# Cholinergic Modulation of Exposure Disrupts Hippocampal Processes and Augments Extinction: Proof-of-Concept Study With Social Anxiety Disorder

## *Supplemental Information*

### Supplemental Methods and Materials

Drug Condition

Scopolamine and placebo solutions were prepared by the University of California's Investigational Drug Service, who also maintained the study blind. Drug or placebo was delivered intranasally, via drops from a syringe. Scopolamine solutions were composed of a) scopolamine hydrobromide, trihydrate, b) sodium phosphate dibasic, heptahydrate, crystal, c) citric acid, monohydrate, granular, d) sodium chloride, granular, e) benzalkonium chloride 17% (w/v) solution, and f) purified water. Placebo solutions were comprised of the same elements minus the scopolamine hydrobromide. Dosages were based upon a review of the empirical literature regarding the mnemonic effects of scopolamine and initial pilot testing. We chose the lower end of doses that have been shown to influence hippocampus dependent tasks. Specifically, .6 mg of scopolamine has been shown to impair hippocampally dependent spatial learning in humans (1). We chose to test a second lower dose (.5mg) given concerns that higher doses of scopolamine can cause significant attenuation of extinction (2,3). Peak effects from nasal administration of .4mg scopolamine in healthy subjects occur at .37 $\pm$ 0.05 hours (19.2 to 25.2 mins), where the blood concentration was reported as 1680 $\pm$ 230 pg/ml with 83% $\pm$ 10 absolute bioavailability, and the mean residence time (average time a molecule spends in the body) was 1.57 ± 0.12 hours (4). Thus, in this study, the absorption of intranasally administered scopolamine was rapid and bioavailability was very similar to intravenous administration. The temporal effects of cognitive

impairment have not been reported with respect to nasal administration, but significant cognitive impairment is observed at one hour and maximal cognitive impairment (on measures of executive functioning problem solving and reasoning) was observed at two hours (with effects lasting six hours) following .5mg scopolamine intravenously (5).

Screening and Sample Description

Anxiety Disorder Interview Schedule-IV/5 (ADIS-IV/5, (6)). The ADIS-IV/5 was used to determine entry criteria, administered by assessors trained to reliability. Assessors were either doctoral students in clinical psychology or BA level, experienced research assistants who completed 15–20 hrs of training and demonstrated inter-rater diagnostic reliability and clinical severity ratings on three consecutive interviews prior to conducting study assessments. "Clinical severity ratings" (CSRs) were assigned to the diagnosis of social anxiety disorder on a 0 to 8 scale (0 = *none*, 3 = *probable clinically significant distress and impairment*, 4 = *clinically significant distress and impairment, 8 = extreme distress and impairment*).

Skin Conductance

Physiological responses were recorded using J&J Engineering I-330-C2 and Physiolab. Skin conductance was recorded from two 3-mm diameter Ag/AgCl electrodes placed on the distal phalanx of the index and middle fingers of the non-dominant hand. Participants were instructed to keep their arms still during anticipation and recovery from speaking and were reminded to do so as appropriate.

Side Effects

At the end of each exposure session, participants rated intensity and level of distress of drug side effects, each on 0-3 scales (0 = absent, 3 = severe). The assessment included thirteen

side effects associated with scopolamine (e.g., feeling drowsy) and twenty foils not associated with scopolamine (e.g., back pain).

Hippocampal Target Engagement Measures

Continuous Paired Associate Learning Task (CPAL) is a hippocampal dependent measure of cue-context learning. After presentation of a set of shapes in different locations, the shapes are covered, and participants are asked to recall and identify the location of the abstract shape when it is presented in the center of the screen, continuing until they correctly identify the location. The dependent variable is number of errors in recall of shape location on a screen. The CPAL reliably taxes the hippocampus and is affected by scopolamine (7). The CPAL was administered at baseline, and at exposure sessions 2 and 6, under drug influence, to test target engagement (i.e., hippocampal processes). Different stimuli were used for each CPAL administration to avoid confounds with stimulus familiarity.

Mnemonic Similarity Task (MST). In the initial learning phase, participants classified a series of pictures (e.g., a shoe) as either "indoor" or "outdoor" items. In the recall phase, participants were presented with items that a) matched a previous item, b) were similar to a previous item, or c) were completely new. They classified the items as either "old" (previously seen), "similar", or "new". The MST reliably taxes the dentate gyrus/CA-3 and measures processes including pattern separation and pattern completion (8). The MST was administered at exposure session 5, under drug influence, to test target engagement.

Procedure

Following informed consent, participants were assessed for eligibility using the ADIS-5 and a medical evaluation. They then completed baseline questionnaires and the CPAL task. After 19 days on average since baseline assessment (range 5-60 days), participants began seven sessions

of virtual reality exposure to public speaking (see Figure 1). With the exception of the first session, each session began with intranasal administration of the assigned scopolamine dose or placebo, followed by 30 min of drug absorption, attachment of virtual reality equipment, and delivery of seven speeches to a virtual audience. The first exposure session included eight speeches: one baseline speech drug-free to assess fear reactivity in the exposure context, and seven speeches under the influence of drug/placebo. Different topics were assigned for each speech.

A VFX 3D Interactive Personal Display combined with a smartphone or a Vuzix Wrap 1200 was used to deliver immersive virtual reality (VR) environments. VR scenes involved audiences (neutral facial expressions) in a conference room, auditorium, or large office, provided by Psious and the Virtual Reality Medical Center.

Each speech began with a 30-second anticipation period during which participants prepared an assigned speech topic while seated facing away from the virtual audience. Next, cued by the onset auditory cue, participants stood, turned towards the virtual audience and spoke for one minute. After the termination auditory cue, participants returned to a seated position, facing away from the virtual audience, for a two-minute inter-trial interval. This sequence was repeated seven times. Following the seventh speech, equipment was removed and participants were monitored for one hour by study physicians. Participants were instructed to keep their limbs still at all times.

During the anticipation period, two startle probes (50 ms burst of white noise) were delivered at either 5 and 20 seconds or 10 and 25 seconds. During the speech, two startle probes were delivered at either 5 and 35 seconds or 15 and 45 seconds. During the recovery period, three startle probes were delivered at either 25, 60, and 90 seconds or 15, 50, and 80 seconds. However, due to technical difficulties, startle eye blink was not analyzed.

Participants completed two exposure sessions per week, with an average of 4.22 days (range 2.5 to 7.5) between sessions. Each session was conducted in the same VR context, in the same physical room, with the same experimenter, and the same auditory and olfactory cues.

Participants returned for context renewal and extinction retest (counterbalanced) on average 5.5 days (range 1-14 days) following their seventh exposure session. Context renewal and extinction retest each included one VR speech using the exposure session format separated by approximately 30 minutes. Context renewal differed from exposure sessions in the following ways: VR audience scene, physical room, experimenter, olfactory cue (air freshener scent or not) and auditory cue (bell or gong) to indicate CS onset and CS termination. VR scenes and olfactory cues were counterbalanced to exposure context or context renewal. The room, experimenter and auditory cue were not counterbalanced. Manipulation of multiple contextual elements heightens context renewal, providing a more stringent test of the effect of scopolamine, while simultaneously increasing external validity (9).

Data Analytic Plan

Following ANOVAs to evaluate baseline differences between groups, the major analyses used multilevel modeling (MLM) in Stata 14 to examine the impact of scopolamine on SCR to CS onset, SCR to CS termination, anticipation SCL, recovery SCL and SUDS. Multilevel modeling has several advantages over traditional repeated measure ANOVA designs including accounting for missing data and uneven spacing between assessment points, and is more appropriate for smaller sample sizes (10).

Time points in extinction models were the first speech of the first exposure session (drug free) and the last (seventh) speech of exposure sessions 2 through 7.

As implemented in other studies (11), participants who did not extinguish on a given index (defined as a decrease of $\leq$ .01 microsiemens for SCR and SCL and $\leq$ 0 SUDS scores from the first speech of the first exposure session to the last (seventh) speech of the last (seventh) exposure session) were excluded from analyses of context renewal, extinction retest, and long-term extinction retest.

MLM for tests of context renewal and extinction retest utilized the full model that included data from the seven extinction/exposure sessions, since the parameters in question, the interaction and simple effects, are defined in exactly the same way in both the full model and a reduced model restricted to only end of extinction, context renewal and extinction retest. As the specific comparisons that are made at the end of extinction, renewal and retest are not influenced by the patterns of data over the course of extinction in the full model, there is no reason to prefer the reduced model. There is reason to prefer the full model to the extent that the more frequent observations provide greater power, and power becomes especially relevant given that our analyses were based on a smaller than expected sample size due to the number of individuals who did not exhibit extinction. Hence, time points in the models examining context renewal and extinction retest included the first speech of the first exposure session (drug free) and the last (seventh) speech of exposure sessions 2 through 7. Simple effects compared (a) group differences at end of extinction, context renewal, and extinction retest, and (b) differences between context renewal and extinction retest within each group. Order of context renewal and extinction retest (which were counterbalanced), as well as the type of VR equipment, were included as covariates and later dropped if there were no significant main effects or interactions.

For the same reasons as described above, MLM models examining long-term extinction retest included the first speech of the first exposure session, the last (seventh) speech of exposure

sessions 2 through 7, and long-term extinction retest. Simple effects only analyzed differences between groups at long-term extinction retest.

Time points were nested within participants in all multilevel models.

## Supplemental Results

### Side Effects

There was a significant effect of Group on the intensity of scopolamine-related side effects ($F(2, 57) = 10.48$, $p < .001$) and associated distress ($F(2, 56) = 8.34$, $p < .01$). The placebo group reported less intense scopolamine-related side effects (M = .05, SD = .06) and less distress (M = .03, SD = .04) than either SCOP.5mg (*ps* < .05; M = .28, SD = .23; M = .17, SD = .18) or SCOP.6mg (*ps* < .05; M = .26, SD = .19; M = .21, SD = .18)[1]. There were no significant differences between SCOP.5mg and SCOP.6mg (*ps* > .67). No adverse or serious adverse incidents were reported.

### Extinction[2]

SCR-to-CS-Onset. To further examine effects, MLM was conducted across speeches during the first exposure session and across speeches during the final exposure session. At session 1, there was no main effect of Group ($X^2$=1.06, $p$=.59) or Time x Group interaction ($X^2$=9.52, $p$=.66). However at session 7, there was a main effect of Group ($X^2$=10.17, $p$<.01). Simple effects showed that, SCR-to-CS-onset was lower in both SCOP.5mg ($b$=-.66, $p$<.01, CI=-1.09 to -.22) and

[1] There was also a significant effect of Group on intensity of non-scopolamine-related side effects ($F(2, 57) = 3.69$, $p < .05$) with placebo (M = .03, SD = .03) reporting less intense effects than SCOP.5mg ($p < .05$; M = .09, SD = .10) but no significant differences between placebo and SCOP.6mg ($p$ =.91; M = .04, SD = .06) or between SCOP.6mg and SCOP.5mg ($p$ =.09).

[2] There was no main effect of type of VR equipment (VFX 3D Interactive Personal Display combined with a smartphone or a Vuzix Wrap 1200) on any dependent variable (*ps* > .29).

SCOP.6mg (*b*=-.54, *p*< .05, CI=-1.00 to -.09) compared to placebo with no difference between SCOP.6mg and SCOP.5mg (*b*=.11, *p*=.64, CI=-.37 to .59).

SCR-to-CS-Termination. At session 1, there was no main effect of Group ($X^2$=1.83, *p*=.40) or Time x Group interaction ($X^2$=11.87, *p*=.46). However at session 7, there was a main effect of Group ($X^2$=19.94, *p*<.001). Simple effects showed that SCR-to-CS-Termination was lower in both SCOP.5mg (*b*=-.70, *p*<.001, CI=-1.07 to -.32) and SCOP.6mg (*b*=-.78, *p*< .001, CI=-1.18 to -.38) compared to placebo with no difference between SCOP.6mg and SCOP.5mg (*b*=-.08, *p*=.69, CI= -.50 to .33).

SCL-Anticipation. At session 1, there was no main effect of Group ($X^2$=3.5, *p*=.17) or Time x Group interaction ($X^2$=15.13, *p*=.23). However, at session 7, there was a trend for a main effect of Group ($X^2$=5.4, *p*=.07). Simple effects revealed that SCL-Anticipation was lower in SCOP.6mg compared to placebo (*b*=-3.45, *p*<.05, CI=-6.5 to -.40). There were no differences between SCOP.5mg and placebo (*b*=-2.25, *p*=.12, CI=-5.11 to .10) or between SCOP.6mg and SCOP.5mg (*b*=-1.2, *p*=.46, CI=-4.39 to 1.2).

SCL-Recovery. There was a main effect of Group ($X^2$=12.50, *p*<.01) and Time ($X^2$=49.30, *p*<.001), but no Time X Group interaction ($X^2$=15.83, *p*=.20) (Figure 3b, Main Text). Overall, SCL-recovery decreased over extinction sessions (*b*=-4.86, *p*< .001, CI=-6.49 to -3.23). SCL-recovery was lower in both SCOP.5mg (*b*=-2.89, *p*< .05, CI=-5.08 to -.70) and SCOP.6mg (*b*= -3.75, *p*< .01, CI=-5.96 to -1.54) compared to placebo with no difference between SCOP.5mg and SCOP.6mg (*b*=-.86, *p*=.46, CI=-3.16 to 1.43). At session 1, there was no main effect of Group ($X^2$=3.26, *p*=.20) or Time x Group interaction ($X^2$=10.32, *p*=.59). At session 7, there no main effect of Group ($X^2$=4.33, *p*=.11).

Context Renewal and Extinction Retest

SCL-Recovery[3,4]. There was a main effect of Time ($X^2$=78.53, $p$<.001) and a Time x Group interaction ($X^2$=34.13, $p$<.01) (Figure 2b). SCL-Recovery was lower at end of extinction in SCOP.6mg compared to placebo ($b$=-3.66, $p$=.05, CI=-5.16 to 2.26) with no difference between SCOP.5mg and placebo or SCOP.5mg and SCOP.6mg ($ps$>.22). There were no significant group differences at context renewal or extinction retest ($ps$>.11). There were no significant differences between context renewal and extinction retest within any group ($ps$>.24).

Long-Term Extinction Retest[5]

SCR-to-CS-onset. There was a main effect of Group ($X^2$=13.24, $p$<.01), Time ($X^2$=100.01, $p$ <.001) and a Time x Group interaction ($X^2$=23.77, $p$<.05). SCR-to-CS-onset increased from end of extinction (final speech of session 7) to long-term extinction retest (one-month follow-up) ($p$<.001). There were no significant group differences at long-term extinction retest ($ps$>.24).

SCL-Anticipation. There was a main effect of Group ($X^2$=13.74, $p$<.01), Time ($X^2$=81.21, $p$ <.001) but no Time x Group interaction ($X^2$=18.46, $p$=.19). SCL-anticipation increased from end of extinction to long-term extinction retest ($p$<.001). There were no significant group differences at long-term extinction retest ($ps$>.66).

SCL-Recovery. There was a main effect of Group ($X^2$=7.27, $p$<.05), Time ($X^2$=62.83, $p$ <.001) but no Time x Group interaction ($X^2$=13.80, $p$=.46). SCL-recovery increased from end of

[3] Four individuals (Placebo = 3, SCOP.5mg =1) did not extinguish and were excluded from analyses.

[4] There was no main effect of type of VR equipment or main effect of order of renewal and extinction retest ($ps$ > .13).

[5] These analyses excluded the same participants who did not exhibit extinction and were excluded from analyses of context renewal and extinction retest.

extinction to long-term extinction retest ($p$<.001). There were no significant group differences at long-term extinction retest ($ps$>.50).

SUDS. There was a main effect of Time ($X^2$=227.98, $p$ <.001) but no Group ($X^2$=1.22, $p$=.54), or Time x Group interaction ($X^2$=7.34, $p$=.92). SUDS did not increase from end of extinction to long-term extinction retest ($p$=.16). In the absence of main effects of Group or interaction effects, simple effects were not explored.

Hippocampal Dependent Tasks

There was a main effect of Group on classifying "similar" items as "old", F(2, 53)=3.43, $p$<.05: SCOP.5mg more often committed this error than placebo ($p$<.05) whereas SCOP.6mg did not differ from placebo or from SCOP.5mg ($ps$>.15). There was a main effect of Group on classifying "new" items as "old", F(2, 53)=4.87, $p$<.05: SCOP.5mg more often committed this error than placebo ($p$<.05) with a similar trend for SCOP.6mg compared to placebo (p=.052), and no difference between SCOP.5mg and SCOP.6mg ($p$=.91). There was a main effect of Group on classifying "new" items as "similar", F(2, 53)=3.67, $p$<.05: SCOP.6mg more often committed this error than placebo ($p$<.05), with no difference between SCOP.5mg and placebo ($p$=.13) or between SCOP.5mg and SCOP.6mg ($p$=.83). There was a main effect of Group on classifying "similar" items as "similar", F(2, 53)=5.02, $p$<.05: Placebo was more often correct than SCOP.5mg ($p$<.05) with the same trend in comparison to SCOP.6mg ($p$=.06), and no difference between SCOP.5mg and SCOP.6mg ($p$=.85).

**Table S1**. Long-Term Extinction Retest (Mean, SD)

| | Placebo | SCOP.5mg | SCOP.6mg |
|---|---|---|---|
| SCR to CS Onset | .45 (.20) | .63 (.13) | .73 (.13) |
| SCR to CS Termination | 1.61 (.22) | *1.03 (.18) | **.71 (.18) |
| Anticipation SCL | 9.47 (1.75) | 10.44 (1.43) | 9.55 (1.43) |
| Recovery SCL | 8.23 (1.65) | 9.67 (1.35) | 9.13 (1.35) |
| SUDS | 20.60 (4.92) | 22.35 (4.67) | 23.54 (4.51) |

*$p < .05$. **$p < .01$. SCOP.6mg and SCOP.5mg demonstrated significantly lower SCRs to CS termination at long-term extinction retest compared to placebo. There were no differences between SCOP.5mg and SCOP .6mg.

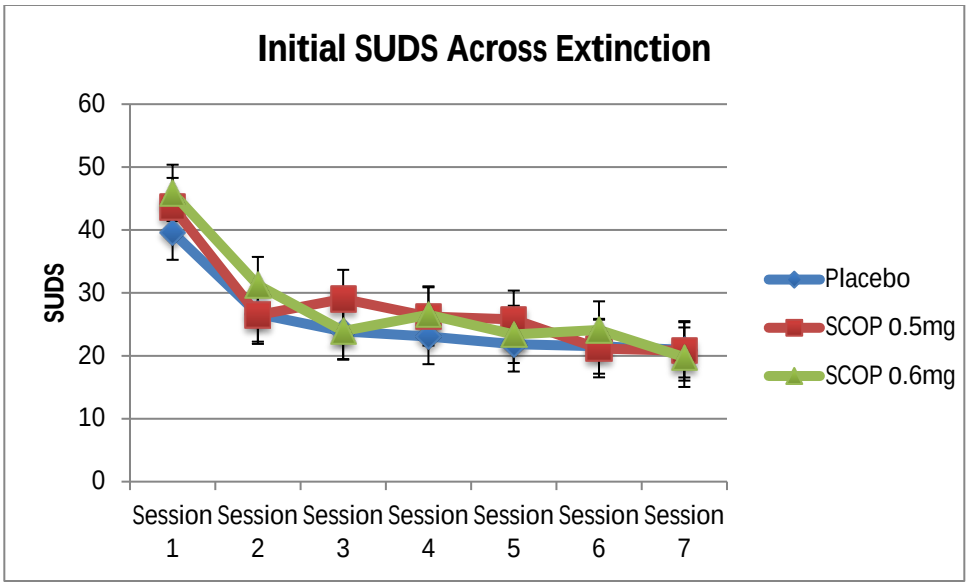


**Figure S1.** SUDS across extinction. SUDS decreased across sessions ($p$<.001) but there were no differences between placebo, SCOP.5mg and SCOP .6mg.

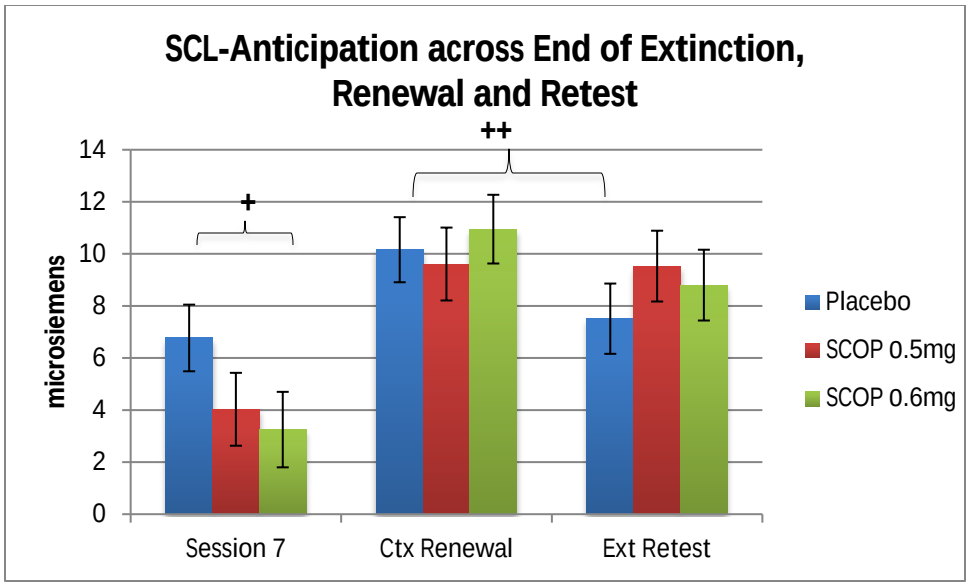


**Figure S2a.** SCL-Anticipation across experimental phases: Ctx Renewal and Ext Retest counterbalanced (in participants who extinguished). +$p$ = .06, ++$p$ = .07. There was a trend for SCOP.6mg to demonstrate lower SCL-anticipation at end of extinction (session 7) compared to placebo ($p$=.07). There were no group differences at context renewal or extinction retest. Placebo demonstrated lower scores at extinction retest than context renewal ($p$=.06), whereas there were no differences within either SCOP.5mg or SCOP.6mg.

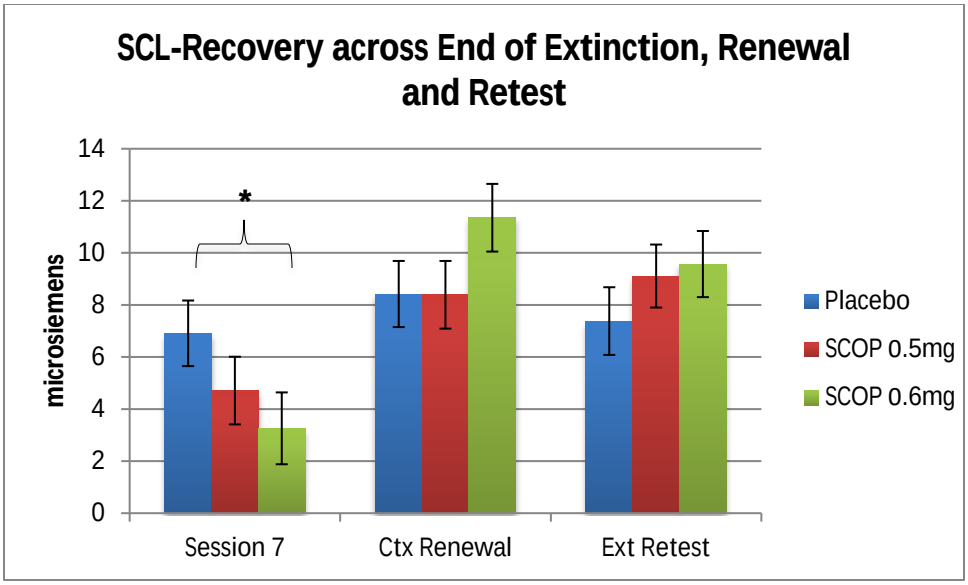


**Figure S2b.** SCL-Recovery across experimental phases: Ctx Renewal and Ext Retest counterbalanced (in participants who extinguished). *$p$ = .05. At the end of extinction (session 7), SCOP.6mg showed lower SCL-recovery than placebo ($p$=.05). There were no group differences at context renewal or extinction retest. There were no significant differences between context renewal and extinction retest within any group.

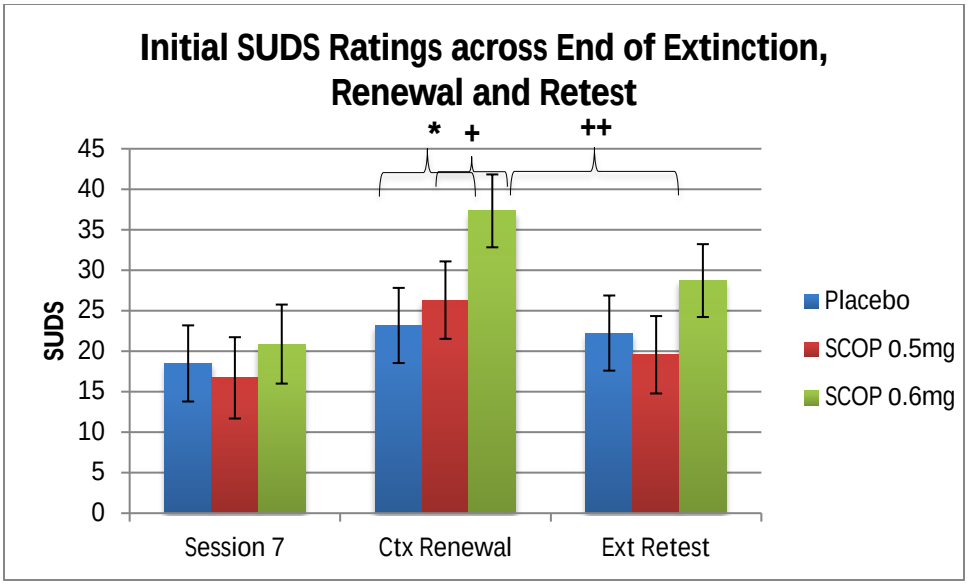


**Figure S3.** Initial SUDS ratings across experimental phases: Ctx Renewal and Ext Retest counterbalanced (in participants who extinguished). +$p$=.09, ++$p$=.08, *$p$< .05. There were no group differences at end of extinction (session 7). At context renewal, SCOP.6mg demonstrated higher scores than placebo ($p$<.05), and showed trends for higher scores than SCOP.5mg ($p$=.09), with no differences between SCOP.5mg and placebo. There were no group differences at extinction retest. There was a trend for SCOP.6mg to demonstrate lower SUDS at extinction retest than context renewal ($p$=.08) whereas there were no significant differences between context renewal and extinction retest within either SCOP.5mg or placebo.

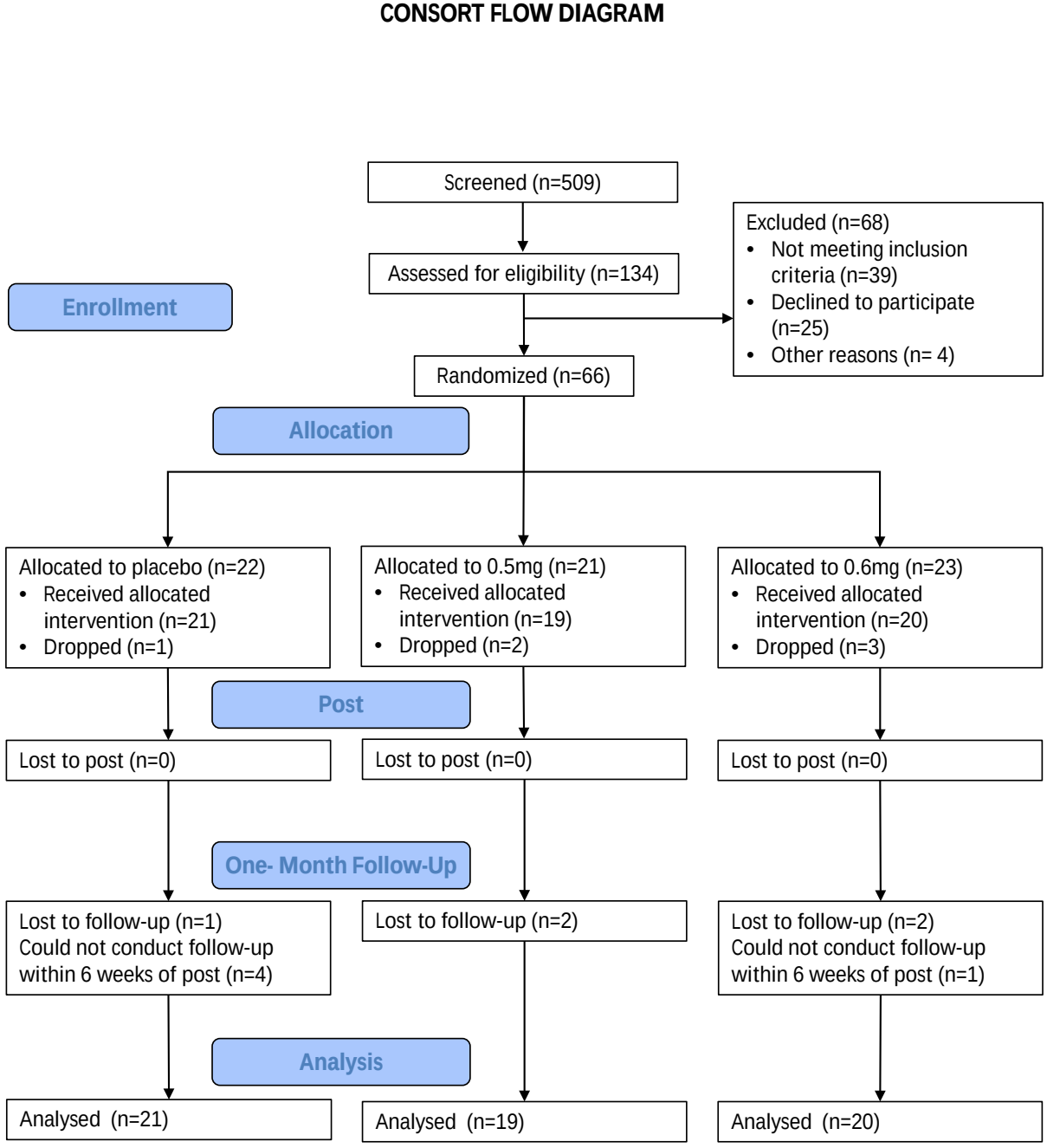


**Figure S4:** CONSORT Diagram

## Supplemental References


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