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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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Supplementary Material

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Data Monitoring and Ethics Committee Chair: Prof Emeritus Stephen Cooper (University of Belfast) and members: Dr Carol Gamble (Reader in Medical Statistics, University of Liverpool); Prof Peter Liddle (University of Nottingham); Graham Dunn (University of Manchester, Professor of Biomedical statistics).

Trial Steering Committee Chair: Prof John Geddes (University of Oxford); Prof Robin Jacoby (University of Oxford); Prof Max Birchwood (University of Birmingham); Gerald Wright (service user rep).

Abigail Gee analysed the N-back imaging data as part of her MSc in Neuroimaging for Clinical and Cognitive Neuroscience at the University of Manchester

Table S 1 Schedule of Assessments

Assessment	Who				When						
	CSO	RA	RMO team		Screening	Randomisation	Month 2	Month 6	Month 9	Month 12	Post trial f/u
Case note diagnosis checklist	x	x			x						
Diagnostic and eligibility c'list		x	x		x					x	
DUP			x		x						
MINI interview for psychosis		x			x					x	
Drug treatment history	x	x			x	x	x	x	x	x	x
Body weight & BMI	x	x				x				x	x
BP & HR	x	x			x					x	x
Lab screen	x	x	x		x					x	
Drug screen (urine)	x	x			x			x			
Drug use questionnaire	x	x			x	x	x	x	x	x	x
Pregnancy screen (urine)	x	x			x	x	x	x	x	x	
Inclusion criteria	x	x	x		x						
Exclusion criteria	x	x	x		x						
Withdrawal criteria		x				x	x	x	x	x	
Consent	x	x	x		x						
Consent genetic	x	x	x		x						
Saliva Oragene kit		x				x					
Blood cytokine screen		x	x			x		x		x	x
PANSS		x			x	x	x	x	x	x	x
GAF		x				x	x	x	x	x	x
Social Function Scale	x	x				x		x		x	x
WAIS III (current IQ)	x	x			x					x	x
WTAR (IQ decline)	x	x			x						
Other cognitive tasks	x	x			x					x	x
EPS scales		x				x		x		x	x
Calgary depression scale	x	x				x	x	x	x	x	x
ANNSERS scale (side effects)	x	x				x	x	x	x	x	x
7 point compliance scale		x					x	x	x	x	
MRI screening questionnaire	x	x			x						
MRI scanning		x				x				x	

References for Schedule of Assessments

Sheehan D V, Lecrubier Y, Sheehan KH, *et al.* The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 1998; **59 Suppl 2**: 22-33;quiz 34-57.

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Table S 2 Means and Standard Deviations for main outcome variables

Range of possible scores in brackets

	Placebo			Minocycline		
Measure	**n**	**mean**	**SD**	**n**	**mean**	**SD**
Negative Symptoms (7-49)						
Baseline	104	16.8	5.5	103	17.7	5.9
2m	85	15.1	5.8	83	16.4	5.6
6m	67	15.7	5.8	69	15.8	6.5
9m	62	14.5	4.9	68	15.9	6.3
12m	65	14.2	5.2	62	16.4	6.2
Follow-up	48	14.0	4.9	41	15.6	6.6
Positive Symptoms (7-49)						
Baseline	104	17.3	5.3	103	16.3	4.1
2 m	86	14.5	4.8	83	13.8	4.5
6 m	67	14.4	5.2	69	13.4	5.0
9 m	63	13.6	5.0	68	12.8	4.6
12 m	65	14.0	4.8	63	13.4	6.1
Follow-up	48	13.8	5.2	41	13.2	5.3
Total Symptoms (32-210)						
Baseline	103	69.3	15.4	103	67.1	13.2
2 m	85	60.1	15.7	83	59.6	14.9
6 m	66	59.4	16.8	69	57.5	15.7
9 m	62	56.8	14.7	68	57.0	14.7
12 m	65	57.1	17.3	62	59.0	17.3
Follow-up	48	55.8	15.4	41	57.7	16.5
CDSS (0-27)						
Baseline	103	5.50	4.96	103	5.17	4.27
2m	95	3.40	3.99	94	3.31	3.85
6m	83	3.05	4.17	83	2.60	3.59
9m	80	2.73	3.77	79	3.25	3.78
12m	78	3.12	4.28	76	3.09	3.98
Follow-up	66	2.88	4.43	57	2.49	3.53
GAF (0-100)						
Baseline	103	56.2	11.6	102	55.5	9.1
2 m	85	59.5	11.4	83	58.1	11.6
6 m	65	59.6	12.1	68	60.2	13.2
9 m	63	60.8	12.0	67	58.5	12.7
12 m	64	60.4	13.4	60	56.3	14.1

Table S 2 Means and Standard Deviations for main outcome variables (cont'd)

	Placebo			Minocycline		
Measure	**n**	**mean**	**SD**	**n**	**mean**	**SD**
Weight (kg)						
Baseline	101	86.8	25.3	97	82.6	19.6
12m	58	91.8	28.5	53	88.0	18.2
BMI						
Baseline	101	28.7	7.6	96	27.1	6.2
12m	58	30.1	8.5	53	28.7	5.5
SFS1: Social Engagement / Withdrawal (0-15)						
Baseline	101	10.2	2.9	103	10.5	3.1
6m	65	11.0	3.2	65	11.0	3.2
12m	63	10.9	3.4	61	10.7	3.7
Follow-up	48	11.5	3.5	41	10.6	3.2
SFS2: Interpersonal Behaviour/Relations (0-9)						
Baseline	102	6.6	1.9	103	6.4	1.8
6m	65	7.2	2.0	65	6.8	2.0
12m	63	7.1	2.0	61	6.6	2.2
Follow-up	48	7.1	2.0	41	6.9	2.1
SFS3: Independence-Performance (0-39)						
Baseline	102	26.1	6.4	103	26.3	7.5
6m	65	27.4	7.2	65	26.7	8.2
12m	63	27.4	7.0	61	26.3	6.8
Follow-up	48	26.6	6.8	41	26.0	7.4
SFS4:Recreation (0-45)						
Baseline	102	17.7	6.01	103	18.2	7.8
6m	65	18.4	7.8	65	17.6	7.3
12m	63	18.4	7.0	61	17.4	7.1
Follow-up	48	17.1	6.8	41	17.4	7.6
SFS5:Pro-social Activities (0-69)						
Baseline	102	16.7	10.5	103	16.6	10.3
6m	65	17.3	11.6	65	16.3	9.6
12m	63	17.2	10.8	61	16.5	10.1
Follow-up	48	18.9	11.5	41	15.9	10.0
SFS6:Independence-Competence (0-39)						
Baseline	102	34.0	6.2	103	34.8	4.9
6m	65	35.0	4.8	65	34.7	5.4
12m	63	34.8	5.0	61	34.0	5.1
Follow-up	48	35.5	3.9	41	34.0	7.1
SFS7:Employment/ Occupation (0-10)						
Baseline	102	4.9	3.0	103	4.7	3.1
6m	65	5.6	3.4	64	4.9	3.3
12m	63	5.9	3.1	61	5.3	3.3
Follow-up	47	5.3	3.3	41	5.4	3,7

Table S 2 Means and Standard Deviations for main outcome variables (cont'd)

	Placebo			Minocycline		
Measure	**n**	**mean**	**SD**	**n**	**mean**	**SD**
Digit Symbol (Raw)						
Baseline	91	52.8	16.8	95	58.0	16.7
12m	59	58.2	15.8	58	56.1	16.2
Follow-up	47	61.2	15.9	36	62.6	16.2
Digit Symbol (Scaled)						
Baseline	91	6.0	2.4	95	6.7	2.7
12m	59	6.8	2.3	58	6.5	2.6
Follow-up	47	7.4	2.4	36	7.4	2.6
Full-Scale IQ (FSIQ)						
Baseline	100	89.2	15.9	101	91.2	14.0
12m	61	94.6	16.6	59	93.7	14.2
Follow-up	49	97.0	17.5	38	98.2	16.1
GMV Left (mm^3)						
Baseline	88	5669	786	94	5644	723
12m	54	5509	787	45	5593	70
GMV Right (mm^3)						
Baseline	88	4581	658	94	4574	583
12m	54	4425	680	45	4543	551
IL6 (pg/ml)						
Baseline	100	0.840	0.639	101	0.690	0.458
6m	65	0.902	0.754	57	0.843	0.926
12m	56	0.811	0.623	53	0.793	0.570
CRP (pg/ml)						
Baseline	100	3.83	5.45	101	3.08	3.82
6m	65	5.33	9.54	57	4.56	11.23
12m	56	4.40	5.30	51	6.01	18.91

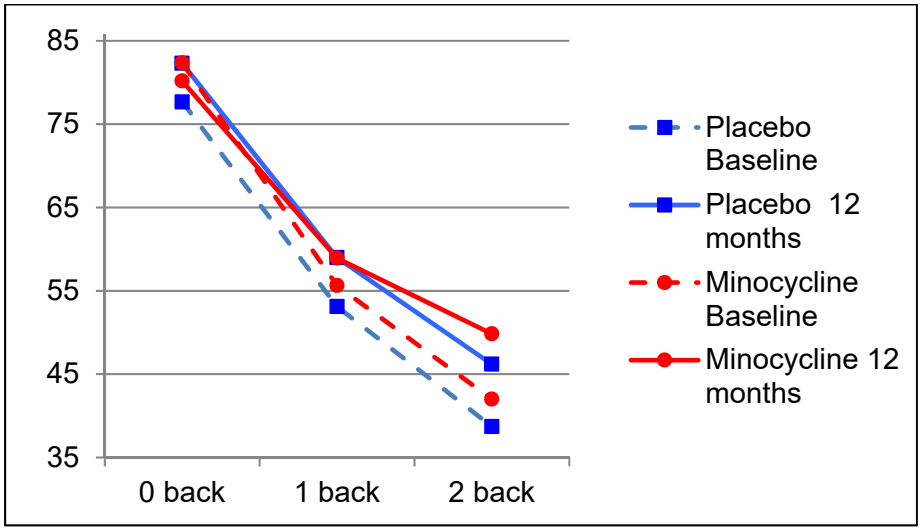
N-Back task performance

Accuracy (percent correct) deteriorated from 0 to 2 back producing a significant main effect of difficulty in a repeated measures ANOVA (table S3 and figure S1). Accuracy improved over 12 months by about 10% at the 2-back level in both groups producing a significant main effect of time. There was no significant interaction between time and treatment

Table S 3 Percent correct responses in N-back task

	Placebo % correct			Minocycline % correct		
Baseline	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**
0 back	36	77.7	25.4	35	82.3	16.9
1 back	36	53.1	27.4	35	55.6	30.1
2 back	36	38.7	23.2	35	42.0	26.0
12 months						
0 back	36	82.3	24.3	35	80.2	26.3
1 back	36	59.0	30.4	35	58.9	31.9
2 back	36	46.2	26.9	35	49.8	25.9

Figure S 1 Percent correct responses in N-back task



N-Back functional imaging

MRI Pre-processing for VBM and BOLD

i) MPRAGE/SPGR and PD/T2: brain tissue extraction, segmentation via scan-specific tissue priors, non-linear registration to the Montreal Neurological Institute 152 (MNI) standard space, smoothing with an isotropic Gaussian kernel; ii) *EPI:* motion and slice-timing correction, brain tissue extraction, non-linear co-registration with MNI-registered MPRAGE/SPGR, smoothing with an isotropic Gaussian kernel, intensity normalisation, high pass temporal filter. For both MPRAGE/SPGR and PD/T2 images were analysed with sequence-specific voxel-based morphometry (VBM)[1,2]

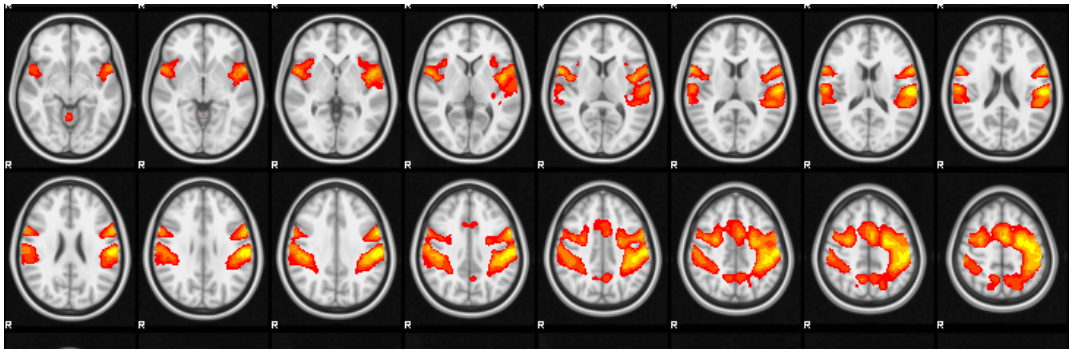
N-back BOLD MRI Methods

Paired scans (baseline and 12 months) were available for 34 in the placebo group and 36 from the minocycline group. After pre-processing, BOLD response to the 0-,1- and 2-back was estimated at every intracerebral voxel. The following contrasts were then estimated: 1-back and 2-back > 0-back, the overall effect of the working memory task in comparison to the control condition (0-back); and 2-back > 1-back, the differential effect of working memory task difficulty.

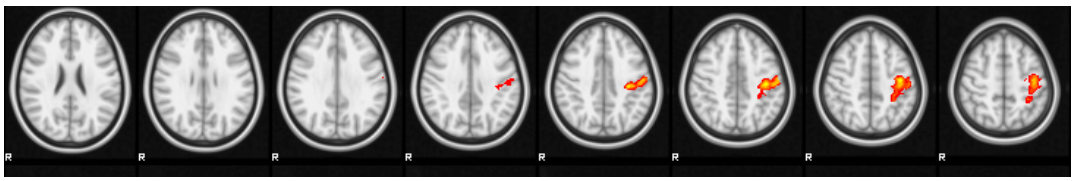
Maps of within-group activation for each contrast from all participants at baseline were derived, and demonstrate patterns of activation typical for this well-established task (Figure S2). The regions of interest (ROIs) to test for treatment effects were selected from two meta-analyses of WM in schizophrenia [3,4], focusing on bilateral dorsolateral prefrontal cortex.

Figure S2: Within-group activation patterns at baseline for: (a) 1-back and 2-back > 0-back; (b) 2-back > 1-back.

(a)



(b)



Results

Within-group activation patterns (Figure S2) included the predicted bilateral regions of interest in dorsolateral pre-frontal cortex, anterior cingulate, and inferior parietal lobule.

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1 Douaud G, Smith S, Jenkinson M, *et al.* Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. *Brain* 2007; **130**: 2375–86.

2 Diaz-de-Grenu LZ, Acosta-Cabronero J, Pereira JMS, Pengas G, Williams GB, Nestor PJ. MRI detection of tissue pathology beyond atrophy in Alzheimer's disease: Introducing T2-VBM. *Neuroimage* 2011; **56**: 1946–53.

3 Glahn DC, Ragland JD, Abramoff A, *et al.* Beyond hypofrontality: A quantitative meta-analysis of functional neuroimaging studies of working memory in schizophrenia. *Hum Brain Mapp* 2005; **25**: 60–9.

4 Minzenberg MJ, Laird AR, Thelen S, Carter CS, Glahn DC. Meta-analysis of 41 Functional Neuroimaging Studies of Executive Function in Schizophrenia. *Arch Gen Psychiatry* 2009; **66**: 811.

7-Point Compliance Scale

The rating was carried out by the RA in a semi-structured interview. The percentage scoring less than 1 or 2 are likely to be non-adherent (see key) and %<3 is the percent of the total number of scores that are 1 or 2. Similarly %> 5 is the percent of the scores that are 6 or 7 which will include adherent participants and some who are over-reporting adherence.

Table S 4 7-Point Compliance Scale

Allocation	**Frequency of total adherence scores**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**%<3**	**%>5**
2 months									
Placebo	5	2	0	1	4	9	68	8%	87%
Minocycline	8	0	0	1	0	7	72	9%	90%
6 months									
Placebo	7	1	1	1	5	8	47	11%	79%
Minocycline	5	1	1	2	3	8	51	8%	83%
9 months									
Placebo	8	3	2	2	4	7	42	16%	72%
Minocycline	10	4	0	0	1	6	48	20%	78%
12 months									
Placebo	14	3	2	2	3	4	41	25%	65%
Minocycline	11	4	0	0	1	3	46	23**%**	75%

Key

1 = Complete refusal
2 = Partial refusal or accepts only minimal dose
3 = Accepts only because compulsory, or very reluctant/requires persuasion, or questions need often (e.g. once every two days)
4 = Occasional reluctance (e.g. questions need once a week)
5 = Passive acceptance
6 = Moderate participation, some knowledge and interest in medication and no prompting required
7 = Active participation, readily accepts, and shows some responsibility for regimen

EPSE and Adverse Events

Table S 5 Parkinsonism: mean Simpson Angus EPSE scale total scores (0-40)

	Placebo					Minocycline				
Measure	**n**	**mean**	**SD**	**Min**	**Max**	**n**	**mean**	**SD**	**Min**	**Max**
Baseline	102	1.60	2.42	0	15	101	1.60	2.42	0	13
6m	66	1.29	2.01	0	10	65	1.60	2.07	0	7
12m	61	1.25	2.59	0	13	61	1.72	2.46	0	10
follow-up	47	1.04	2.27	0	13	41	1.83	2.52	0	9

Table S 6 Antipsychotic Non-Neurological Side-Effects Rating Scale (ANNSERS)

	Placebo			Minocycline		
Measure	**n**	**mean**	**SD**	**n**	**mean**	**SD**
Baseline	103	13.36	7.09	102	11.49	7.30
2 months	80	7.62	6.96	80	6.39	5.97
6 months	59	6.75	6.92	67	6.57	5.90
9 months	58	6.10	6.19	62	6.70	6.06
12 months	60	6.90	6.62	58	7.29	6.77
follow up	44	5.51	5.96	38	6.70	7.34

Table S 7 Akathisia: distribution of BARS global scores

	Placebo					Minocycline				
Score	**0**	**1**	**2**	**3**	**n**	**0**	**1**	**2**	**3**	**n**
Baseline	83	7	12	1	103	77	13	9	1	100
12 mth	51	3	9	0	63	49	6	4	1	60

Table S 8 Tardive dyskinesia: distribution of AIMS scores

	Placebo							Minocycline						
Score	**0**	**1**	**2**	**3**	**4**	**5**	**n**	**0**	**1**	**2**	**3**	**4**	**5**	**n**
Baseline	89	9	1	2	0	0	103	86	8	1	1	0	3	100
12 mth	58	2	3	0	0	0	63	54	2	2	0	0	1	60

Table S 9 Adverse events and reactions, and serious adverse events

Event Type	Placebo	Minocycline
Adverse Events (AEs)		
Total number of AEs	67	60
Psychiatric events (MedDRA cat 19)	16	8
Adverse Reactions (ARs)		
Rash	3	2
GI upset	6	2
Headache	2	1
Total ARs	11	5
Severe Adverse Events (SAEs)		
Total number of SAEs	11	18
Psychiatric hospitalisation events (patients)	10 (6)	15 (10)
Admission for abdominal pain (patients)	0 (0)	3 (2)
DVT	1	0

NB Effect of treatment on PANSS negative and positive in univariate ANOVAs for each visit controlling for baseline did not reveal significant effects when relapsers were excluded.