NMDA hypofunction in the posterior cingulate as a model for schizophrenia: an exploratory ketamine administration study in fMRI

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Abstract

Background: Based on animal data, NMDA receptor hypofunction has been suggested as a model for positive symptoms in schizophrenia. NMDA receptor hypofunction affects several corticolimbic brain regions, of which the posterior cingulate seems to be the most sensitive. However, empirical support for a crucial role of posterior cingulate NMDA hypofunction in the pathophysiology of positive symptoms is still missing in humans. We therefore conducted an fMRI study using the NMDA antagonist ketamine in healthy human subjects during episodic memory retrieval, which is supposed to activate the posterior cingulate.

Methods: We investigated 16 healthy subjects which were assigned to either placebo (*n*=7; saline) or ketamine (*n*=9; 0.6 mg/kg/ h) group in a double-blind study design. All subjects received their infusion while performing an episodic memory retrieval task in the scanner. Immediately after the fMRI session, psychopathological effects of ketamine were measured using the Altered States of Consciousness Questionnaire.

Results: The placebo group showed BOLD signal increases in the posterior and anterior cingulate during retrieval. Signal increases were significantly lower in the ketamine group. Lower signal increases in the posterior cingulate correlated significantly with positive (i.e. psychosis-like) symptoms induced by ketamine.

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Conclusion: The present study for the first time demonstrates a relationship between NMDA receptors, posterior cingulate and positive (i.e. psychosis-like) symptoms in humans. Confirming findings from animal studies, it supports the hypothesis of a pathophysiological role of NMDA receptor hypofunction in the posterior cingulate in schizophrenia.

1. Introduction

The pathophysiology of psychotic symptoms such as auditory perceptual alterations and disturbances in self-perception remains unclear in schizophrenia. Based on animal and human data, it has been postulated that the glutamatergic system and especially NMDA receptors might be involved in the pathophysiology of schizophrenia. NMDA receptor hypofunction was shown to affect multiple corticolimbic brain regions. Among these regions the posterior cingulate appears to be most susceptible to the disinhibitory effects induced by NMDA receptor hypofunction. Accordingly, a special role of the posterior cingulate in the pathophysiology of psychotic symptoms has been assumed (Olney and Farber, 1995). Studies in rats and mice demonstrate structural damage, reversible neuronal alterations, neural disinhibition, and heat shock protein expression particularly in the posterior cingulate during systemic administration of NMDA receptor antagonists like ketamine (Olney et al., 1989, 1991, 1999; Sharp et al., 1991; Olney and Farber, 1995; Nakki et al., 1996; Nishizawa et al., 2000; Brosnan-Watters et al., 2000; Li et al., 2002). However, neither the modulation of the posterior cingulate by NMDA receptor antagonists like ketamine nor the posterior cingulate NMDA receptor function in schizophrenia has yet been investigated in humans. Accordingly, studies bridging the gap between animal data and psychotic symptoms in humans are still lacking.

While the modulation of the posterior cingulate by NMDA receptor antagonists has not yet been investigated in humans, several studies demonstrated induction or exacerbation of positive (i.e. psychosis-like) symptoms in both healthy and schizophrenic subjects by ketamine (Krystal et al., 1994; Hetem et al., 2000; Malhotra et al., 1996; Lahti et al., 2001; Vollenweider et al., 1997, 2000). Though fMRI studies using ketamine administration have been reported (Abel et al., 2003a,b), imaging studies focusing on ketamine-induced modulation of tasks which specifically activate the posterior cingulate have not yet been reported for healthy or schizophrenic subjects. The posterior cingulate and the adjacent precuneus have been implicated in episodic memory retrieval in healthy human subjects (Fink et al., 1996; Maddock et al., 2001; Krause et al., 1999; Duzel et al., 1999; Andreasen et al., 1995; Maguire et al., 1999; Ragland et al., 2001). Though NMDA antagonists like ketamine are known to impair episodic memory retrieval (see Hetem et al., 2000; Newcomer et al., 1999; Grunwald et al., 1999; Krystal et al., 1994; Malhotra et al., 1996; Newcomer and Krystal, 2001), no imaging study on the posterior cingulate/precuneus during ketamine administration has been reported so far for humans. Ketamine-induced modulation of posterior cingulate activity in a specific activation task and concurrent induction of psychosis-like symptoms would provide first evidence for the close relationship between posterior cingulate NMDA hypofunction and psychotic symptoms in humans, as suggested by Olney and Farber (1995), and Olney et al. (1999), predominantly based on animal data.

In order to investigate the relationship between NMDA receptors, posterior cingulate, and positive (i.e. psychosis-like) symptoms, we conducted an exploratory fMRI study using the NMDA receptor antagonist ketamine in a double-blind placebo-controlled design. The feasibility of pharmacological intervention in fMRI has been demonstrated with ketamine (Abel et al., 2003a,b) as well as with scopolamine and lorazepam (Sperling et al., 2002; Thiel et al., 2001; see discussion for methodological problems). During ketamine administration, subjects

were asked to perform an episodic memory retrieval task, because (i) this task produces reliable activation in the posterior cingulate in healthy subjects and (ii) schizophrenic patients with positive (i.e. psychosis-like) symptoms show deficits in this task (see above and Discussion for further details). Accordingly, investigation of ketamine administration during episodic memory retrieval in fMRI allows for elucidation of the interaction between NMDA receptors, posterior cingulate, and positive (i.e. psychosis-like) symptoms. Based on these considerations, we hypothesized: (1) ketamine is associated with significantly smaller BOLD-signal changes in the posterior cingulate during retrieval of episodic memory; (2) based on animal data and the hypothesis of Olney and Farber (see above), we assumed a significant correlation between signal intensity in posterior cingulate and the positive (i.e. psychosis-like) symptoms induced by ketamine.

2. Methods

2.1. Subjects

Sixteen subjects (eight women, eight men) participated in the study as paid volunteers. Before study participation, they underwent a semi-structured psychiatric interview, physical examination, ECG, and laboratory testing. Exclusion criteria were history of psychiatric disorders or serious medical-neurologic illness as well as a psychiatric disorder in a first- or second-degree relative. Further exclusion criteria were the history of substance abuse (any glutamatergic substances, alcohol or other addictive substances), pregnancy, and prior exposure to psychotropic medication. The study was approved by the Regional Ethics Committee and conducted at the Department of Psychiatry of the University of Zurich. Written informed consent was obtained after complete explanation of the study (see also Carpenter, 1999).

Subjects were randomly assigned to one of two groups receiving ketamine or placebo (saline). They were asked to perform a memory task during administration of either ketamine or placebo while being in the fMRI scanner. We excluded two participants from the study. Exclusion reasons were technical failure ($n=1$) and retrospectively discovered

deformity ($n=1$). The effective group size was $n=6$ for placebo (two women, four men; mean age: 24.5 years) and $n=8$ for ketamine (five women, three men; mean age: 27.4 years).

2.2. Drugs

A double-blind placebo-controlled drug administration technique was used, so that each subject received an injection intravenously at the onset of the study phase, i.e. the fMRI. The ketamine group received a ketamine infusion (0.6 mg/kg/h) during anatomical and functional image acquisition (90 min). Accordingly, subjects had already been receiving a continuous infusion of ketamine for 20 min (duration of anatomical image acquisition), when functional imaging and task performance started. Throughout functional image acquisition, subjects continuously received ketamine in the above described dose. This dose is known to reliably induce episodic memory impairments and positive (i.e. psychosis-like) symptoms (Hetem et al., 2000; Vollenweider et al., 1997, 2000; Krystal et al., 1994, 1998, 1999). The placebo group received saline (0.9% NaCl) over the same period of time.

2.3. Experimental paradigm

The experimental paradigm consisted of an episodic memory retrieval task. Subjects encoded words from a study list which they were asked to retrieve on the subsequent day during ketamine/placebo administration in fMRI.

2.3.1. Items/words

We used 30 sets of semantically associated German nouns, similar to those used in previous studies (see Ullsperger et al., 2000). Lists were created in a categorical noun generation experiment, in which 139 participants were asked to give words related to a proposed 'theme word' (see Ullsperger et al., 2000 for further details). Each set consisted of 10 semantic associates (e.g. rose, tulip, viola) of a 'theme word' (e.g. flower), matched for mean word typicality across categories. The study comprised words with medium mean word typicality selected from all 30 sets. Four types of target words were distinguished for retrieval (see below and Fig. 1). True target words were items,
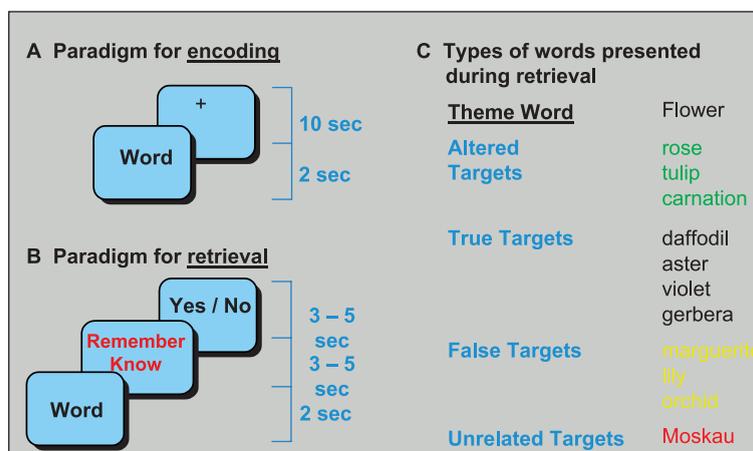
Fig. 1. Paradigms for encoding (A) and retrieval (B) of words and types of words presented during retrieval (C). One day after encoding (A) subjects underwent fMRI while performing a retrieval task (B). During fMRI, they received either placebo (n=6) or ketamine (n=8). (A) Presentation of 66 words separated by a fixation cross. These words would represent 'True targets' during retrieval. (B) Words of four different types were presented during fMRI. Each word was followed by both tasks. 'Remember': conscious recollection. 'Know': no conscious recollection, but feeling of familiarity. 'Yes': word was presented during encoding. 'No': word was not presented during encoding. (C) Four different types of words were presented during retrieval. The figure shows examples for each type. 'True targets': words which were presented during encoding. 'Altered targets': words which were not presented during encoding but are semantically stronger associated with the theme word than 'True targets'. 'False targets': words which were not presented during encoding and are not strong semantic associates of the theme word. 'Unrelated targets': words which were unrelated to the theme word.

which had been presented in the study list during encoding. Altered target words were items, which had not been presented during encoding, but were related to true targets as semantically related lures with high mean word typicality. False target words were items which had not been presented during encoding and were only slightly semantically related to the theme word. Unrelated target words were items, which had not been presented during encoding and were semantically unrelated to true, altered, and false targets. All types of target words were balanced for order across subjects, word length, frequency of occurrence in the language, and frequency of occurrence of first and second associate position.

2.3.2. Encoding

One day before scanning participants studied a list of 66 items (later true targets). Words were presented visually for 2 s on a computer screen using special stimulus presentation software (e-prime). After each word, a fixation cross appeared for a duration of 10 s. Subjects were instructed to read the word list attentively and try to remember the words in preparation for a later memory test. To control for

attention during encoding six non-sense words (e.g. Hysfhz) were included. When a non-sense word appeared, they had to press a button. The order of words was randomized across subjects.

2.3.3. Retrieval

All subjects underwent fMRI during retrieval. During fMRI stimuli were presented visually, digitally projected on a screen behind the scanner, visible through a mirror adjusted on the head coil. Each trial consisted of the word presented for 2 s, followed by the questions 'Remember'/'Know' and 'Yes'/'No' both with variable duration (3–5 s; see Fig. 1). Subjects had to click either the right ('Remember'/'Yes') or left ('Know'/'No') mouse button. Reaction times were taken for every button press using the presentation software. For each of two blocks, 120 trials were presented (30 words for each of the four targets). All targets were presented in randomized and counterbalanced order within and across subjects. Subjects were asked to recognize the previously encoded items and to decide whether they 'Remember' or 'Know' them. A 'Remember' response was defined as the ability to become consciously aware of some aspect of

what had happened or had been experienced when the word was presented (see also Wheeler et al., 1997). Examples included an association with another list word, an image that came to mind, something of personal significance in autobiographical memory, or something that happened in the room. A 'Know' response was described as the knowledge of an items' presence in the study lists, but without any conscious recollection, the recognition being based primarily on feelings of familiarity (see also Wheeler et al., 1997). In addition to the 'Remember' versus 'Know' response, subjects had to decide whether they had seen the presented word before or not by giving the answer 'Yes' or 'No' (see also Fig. 1). The cases with the absence of any answer were subsumed under the term 'missings'. The response should be given immediately.

2.3.4. Behavioral and psychopathological parameters

We calculated reaction times for all four types of answers ('Remember', 'Know', 'Yes', 'No') and compared them between both groups using a *t*-test for independent groups for comparison of equality of the mean values. Furthermore, the number of correctly and incorrectly (i.e. mistakes) recognized words was calculated for 'Remember'/'Know' (excluding all missings) and 'Yes'/'No' and compared between both groups (placebo, ketamine) using a non-parametric test (Chi-square test) with SPSS 10. Psychological effects were measured using the Altered States of Consciousness Questionnaire (OAVAV; Dittrich, 1998) immediately after the fMRI session. This scale is a visual analogue scale in which subjects have to rate their subjective experience with regard to psychosis-like symptoms. The OAVAV includes a total number of 94 items which are categorized into five subscales. Positive (i.e. psychosis-like) symptoms are reflected in the subscales for 'anxious ego-dissolution' (AED), 'oceanic boundlessness' (OCB), the former accounting for thought disorder and anxiety while the latter focuses especially on ego-disturbances. Two further subscales assessing positive (i.e. psychosis-like) symptoms include 'visionary restructuration' (VR) and 'auditory perceptual alter-ation' (APA), which measure changes in perceptual experience including visual and auditory illusions/hallucinations, respectively. The OAVAV also includes a subscale for 'vigilance' (VIG); the OAVAV scores

were compared between groups (placebo, ketamine) using a *t*-test for independent groups for comparison of equality of the mean values. Previous studies demonstrated that ketamine induces significant changes in these subscales in healthy subjects (Vollenweider et al., 1997, 2000).

2.4. fMRI

2.4.1. Imaging methods

A 2 T MRI system (Tomikon S200A, BRUKER Medical) with quadrature head coil was used for all measurements. We acquired 14 interleaved slices adjusted to the AC-PC line (5 mm slice thickness, 1.5 mm interslice interval) using a T2* gradient echo, BOLD contrast sensitive sequence (TR=2000 ms, TE 40 ms, FOV=25 cm). Each slice was recorded with a matrix resolution of 128×64 and later reconstructed into a 128×128 matrix after zero filling. The first eight volumes were discarded to allow for T1 equilibrium effects. Images were realigned to the first slice for interscan movement correction, synchronized to the middle slice to correct for differences in image acquisition time and normalized to a standard EPI template volume using SPM99 software. The data were then smoothed with a Gaussian kernel of 12 mm FWHM to accommodate intersubject anatomical variability.

2.4.2. Imaging processing and analysis

Data were analysed with statistical parametric mapping software (SPM 99). The realignment parameters were included as additional regressors. The hemodynamic response to stimulus onset for each event type was modelled by a canonical synthetic hemodynamic response function (HRF) and its first-order temporal derivative. Data were globally scaled within sessions and temporally smoothed. Linear contrasts of parameter estimates for the canonical HRF were taken to generate statistical parametric maps (SPM's) of the *t*-statistic. SPM's (thresholded at $p<0.001$, uncorrected on a voxel level) were created for both groups (ketamine and placebo) and used as a mask to identify brain regions showing differential involvement during both types of answers 'Remember'/'Know' and Yes/No excluding all missing answers. To elucidate differences in neuronal activity between placebo and

ketamine single contrasts were taken to a second level analysis (random effects model). Based on significant behavioral effects of ketamine on 'auditory perceptual alteration' (APA) and 'oceanic boundlessness' (OCB), we calculated correlation maps to investigate the relationships between these two subscores and regional signal increases in fMRI; voxel-by-voxel correlation maps were transformed to Z-maps. Since these two correlation analyses arose from predictions based on behavioral data (a priori comparisons), we did not correct for multiple comparisons.

3. Results

3.1. Behavioral data

The ketamine group (1093.7±132.2 ms) did not show significantly longer reaction times compared to the placebo group (905.1±128.8 ms) in the answer 'Remember' (*t*-test for independent groups). There were also no significant differences in reaction times between both groups in the answer 'Know' (1042.3±166.3 ms placebo; 994.2±174.1 ms ketamine). Moreover, there were no significant differences in reaction times between placebo and ketamine group in both answers 'Yes' (887.67±142.38 ms placebo; 972.63±164.74 ms ketamine) and 'No' (849.17±145.23 ms placebo; 821.00±201.43 ms ketamine). The ketamine group (16.4±24.83; means±SD) showed a significantly (X=8.28, *df*=1, p=0.0041; X=8.14, *df*=1, p=0.0042) higher number of mistakes in 'Remember'/'Know' compared to the placebo group (13.4±8.41; means±SD) (Chi-Square test). The number of mistakes did not differ in the 'Yes'/'No' task between both groups.

Psychological effects of ketamine were assessed by comparing the OAVAV scores between placebo and ketamine group using *t*-tests for independent groups. Ketamine induced significant effects in the subscales 'auditory perceptual alterations' (4±9 placebo; 119±116 ketamine; p=0.027) and 'oceanic boundlessness' (11±16 placebo; 704±620 ketamine; p=0.016) (see also Fig. 2). Though not statistically significant, subjects of the ketamine group showed a trend to higher values in the subscales for 'anxious ego dissolution' (176±137 placebo; 583±502 ket-
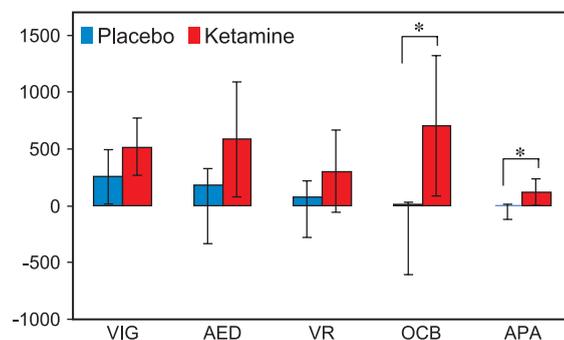
Fig. 2. Psychological effects of ketamine. Scores for positive (i.e. psychosis-like) symptoms and vigilance: Comparison of placebo (six subjects) and ketamine (eight subjects) group. Using the Altered States of Consciousness Questionnaire (Dittrich, 1998), scores were acquired for vigilance (VIG), oceanic boundlessness (OCB), anxious ego-dissolution (AED), visionary restructuration (VR), and auditory perceptual alterations (APA). Error bars represent the standard deviation. The asterisks (*) indicate statistical significant differences at p<0.05.

amine) and 'visionary restructuralization' (72±127 placebo; 299±347 ketamine). Finally, we compared the 'vigilance' subscale between both groups and found no differences (254±236 placebo; 477±244 ketamine) (see Fig. 2). In order to further exclude effects of vigilance, we correlated (using Spearman correlation) the 'vigilance' score with the number of mistakes and the reaction times during 'Know' and 'Remember' in both groups and obtained no significant correlations in either group. Therefore, differences between ketamine and placebo in the number of mistakes and reaction time cannot be related to vigilance changes.

In summary, ketamine induced significant impairment in episodic memory retrieval (i.e. in 'Remember'/'Know') as well as positive (i.e. psychosis-like) symptoms.

3.2. fMRI

In the placebo group, signal increases during 'Know' versus 'Remember' were observed in the posterior cingulate/precuneus (BA 31; p<0.001) and anterior cingulate (BA 32; p<0.001; see Fig. 3A and Table 1). In the ketamine group, comparison between 'Know' and 'Remember' revealed signal increases in the posterior cingulate (BA 30; p<0.001) though on a lower level of significance than in the placebo group (see Fig. 3B and Table 1). In contrast, no
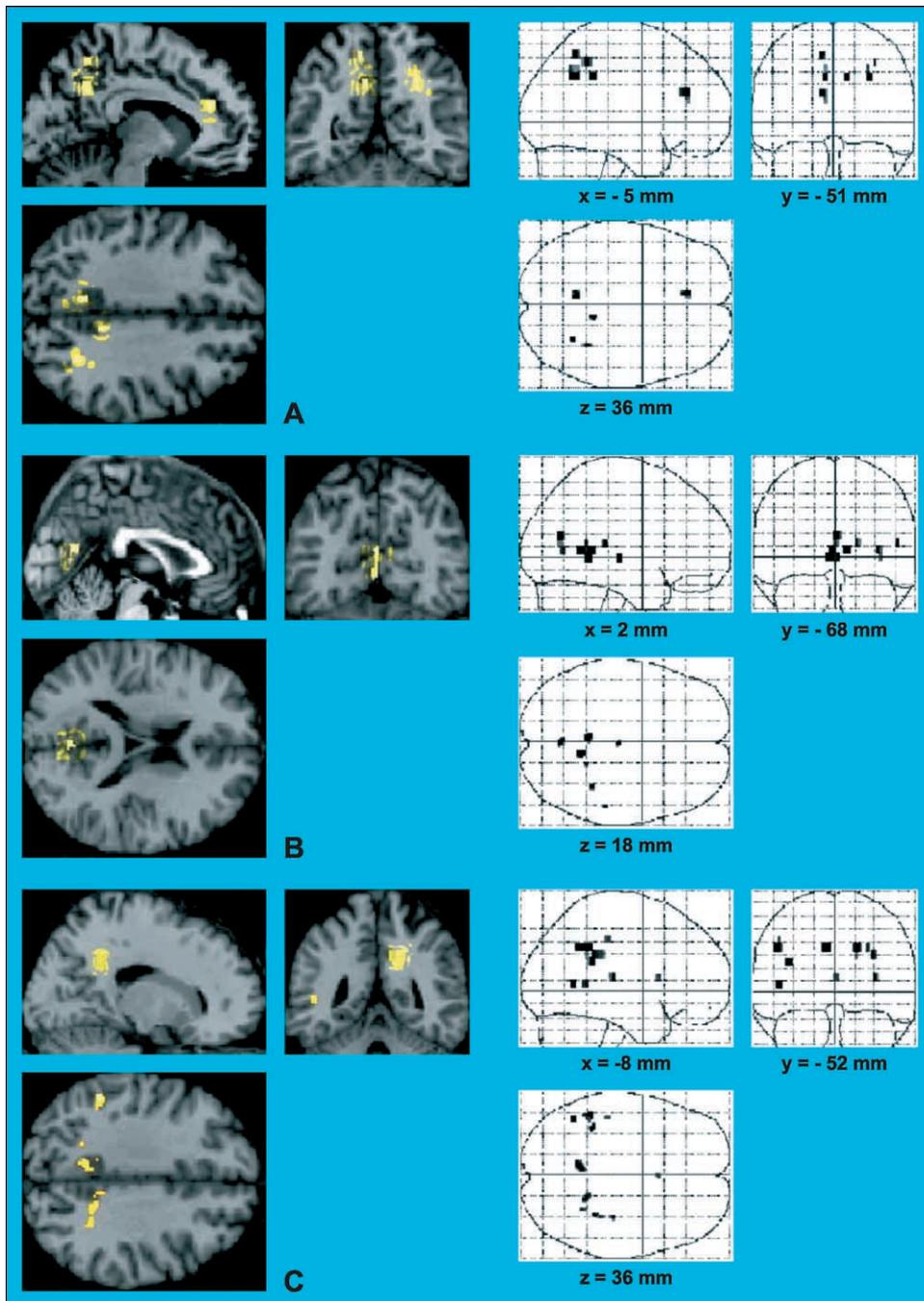
Fig. 3. Significant BOLD signals in ketamine and placebo group (all 14 subjects): 'Know'>'Remember'. Left side: cortical activations displayed on a normalized EPI structural mean image. Right side: statistical parametric maps in axial, coronal, and sagittal view (Talairach coordinates). (A) Placebo group. Clusters of >three voxels, T=3. (B) Ketamine group. Clusters of >one voxel, T=2.33. (C) Second level analysis: placebo versus ketamine (two sample t-test; T=3).

Table 1

Anatomical locations and coordinates of signal changes in placebo and ketamine

Description	BA	Side	Talairach coordinates			Z-score	p-value	Extent
			x	y	z			
Placebo								
Know > Remember								
Anterior cingulate	32	L	−8	36	24	3.45	0.0002	39
	24	R	4	−6	42	2.90	0.0016	86
Posterior cingulate	31	R	10	−40	36	3.55	0.0002	50
Precuneus	31	L	−6	−54	36	3.40	0.0003	103
Parietal lobe	39	R	28	−56	36	3.32	0.0005	147
	40	L	−46	−40	36	2.72	0.0033	26
Yes/No								
Temporal pole	38	R	46	4	−18	3.29	0.0005	9
Temporoparietal junction	39	R	46	−70	18	3.12	0.0009	11
Posterior cingulate	31	L	−16	−62	18	2.72	0.0033	5
Superior temporal gyrus	22	R	56	−32	6	2.71	0.0034	6
Ketamine								
Know > Remember								
Posterior cingulate	30	R	10	−50	6	3.03	0.0012	8
Precuneus	31	R	2	−68	18	2.92	0.0018	11
Superior temporal gyrus	42	R	52	−30	12	2.55	0.0054	16
Cerebellum		L	−4	−44	0	2.88	0.0020	13
Yes/No								
Parahippocampal gyrus	36	L	−30	−38	−6	3.29	0.0005	22
	27	R	14	−38	0	2.76	0.0029	3
Hippocampus		L	−34	−10	−18	2.76	0.0029	3
Lentiform nucleus/putamen		R	30	−26	0	2.86	0.0021	15
Insula	13	L	−44	0	0	2.82	0.0024	11
Placebo > Ketamine								
Know > Remember								
Temporoparietal junction	39	L	−46	−56	6	3.39	0.0003	6
Precuneus	31	R	18	−46	36	3.07	0.0011	12
	7	R	20	−72	42	2.75	0.0030	3
Anterior cingulate	32	L	−14	32	24	2.27	0.0116	11
Insula	13	R	34	−24	12	2.86	0.0021	3
Middle frontal gyrus	9	L	−32	16	30	2.82	0.0025	4
Yes/No								
Posterior cingulate	23	R	4	−22	30	2.71	0.0034	36
Precuneus	31	L	−4	−54	30	2.61	0.0045	76
	7	R	22	−62	30	2.50	0.0062	61
Superior temporal gyrus	22	R	42	−26	4	2.48	0.0066	87
Hippocampus		L	−32	−36	0	2.14	0.0162	35

Coordinates (in mm^3) are given for the peak voxel and refer to the standard stereotaxic. Only the most significant cluster of each region is listed.
BA = Brodmann's Area, R = right, L = left hemisphere, Extent = cluster size in number of voxels.
No significant signal changes were revealed in the comparison ketamine > placebo.

signal increases were observed in the anterior cingulate in the ketamine group. Comparison of the 'Know'/'Remember' effects between placebo and ketamine group revealed a significant interaction in both posterior cingulate and precuneus: compared to the ketamine group, the placebo group showed significantly larger signal increases in the posterior cingulate/precuneus (BA 31; $p=0.001$) and a trend towards signal increases in the anterior cingulate (BA 32; $p=0.012$) (see Fig. 3C and Table 1). Other

regions showing significantly more activation with placebo (compared to ketamine) included insula, middle frontal gyrus, and temporoparietal junction (see Table 1).

The comparison between 'Yes' and 'No' responses within the placebo and ketamine group, respectively (using F-statistics) revealed patterns of activity in temporal regions and the hippocampal/parahippocampal area (see Table 1). Comparing both groups in a second level analysis, the placebo group showed significantly larger signal increases in the posterior cingulate, precuneus, and temporal regions including the hippocampus (see Table 1). Neither in 'Know' versus 'Remember' nor in 'Yes' versus 'No' significantly larger signal increases were found in ketamine compared to placebo.

Based on the significant behavioral effects of ketamine on 'auditory perceptual alteration' (APA) and 'oceanic boundlessness' (OCB), we correlated the signal changes revealed in the contrast 'Know' versus 'Remember' with the OAVAV scores for APA and OCB in a voxel-by-voxel analysis. The scores of the 'auditory perceptual alterations' (APA) subscale correlated significantly with the signal intensity in the precuneus ($x=-8$, $y=-72$, $z=24$, $Z=3.01$; BA=31; $p<0.001$) and two spots in the cuneus ($x=-20/-10$, $y=-74/-60$, $z=12/6$, $Z=3.67/2.86$; BA=17/30; $p<0.001$; see Fig. 4). The scores for 'oceanic boundlessness' (OCB) correlated significantly with the signal intensity in the posterior cingulate ($x=2$, $y=-76$, $z=12$, $Z=2.22$; BA=23; $p<0.001$), the left parietal lobe ($x=-36$, $y=-34$, $z=42$, $Z=2.59$; BA=40; $p<0.001$), and the right lateral globus pallidus ($x=24$, $y=-6$, $z=-6$, $Z=2.83$; $p<0.001$).

One concern was that ketamine might have affected vigilance during the task. To determine whether altered vigilance contributed to the above described signal changes, we correlated the OAVAV scores for 'vigilance' (Dittrich, 1998) with the signal changes revealed by the contrasts 'Know' versus 'Remember' and 'Yes' versus 'No'. We did not find significant correlation with either the posterior cingulate/precuneus or the anterior cingulate in both groups, placebo and ketamine. Moreover, no significant differences in correlations between both groups were obtained. Another concern was that ketamine could already have exerted its effects during the presentation
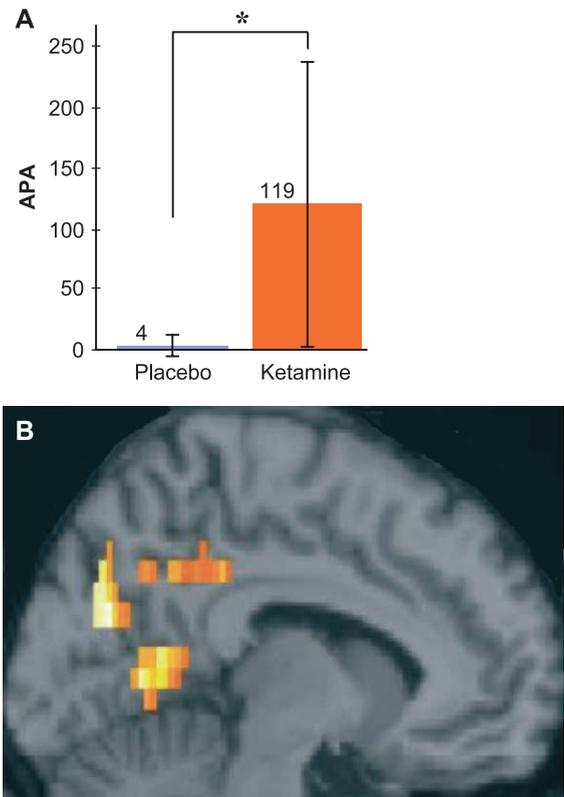
Fig. 4. Effects of ketamine on Auditory Perceptual Alterations (AUD) and their correlation to regional signal changes. (A) Mean data for AUD in subjects with placebo (six subjects) and ketamine (eight subjects). The asterisk (*) indicates statistical significant differences at $p<0.05$. (B) Brain regions significantly correlated with Auditory Perceptual Alterations (AUD) in the contrast 'Know' versus 'Remember' in the ketamine group (eight subjects). Sagittal view ($x=-6$) of significantly correlated voxels ($T=2$, clusters >25 voxels) in the posterior cingulate (and adjacent posterior regions like the precuneus) displayed on the MNI template.

of the words before the actual task, i.e. 'Know'/'Remember' (see Methods and Fig. 1 for description of the paradigm). The observed signal changes in the posterior cingulate revealed by the contrast 'Know' versus 'Remember' would then have been due to effects of ketamine on the preceding word presentation. The effects of ketamine would thus have been item-related rather than task-related (see also Duzel et al., 1999 for a similar distinction). To exclude this possibility, 'presentation of words' was compared with all other conditions for both groups, ketamine and placebo. No signal increases in posterior cingulate and precuneus were observed in either group. More-

over, the comparison between ketamine and placebo revealed no significant drug-word presentation interaction in either region.

In summary, ketamine induced significantly lower signal changes in the posterior cingulate during episodic memory retrieval which, in addition, correlated specifically with psychosis-like symptoms.

4. Discussion

We investigated the modulation of neural activity during episodic memory retrieval by the NMDA receptor antagonist ketamine in healthy subjects. The following main findings were obtained: (i) significantly smaller BOLD-signal changes in the posterior cingulate, precuneus, and anterior cingulate in the ketamine group during the retrieval of episodic memory; (ii) significant correlation between signal changes in the posterior cingulate/precuneus and positive (i.e. psychosis-like) symptoms in the ketamine group.

Our findings reveal (i) NMDA-ergic modulation of neural activity in the posterior cingulate and (ii) the relationship between positive (i.e. psychosis-like) symptoms and NMDA-ergic modulation of the posterior cingulate. Accordingly, our findings demonstrate for the first time a relationship between NMDA receptors, posterior cingulate, and positive (i.e. psychosis-like) symptoms in humans. Our study confirms findings from animal studies in humans and lends support to the hypothesis of a particular role of NMDA receptor hypofunction in the posterior cingulate in the pathophysiology of schizophrenia.

In accordance with previous studies (Fink et al., 1996; Maddock et al., 2001; Krause et al., 1999; Duzel et al., 1999; Andreasen et al., 1995; Maguire et al., 1999), our placebo group showed significant activation in the posterior cingulate during episodic memory retrieval. When ketamine was administered, the posterior cingulate activation was significantly attenuated: we observed significantly lower signal increases during episodic memory retrieval in the ketamine group compared to placebo. Accordingly, our first main finding suggests the modulation of the posterior cingulate activity by NMDA receptors in humans. While the influence of ketamine on the

posterior cingulate activity has not been investigated in humans so far, animal studies revealed a close relationship between NMDA receptors and the posterior cingulate function. In rats and mice, the systemic administration of NMDA receptor antagonists like ketamine was shown to affect multiple corticolimbic brain regions. Among these regions, the posterior cingulate was most sensitive to the disinhibitory effects induced by NMDA receptor hypofunction. NMDA receptor antagonists led to structural damage, reversible neuronal alterations, neural disinhibition, and heat shock protein expression particularly in the posterior cingulate (Olney et al., 1989, 1991, 1999; Sharp et al., 1991; Olney and Farber, 1995; Nakki et al., 1996; Nishizawa et al., 2000; Brosnan-Watters et al., 2000; Li et al., 2002). The present findings demonstrate a similar relationship between NMDA receptors and the posterior cingulate activity in humans.

Based on animal data (see above) and the observation of ketamine-induced positive symptoms in humans, Olney and Farber (1995) and Olney et al. (1999) suggested that NMDA receptor hypofunction in the posterior cingulate might play a special role in the pathophysiology of schizophrenia. Our second main finding provides support for this hypothesis in humans: Signal intensity in the posterior cingulate correlated significantly with positive (i.e. psychosis-like) symptoms. While ketamine-induced positive (i.e. psychosis-like) symptoms have previously been reported (Malhotra et al., 1996; Krystal et al., 1994; Lahti et al., 2001; Vollenweider et al., 1997, 2000), these symptoms have not specifically been related to NMDA-ergic modulation of neural activity in the posterior cingulate. However, our findings are in accordance with recent reports on structural and functional alterations in the posterior cingulate in schizophrenic patients (Haznedar et al., 1997; Leonard et al., 1999; Holcomb et al., 2000; Kiehl and Liddle, 2001; Hulshoff Pol et al., 2001; Miller et al., 2001; Franck et al., 2002; Northoff et al., in press). Our findings extend these results in that they demonstrate a direct relationship between positive (i.e. psychosis-like) symptoms and NMDA receptor function in the posterior cingulate in healthy subjects. Future studies might employ an analogous paradigm for acute schizophrenic patients with positive symptoms.

Besides the posterior cingulate, the anterior cingulate was significantly modulated by ketamine. This is in full accordance with previous studies on the relationship between NMDA-ergic modulation of the anterior cingulate and positive symptoms in both healthy and schizophrenic subjects. Vollenweider et al. (1997) demonstrated a significant relationship between ketamine-induced metabolic changes in the anterior cingulate and ego-pathology in healthy subjects. Lahti et al. (1995) observed the exacerbation of positive symptoms and the modulation of r-CBF in the anterior cingulate when ketamine was administered to schizophrenic patients (see also Tamminga et al., 2000). Ragland et al. (2001) found significant signal decreases in the anterior cingulate in schizophrenic patients during word recognition. As a result, both anterior and posterior cingulate seem to be specifically modulated by NMDA receptors. This suggests NMDA receptor mediated hypofunction in the anterior and posterior cingulate in positive (i.e. psychosis-like) symptoms.

Our study employed an activation paradigm (episodic memory retrieval task) that was specifically designed to activate the posterior cingulate. In contrast, most previous studies investigating the effects of ketamine were resting state studies. This might explain why previous studies did not reveal an influence of ketamine on posterior cingulate activity. Since recruitment of the posterior cingulate was not required in these studies, the modulation of the posterior cingulate activity by ketamine might have been missed. Differences in the experimental design might also explain differing findings reported by Abel et al. (2003a,b). Relying on a gender discrimination task, they did not observe ketamine-induced signal changes in the posterior cingulate. In addition, differing findings might be due to differences in the application of ketamine. Abel et al. (2003a,b) applied a lower dose of ketamine (0.5 mg as compared to 0.6 mg per kg body weight) over a shorter time period (45 min as compared to 90 min in our case).

Previous studies have reported NMDA-ergic modulation of hippocampal function by ketamine (Lahti et al., 1995; Grunwald et al., 1999; Tamminga et al., 2000). Altered hippocampal activity was also observed in schizophrenia (Bogerts, 1997; Heckers et al., 1998; Heckers, 2001). In accordance with previous studies (see Maguire, 2001) we found

significant signal changes in medial temporal regions (see Table 1) during word recognition ('Yes'/'No'). However, we did not observe a modulation of hippocampal activity by ketamine during episodic memory retrieval (i.e. 'know' versus 'remember'). Neuroimaging studies on memory retrieval frequently fail to observe activation in medial temporal structures. This may be due to ongoing metabolic activity in these structures during the control conditions, rather than lack of involvement in memory retrieval. Another point to mention is that we observed a large amount of inter-individual variability in OAVAV scores. This is probably due to the fact that the OAVAV is a subjective scale. We did not use objective or investigator-scored ratings because the processes we wanted to account for are largely subjective in nature and seem to be best measured with the OAVAV. Furthermore, we did not use a within-subject design in the study in order to avoid the problem of repetition effects in both task performance and fMRI.

Various factors may have confounded the attenuation of signal increases in the posterior cingulate in the ketamine group. One factor to consider is a potential non-specific effect of ketamine on vigilance. However, there were no significant differences between placebo and ketamine in the vigilance subscale. In addition, no significant correlation between vigilance scores and regional signal changes could be observed (see also Abel et al., 2003a,b). Another factor to consider is a potential influence of ketamine on the period of word presentation prior to the actual retrieval task. To exclude this confounder, we distinguished between item- and task-related signal increases (see Results and also Duzel et al., 1999). The posterior cingulate showed signal increases and ketamine-induced attenuation exclusively during the task ('Remember'/'Know'; Yes/No), while it was not activated during the period of word presentation.

It should be noted that ketamine has effects on several receptor systems. It increases dopamine concentrations in the striatum (Vollenweider et al., 2000; Kapur and Seeman, 2002), inhibits NMDA receptor-mediated acetylcholine release (see Grunwald et al., 1999), and shows an affinity for 5-HT(2) receptors (Kapur and Seeman, 2002). Moreover, it also exerts influence on sigma receptors (Nakao et al., 2002). Thus, ketamine may not induce a selective

hypoglutamatergic state, but more likely produce non-selective neurochemical changes via direct and indirect effects. Accordingly, it cannot be excluded that the attenuation of activation in the posterior cingulate, as observed in the present study, might not only be due to NMDA receptor hypofunction, but also be related to other receptor systems.

It could be argued that our findings might be related to an unspecific effect of ketamine on cerebral blood flow. However, both our results and those of Abel et al. (2003a,b) suggest that the influence of ketamine on BOLD-signal change (and thus on blood flow) is rather dependent on the specific cognitive task carried out rather than related to a large independent effect of ketamine on cerebral blood flow or neurovascular coupling per se. This assumption is supported by animal studies showing that ketamine does not influence vascular mechanisms independently of its effects on neuronal activation (Burdett et al., 1995). One might consequently assume that there are no global and task-independent effects of ketamine on cerebral blood flow, which is consistent with the study of Breier et al. (1997). Using FDG-PET, they found no significant difference in global metabolic activity between ketamine and placebo infusion.

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