

REGULAR RESEARCH ARTICLE

Ketamine Effects on EEG during Therapy of Treatment-Resistant Generalized Anxiety and Social Anxiety

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Abstract

Background: Ketamine is swiftly effective in a range of neurotic disorders that are resistant to conventional antidepressant and anxiolytic drugs. The neural basis for its therapeutic action is unknown. Here we report the effects of ketamine on the EEG of patients with treatment-resistant generalized anxiety and social anxiety disorders.

Methods: Twelve patients with refractory DSM-IV generalized anxiety disorder and/or social anxiety disorder provided EEG during 10 minutes of relaxation before and 2 hours after receiving double-blind drug administration. Three ascending ketamine dose levels (0.25, 0.5, and 1 mg/kg) and midazolam (0.01 mg/kg) were given at 1-week intervals to each patient, with the midazolam counterbalanced in dosing position across patients. Anxiety was assessed pre- and postdose with the Fear Questionnaire and HAM-A.

Results: Ketamine dose-dependently improved Fear Questionnaire but not HAM-A scores, decreased EEG power most at low (delta) frequency, and increased it most at high (gamma) frequency. Only the decrease in medium-low (theta) frequency at right frontal sites predicted the effect of ketamine on the Fear Questionnaire. Ketamine produced no improvement in Higuchi's fractal dimension at any dose or systematic changes in frontal alpha asymmetry.

Conclusions: Ketamine may achieve its effects on treatment-resistant generalized anxiety disorder and social anxiety disorder through related mechanisms to the common reduction by conventional anxiolytic drugs in right frontal theta. However, in the current study midazolam did not have such an effect, and it remains to be determined whether, unlike conventional anxiolytics, ketamine changes right frontal theta when it is effective in treatment-resistant depression.

Keywords: anxiety disorder, electroencephalography, generalized anxiety disorder, ketamine, social anxiety disorder; treatment resistance

Introduction

A wide range of “neurotic” disorders (Andrews et al., 1990), even when these are resistant to conventional treatment, respond to ketamine. The neural basis for this therapeutic effect of ketamine is not known. Here, we report widespread effects of

ketamine on brain activity in patients resistant to other treatments for generalized anxiety and social anxiety; and suggest that changes in right-frontal theta band rhythmicity may underlie changes in anxiety ratings.

Received: February 6, 2018; Revised: March 15, 2018; Accepted: April 12, 2018

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Significance Statement

We report that ketamine decreases low-frequency brain rhythms and increases high ones in patients with treatment-resistant generalized anxiety and social anxiety disorders. Only the decrease in medium-low frequency (“theta”) power at right frontal sites predicted the improvement by ketamine in fear questionnaire scores. This is the first report of the effects of ketamine on brain rhythms and treatment-resistant anxiety and suggests that right frontal “theta” rhythmicity may be important for all types of anxiolytic action.

Anxiety disorders such as generalized anxiety disorder (GAD) and social anxiety disorder (SAD) are among the most prevalent of mental health problems (Stein and Sareen, 2015). In the United States, the prevalence of GAD has been reported to be as high as 3.1% per year, and 5.7% over a patient’s lifetime (Stein and Sareen, 2015). Further, 12% of the population is affected by SAD, making it a leading cause of impairment and distress (Lipsitz and Schneier, 2000; Kessler et al., 2005). SAD, in particular, has high economic burden, because it causes social impairment, poor academic achievement, reduced work productivity, and increased financial dependence on the government (Lipsitz and Schneier, 2000). Conventional treatments can take weeks to produce their full effects and, worse, one-third of SAD patients are treatment resistant (Kelly et al., 2015; Taylor et al., 2015, 2018), which increases outpatient costs, doubles hospitalizations, and produces substantial morbidity (Liebowitz et al., 2003). We urgently need novel pharmaceutical agents that are both more effective and act quickly (Liebowitz et al., 2003; Taylor et al., 2018).

Ketamine is an N-methyl-D-aspartate (NMDA) receptor antagonist that has been found to be rapidly effective in treating treatment-resistant depression (Zarate et al., 2006), possibly via a non-NMDA route (Zanos et al., 2016). Initial clinical studies have also demonstrated rapid improvement in obsessive-compulsive disorder (Rodriguez et al., 2013) and posttraumatic stress disorder (Feder et al., 2014). Converging neuroimaging and pharmacological evidence implicates glutamate abnormalities in the pathophysiology of SAD (Freitas-Ferrari et al., 2010; Averill et al., 2017), and we have previously reported dose-related effects of ketamine on SAD and GAD in treatment-refractory patients (Glue et al., 2017). Taken together, these data suggest that ketamine may be acting on a single fundamental mechanism to produce rapid changes in the broad class of “neurotic, stress-related and somatoform disorders” (World Health Organization, 1992) even when these are resistant to conventional treatments.

Anxiety and depression appear to share common changes in brain network activity (Pannekoek et al., 2015) and regional grey matter (Van Tol et al., 2010). In depressed patients, ketamine specifically increases slow wave activity during sleep, especially in those with low baseline slow waves, and this may mediate its antidepressant effects (see Duncan and Zarate, 2013). In healthy participants, it can reduce delta (1–3 Hz), theta (4–7 Hz), and alpha (8–15 Hz) band power, while increasing gamma (>32 Hz) band power (Hong et al., 2010; de la Salle et al., 2016). But it can also increase theta power while decreasing alpha power (Domino et al., 1965; Schüttler et al., 1987; Kochs et al., 1996), particularly at frontal sites (Muthukumaraswamy et al., 2015); so changes in bands can be interleaved, with decreased delta, alpha, and beta (16–31 Hz) mixed with increased theta and gamma (Muthukumaraswamy et al., 2015; Rivolta et al., 2015).

We therefore evaluated the effects of ketamine concurrently on symptoms of anxiety and EEG in treatment-resistant Diagnostic and Statistical Manual of Mental Disorders, Volume IV (American Psychiatric Association, 2000) SAD and GAD patients using an active-control double-blinded design. We assessed GAD with the Hamilton Anxiety Scale/HAM-A

(Hamilton, 1959) and SAD with the Fear Questionnaire (FQ; Marks and Mathews, 1979). We assessed EEG by quantitation of power in specific frequency bands and by measures that show depression-related changes: frontal alpha asymmetry (FAA; Allen et al., 2004; Stewart et al., 2014; Mennella et al., 2017) and increased Higuchi’s fractal dimension (HFD; Higuchi, 1988; Bachmann et al., 2013; Akar et al., 2015). We predicted that ketamine would produce dose-related improvements in symptoms, FAA, and HFD; show dose-related power decreases in the delta, alpha, and beta bands; and power increases in the theta and gamma bands (Muthukumaraswamy et al., 2015).

Methods and Materials

Participants

We recruited 12 patients with refractory DSM-IV GAD and/or SAD. The Southern Health and Disabilities Ethics Committee approved this study (15/STH/86). Patient inclusion criteria included having a HAM-A score of ≥ 20 , and/or an LSAS (Liebowitz, 1987) score of ≥ 60 at screening, and being aged 18 years or older. All had failed to respond to 2 courses of antidepressants. Patients were excluded if there was evidence of severe acute or chronic medical disorders or if they were pregnant or lactating; taking monoamine oxidase inhibitors, thyroxine, or stimulants, or had active suicidal ideation. To reduce the risk that changes in anxiety ratings were confounded by comorbid depression, we excluded patients with Montgomery-Asberg Depression Rating Scale (MADRS; Montgomery and Asberg, 1979) scores of ≥ 20 at screening. All patients provided signed informed consent prior to enrollment and were assessed as suitable to participate based on review of medical history, safety laboratory tests, and vital signs. Patients remained on their current medication regimens and continued with ongoing psychotherapy. However, they started no new treatments and did not change doses/visit schedules. There were 3 ascending ketamine dose levels (0.25, 0.5, and 1 mg/kg) and midazolam (0.01 mg/kg), administered double blind. Justification of the 3 ketamine doses is provided in Glue et al. (2017) and for the midazolam dose in Loo et al. (2016). The choice of control treatment for ketamine studies in mood disorders is complicated. Saline placebo has been criticized for its lack of psychoactive effects, which essentially is unblinding. Therefore, we chose to use midazolam, which is psychoactive, as an active control for ketamine. The 3 ketamine doses were administered in ascending order, with midazolam dosing randomly inserted into the dosing schedule. All medications were injected subcutaneously in the upper arm, with 1 week between doses. The study was registered prospectively with the Australian New Zealand Clinical Trial Registry (ACTRN 12615000617561; <http://www.anzctr.org.au/>).

Assessments

We monitored patients in the clinic for 2 hours postdose, with vital signs obtained predose, and 15, 30, 60, 90, and 120 minutes

postdosing (data not reported). Anxiety assessments included the FQ (score range 0–136; Marks and Mathews, 1979) and the HAM-A (range 0–52; Hamilton, 1959) predose, at 1, 2, 24, 72, and 168 hours postdose. Tolerability assessments included reported adverse events throughout the study, and Clinician Administered Dissociative States Scale (Bremner et al., 1998) predose, 30, and 60 minutes postdose. Summary statistics were calculated and reported for demographic, vital signs, and rating scale data. As EEG was only recorded predose and at 2 hours postdose, we report only the predose and 2-hour postdose anxiety scale data here.

Electroencephalography

A Waveguard EEG cap (ANT Neurotechnology) using the 10:20 system was used to record brain activity across the frontal lobes of the participants, specifically using channels Fp1, Fp2, F7, F3, Fz, F4, F8, and Cz, with left mastoid as the reference electrode. Depending on their head circumference, each participant was fitted with one of 3 appropriate cap sizes: large (head circumference 57–64 cm), medium (53–57 cm), and small (47–53 cm). The EEG cap was connected to a Bioradio (CleveMed: Cleveland Medical Devices Inc.). The Bioradio used Bluetooth to stream the recorded data (sampled at 256 Hz) to a computer that stored the data for later offline analysis using BioCapture (CleveMed, Cleveland Medical Devices Inc.). Participants were fitted with the EEG cap and a recording was made prior to study drug administration (predose recording). For the predose recording, participants were asked to sit still to reduce any noise interference and were then instructed to have their eyes open and then closed for alternating 1-minute intervals on request for the next 10 minutes. There were, therefore, 5 recorded minutes of eyes open and 5 recorded minutes of eyes closed, with marks in the EEG file indicating the point of changeover. After the EEG predose recording, the participants received their SC study drug dosing and were supervised by registered nurses and psychiatrists for the next 2 hours, after which participants underwent another EEG recording (postdose recording) identical to the predose recording.

EEG Processing

The EEG data were analyzed using custom software written in Visual Basic 6. The data were down-sampled to 128 Hz and submitted to a 3-point running mean as a low pass filter (effective 46 Hz cut off) and then submitted to an automated procedure for eye blink removal, based on the ballistic components of the eye blink, which left residual EEG (Zhang et al., 2017).

For simple power analysis, the files were then manually processed and any remaining artefacts were replaced with missing values. The recordings were separated into single open/closed minute segments and a serial Fast Fourier Transform with a 1-second overlapping Hanning window was applied. The resultant power spectra were log transformed to normalize error variance and averaged. This segmented the file into 10 spectra, 5 of which eyes were open and 5 of which eyes were closed. For the current analyses, these were then averaged over minutes to produce a single spectrum for each testing occasion for each participant. Power was then averaged across frequencies within each of the conventional bands to give a single power value for each of delta (1–3 Hz), theta (4–6 Hz), alpha1 (7–9 Hz), alpha2 (10–12 Hz), beta (25–34 Hz), and gamma (41–53 Hz).

Alpha asymmetry was calculated for both the alpha1 and alpha2 bands by subtracting logarithmic power at left electrodes from their right-most counterparts $[\ln(R) - \ln(L)]$ for each of F8:F7 and F4:F3.

Fractal dimension was calculated using Higuchi's algorithm with a k_{max} of 8 (Higuchi, 1988). After the eye-blink removal stage, the data were subjected to an additional 2- to 36-Hz bandpass filter, and sections with artefacts were manually removed. The continuous data were then split into 2-second (256 sample) epochs with 50% overlap. Higuchi's algorithm creates k_{max} number of new time series (with k running from 1 to k_{max}), each obtained by taking every k th sample of the original epoch. The length of the curve of each series is calculated and plotted against k on a double logarithmic graph. If the length of the curve and k are proportional, then the plotted data will fall on a straight line. The slope of this line is the fractal dimension.

Statistical Analysis

The data were submitted to ANOVA in SPSS with channel, frequency, and dose as within-subjects variables. Polynomial components of all factors were extracted with the MDZ active control treated as 0 mg ketamine.

Results

Participants

The participants were 12 patients (4 male, 8 female; mean age=31 years, range 18–65). Mean duration of their anxiety disorders was 13.8 years. All 12 participants had SAD, 10 had GAD, and 2 panic disorder. Nine had past MDE but none were depressed at the time of enrolment (mean MADRS 6.6). Baseline HAMA score was 28.1 and mean LSAS was 91.3. Demographic and diagnostic details are provided in Table 1, along with information about prior failed treatments for their anxiety disorders.

Changes in Anxiety Ratings

Overall, 8 of 12 patients (67%) reported a >50% reduction in HAM-A and/or FQ scores after the 0.5- or 1-mg/kg doses of ketamine at 2 hours postdose. Scores are shown in Table 2 and postdose improvement relative to predose is shown in Figure 1A. There was a clear dose-related improvement in FQ scores with ketamine dose (dose, $F(2.67, 29.41)=3.80, P=.024$, Greenhouse-Geisser corrected; dose[lin], $F(1, 11)=7.12, P=.022$) with a trend to a ceiling effect or perhaps even reduction at 1.0 mg of ketamine (dose[quad], $F(1, 11)=4.68, P=.053$). The very slight apparently similar trend in HAM-A scores (Figure 1A) was not supported statistically (all $F \leq 1.2$, all $P \geq 0.3$).

Ketamine Reduced Low-Frequency and Increased High-Frequency EEG Power but Did Not Improve HFD or FAA

For all analyses, we treated MDZ as equivalent to 0 mg of ketamine. To simplify EEG power analysis, we averaged across frequencies within each band. Figure 2A–B shows the effects (with statistics in the legend) of varying doses of ketamine on the post:pre difference in EEG power for the different frequency bands and channels. Across frontal sites (Figure 2A), higher doses of ketamine significantly but nonlinearly reduced delta, and sometimes theta, power at the lateral sites F7, F4, and particularly F8, while generally increasing beta and particularly delta.

From anterior to posterior (Figure 2B), there was a clear dose- and band-related (largest in the delta band) reduction in power at lower frequencies with ketamine at the fronto-polar site, which diminished across the mid-frontal and central sites.

Table 1. General Demographic and Treatment Details for Participants

No.	Age	Gender	Employed	Anxiety Duration (y)	Baseline Score			Prior Ineffective Treatments		Current Meds (mg/d)	Diagnoses				
					HAMA	LSAS	MADRS	Medication	Psycho-therapy		GAD	SAD	PD	Past MDE	Other
1	24	f	y	8	39	78	6	SSRIs, TCAs, ven	CBT, PRx	Doth 50 mg, loraz 4 mg	X	x	x		
2	22	f	n	7	22	97	6	Sert	-	Ven 150 mg	X	x			
3	25	m	n	5	26	118	6	Fluox,quet	CBT, PRx	Fluox 40 mg	X	x	x		
4	24	f	n	12	33	105	6	Fluox,sert,cital	PRx	Ven 300 mg	X	x	x		
5	25	f	n	12	32	68	5	SSRIs,mocl	CBT,	Ven 400 mg,VPA 400 mg,clon 2 mg	X	x	x	x	Past polysubstance use (opioids, cannabis)
6	33	m	y	20	27	87	8	Fluox,cital	CBT, PRx	Ven 75 mg	x	x			
7	29	f	y	19	36	80	8	SSRIs,mirt,busp	-	Ami 150 mg	X	x	x		
8	26	f	n	16	14	88	5	SSRIs,mirt	PRx	Ven 300 mg,bup 300 mg,diaz 10 mg	X	x	x		
9	65	m	n	15	38	101	8	SSRIs,ven,mirt,TCAs	PRx	Dox 75 mg,diaz 2 mg	X	x	x		Past AUD
10	27	f	y	10	36	103	5	Ami	PRx	Cital 10 mg	X	x			
11	18	f	n	5	16	109	4	Parox,mirt	PRx	None	x	x	x		EDNOS
12	52	m	y	37	18	61	12	SSRIs	PRx	Parox 20 mg	x	x	x		

Abbreviations: ami, amitriptyline; AUD, alcohol use disorder; bup, bupropion; busp, buspirone; CBT, cognitive behavioural therapy; cital, citalopram; clon, clonazepam; diaz, diazepam; doth, dothiepin; dox, doxepin; EDNOS, eating disorder not otherwise specified; fluox, fluoxetine; GAD, Generalised Anxiety Disorder; HAMA, Hamilton Anxiety; LSAS, Liebowitz Social Anxiety Scale; loraz, lorazepam; MADRS, Montgomery Asberg Depression Rating Scale; mirt, mirtazapine; mocl, moclobemide; parox, paroxetine; PD, Panic Disorder; PRx, other psychotherapy; Quet, quetiapine; SAD, Social Anxiety Disorder; sert, sertraline; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant; ven, venlafaxine; VPA, sodium valproate.

Table 2. Fear Questionnaire (FQ) and Hamilton Anxiety (HAM-A) Questionnaire Means Predose and 2 Hours Postdose for Midazolam (MDZ) and Ketamine (K), with Values Showing Dose in mg

Scale	Time	MDZ	K0.25	K0.50	K1.00
FQ	Predose	45.33	54.33	49.67	42.00
	+2h	35.92	37.75	28.13	24.17
HAM-A	Predose	16.25	19.92	16.17	13.75
	+2h	8.83	11.58	4.92	4.58

Whereas, there was increased power at higher frequencies that tended to increase from frontal to central sites. Changes in HFD (Figure 2C) were minimal, nonsignificant, and in the opposite direction to that predicted. There were no systematic changes in FAA for either F8:F7 or F4:F3. As there were no significant effects differentiating alpha1 and alpha2, the results are averaged across band in Figure 2D.

Posthoc calculation of statistical power for the mean differences between ketamine 1 mg/kg and midazolam for each of the

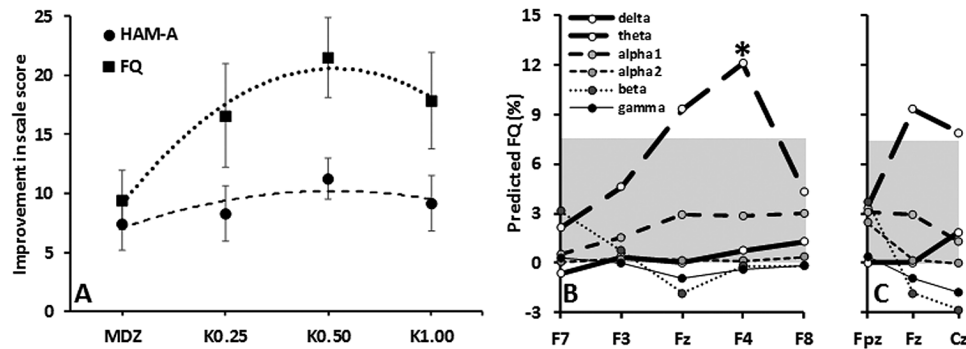


Figure 1. Predose vs postdose improvements in scale scores. (A) Variation with ketamine dose (K, mg) relative to midazolam (MDZ) for Fear (FQ) and Hamilton Anxiety (HAM-A) Questionnaires subjected to separate analyses. Curves are linear+quadratic trend lines (significant for FQ but not HAM-A). Bars are \pm SEM and are approximately equal (2.5 vs 2.2, respectively) for the 2 questionnaires in the case of MDZ. (B) Correlation of FQ change with power change in different frequency bands at different electrode sites. Values are signed (\pm) percent of variance accounted for ($r^2 \times 100$). Height of the grey zone represents the 95% CI uncorrected for multiple comparisons. *Significant effect within stepwise regression ($P < .05$).

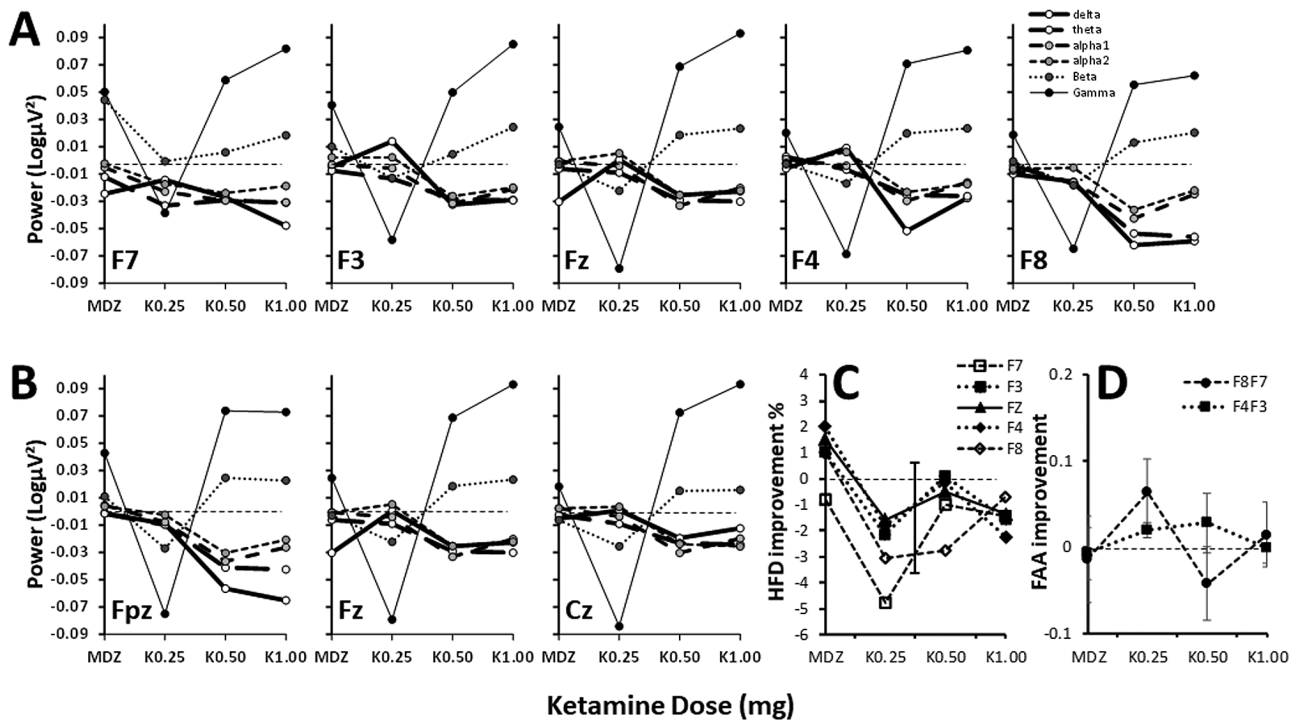


Figure 2. Post-pre effects (difference scores) for different doses of ketamine and midazolam (MDZ) on power in different frequency bands, on Higuchi's fractal dimension, and on alpha asymmetry at frontal-central electrodes. (A) Power data subjected to ANOVA for left-right effects across the frontal sites. The strongest reductions in power were at lateral sites and lower frequencies: dose[lin] x band[quad] x channel[quad], $F(4, 7)=5.04, P=.05$; dose[cub] x band[lin] x channel[quad], $F(4, 7)=8.51, P=.022$; dose[cub] x band[quad] x channel[cub], $F(4, 7)=79.37, P<.001$; dose[cub] x band[quad] x channel[quad], $F(4, 7)=30.52, P=.001$. (B) Power data subjected to ANOVA for anterior-posterior effects. The strongest reduction was at Fp1 and in the delta band: dose[lin] x band[cub], $F(4, 7)=11.65, P=.011$; dose[cub] x band[cub] x channel[lin], $F(4, 7)=4.25, P=.077$. (C) Higuchi's Fractal Dimension (HFD, percent) shown for each of the 5 frontal electrode sites (bar is $2 \times$ maximum SE for the set of means). There were no reliable effects of ketamine. (D) Frontal Alpha Asymmetry (FAA) shown separately for the F8:F7 and F4:F3 pairs. Values are averaged across alpha1 and alpha2 as there were no significant effects associated with sub-band. There were no systematic effects of ketamine (bars are \pm SEM).

theta and gamma bands and at all 5 electrode positions showed that with sample sizes ranging from 3 to 11, there was >80% power at $\alpha=0.05$.

Ketamine Effects on FQ Appear Related to Right-Frontal Theta Power

For each of the electrode sites, separately, we carried out a stepwise regression of FQ change score with power-change scores for all the bands as predictors. The bulk of the simple correlations (uncorrected for multiple comparisons) were well within 95% confidence limits (Figure 1B). The lack of any obvious contribution to FQ change was particularly clear for the delta and gamma bands despite the fact that they were most affected by ketamine (Figure 2A–B). All the highest correlations were obtained with the theta band with Fz and Cz achieving values that would have been significant uncorrected. F4 theta was the only power change that was extracted as a significant predictor by the stepwise analysis, with the other high values surrounding it. To test the structure of these adjacent values we forced F3, Fz, F4, F8, and Cz into a multiple regression on FQ. The total predictive power of the equation as a whole was 17%, about 5% greater than F4 alone, with the bulk of the additional explanatory power coming from a unique contribution (3%) from the contralateral site F3. Of the remaining 14%, 9% was variance shared among Fz, F4, F8, and Cz and 5% was unique to F4, with F8 and Cz having no unique contribution. These results are consistent with the bulk of the effect of ketamine on FQ being mediated by a single source close to F4, with some spread of activity to the immediately adjacent electrodes, and a weak contribution from an independent source in a similar location in the opposite hemisphere.

Discussion

Our main finding is that ketamine produced a dose-related decrease, maximal at 0.5 mg, in theta frequency frontal power at the right frontal site F4 that appears to mediate its therapeutic effects on GAD and SAD, as measured by the FQ. Similar power changes in the theta range at adjacent sites appeared to be less involved in controlling FQ, while larger decreases in power in the delta range and large increases in power in the gamma range appeared to make no contribution to changes in FQ. Ketamine produced no sign of an improvement in HFD scores at any dose and no systematic or reliable changes in FAA. Reduced anxiety has previously been reported with ketamine (Glue et al., 2017; Taylor et al., 2018); however, we saw significant changes only in FQ and no large changes in HAM-A scores.

Our alpha asymmetry results are against our prediction but not entirely surprising. FAA has previously been linked to aversion/withdrawal/pessimism/introversion in general (Wacker et al., 2010; De Pascalis et al., 2013; Wacker, 2017) and not depression or anxiety (Bruder et al., 1997; Mathersul et al., 2008; Adolph and Margraf, 2017) in particular. It shows a trait-like reliability and stability that (over months) is not related to changes in depressed state in patients with major depression (Debener et al., 2000; Allen et al., 2004) and may be a predictor of future disorder rather than a biomarker of current disorder (Smith and Bell, 2010).

Our fractal dimension results are also against our prediction. This measure has so far been linked only to depression (Bachmann et al., 2013; Akar et al., 2015), and it is possible that it is specifically linked to this rather than more generally linked to the neurotic spectrum. This may also be true of alpha asymmetry (Gordon et al., 2010). Alternatively, like alpha

asymmetry, it may be a characteristic that is linked to depressed people but not to the depressed state itself. Benzodiazepines, such as diazepam and lorazepam, often used to treat anxiety disorders but not depressive disorders, increase HFD in healthy humans (Chouvarda et al., 2009; Michail et al., 2010). We found no such effect with midazolam in the current GAD and SAD patient group.

Our findings that ketamine rapidly reduces power in the alpha1, alpha2, and particularly delta bands in GAD and SAD patients are broadly similar to previous findings (Hong et al., 2010; de la Salle et al., 2016). However, the observed reduction in delta might seem opposite to the previously reported increase in slow wave sleep activity (Duncan and Zarate, 2013). Given the consistent previous reduced waking delta and the very distinctive EEG state occurring in deep slow wave sleep, it is possible that sleep delta is functionally distinct from waking delta. An alternative is that the increase in sleep delta (which occurs during the first night after dosing) is a rebound from the immediate decrease (reported here 2 hours after dosing).

Our increased gamma, unlike our increased beta, is as predicted. It seems likely that the variations in previous results and between our specific findings and our predicted pattern is due to dose- and testing-related variations (note the decrease in gamma at 0.25 mg) but could also be due to our use of a particular patient population (GAD/SAD) and also our small sample number. Other limitations include the lack of a placebo control group, although there was an active control group; while we obtained blood levels of drug, they were not analyzed. Further work with carefully matched healthy controls is required to clarify these points. However, if we take our data at face value, they suggest that, at least under some conditions, ketamine produces a decrease that is greatest at lower frequencies and an increase that is greatest at higher frequencies.

Our observation of reduced theta power is consistent with some previous reports and opposite to others. There is a suggestion in Figure 2 that the theta decrease is greater at F8 than other sites, and so the variable results reported in this band may depend on site of recording (Muthukumaraswamy et al., 2015), method of testing (Kochs et al., 1996), and the dose of ketamine. Given our inverse-U dose-response curve with the FQ and the largely linear dose-response for most bands and electrode sites, our current data suggest that the observed effect of ketamine most likely to be related to its therapeutic effect is at right frontal sites, particularly F4. Critically, F4 is the only site for which we have clear evidence that changes in the theta band (and no other) relate to FQ changes. Despite large dose-related changes in power in the delta and gamma bands, there was no evidence that these changes were linked to therapeutic action (as opposed, say, to residual effects of dissociation). A much larger sample and much more detailed analysis would be needed to confirm these observations.

Our recently developed human anxiolytic biomarker (McNaughton, 2017), goal-conflict rhythmicity, is obtained in the theta (spreading to alpha1) band at right frontal sites (McNaughton et al., 2013; Shadli et al., 2015). It is possible, therefore, that the therapeutic effects reported with ketamine here reflect an action on the same brain system, which is potentially homologous with the rodent theta that is a uniquely reliable test of anxiolytic action (McNaughton et al., 2007) and is known to be reduced by ketamine (Engin et al., 2009). However, in healthy humans and rats, this biomarker has been defined by conventional anxiolytics given in single, low, doses. In our GAD and SAD patients, MDZ had little effect on rhythmicity and no effect at all on theta at right frontal sites. This lack of effect could be

an explanation of the patients' resistance to such treatments. However, it is just as likely that ketamine in the current experiments is acting on a quite distinct right frontal system, which also requires theta-frequency rhythmicity, to that activated by our existing biomarker paradigm

We have reported a dose-related effect of ketamine on ratings of anxiety and EEG recordings in patients with treatment refractory anxiety disorders. In particular, we found that right frontal slow-wave (theta) EEG changes predicted reduced intensity of phobic anxiety ratings. These novel double-blind findings in patients are consistent with earlier preclinical and human data that link diverse anxiolytic treatments with right frontal EEG changes, which may represent a plausible biomarker of anxiolytic action.

Acknowledgments

Shabah Shadli was supported by funding from the Health Research Council of New Zealand (14/129).

Statement of Interest

The authors declare the following potential conflicts of interest: P. Glue has a contract with Douglas Pharmaceuticals to develop novel ketamine formulations. Within the last 3 years, P. Glue has participated in an advisory board for Janssen Pharma and N. McNaughton has had a confidential disclosure and consulting agreement with Janssen Research & Development, LLC. The other authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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