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Supplementary Material

Article Title: A Randomized Controlled Trial of Intravenous Scopolamine Versus Active-Placebo Glycopyrrolate in Patients With Major Depressive Disorder

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Supplementary Materials

Supplementary Appendix 1. Participant Medication Washout

As can be seen from the Participant Advertisements (Supplementary Figure 4), we advertised for participants who were explicitly not medicated. As such washout of medications, as happens in many antidepressant trials was not needed. This is a more stable population to study than those who patients who are explicitly washed out of medication prior to entering a trial. The overwhelming majority of participants who presented to our screening session had either been off medication for substantial amounts of time or completely treatment-naïve. In two known cases, one participant was already weaning off an antidepressant (nortriptyline) with medical supervision and they were screened two weeks after they were off medication. They did not take part in the study session until the following week. Another participant contacted us whilst on-medication, but we stated we could not screen them if they did not present at the screening interview with at least two weeks medication-free. They later contacted us 10 weeks later and was screened then.

Supplementary Appendix 2. Conduct of the Trial

It is known that expectancy, de-blinding through side-effects, the clinical trial environment, and the information provided to participants all mediate antidepressant responses (1–5). Regarding the clinical trial environment, participants were made aware that their comfort, consent, and well-being remained the top priority over the experiment with no procedure being possible without their continuing verbal consent. Regarding expectancy effects, participants were told that they would either receive the drug being investigated for depression (scopolamine), or an active placebo (glycopyrronium). Participants were told that both drugs cause similar effects – most likely sedative effects and a dry mouth; however, only one drug was expected to improve their mood symptoms. Participants were told that the drowsiness should wear off within a few hours, and by the time they left (four hours after administration), they would physically feel "90% back to normal". If asked, participants were told there was an approximately 50% chance of receiving either drug, but if participants explicitly asked, they were told there was a 60% chance of receiving scopolamine, and 40% chance of receiving the active placebo according to the randomisation procedure. Participants were told they would find out the identity of the drug they received, together with their MADRS rater six weeks after administration. Given this information, participants were reasonably well-informed of the process before providing initial consent and remained well-engaged with the trial procedures with little loss to follow-up.

Supplementary Appendix 3. A Comment on Therapeutic Study Staff – Participant Relationships

We were conscious of the fact that therapeutic alliance between study staff (in the form of repeated phone interviews and study staff engagement) can contain intrinsically therapeutic elements. Notably, the number of 'other treatments tried' (i.e. psychotherapy, counsellor appointments, psychologist appointments) were roughly equal in both scopolamine and glycopyrrolate groups and randomisation did not appear to have created an imbalance. Furthermore, in viewing all the cases (less than 10% of the study sample) where the individual had disclosed no prior interactions with mental health professional, they were evenly distributed in both scopolamine and glycopyrrolate groups. Therefore, we believe this effect was balanced out in both groups overall and did not unduly affect our main conclusions.

Supplementary Appendix 4. MADRS Reliability

The MADRS was performed using the Structured Interview Guide for the Montgomery-Asberg Depression Rating Scale (SIGMA) (6). The inter-rater calibration exercises we conducted for the MADRS showed that raters were congruent in their MADRS rating of participants in all domains of the MADRS. That is, the scores either agreed or differed by a maximum of 1 and the total MADRS also either agreed or differed by a maximum of 1.

Supplementary Appendix 5. Deviations from Protocol and Analysis

Overall, the present trial had good adherence to protocol. Only four deviations to protocol were recorded. One participant (who received scopolamine 6 μg/kg) was not followed-up with at the week 6 follow-up. Their

MADRS score was carried forward from week 4. One participant (who received scopolamine 6 μg/kg) reported severely worsened depressive symptoms noted during their day 3 assessment. To be clear, the day 3 assessment was still blinded. For the purposes of clinical management, both the participant and the rater were de-blinded to the drug received and the participant received further follow-ups. Remaining MADRS were performed but these were de-blinded. One participant (who had received glycopyrronium) was not able to be followed up with at day 3 and 2 weeks (but was followed up with at all other time-points). The two missing MADRS scores were imputed by taking the average of the neighbouring timepoints. One participant (who received glycopyrronium bromide) reported worsened depressive symptoms at day 3. This participant resumed standard antidepressant treatments with their usual care provider at 4 days post-administration. To ensure no undue influence of missing data, the linear mixed model was re-analysed with protocol-deviated data as "missing" and with "case wide removal" (Supplementary Table 3 – complete case analysis) with no changes to the main interpretation.

Supplementary Appendix 6. Timing of Blinding Guesses

In this trial we asked for participants (and outcome assessors) to guess their allocation at the end of the trial period – as is generally done when this practise is employed. More recently, we have argued (as we conducted this trial), that it is better to practise to obtain such guesses immediately after the administration of any psychoactive drug – but prior to any therapeutic effect being observed (5). This helps to separate guesses based on therapeutic (antidepressant) effects from malicious (psychoactive effects). However, given the result of the trial that was obtained (a null result), indicating no therapeutic effect, this limitation would not have affected the present de-blinding guess data.

Supplementary Appendix 7. Potential Covariates of Antidepressant Responses

There does not appear to be a dose response with scopolamine (at 4, 5, and 6 μg/kg). On average, participants exhibited 11.2, 13.6, 14.3, and 9.9 MADRS improvements from baseline to day 3 in the glycopyrronium, scopolamine 4 μg/kg, 5 μg/kg, and 6 μg/kg groups respectively (Supplementary Figure 3A). Given the mean MADRS improvements observed in the present study being the highest for the middle dose, it is possible that the antidepressant response to scopolamine has an inverted U-shape (or, a "Goldilocks" effect) such that the most efficacious dose is found in a dosage that is not too low nor too high. However, the omnibus dose fixed effect was not statistically significant and visual inspection of the results did not show major differences between these three doses (Supplementary Figure 3A). Individual statistical analyses between the three scopolamine doses (4, 5, 6 μg/kg) against glycopyrrolate at day three showed non-significant effect sizes of 0.29, 0.43, and -0.12 in favour of scopolamine respectively. A power calculation showed that with $\alpha = 0.05$ and $1-\beta = 0.8$, our study is powered to detect individual dose comparisons of 1.27 – which is well within the range of prior scopolamine studies' effect sizes of 1.2, 1.7, 2.2, and 3.4 (7,8). No significant omnibus gender effects were observed (Supplementary Figure 3B). Regardless, at day 3 post glycopyrronium infusion, MADRS improvements of 10.2 and 15.3 were observed for females and males respectively. At day 3 post scopolamine infusion, MADRS improvements of 12.5 and 12.7 were observed for females and males respectively. The number of treatments tried and the baseline MADRS severity were not statistically significant as covariates (Supplementary Figure 3C,D).

Previously identified confounding variables such as dose (7), gender (9), treatment-naivety (10), and depressive severity (10) were unable to be replicated in this study due to insufficient power. The initial study tested scopolamine doses at 2, 3, and 4 μg/kg with only 4 μg/kg showing statistically significant mood improvements (7) and subsequent studies continued using this dose without exploring whether higher doses exhibited larger mood improvements (8–11). The present study was unable to identify a significant omnibus dose effect, though just by average MADRS improvement, the 5μg/kg dose yielded the largest absolute dose. Females have been shown to exhibit a stronger antidepressant response to scopolamine (9). It was perhaps surprising then to see males exhibiting the larger response in the current study, however, with only 12 (30%) males enrolled in this study, this study was not adequately powered to detect sex effects. This is a limitation of the present study and further covariate analysis of gender is required before more convincing conclusions may be drawn about gender effects. Treatment-naïve patients experienced larger antidepressant effects

(10), and higher baseline MADRS scores were thought to contribute to the null effect of scopolamine in the recent study (11). In analysing the number of past treatments and baseline MADRS scores as covariates, the present study also did not show a clear trend as to whether these covariates had any effect on the outcome. The present study is also limited in power to determine whether treatment naivety or depressive severity impacts the antidepressant response of scopolamine.

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Supplementary Table 1 - Full Inclusion and Exclusion Criteria for Participants. Abbreviations: Diagnostic and Statistical Manual (DSM), Montgomery-Asberg Depression Rating Scale (MADRS).

Inclusion Criteria		
Consent		Willing and able to give informed consent for participation in the trial.
Demographics	Age	18 – 60 years
	Sex	Male or female
Mental Health	Diagnosis	Major depressive disorder according to DSM-V criteria
	Duration	Greater than 2 weeks
	MADRS	≥20 (i.e. moderate to severe depression)
	Treatment status	Antidepressant medication free for at least two weeks (or four weeks if previously on fluoxetine)
Exclusion Criteria		
Consent		Inability to speak or read English
Mental Health	Lifetime	History of psychosis
	Current	Any unstable medical or neurologic condition, judged at the discretion of the clinician
		Imminent risk of suicide as determined by the MADRS / clinical interview
		Substance abuse or dependence in previous 3 months
		Stage 3 treatment-resistant depression or higher as determined by Thase and Rush Staging criteria (12)
		Receiving neuromodulation treatment
		Undergoing planned changes to psychotropic medication
Drug contraindications		Significant renal or hepatic impairment
		Cardiovascular conditions including abnormal heart rate and blood pressure checked at screening
		Glaucoma
		Female participants who are pregnant, lactating or planning pregnancy during the course of the trial
		Contraindication to the use of scopolamine or glycopyrronium according to manufacturer guidelines
		Regular use of any medication deemed to be contraindicated as judged by the attending trial physicians
Other safety criteria		Inability to fast for two hours prior to each administration of trial drug
		Any other condition judged by the trial clinicians as likely to impact on the ability of the participant to complete the trial
		Are currently attending a New Zealand specialist mental health or addiction service

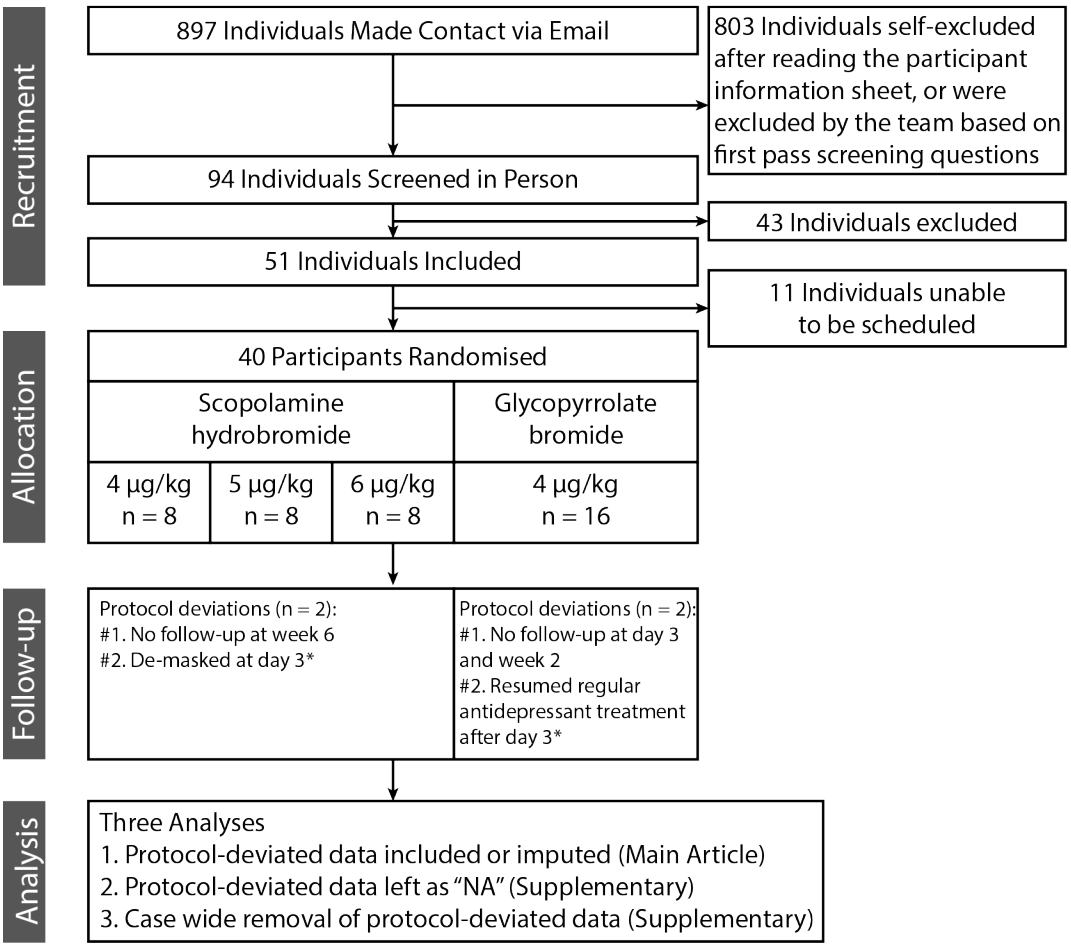
Supplementary Table 2 – Summary demographic details of participants enrolled into this trial. Summary details represented as mean ± standard deviation or the count of cases (with percentage of participants in brackets). Statistical tests are either Fisher's Exact Test or Welch t-test. Abbreviations: Middle Eastern / Latin American / African (MELAA), Alcohol Use Disorders Identification Test (AUDIT), Credibility Expectancy Questionnaire (CEQ), Middle Eastern / Latin American / African (MELAA).

Demographics	Glycopyrronium (*n* = 16)	Scopolamine (*n* = 24)	*
Age	37.8 ± 11.1	33.0 ± 10.7	ns
Male	3 (19%)	9 (38%)	ns
Ethnicity			
European	10 (63%)	14 (58%)	ns
Māori	1 (6%)	4 (17%)	ns
Pacific Peoples	1 (6%)	2 (8%)	ns
Asian	2 (13%)	3 (13%)	ns
MELAA	2 (13%)	1 (4%)	ns
Right-Handed	14 (88%)	20 (83%)	ns
Years in Education	17.2 ± 4.7	16.5 ± 3.6	ns
Height (cm)	167.3 ± 6.0	171.2 ± 8.6	ns
Weight (kg)	78.6 ± 15.1	82.6 ± 23.6	ns
Body mass index (kg m^{-2})	28.0 ± 4.8	27.8 ± 6.4	ns
Systolic Blood Pressure (mmHg)	131.4 ± 13.3	127.1 ± 13.5	ns
Diastolic Blood Pressure (mmHg)	84.4 ± 6.1	83.6 ± 9.9	ns
Heart Rate (bpm)	77.9 ± 15.0	71.0 ± 11.0	ns
Drinks per week	0.8 ± 1.1	1.8 ± 2.2	ns
Cigarettes per week	0.6 ± 2.0	0 ± 0	ns
AUDIT Score	4.8 ± 3.5	3.9 ± 3.4	ns
Previous Antidepressants Tried	0.9 ± 0.9	1.4 ± 1.3	ns
Other Treatments Tried	0.8 ± 0.7	0.7 ± 0.6	ns
Depression Diagnosis (years)	12.9 ± 15.4	7.8 ± 7.6	ns
Depression Self-report (years)	19.5 ± 15.7	7.8 ± 7.6	ns
CEQ score	11.38 ± 2.9	11.06 ± 3.9	ns
MADRS Score at Baseline	27.7 ± 4.4	28.3 ± 4.3	ns
Co-morbidities	2.0 ± 1.5	1.6 ± 1.5	ns
Anxiety Disorders	14 (88%)	17 (71%)	ns
Any Psychotic Disorder	0 (0%)	1 (4%)	ns
Substance Use Disorder	1 (6%)	2 (8%)	ns
Any Eating Disorder	0 (0%)	0 (0%)	ns

*Supplementary Table 3 – Frequentist and Bayesian Summaries of Mood Scores with the equation: MADRS ~ (Drug * Time) + (1|ID). Frequentist analysis denotes linear mixed model. Analysis Abbreviations: parameter estimate (β), t-statistic (t), Cohen's effect size (d).*

Parameters	1. Protocol Deviated Data Included / Imputed (Same as Main Article)			2. Protocol Deviated Data left as "NA"			3. Protocol Deviated Data Case Wide Removal		
	β	t	d	β	t	d	β	t	d
Intercept	27.69	13.41	4.33	27.69	13.97	4.51	27.45	13.00	4.19
Drug	0.56	0.21	0.07	0.56	0.22	0.07	0.92	0.34	0.11
Time (3 hour)	-8.25	-4.03	-1.30	-8.25	-4.07	-1.31	-6.91	-2.88	-0.93
Time (1 day)	-11.44	-5.58	-1.80	-11.4	-5.64	-1.82	-11.09	-4.63	-1.49
Time (3 day)	-11.19	-5.46	-1.76	-11.50	-5.45	-1.76	-12.00	-5.01	-1.62
Time (1 week)	-10.06	-4.91	-1.59	-10.00	-4.73	-1.53	-11.00	-4.59	-1.48
Time (2 weeks)	-7.69	-3.75	-1.21	-7.18	-3.34	-1.10	-6.55	-2.73	-0.88
Time (4 weeks)	-7.00	-3.42	-1.10	-5.63	-2.60	-0.84	-4.18	-1.75	-0.56
Time (6 weeks)	-7.88	-3.85	-1.24	-7.75	-3.67	-1.18	-7.64	-3.19	-1.03
Drug:Time (3 hour)	2.83	1.07	0.35	2.83	1.08	0.35	1.91	0.61	0.2
Drug:Time (1 day)	-2.69	-1.02	-0.33	-2.69	-1.02	-0.33	-1.60	-0.51	-0.17
Drug:Time (3 day)	-1.40	-0.53	-0.17	-1.19	-0.44	-0.14	-0.75	-0.24	-0.08
Drug:Time (1 week)	1.10	0.42	0.13	0.50	0.19	0.06	0.81	0.26	0.08
Drug:Time (2 weeks)	0.23	0.09	0.03	-0.76	-0.28	-0.09	-0.83	-0.27	-0.09
Drug:Time (4 weeks)	-0.25	-0.10	-0.03	-1.43	-0.52	-0.17	-2.63	-0.85	-0.27
Drug:Time (6 weeks)	-0.50	-0.19	-0.06	-0.46	-0.17	-0.05	0.20	0.06	0.02

Supplementary Figure 1



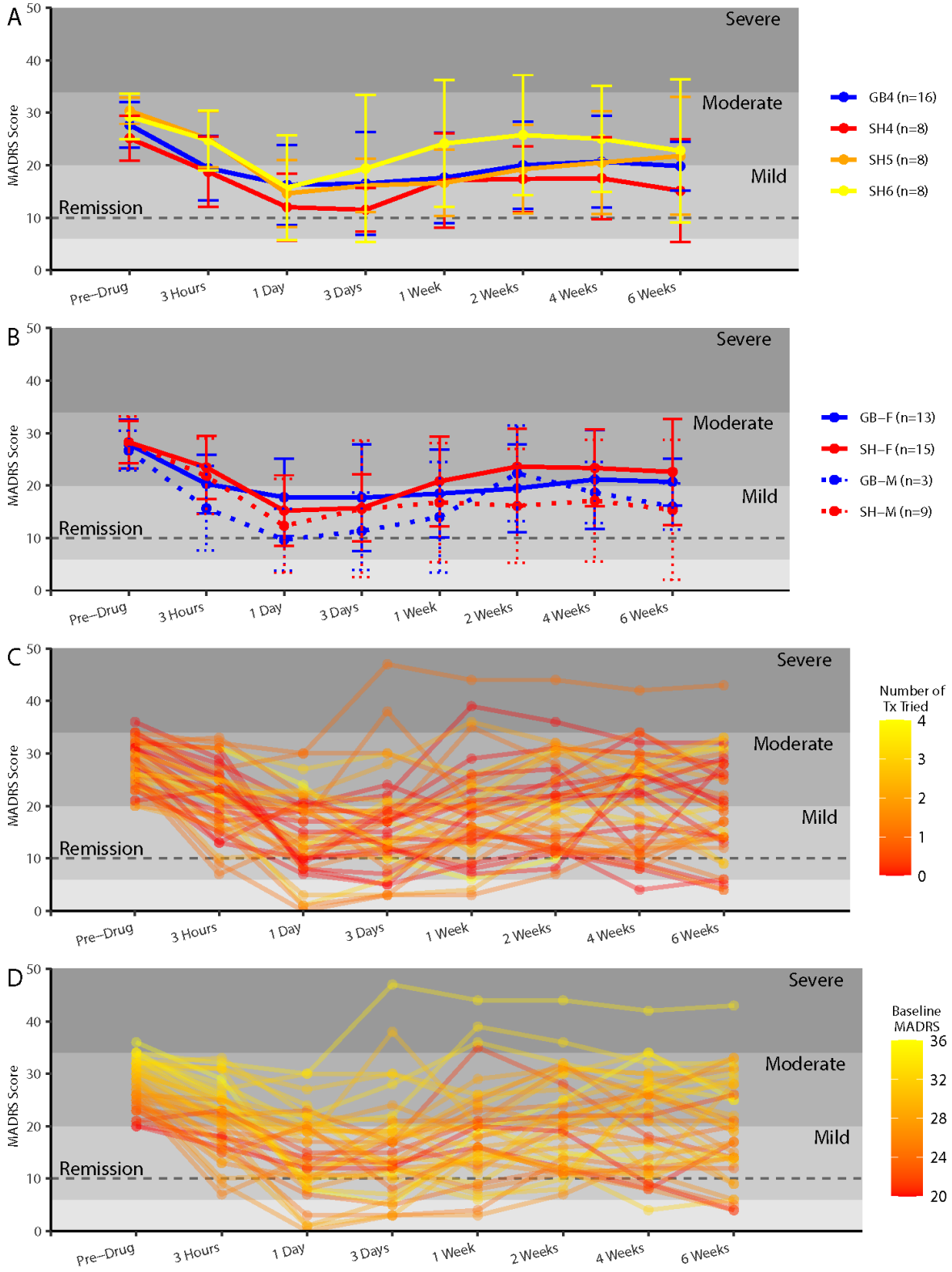
*Supplementary Figure 1 – CONSORT diagram detailing the numbers of participants in each phase of the Trial. *Please refer to Supplementary text for more details.*

Supplementary Figure 2

	STUDY PERIOD															
	Enrolment	**Allocation**	**Study Day**									**Follow-Up**				
Time-point			**Baseline**	**5m**	**10m**	**15m**	**20m**	**30m**	**60m**	**120m**	**240m**	**1d**	**3d**	**1w**	**2w**	**4w**	**6w**
ENROLMENT:																	
Eligibility screen	X																
Informed Consent	X																
Randomisation		X															
INTERVENTIONS:																	
Scopolamine (4,5,6µg/kg) or Glycopyrronium 4µg/kg			●	—	—	●											
ASSESSMENTS:																	
Psychiatric:																	
MADRS + QIDS			X								X	X	X	X	X	X	X
Bowdle VAS			X	X	X	X	X	X	X	X	X						
SHAS, BAES, CADSS								X									
Qualitative Interview										X							
CEQ			X														
5D-ASC										X							
GASE										X				X			
Physiological:																	
Blood sample			X	X	X	X	X	X	X	X	X						
EEG			●	—	—	—	—	●	X	X	X						

Supplementary Figure 2 - SPIRIT Figure describing the timeline of outcomes.

Supplementary Figure 3



Supplementary Figure 3 – Covariates of Mood Responses – (A) Dose Response, (B) Sex Effects, (C) Number of Treatments Tried, and (D) Depressive Severity. Abbreviations: Glycopyrrolate Bromide at 4µg/kg (GB4), Scopolamine Hydrobromide at 4/5/6µg/kg (SH4/5/6), Montgomery Asberg Depression Rating Scale (MADRS), Treatments (Tx).

Supplementary Figure 4

Supplementary Figure 4 – The advertisement placed in public areas for participants to respond to.