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Dissociating neuromodulatory effects of diazepam on episodic memory encoding and executive function

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Abstract Rationale: Diazepam and other benzodiazepines impair episodic memory encoding. Deficits in tests of executive function are also reported. In this study, we ask whether the latter effects are secondary to mnemonic impairment, or reflect specific and distinct effects of benzodiazepines on executive function. **Objectives:** Using positron emission tomography in healthy human volunteers, we examined similarities in the neuroanatomical correlates of the effect of diazepam on performance of executive compared to episodic memory tasks. Close similarities are proposed to reflect commonalities in the functional effects of the drug. Conversely, any evidence of task-specific regional changes in activity is proposed to reflect distinct functional effects of DZP on the two tasks. **Methods:** Twelve volunteers received placebo or 10 mg diazepam in a between-subjects design. During scanning, subjects performed one of four experimental conditions, corresponding to a 2×2 factorial design, with memory encoding and executive function (on-line ordering of stimuli) as the two factors. Drug- or task-induced changes in brain activation indexed the neuroanatomical correlates of each condition. **Results:** Averaged across all conditions, and compared to placebo, diazepam decreased activity bilaterally in prefrontal and temporal cortices. Within this network of deactivation, left dorsal prefrontal cortex activity was attenuated by diazepam during memory encoding, while left frontal opercular activity was attenuated during ordering. **Conclusion:** This neuroanatomical dissociation reflects distinct functional effects of diazepam on encoding versus ordering tasks. Therefore, the effects of diazepam on ordering tasks are not simply secondary to diazepam effects on episodic memory, but reflect real and distinct effects of the drug on executive function.

Key words Human · PET · Benzodiazepine · Working memory · Left prefrontal

Introduction

The influence of benzodiazepines (BZ) on cognition has been studied principally in relation to effects on memory (see Curran 1991 for a review). A consensus is that BZs do not affect semantic memory, but cause significant impairments in episodic memory function. This deficit is thought to be due to an effect at encoding rather than retrieval (see Curran 1986, 1991 for reviews). For example, Ghoneim et al. (1984) found a dose-dependent (0.1, 0.2 and 0.3 mg/kg diazepam) decrease in the recall of early items in a free recall verbal task, while later items that could be maintained in short-term memory were less affected (see also Frith et al. 1984; Roy-Byrne et al. 1987; Rodrigo and Lusiardo 1988; Hennessey et al. 1991; Rosier et al. 1996; Robbins et al. 1997). Diazepam-induced encoding deficits on a test of visuo-spatial episodic memory and learning have also been reported (Coull et al. 1995a). Importantly, in the same study, DZP-induced deficits in tests of frontal lobe function were also observed (Coull et al. 1995b). Specifically, DZP-treated subjects showed exactly the same profile of performance as frontal-lesion patients, both within a particular task and also across a whole range of tasks (e.g. planning, attentional set-shifting, spatial working memory). [Using a higher (15 mg) dose of DZP, Gorissen et al. (1998) failed to replicate our previously reported (Coull et al. 1995b) DZP-induced impairment on Tower of London accuracy. However, the measure of accuracy used in the study of Gorissen et al. (1998) (number of trials to criterion) may have been less sensitive than that used in our own study (total number of excess moves) since a single trial attempt could fail equally after making one wrong move or, e.g. six wrong moves.] Collectively, these deficits can be thought to reflect an impairment in either working memory or, more specifically, “executive” function, whereby a high-order “central executive” (Baddeley 1986) or Super-

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visory Attentional System (SAS) (Norman and Shallice 1986) exerts conscious attentional control over behaviour. Evidence from Rusted et al. (1991) suggests that DZP impairs central executive function, but leaves the auditory and visual components (the so-called “slave systems”) of working memory intact. However, it should be noted that “executive function” is a rather all-encompassing term, and the specificity of the DZP-induced executive dysfunction is still unclear.

The evidence that DZP exerts effects on these higher-order processes extends beyond its well-known effects on episodic memory. Importantly, these results are unlikely to be explained solely by sedative effects of the drug, since double dissociations in performance of learning and sustained attention tasks were noted with DZP and the noradrenergic α -receptor agonist, clonidine (Coull et al. 1995a). One explanation for the effect of DZP on tests of executive function is that rather than affecting executive function per se, the drug is primarily affecting mnemonic function necessary for executive task performance. In other words, the effects of DZP on “executive” tasks might solely reflect the well-documented effects of BZs on components of episodic memory. Functional neuroimaging provides a means of testing this prediction. Neuroimaging studies are increasingly used to test psychological theories, where perfusion measures act as a psychophysiological index of brain function in much the same way that reaction times have done for decades (Coull 1998). Our study was based on the null hypothesis that the effects of DZP on executive tasks would have exactly the same neuroanatomical correlates as the effects of DZP on memory tasks. This result would support a common neural, and functional, basis for the effects of DZP on both processes, suggesting that the effect of DZP on executive processes may be a consequence of a more fundamental effect on memory processes. Conversely, *task-specific* effects of DZP on neuroanatomically distinct brain areas would support the hypothesis that effects of DZP on executive processes can be distinguished from the effects on mnemonic processes.

Materials and methods

Subjects

Twelve healthy male right-handed volunteers (mean age=30.2 years, range=21–41) took part. Six of these subjects were randomly assigned (double blind for both the subjects and the experimenter) to the placebo group and six to the drug group. Subjects were physically fit, and none was taking medication. The study was approved by the local hospital ethics committee, and permission to administer radioactive substances was obtained from the Administration of Radioactive Substances Advisory Committee (ARSAC) UK. Written informed consent was obtained prior to the study.

PET scanning

Scans of the distribution of cerebral blood flow (CBF) were obtained for each subject in a quiet room, using a Siemens/CPS ECAT EXACT HR+ (model 962) PET scanner (CTI, Knoxville,

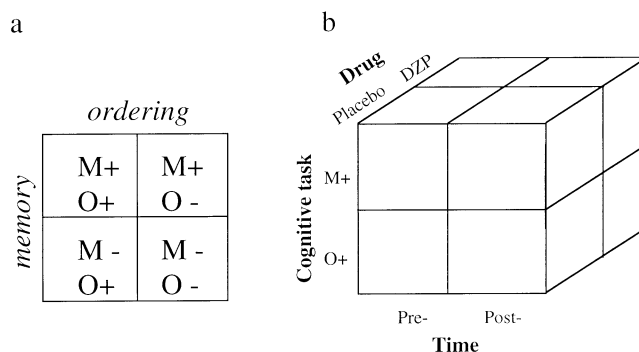


Fig. 1 **a** The two cognitive factors of interest were episodic memory encoding (*M*) and stimulus re-ordering (*O*). We measured the main effect of each factor and, when necessary, the simple main effect of each active condition compared to baseline. **b** Schematic representation of the 2×2×2 experimental design. There were three factors, each with two levels: cognitive state [encoding (*M*) or re-ordering (*O*)]; time of scan relative to drug administration (*T*) (four scans pre-administration, and eight scans post-administration); and drug state (*D*) (placebo or diazepam). The latter factor was a between-subjects factor, while the other two were within-subjects factors. In order to test our experimental hypothesis we analysed the interactions $M \times D \times T$ and $O \times D \times T$. There were not enough scans per condition to satisfactorily analyse the four-way interaction $M \times O \times D \times T$

Tenn., USA), with septa retracted. A neuro insert positioned at the front of the scanner attenuated the contribution of radioactivity counts from the rest of the body. Radioactivity was administered as a $H_2^{15}O$ bolus, infused over 20 s followed by a 20-s saline flush. The total effective dose equivalent of radioactivity per subject was 5.0 mSv. Twelve PET emission scans were collected over 96 min, with an 8-min interval between scans except for the interval between scans 4 and 5, which was 30 min. Integrated radioactivity counts accumulated over a 90-s acquisition period, beginning with the rising phase of radioactivity counts in the head, were used as an index of perfusion. A transmission scan was collected prior to the emission scans to check the position of the subject and to correct for attenuation effects.

Drug administration and experimental design

A 10 mg dose of DZP (2×5 mg tablets) was given orally to six of the subjects and a placebo, similar in colour and size to DZP, was given to six other subjects. This is the same dose used by Coull et al. (1995a, b). For each of the 12 subjects, the first four scans were acquired in the absence of either drug or placebo. The drug (or placebo) was administered directly after completion of the fourth scan, which was followed by a half-hour waiting period before the start of the fifth scan. Therefore, each subject had four scans pre-administration and eight scans post-administration, thus incorporating a within-subjects comparison into the experimental design. The crucial comparison in this type of study is the effect of DZP on the distribution of task-induced activity pre- versus post-administration. However, any observed changes in activity may be confounded with the effects of time. For this reason, a placebo group, tested under exactly the same experimental conditions but without active drug, was also studied and compared directly to the drug group.

There were four psychological tasks, repeated 3 times across 12 scans. These tasks were presented in a randomised manner both within- and between-subjects, with the proviso that each task was presented once pre-infusion and twice post-infusion. The psychological conditions varied along two experimental factors of interest: memory encoding and executive function (Fig. 1a). Along with the between-subjects factor of drug administration (DZP or

placebo) and the within-subjects factor of pre- versus post-administration, this constitutes a four-way factorial design. However, our experimental hypothesis is based on a comparison of the neuroanatomical correlates of memory encoding and executive function, not on an interaction between the two. Therefore, we confine the analysis to the effects of the drug on the main effects of memory encoding and executive tasks (and on the simple main effects of each active condition minus baseline). This, in practice, constitutes a three-way factorial design (Fig. 1b). The two-way factorial design of the neuropsychological tasks was used to match encoding and executive tasks as closely as possible for non-specific factors. This also allowed examination of the effects of DZP on an executive task which has an explicit episodic memory component (anagrams) versus one which does not (alphabetical ordering). Visual analogue scales measuring stress and arousal were given to subjects immediately after half of the scans (specifically, after those which required a memory component to act as a distractor between encoding and retrieval).

Task details

Cognitive tasks

The four tasks were derived from a 2×2 factorial design, the two factors being episodic memory encoding (M) and ordering (O). The latter factor reflects executive function: the ordering tasks required subjects to monitor up to five stimuli on-line in working memory (WM), and then to rearrange these according to a pre-specified scheme or goal (see Baddeley 1986; Shallice 1988). Visual and motor requirements were equated for each of the four tasks. All tasks comprised visual presentation of letter strings, five letters in length, in the centre of a computer monitor. Subjects were asked to read aloud the five presented letters. Presentation of the letter strings was serial, with a total of 15 stimuli (Fig. 2) displayed throughout the task with an inter-stimulus interval of 4 s. The stimuli remained on the screen for the duration of the 4-s interval (pilot testing suggested that 4 s was the optimal time for subjects to solve the simple anagrams presented).

The *memory encoding* task (M+O−) was based on one used previously in functional imaging studies (Grasby et al. 1993; Fletcher et al. 1995). Subjects were shown five-letter words during the scanning time and asked to spell the word aloud (reading from left to right on the screen) and, crucially, to remember the words for later recall and recognition. The *alphabetical ordering* task (M−O+) presented subjects with five-letter non-words (consonant-vowel strings). Subjects were asked to rearrange, and then to read aloud, the presented letters in alphabetical order. For example, if presented with the letter string L-V-A-P-Z, the subject would respond A-L-P-V-Z. There was no long-term episodic memory requirement in this task. The *anagrams* task (M+O+) taxed both encoding and ordering processes. Subjects were presented with anagrams, asked to rearrange the letters to form a real word, and then to spell that word aloud using the corrected letter order. Subjects were also asked to remember the solved words for later recall and recognition. The *baseline* task (M−O−) taxed neither memory or ordering processes. Subjects were presented with alphabetically ordered five-letter non-words (consonant-vowel strings) and asked to read the letters aloud (as they appeared from left to right on the screen). There was no memory requirement in this task. The words in the memory encoding and the anagrams conditions were matched for frequency and imageability, according to the MRC Psycholinguistic database (Coltheart 1981). The solutions for the anagrams and for the alphabetical letter ordering were matched for the number of letter swaps required to reorder the letters correctly (Dominowski 1966).

The cognitive tasks began approximately 20 s before the start of the scanning data acquisition period, and lasted for its duration (each task lasted for 60 s in total). All verbal responses during task performance were recorded which allowed later measurement of the number of anagrams correctly solved (for the anagrams task), and the number of non-words correctly reorganised into alphabetical order (for the alphabetical ordering task). For the two tasks requiring mem-

Baseline	Alphabetical Ordering	Memory encoding	Anagrams
dfhmp	masyh	small	uhseo
ikpwx	blzwt	scene	wreti
gnsuy	cjswp	worry	enoyj
adgik	fkxto	watch	trnia
kmruw	lafqu	toast	ocrwn
bchot	bhsxo	cloud	hoeyn
jlpuz	rvcin	ocean	chlun
bdgil	dmzqt	beach	prneu
fjkvy	wyens	hedge	ircha
dkmqr	rvzfo	hotel	antpl
cetwz	lbgqv	gravy	tmach
achnt	mcirw	woman	oclwn
eitr x	xtdjo	smoke	dlchi
hkosu	zuekp	steam	emfla
efjoq	fmywr	image	dalyi

Fig. 2 An example stimulus set showing the 15 stimuli used for each of the four conditions. Conditions were presented in randomised order within-subjects. There were three such sets, presented in randomised order between-subjects. The baseline condition required neither episodic memory (M) nor re-ordering (O); the alphabetical ordering condition required O but not M; the memory encoding condition required M but not O; and the anagrams condition required both M and O

ory encoding, VASs of stress and arousal were given immediately after completion of the task. This served to prevent rehearsal of the word-list in WM, prior to free recall. Subjects were then asked to remember as many words (or solved anagrams) as they could from the list which had been presented during the scan. After free recall, subjects were shown 30 words on the computer screen, 15 of which were entirely novel and 15 of which had been part of the list presented during scanning. Subjects pressed a button if they recognised a word (or solved anagram) as having been part of the previously presented list.

Visual analogue scales (VAS)

Subjective ratings of stress and arousal were measured using the 24-item check-list of Mackay et al. (1978). Subjects were asked verbally to report how they felt, on a scale of 1–4, according to a list of 24 adjectives, immediately after presentation of M+ word lists and before their active recall. This had the dual benefit of preventing subjects from rehearsing word lists prior to recall, and also allowed us to monitor changes in levels of anxiety or drowsiness due to administration of the drug.

Statistical analysis

Behavioural data

Performance data and VAS ratings were analysed using a repeated-measures ANOVA, with pre- or post-infusion and task-type as within-subjects factors, and administration of drug or placebo as a between-subjects factor.

PET scanning data

Images of radioactivity counts were analysed using statistical parametric mapping (SPM97d; Wellcome Dept of Cognitive Neurology, London, UK) implemented in MATLAB (Mathworks Inc., Sherborn, Mass., USA). Integrated counts accumulated during the emission scan were used as an index of rCBF.

Image pre-processing. Each subjects' images were realigned to the first, in order to correct for head movement between scans, then spatially normalised into a standard space (Talairach and Tournoux 1988) by matching each image to a standardised template (Montreal Neurological Institute, Quebec, Canada) using both linear and non-linear three-dimensional transformations (Friston et al. 1995a). Each image was then smoothed using an isotropic Gaussian kernel of

16 mm FWHM to accommodate inter-subject differences in anatomy. A high resolution anatomical MRI (Siemens Vision scanner operating at 2 T) was co-registered to the same stereotactic space as the PET images. The resulting MRI images were averaged across all subjects, and the mean image was used to display the anatomical location of areas of significant blood flow change.

Statistical analysis Condition, covariate and subject effects were estimated according to the general linear model at each voxel in brain space (Friston et al. 1995b), with global activity as a confounding co-variate (normalised to 50 ml/100 ml.min.) (Friston et al. 1990). Linear contrasts were used to test hypotheses about regionally specific condition and covariate effects, which produced a statistical parametric map of the t statistic generated for each voxel (SPM{t}). The SPM{t} was transformed to a map of corresponding Z values, thresholded at a Z value of 3.09 ($P=0.001$ uncorrected for multiple comparisons), and the resulting foci were characterised in terms of peak height. The significance of each region corrected for multiple comparisons was estimated using distributional approximations from the theory of Gaussian fields. Corrected P values are reported in order to avoid making type I errors. However, it is also necessary to avoid making type II errors (incorrectly rejecting a significant result) and so a less conservative threshold for significance ($P<0.001$ uncorrected for multiple comparisons) was adopted for areas in which activations were expected, based on existing literature. Specifically, these areas comprise left prefrontal cortex for memory encoding tasks (Tulving et al. 1994; Fletcher et al. 1995; Dolan and Fletcher 1997), bilateral dorsal and ventral prefrontal and parietal cortices for ordering (Paulesu et al. 1993; Petrides 1993; Smith et al. 1996; Owen 1997), bilateral visual cortex for DZP-induced rCBF increases (Veselis et al. 1997) and bilateral frontal and temporal cortices for DZP-induced rCBF decreases (Veselis et al. 1997).

With the experimental design illustrated in Fig. 1, we measured:

- (i) The main effect of the cognitive tasks (subtracting M- tasks from M+ tasks to measure memory encoding, and subtracting O- tasks from O+ tasks to measure ordering), using data from the placebo subjects only, so as to avoid any confounding effects of the drug.
- (ii) The main effect of the drug (DZP post-administration scans versus DZP pre-administration scans contrasted directly with the same comparison in the placebo group) averaged across all experimental conditions.
- (iii) The interaction between drug and cognitive tasks, which represents the modulatory influence of the drug on the magnitude of the task-induced changes in activity. This interaction is used to define neural correlates of the modulation of mnemonic or executive processes by DZP. We can measure the effect of DZP both on the main effect of each cognitive factor (M or O), and also on each active condition compared to baseline.

Results

Behavioural results

Visual analogue scales

There were no significant effects of DZP on measures of either stress or arousal, as compared to the placebo group.

Cognitive tasks

Effect of DZP on memory encoding. Data from memory encoding and anagrams tasks were included in this analysis.

Table 1 Effects of 10 mg DZP and placebo on accuracy of memory encoding and ordering. Scores shown are mean number correct, out of a possible total of 15 (SEs are shown in parentheses). Pre-administration scores reflect a baseline, treatment-free state for both placebo and diazepam groups

	Pre-administration	Post-administration
<i>Memory encoding</i>		
Free recall		
Placebo	5.4 (0.9)	5.0 (0.8)
10 mg diazepam	7.0 (1.7)	5.7 (1.6)
Recognition		
Placebo	12.2 (0.2)	12.7 (0.2)
10 mg diazepam	12.4 (0.8)	11.1 (1.3)
<i>Ordering</i>		
Placebo	11.5 (0.5)	12.0 (0.7)
10 mg diazepam	12.1 (1.2)	11.7 (1.3)

There were no significant main effects of DZP on *free recall* [$F(1,9)=0.42$, $P>0.1$], although there was a trend for an interaction between DZP and task-type [$F(1,9)=4.07$, $P=0.75$] such that DZP tended to impair recall during the anagrams task (% change from pre- to post-administration was 107% (± 0.3) for placebo group and 75% (± 0.2) for DZP group) but not during the straightforward memory encoding task (% change from pre- to post-administration was 75% (± 0.1) for placebo group and 78% (± 0.1) for DZP group).

There was a significant main effect of DZP on *recognition* [$F(1,10)=5.77$, $P<0.05$] such that fewer words were recognised following administration of DZP compared to placebo (Table 1). There was no significant interaction with task-type [$F(1,10)=0.001$, $P>0.1$], suggesting that this effect was equivalent for the memory encoding and anagrams tasks.

Effect of DZP on ordering. Data from alphabetical ordering and anagrams tasks were included in this analysis. There was no significant effect of DZP on ordering [$F(1,10)=2.65$, $P>0.1$] although mean values showed that, compared to pre-administration scans, fewer words were correctly ordered following administration of DZP compared to placebo (Table 1). The lack of significance of this result may reflect a combination of the subtlety of the DZP effect on executive compared to mnemonic function, and the relatively small sample size of six subjects per group. There were no significant interactions with task-type.

PET data

Main effect of cognitive tasks

In order not to confound drug effects with task effects, the effects of the cognitive tasks were measured in the placebo subjects only.

Memory encoding. The subtraction of the average of the baseline and alphabetical tasks from the average of the

Table 2 Areas of significant activation during (a) episodic memory encoding and (b) stimulus re-ordering. The co-ordinates are equivalent to those in the stereotactic atlas of Talairach and Tournoux (1988). All postulated areas (see Materials and methods) are significant to a value of at least $P \leq 0.001$ (uncorrected for multiple comparisons). Areas indicated by * are significant at $P < 0.05$ (corrected for multiple comparisons) (*L*=left, *R*=right)

Anatomical area		<i>x, y, z</i> co-ordinates (mm)	Brodmann's area	Z score
<i>a Memory encoding</i>				
L dorsolateral prefrontal		-38, 48, 14	46/10	3.43
<i>b Ordering</i>				
L dorsolateral prefrontal		-50, 40, 22	46	3.33
L ventrolateral prefrontal		-42, 56, -6	10	3.69
Inferior frontal gyrus	L	-52, 10, 28	44	4.02
	R	62, 14, 30	44	3.43
Frontal operculum	L	-32, 18, 2	47	3.33
	R	28, 14, -4	47	4.90*
Superior parietal	L	-18, -72, 50	7	6.07*
	R	24, -72, 60	7	4.99*
L dorsal extrastriate		-32, -84, 18	18	6.29*
L ventral extrastriate		-36, -90, 0	18	4.76*
Cerebellum	L	-38, -72, -16	-	4.45*
	R	46, -72, -18	-	5.26*

Table 3 Areas of significant rCBF change after administration of DZP. Both increases and decreases in rCBF, averaged across all task types, are shown. The co-ordinates are equivalent to those in the stereotactic atlas of Talairach and Tournoux (1988). All postulated areas (see Materials and methods) are significant to at least a value of $P \leq 0.001$ (uncorrected for multiple comparisons). Areas indicated by * are significant at $P < 0.05$ (corrected for multiple comparisons) (*L*=left, *R*=right)

Anatomical area		<i>x, y, z</i> co-ordinates	Brodmann's area	Z score
<i>a rCBF increases</i>				
Visual extrastriate	L	-16, -100, 6	17/18	4.69*
		-40, -88, -10	18	4.48*
	R	40, -68, 6	37/19	5.62*
		50, -78, -2	18/19	5.01*
R middle temporal gyrus		54, -14, -10	21	4.77*
L thalamus		-20, -22, 18	-	5.41*
L substantia nigra		-14, -22, -12	-	5.12*
R caudate nucleus		10, 16, -2	-	4.90*
<i>b rCBF decreases</i>				
L inferior frontal gyrus		-64, 10, 16	44	3.34
L frontal operculum		-54, 26, -8	47	5.18*
Dorsal prefrontal	L	-24, 26, 44	8	3.99
	R	60, 14, 34	9	3.75
Lateral orbitofrontal	L	-32, 30, -16	11	4.44*
	R	26, 44, -26	11	3.61
R anterior cingulate		18, 34, 32	32	4.03
L temporal pole		-40, 16, -34	38	3.86
L medial and lateral temporal		-32, -12, -28	35	5.42*
		-52, -26, -30	20	4.87*
Medial cerebellum		0, -86, -22	-	4.48*

memory encoding and anagrams tasks produced activations in left prefrontal cortex (Table 2a).

Ordering (executive function). The subtraction of the average of the baseline and memory encoding tasks from the average of the alphabetical and anagrams tasks revealed lateralised activation of left dorsolateral and ventrolateral prefrontal cortex and left extrastriate cortex, and bilateral activation of inferior frontal gyri, frontal opercula, superior parietal cortices and cerebellum (Table 2b).

Main effect of drug

In order not to confound the effect of drug with task-specific activations, these results are averaged across all cognitive conditions. The DZP group are compared directly to the placebo group. DZP-induced rCBF increases were

found in bilateral extrastriate cortex and right temporal cortex, and subcortically in thalamus, substantia nigra and caudate nucleus (Table 3a). Conversely, DZP-induced rCBF decreases were found in left inferior frontal gyrus, left frontal operculum, bilateral dorsal prefrontal and orbitofrontal cortices, right anterior cingulate, left temporal cortex and medial cerebellum (Table 3b and Fig. 3).

Task \times drug interaction (modulation of task-induced activations by DZP)

Because of the significant effect of DZP on recognition memory, recognition score was modelled as a confound in the interaction analysis. This ensures that changes in activity reflect an effect of the *drug* on discrete brain regions, and not of poor task performance. Additionally, we only report brain regions revealed by the interaction term that were also significantly altered by the appropriately

weighted main effect of the drug. This safeguards against reporting false positives.

Memory encoding. The interaction between drug administration and memory encoding was measured by examining the differential effects of placebo and DZP on those tasks with a memory component (memory encoding and anagrams) minus those without (alphabetical ordering and baseline). During tasks with an episodic memory component, DZP increased activity in bilateral extrastriate cortex and deactivated left antero-dorsal prefrontal cortex ($x,y,z=-18, 32, 52$; $Z=3.97$; $P<0.001$; and $x,y,z=-14, 56, 22$; $Z=3.19$; $P<0.001$). Further analysis using the simple main effect of memory encoding (compared to baseline) confirmed this finding (Table 4a and Fig. 4a).

Ordering. The interaction between drug administration and ordering was measured initially by examining the

differential effects of placebo and DZP on those tasks with an ordering component (alphabetical ordering and anagrams) minus those without (memory encoding and baseline). There were no significant DZP-induced changes in activity during tasks with an ordering component compared to those without, but there was a single deactivation in left frontal operculum ($x,y,z=-48, 20, -10$; $Z=2.66$; $P=0.004$) at a sub-threshold level. Further analyses using the simple main effects of ordering were more informative. In the interaction between drug and alphabetical ordering (compared to baseline), DZP-induced activations were noted in right extrastriate cortex during the alphabetical ordering condition, and corresponding DZP-induced deactivations in left frontal operculum (Table 4b and Fig. 4b). However, in the interaction between drug and anagrams (compared to baseline), a different pattern of results was observed. During the anagram condition, DZP-induced activations were noted in extrastriate cortex bilaterally, and DZP-induced deactivations were seen in left dorsal and anterior frontal cortex (Table 4c).

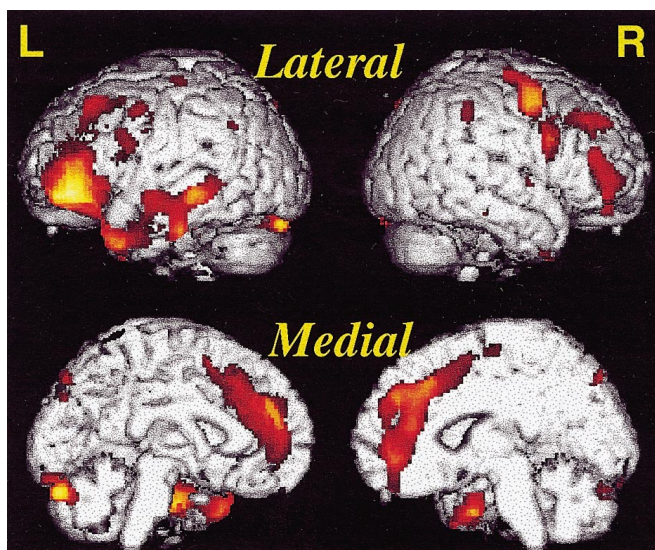
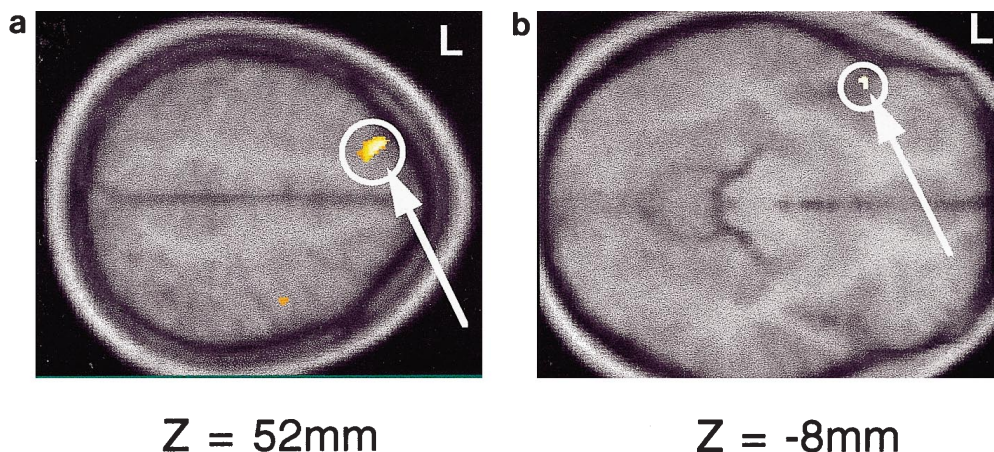


Fig. 3 Decreases in rCBF following administration of DZP, as compared to placebo, averaged across all task types. Areas of significant change are rendered onto a standard template brain (L =left, R =right)

Correlation between recognition score and rCBF

Because recognition performance was significantly impaired by DZP, recognition score was correlated with activity to reveal the brain regions associated with changes in memory performance. As performance improved, we expected changes in areas generally associated with episodic memory (prefrontal cortex and precuneus; see Fletcher et al. 1997 for a review), and also, more specifically, in areas implicated in retrieval success [right prefrontal cortex and anterior cingulate (Rugg et al. 1996)]. We found that in the placebo group, activity increased in left prefrontal and right anterior cingulate cortices (Table 5a) as recognition score increased. Similar results were seen in the DZP group, but with additional activation of right prefrontal cortex, left inferior parietal cortex and bilateral precuneus (Table 5b). A comparison of the two groups revealed that the correlation between performance and activity was greater in the DZP group than

Fig. 4 a,b DZP-induced deactivations during performance of **a** the memory encoding task and **b** the alphabetical ordering task, both compared to baseline. Areas of significant interaction are identified by the arrow, and are superimposed upon transverse sections of the averaged MRI of all subjects



$Z = 52\text{mm}$

$Z = -8\text{mm}$

Table 4 Areas showing significant change in activity following administration of DZP in the three experimental tasks, each compared to baseline. The co-ordinates are equivalent to those in the stereotactic atlas of Talairach and Tournoux (1988). All postulated areas (see Materials and methods) are significant to a value of at least $P \leq 0.001$ (uncorrected for multiple comparisons) (*L*=left, *R*=right)

Anatomical area		<i>x, y, z</i> co-ordinates	Brodmann's area	Z score
<i>a Memory encoding</i>				
Activations				
Visual extrastriate	L	-30, -92, 14	18	3.71
	R	14, -92, 14	18	4.14
Deactivations				
L dorsal prefrontal		-20, 34, 52	8	3.87
L anterior prefrontal		-14, 56, 22	10	3.34
<i>b Alphabetical ordering</i>				
Activations				
R visual extrastriate		32, -70, 6	18	4.04
Deactivations				
L frontal operculum		-48, 22, -8	47	3.18
<i>c Anagrams</i>				
Activations				
Visual extrastriate	L	-10, -74, -16	18	4.17
	R	18, -90, 16	18	3.54
Deactivations				
L dorsal prefrontal		-20, 34, 52	8	3.38
L anterior prefrontal		-10, 46, 36	9	3.22

Table 5 Significant positive correlations between rCBF and recognition score in subjects receiving either placebo or DZP. The co-ordinates are equivalent to those in the stereotactic atlas of Talairach and Tournoux (1988). All postulated areas (see Results) are significant to a value of at least $P \leq 0.001$ (uncorrected for multiple comparisons). Areas indicated by * are significant at $P < 0.05$ (corrected for multiple comparisons) (*L*=left, *R*=right)

Anatomical area		<i>x, y, z</i> co-ordinates	Brodmann's area	Z score
<i>a Placebo</i>				
L dorsolateral prefrontal		-42, 50, 14	46	3.93
R anterior cingulate		2, 30, 26	32	3.46
<i>b DZP</i>				
L dorsolateral prefrontal		-34, 38, 26	9/46	5.44*
R dorsolateral prefrontal		34, 30, 34	9	4.83*
R anterior cingulate		6, 26, 24	32	3.41
L inferior parietal		-54, -52, 40	40	5.18*
L precuneus		-8, -70, 40	7	3.93
R precuneus		12, -30, 46	7	3.13
<i>c DZP > placebo</i>				
L dorsolateral prefrontal		-34, 30, 36	9	3.06
R dorsolateral prefrontal		32, 32, 36	9	3.92
R precuneus		12, -32, 46	7	3.25

the placebo group in bilateral dorsolateral prefrontal cortex and right precuneus (Table 5c). There were no areas of greater activation in the placebo group than the DZP group as recognition score increased.

Discussion

This study examined neural correlates of diazepam (DZP) effects on memory encoding and executive function. We examined the hypothesis that effects of DZP on executive function are a consequence of drug effects on underlying memory processes. We reasoned that differential neuroanatomical bases for the effect of DZP on encoding versus executive tasks would indicate distinct functional bases for the drug effect. Our data support this position in that DZP modulated unique areas of left prefrontal cortex activity during performance of an encoding task as compared to an executive task (on-line reor-

dering). This suggests that the effect of DZP on executive tasks has a dissociable neurobiological basis to that of episodic memory tasks, and so impairment of executive function by DZP cannot be dismissed simply as a consequence of an effect of the drug on memory encoding. Rather, DZP has distinct neuroanatomical, and functional, effects on each of these two processes.

Detrimental effects of diazepam on memory performance

DZP has previously been shown to affect both memory encoding and, to a lesser extent, executive function (Curran 1986, 1991; Rusted et al. 1991; Coull et al. 1995b). The behavioural results of our study confirm this: there were impairments in verbal memory following DZP administration, with more subtle effects on stimulus reordering. In terms of the memory effect, DZP significantly

impaired recognition memory for regular words (M+O–task) and for solved anagrams (M+O+ task). Additionally, DZP tended to impair free recall of solved anagrams but not of regular words. We suggest that this dissociation reflects the greater degree of effort required to encode solved anagrams, which may be more sensitive to the effects of BZs (see Joyce and File 1995). This task requires subjects both to solve the anagram and then encode it for later recall, all within the 4-s presentation time. This is compared to the memory encoding task which allows the subject 4 s simply for the process of encoding. It is notable that the greater effect of DZP on recognition memory rather than free recall is somewhat atypical (Curran 1986), but may be partially explained by the relatively large pre-administration (baseline) group differences in free recall performance (Table 1).

Non-specific deactivation of anterior cortical regions by diazepam

There have been several imaging studies of benzodiazepines (BZ) and brain function. Most of these employ radioactive ligands, such as ^{11}C -flumazenil to image the distribution of benzodiazepine receptors in various clinical conditions (see Malizia and Richardson 1995). However, the number of imaging studies examining cognitive consequences of BZ administration in healthy subjects is small. Veselis et al. (1997) examined the effects of midazolam (a short-acting BZ agonist) on perfusion in healthy volunteers who were scanned while listening, non-attentively, to binaural auditory tones. Drug-induced deactivations were noted mostly in anterior structures (e.g. frontal and anterior cingulate cortex) with a lateralisation to the left hemisphere, while activations were all posterior (e.g. occipital cortex, fusiform gyrus) with a lateralisation to the right hemisphere. These activity changes are consistent with the results of our own study using more specific cognitive tasks. In particular, the left-sided frontal deactivations of Veselis et al. (1997) suggest that the left-sided deactivations seen in our study were not simply a by-product of the fact that we were measuring the main effect of the drug during conditions in which subjects were performing verbal tasks. The study of Veselis et al. (1997), together with our own (which was placebo-controlled to monitor for non-specific time effects), suggests that there is a preferential deactivation of left-sided anterior areas by BZs.

Diazepam has distinct neuroanatomical and functional effects on memory encoding and stimulus re-ordering

Measures of brain activity in the placebo subjects were consistent with previous findings of left dorsal prefrontal cortex activation during episodic memory encoding (Shallice et al. 1994; Tulving et al. 1994; Fletcher et al. 1995; also see Fletcher et al. 1997 for a review) and of more extensive left-sided dorsolateral and ventrolateral

prefrontal cortex for verbal working memory (WM) (Petrides et al. 1993; Jonides et al. 1993; Smith et al. 1996, 1998). However, our main research question was a psychopharmacological one: can the effects of DZP on executive function be explained by a primary effect on memory encoding? Differential deactivation of left dorsal prefrontal cortex during memory encoding, and of left frontal operculum during ordering tasks suggest otherwise, supporting independent functional effects of the drug. However, when a task requires both executive function *and* memory encoding (anagram condition), DZP deactivates left dorsal prefrontal cortex only, suggesting that the predominant effect of the drug is on memory encoding.

Left dorsal frontal cortex is deactivated by DZP during encoding

Veselis et al. (1997) speculated that the BZ-induced left-sided frontal deactivations represented the neural basis for the impairment of memory encoding by BZs. We confirmed this by using specific tests of episodic memory encoding. In addition, we found that activity in the left dorsolateral prefrontal cortex co-varied with recognition performance. This was more significant for subjects receiving DZP than for those receiving placebo, suggesting that DZP-subjects had to make a greater cognitive effort to encode words successfully for later recall. Furthermore, the additional recruitment of *right* prefrontal cortex in these subjects may perhaps be indicative of a functional short-term cortical reorganisation (Chollet et al. 1991). While the left prefrontal cortex has already been strongly implicated in memory encoding, additional activation of right prefrontal cortex in this study suggests that homologous areas in the opposite hemisphere can be recruited when activity in the functionally specialised area is disrupted or compromised in some way (e.g. by administration of drugs).

Left frontal operculum is deactivated by DZP during stimulus ordering: effects on WM rehearsal processes

During alphabetical reordering of letter stimuli, activity in the left frontal operculum was reduced by DZP. This region is close to areas implicated in the phonological rehearsal component of verbal WM (e.g. Broca's area) (see Smith et al. 1998 for a review). By comparison, the "executive" component of WM is thought to be subserved by more anterior dorsolateral prefrontal cortex (see Owen et al. 1996; Petrides 1996). Therefore, we appear to have identified an effect of the drug on the "phonological loop" component of verbal WM, rather than on higher-order executive functions (e.g. on-line monitoring or manipulation of stimuli). This observation conflicts with previous studies which have consistently shown that short-term recall, such as digit-span, is unaffected by DZP administration (e.g. Rusted et al. 1991; see also

Curran 1991 for a review). However, the type of short-term memory tapped by digit span tasks is most likely distinct from that required for tasks such as letter ordering. During the ordering task, stimuli are held on-line in verbal WM for subsequent processing and manipulation. However, stimuli in span tasks are retained in short-term memory, essentially unchanged. The psychopharmacological differentiation in the effect of DZP on these two short-term memory processes further highlights the cognitive distinction between them.

Within the context of verbal WM, a cognitive and neuronal distinction has also been made between maintenance and rehearsal of verbal stimuli in WM. While the former is characterised as a short-term storage buffer and is thought to be subserved by activity in posterior parietal cortex (Paulesu et al. 1993; Jonides et al. 1998), the latter is equivalent to the phonological loop component of WM (Baddeley 1986) and is thought to be subserved by frontal "speech areas" (Paulesu et al. 1993; Smith and Jonides, 1997; Smith et al. 1998 for reviews). Within this framework, our data are consistent with an effect of DZP on brain areas specifically involved in WM rehearsal processes (viz. left frontal operculum) rather than short-term maintenance.

In conclusion, we have demonstrated that the neuro-anatomical correlates of the effect of DZP on WM rehearsal processes are distinct from those underlying the effect of the drug on longer-term memory encoding. We suggest that this provides evidence that the effects of DZP on WM or executive processes can be distinguished from the BZ-effect on episodic memory encoding.

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