effect (F=1.6, P=0.13, df 1,40). When the 3 non-placebo groups were analyzed separately there was no difference between the effect of active drugs (F=1.7, P=0.19, df 2,30) but there was a trend towards rebound in performance on the second post-drug trial (F=3.1, P=0.076, df 1,30). In summary, all three active drug administrations resulted in impaired performance on the first post-drug trial with a rebound on the second post-drug trial (Fig. 1).

The rCBF data were analysed in two stages. First the voxels exhibiting a memory activation were identified. These voxels constituted the 'memory' system. The modulatory effects of the drugs were then assessed for all voxels in the memory system by identifying those regions where memory activation was significantly attenuated following drug administration.

To undertake the above analysis the techniques of statistical parametric mapping were used [7–10]. In brief, all scans were first stereotactically normalized [7, 9] to allow inter-subject averaging of functional images. To define the memory activation all 72 supraspan conditions were compared with the 72 subspan conditions with a comparison of means using the t-statistic [10]. This comparison followed an analysis of covariance (ANCOVA) to remove the confounding effect of inter and intra-subject variation in global flow [8]. The comparison (i.e. subtraction) of subspan scans from supraspan scans removed cognitive components common to both tasks such as auditory stimulation. The resulting profile of rCBF represents the essential difference between scans and reflects auditory verbal memory engagement. A high threshold (P<0.001) was chosen to ensure a memory activation effect that was sufficiently substantial to allow the detection of a modulatory change.

The profile of memory activation is seen in Fig. 2 (top) and as predicted included the DLPFC (Brodmann's areas 44, 45, 46 and 10). In addition, the precuneus (Brodmann's area 31) and retrosplenial regions of the posterior cingulate (Brodmann's areas 29/30) were also activated (circled). In contrast to our prediction no activation was seen in the hippocampus although the retrosplenial activation subsumed the posterior extent of the parahippocampal gyrus.

After identifying the 'memory system', the 4 drug treatment groups were then entered separately into an ANCOVA [8] and the difference in memory activation pre-drug and post-drug was assessed on a voxel-by-voxel basis using the t statistic [10]. The resulting t-values (P<0.05) for each voxel are seen in Fig. 2 (bottom) for apomorphine (10 μg/kg) and for buspirone. As predicted, apomorphine attenuated the memory activation in the DLPFC (maximum t = 3.26, P <0.01 at −42, 20, 20 mm — Brodmann's area 45, 46 according to the atlas of Talairach and Tournoux [22]. No such attenuation was seen in the retrosplenial region. Buspirone, in contrast, attenuated rCBF increases in the retrosplenial region (maximal t = 2.92, P<0.01, at −6, −44, 4 mm — Brodmann's area 29/30) with a less marked effect in the DLPFC.

A striking finding concerning the observed interac-

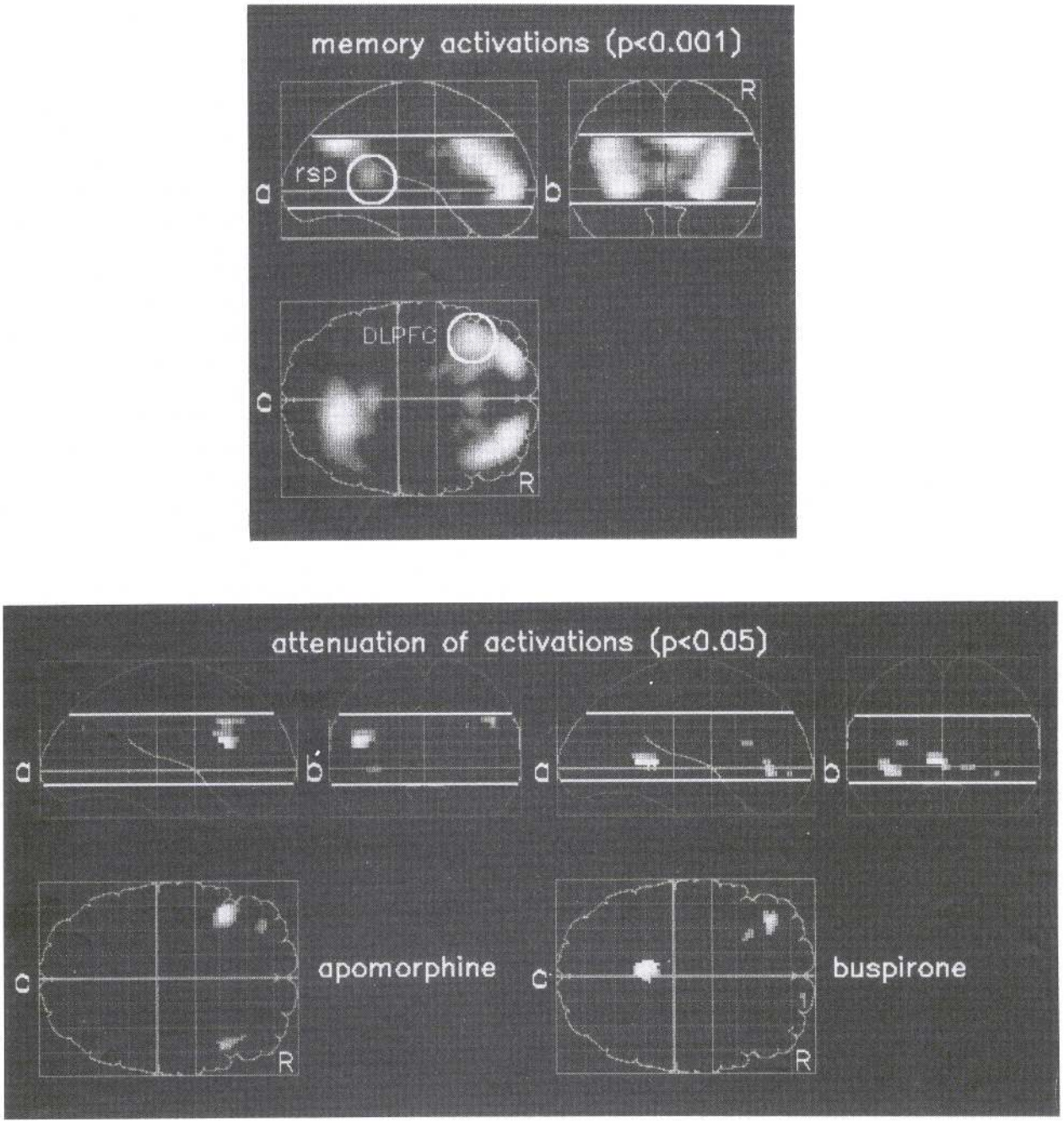

Fig. 2. Statistical parametric maps of the *t*-statistic, displayed as volume images viewing the brain from the right (a), from the back (b) and from above (c). The brightest voxel along any line of view is displayed. R, right. Top: the increases in rCBF during all supraspan memory tasks compared to the subspan conditions. The retrosplenial/parahippocampal region (rsp) and dorsolateral prefrontal cortex. (DLPFC) have been circled for clarity. Only *t*-values corresponding to $P<0.001$ are displayed. Bottom: the attenuation of the above increases on comparing the pre-drug sub/supraspan pair with the first post-drug pair in the apomorphine (10 μg/kg) and buspirone treatment groups. Only *t*-values corresponding to $P<0.05$ are displayed.

tions is that they are localized and discrete. For both doses of apomorphine the attenuation of rCBF activation involved the DLPFC. Though this effect was bilateral, it was more marked on the left. These data support the contention that monoaminergic projections show regional specificity of function [17].

Fig. 3 shows the change in the activation effect for the two regions in question. The left DLPFC at −42, 22, 20 mm (BA 45/46) and the retrosplenial region −6, −42, 8 mm (BA 29/30). These stereotaxic coordinates are precise but the data represents rCBF equivalents in a (weighted) spherical domain of 20 mm diameter because of the smoothing function applied during statistical parametric mapping [10]. In the DLPFC, in contrast to placebo, all drugs attenuated the activation in the first post-drug pair with a rebound in the second post-drug pair.

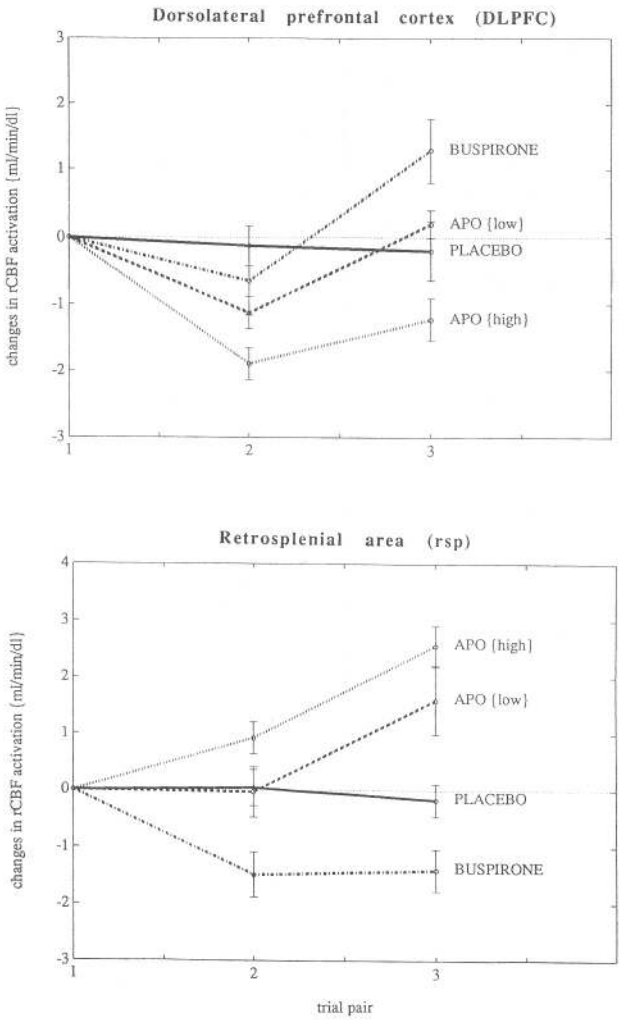

Fig. 3. The effect of drug on memory induced activations expressed as rCBF equivalents (normalized to a whole brain mean of 50 ml/min/dl in the ANCOVA) at the sites of interaction. The change in activation (supraspan minus subspan) is the difference between the post-drug activations and the pre-drug activation for each subject. Solid line, placebo group; broken lines, drug groups; circles, means; bars, S.E.M. APO {high} — 10 μg/kg. APO {low} — 5 μg/kg. Abscissa = trial 1 (pre-drug), trial 2 and 3 (first and second post-drug trials).

The attenuation was significant for both doses of apomorphine but not for buspirone. In the retrosplenial region the memory activation in the placebo group was again stable over time. Both doses of apomorphine augmented the activation effect whilst, conversely, buspirone significantly attenuated the activation. The augmentation of rCBF in the retrospinal region following apomorphine was not predicted. The reciprocal changes in rCBF activation in the DLPFC and retrosplenial region may reflect a functional relationship between these sites that needs further exploration.

We have used rCBF as an indirect index of trans-synaptic neurophysiological activity. However, the observed effects on rCBF might be explained by a direct effect of the drugs on the cerebral vasculature. The marked regional specificity of these results and the attenuation of cognitive (i.e. neurogenic) induced increases in blood flow are strong arguments against such an explanation. It should be noted that the interaction effects may reflect direct drug induced changes in receptor function at the sites of interaction or effects one or more synapses away [15, 19]. Wherever the exact pharmacological site of action of the drugs this does not detract from the conclusion that neuromodulation is occurring at the observed sites.

In conclusion, we have shown that acute interactions between cognitive and pharmacological manipulations of neurophysiology (indexed by rCBF) can be detected with PET. A regionally specific modulatory role for apomorphine has been demonstrated for DLPFC functional activity and similarly for buspirone in the retrosplenial region. This dissociation is in part consistent with predictions from animal studies. These experiments provide a strategy for further assessment of the mechanisms of pharmacologic effects on neuropsychological functions and in addition may provide a way of linking animal work, on the neurotransmitter basis of higher brain function, to in-vivo studies in man. A fuller description of the methodology in this paper will be presented at a later date.

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