NCT04857983: Memantine Augmentation of Targeted Cognitive Training in Schizophrenia

Study Protocol and Statistical Analysis Plan

2/14/2020

7. Project Summary/Abstract:

In response to RFA-MH-18-705, this application develops and tests a novel treatment strategy for improving cognition in patients with schizophrenia (SZ), via **P**harmacologic **A**ugmentation of **C**ognitive **T**herapies (PACTs), and directly addresses a critical need for more effective treatments for these disabling impairments. Cognitive benefits in SZ patients can be achieved via "bottom-up" sensory-based targeted cognitive training (TCT) therapies, but such treatments are time- and resource-intensive, and responses are incomplete and variable. This application tests a rational and empirically supported platform for augmenting the benefits of TCT in antipsychotic medicated SZ patients by adjunctive daily treatment of 20 mg memantine (MEM)**,** an FDA approved medication for the treatment of cognitive dysfunction in Alzheimer's Disease. Recent meta-analyses of MEM augmentation in antipsychotic-medicated SZ patients have demonstrated its safety, tolerability, and effectiveness at improving scores on brief cognitive screening tests. We hypothesize that MEM **will augment TCT learning and hence the clinical gains from TCT, and that this PACT approach will be most effective in biomarker-defined subgroups of patients.** Preliminary support for these hypotheses comes from our proof-of-concept, randomized, controlled studies of single-dose exposure to MEM relative to placebo. In these studies, we found that MEM significantly enhanced learning in auditory discrimination, the key component of the TCT program which is known to drive the cognitive gains in SZ patients following 30-50h of TCT. We also found that a single dose of MEM significantly enhanced several biomarkers of early sensory information processing in antipsychotic medicated SZ patients. Dose-response and time course studies identified the optimal MEM dose (20 mg) for maximal pro-learning effects. This application conducts a careful assessment of this PACT strategy for SZ: **Aim 1**) **Confirmation of target engagement**: 54 SZ patients will be tested to confirm that MEM (20 mg) enhances measures of TCT learning; **Aim 2) Efficient pilot testing**: Subjects from Aim 1 will be randomized into 2 treatment arms (n=27/arm) for a double-blind placebo-controlled 30-session clinical trial of MEM+TCT vs. placebo+TCT, to determine whether daily dosing of MEM augments the magnitude, rate and/or durability of TCT gains, and whether these gains are associated with target engagement, using specific Go/No-Go criteria and outcome measures of symptoms, cognition and real-life function; **Aim 3) Predictive biomarker identification** of the PACT response, based on cognitive, electrophysiological, and performance-based measures assessed pre- and post-TCT. This is a highly novel, high-risk high-reward application to develop a PACT-based treatment paradigm that will enhance cognition, improve recovery and enhance outcomes for patients with schizophrenia, and will determine whether a future, fully-powered "Confirmatory Efficacy trial" of this approach is warranted.

8. Project Narrative

Treatment of schizophrenia currently includes antipsychotic medications and cognitive therapies which improve some symptoms, but do not sufficiently restore cognitive functioning or reduce psychosocial disability. We hypothesize that medications that specifically target sensory information processing deficits, rather than psychotic symptoms per se, will significantly enhance the benefits of a sensory-based targeted cognitive training (TCT) intervention in patients with schizophrenia. We will complete a randomized, double-blind clinical trial to: 1) confirm that the drug memantine augments TCT learning; 2) determine whether memantine enhances the clinical benefits from a full 30 session course of TCT vs. TCT plus placebo in antipsychotic-medicated schizophrenia patients, and 3) determine if memantine's enhancement of TCT is most effective in biomarker-defined subgroups of patients.

SPECIFIC AIMS: Memantine Augmentation of Targeted Cognitive Training in Schizophrenia

Cognitive impairment affects the majority of patients with schizophrenia (**SZ**) and directly contributes to their psychosocial disability. Unfortunately, treatment approaches designed to improve cognition in SZ have been limited. Recent findings demonstrate that cognitive functioning in SZ can be improved by interventions that leverage neuroplasticity-based perceptual learning mechanisms. "Targeted Cognitive Training" (**TCT**) is a computerized intervention designed to sharpen the acuity and fidelity of sensory information processing through specific exercises that systematically increase demands on early perceptual and attentive processes. For many participants, TCT leads to "bottom-up" gains in perceptual functioning that lead to improvements in verbal learning and memory after 20-30h. While these benefits of TCT are evident at the group level, nearly half of all patients fail to show cognitive gains, even after extended 40-100h courses of TCT. For patients, their families, clinicians and health systems, the costs and logistical complexities associated with these time- and resource-intensive interventions, even under optimized conditions, can be prohibitive.

A novel approach to bridge this divide between the therapeutic promise of TCT and its mixed effectiveness is to combine TCT with an intervention that augments the clinical gains of TCT. This application responds to RFA-MH-18-705 by conducting efficient pilot testing of a novel intervention strategy for chronic psychotic disorders - **P**harmacologic **A**ugmentation of **C**ognitive **T**herapy (**PACT**) - via an experimental medicine approach. We propose to test a novel "augmentation strategy" of daily dosing of 20 mg of memantine to enhance the benefits of TCT in stable, antipsychotic medicated SZ patients.

Memantine is a non-competitive NMDA receptor modulator which is currently FDA-approved for the treatment of moderate-to-severe Alzheimer's disease, and is often used adjunctively in a variety of neuropsychiatric illnesses, including SZ. Recent meta-analyses of memantine augmentation in antipsychotic-medicated SZ patients have demonstrated its safety, tolerability and modest effectiveness at improving cognition. **We hypothesize that memantine will augment TCT learning (Aim 1) and hence the clinical gains from TCT (Aim 2), and that this PACT approach will be particularly effective in biomarker-defined subgroups of patients (Aim 3).**

Preliminary support for these hypotheses and the proposed design comes from both laboratory- and clinic-based studies conducted over the past decade. **In our laboratory**, we have tested both dose-effects of a single pill of memantine on neurophysiological measures of early auditory information processing (EAIP) and TCT learning in antipsychotic-medicated SZ patients. In these studies, memantine (20 mg > 10 mg = 0 mg) significantly enhanced several EEG-based measures of EAIP, as well as learning in an auditory discrimination task -- a key component of the TCT suite of exercises with established efficacy in SZ patients. Although memantine-enhanced TCT learning during a 1h Sound Sweeps test provides a valuable assay of "target engagement", we do not yet have evidence that it predicts greater, faster or more lasting clinical, neurocognitive or functional gains with a full course of memantine+TCT.

Our clinic-based studies yielded key findings related to biomarkers, Sound Sweeps performance, and predictors of therapeutic sensitivity to a full course of TCT. **First**, baseline levels of EAIP predicted TCT learning during 1h of Sound Sweeps. **Second**, SZ patients exhibited clinical, neurocognitive and functional gains after 30h of TCT, and changes in EAIP during the first hour of Sound Sweeps testing predicted neurocognitive and clinical gains after a full 30h course of TCT. In total, these findings with memantine, EAIP, TCT learning, and clinical outcomes provide a strong empirical and conceptual framework for a mechanistically-based hypothesis to be tested via the proposed randomized, placebo-controlled 30h pilot trial of (memantine+TCT) vs. (placebo+TCT). Via the below Aims, this application provides efficient pilot testing of this PACT strategy in antipsychotic-medicated SZ patients, using Go/No-Go criteria to inform a future, fully-powered "Confirmatory Efficacy trial":

AIM 1 – Replicate previous findings of memantine target engagement on TCT learning: In two, 1h tests, determine if memantine increases Sound Sweeps TCT learning in SZ patients (n=54) vs. placebo.

AIM 2 – Test clinical, neurocognitive and functional impact of memantine on TCT, and its relationship to target engagement: In a 30-session course of TCT over 10 weeks, determine if memantine augments the magnitude, rate and/or durability of TCT-induced gains, and whether these gains are associated with target engagement.

Aim 3 – Explore biomarkers of memantine-induced gains: Determine whether higher basal levels of EAIP (and related functional and neurophysiological measures) or changes in these measures across the first hour of Sound Sweeps predict greater sensitivity to memantine-enhanced clinical, neurocognitive or functional gains among SZ patients receiving combined memantine+TCT.

3. Research Strategy: A. Significance: Chronic psychotic disorders, including schizophrenia (SZ), affect 1-2% of the world's population, causing suffering and severe disability. Among the consequences of these disorders, psychosocial disability is strongly correlated with neurocognitive impairment[1-4]. The primary treatment for these disorders over the past 60 years has been antipsychotic (AP) medications, which produce only marginal gains in neurocognition[5]; by contrast, specific cognitive therapies significantly improve neurocognition and outcome in SZ patients, with pooled mean effect sizes across studies of d≈0.40 vs. APs alone[6-10]. Many studies document the safety, feasibility and efficacy of cognitive therapies in SZ with benefits often lasting years[7,8,11-23]; benefits are achieved with both "top-down" therapies, that engage higher order cognitive mechanisms, and "bottom-up" that target basic sensory training delivered via computerized cognitive remediation programs[24-27].

Targeted Cognitive Training (TCT): TCT is a "bottom-up," "neuroplasticity-based" computerized approach to cognitive remediation[24]. In TCT, SZ patients train on progressively more difficult auditory processing exercises designed to improve pitch and temporal acuity of processing of low-level sensory information. This bottom-up approach seeks to improve the speed, accuracy and fidelity of auditory information processing, to generate "upstream" gains in cognition and function. TCT aims to foster the recovery of key neurocognitive functions by harnessing mechanisms of neuroplasticity under carefully controlled experimental conditions, rather than to promote the development of compensatory cognitive or behavioral operations. TCT relies on *repetitive practice and procedural learning* - mechanisms that are relatively intact in SZ patients[28,29] - by providing auditory training exercises that are: 1) *intensive*: thousands of trials per exercise; 2) *attentionally engaging*: self-paced initiation of each trial; 3) *adaptive*: the difficulty of each training task adjusts trial-by-trial based on performance; and 4) *rewarding*: entertaining animations reinforce correct responses[30]. In several reports[24-26], Vinogradov and colleagues have shown that after 30-50 hours (h) of TCT, SZ patients have large effect size gains (d≥0.8) in auditory-dependent cognitive domains (verbal learning and memory), global cognition and quality of life that persist for at least 6 months post-TCT.

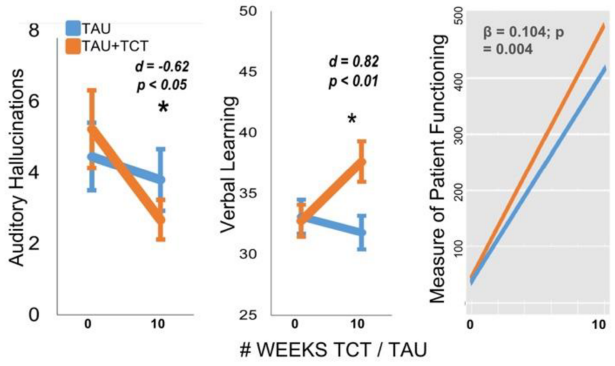

Fig. 1. TCT effects on symptoms (Scale of Positive Symptoms auditory hallucinations p<.05 and voices conversing p<0.01), neurocognition (MCCB verbal learning, p<0.01) and function (participation in groups/activities, p<0.005) in SZ patients over 10 weeks of TAU (n=22; blue) or TAU+TCT (n=24; orange). Findings confirm significant gains from TCT. Larger effects were detected in biomarker-identified subgroups, but there is clearly "room" for MEM-enhanced effects on these and other symptoms, neurocognitive domains and functional metrics, as proposed here, as well as the rate and durability of these changes (not assessed in our previous studies).

We have replicated and extended this work, showing that TCT significantly improved verbal memory as well as reduced the severity of auditory hallucinations in 46 chronic SZ patients[2,31-34]. In brief, patients were tested in measures of neurocognition and early auditory information processing, and then randomly assigned to TCT (1-h, ~3 times/week, total ≈ 30 sessions) or treatment as usual (TAU) groups. As seen in Fig. 1, compared to TAU, TCT patients exhibited significant gains in clinical (reduction in auditory hallucinations), cognitive (verbal learning, the most disabling neurocognitive deficit of SZ), and functional domains (engagement and participation in psychosocial groups and activities). Compared to TAU, gains in function by TCT patients over 10-weeks translated to 1 full extra week of clinical rehabilitation[34]. Importantly, gains in neurocognition and symptoms correlated significantly with (i.e. were predicted by) 3 EEG-based measures (discussed below). As it relates to the feasibility of this application, these findings demonstrate that: 1) a 30-session trial of TCT is feasible in patients with chronic psychotic disorders; 2) TCT is well-tolerated, even among severely disabled, functionally impaired patients; 3) TCT produces quantifiable gains in clinical, neurocognitive and functional metrics; 4) adding high-density EEG testing to a TCT protocol is feasible and well-tolerated; and 5) changes in functional EEG biomarkers predicted TCT-induced gains[31,32].

Unfortunately, however, TCT does not work for all patients: While it is highly efficacious at the group level, individual gains from TCT vary considerably: up to 35-45% of SZ patients fail to benefit (d ≤ 0.2)[2,35,36], even after an extended 100h course of TCT[37]. Moreover, despite significant cognitive gains observed at the group level, TCT-treated patients still exhibit cognitive and functional impairment in our study[38]. Indeed, no significant effects of TCT were detected in patients' self-reports of problems in daily cognition[39]. Given the high rate of TCT "non-response" and the modest overall effect sizes, the costs and logistical impediments associated with getting severely ill SZ patients to complete 100h or even 30h of TCT can be prohibitive. The long-term goal of this program of research is to enhance and accelerate the efficacy of cognitive therapies like TCT in SZ patients. *Per RFA-MH-18-705, this application seeks to "pilot test" a practical means to augment, accelerate and/or prolong the benefits of TCT in SZ patients, in order to inform a future, fully-powered "Confirmatory Efficacy trial".*

Pharmacologic Augmentation of Cognitive Therapy (PACT): In **P**harmacologically **A**ugmented **C**ognitive **T**herapies ("PACTs") for SZ[40-42], as first described by our group[42], drugs with pro-cognitive effects are used specifically, and perhaps synergistically, to augment the clinical benefits of a range of cognitive therapies. These drugs do not replace APs, which remain essential for limiting active psychotic symptoms that impede a patient's ability to participate in cognitive therapy. While suppressing active psychosis with APs can benefit a cognitive intervention, we are trying to identify drugs that more specifically, and perhaps synergistically, enhance the clinical benefits of cognitive therapies. PACT works when a medication engages brain substrates regulating neurocognitive resources that are engaged by the form of learning being applied in cognitive therapy. With enhanced resources, greater learning is possible. With greater learning, the learning-based therapy is presumed to produce an enhanced clinical impact.

Pharmacologic Augmentation of TCT Learning: Towards this end, we reported that in AP-medicated SZ patients, the pro-attention drug, amphetamine, enhances learning of an auditory frequency discrimination task (henceforth, **"auditory learning"**) that is a key component of TCT[43]. This finding provided a crucial PACT "proof of concept": even in severely ill, AP-medicated SZ patients, one pill acutely enhanced learning in a manner that would be predicted to accelerate or increase the clinical benefits of TCT. Clearly, substantial plasticity is retained in TCT learning-relevant neural substrates in SZ patients; leveraging this plasticity is a rational and novel clinical strategy that is the ultimate goal of our work. Towards this end, we are pursuing separate R33 support to extend studies of amphetamine-enhanced TCT in SZ patients (see Budget Justification). Importantly, however, there are theoretical and practical reasons that stimulants might not be optimal for use in some SZ patients, even in the setting of stable antipsychotic medications; thus, while we explore the viability of stimulants for PACT approaches, we are also motivated to pursue alternative classes of drugs for their PACT potential in SZ patients.

Memantine is a strong PACT candidate: Memantine (MEM) is a non-competitive NMDA receptor modulator that is currently FDA-approved for the treatment of moderate-to-severe Alzheimer's disease[44,45], and is often used adjunctively in a variety of neuropsychiatric illnesses, including SZ[46]. A recent meta-analysis of 15 randomized controlled trials (n=512 SZ patients; see also Table 2), showed that daily adjunctive MEM treatment is well-tolerated and modestly effective at reducing negative symptoms and improving mini-mental state exam (MMSE) cognition scores in antipsychotic medicated SZ patients without adverse drug reactions or discontinuation due to any reason relative to comparator conditions[46]. Importantly, *none of these studies utilized MEM in concert with a systematic cognitive intervention*, nor were any specific biomarkers used to predict sensitive patient subgroups.

For the past decade, we have studied the acute neurophysiological effects of MEM, first in laboratory rats[47] and then in healthy human subjects (HS) and schizophrenia (SZ) patients[48-51], with the goal of using MEM or related drugs as pro-cognitive interventions in SZ. [Note: no investigators in this application have financial relationships with the commercialization of MEM.] In the past 5 years, supported by NIMH, these studies assessed the effects of acute MEM challenge (placebo, 10, 20 or 30 mg, po) on measures of early auditory information processing (EAIP) and neurocognition in SZ patients and HS. Prepulse inhibition (PPI), mismatch negativity (MMN) and gamma band auditory steady-state response (ASSR) were used as dependent measures because they: 1) are neurophysiological measures of EAIP, i.e. of the brain's automatic response to a simple sensory event proximal to, or independent of, a point at which it engages conscious or volitional processing; 2) consistently detect deficits in SZ patients; 3) are reliable, objective and quantitative[52,53]; 4) reflect "automatic" vs. volitional processes and are relatively insensitive to motivational or effort-based artifact; 5) are suited to repeated testing in a cross-over design without significant order or carry-over effects 6) are associated with important domains of clinical, cognitive, and psychosocial functioning in SZ[54-60]; 7) are each regulated by NMDA activity, 8) have been previously reported to be increased by MEM[47,61,62], including MEM-enhanced PPI[47] and MMN[62] in HS.

Memantine acutely "moves" cognitively and functionally-relevant neurophysiologic biomarkers: The findings from our previous studies were striking. We detected statistically significant effects of MEM (20 mg po) on PPI, MMN, ASSR, and related measures[49,50,63]. In each case, one pill of MEM significantly "moved" these measures in SZ patients towards "normal" values. These changes could not be explained on the basis of AP medication interactions, or other artifacts related to illness treatment or chronicity, as qualitatively similar changes were also detected in HS. For PPI, these MEM-induced changes were somewhat less robust in HS; for MMN, they were somewhat more robust in HS; and for ASSR, they were roughly comparable in HS and patients (Fig. 2).

To our knowledge, these studies served as a critical first demonstration that deficient PPI, MMN and ASSR in SZ patients – widely viewed to reflect fixed, heritable abnormalities in brain mechanisms regulating auditory information processing – can be significantly enhanced (i.e. brought significantly closer to normal values) via an acute intervention in chronically ill SZ patients. These findings are novel and important, as they demonstrate significant plasticity in brain mechanisms that are thought to contribute to "core" neurocognitive deficits in SZ.

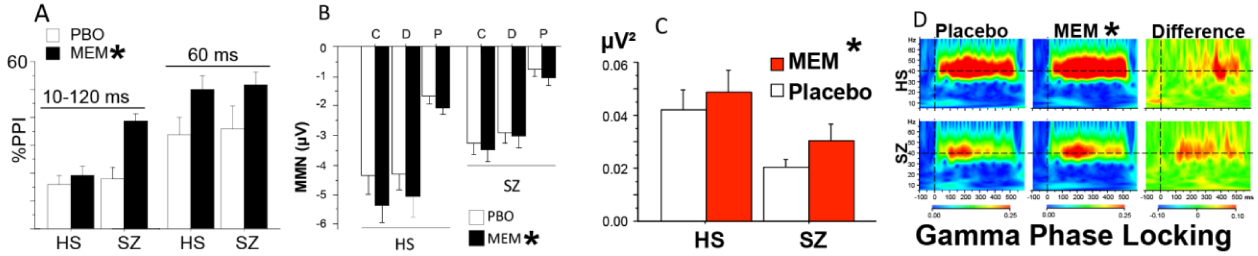

Fig. 2. Effects of MEM on A: PPI; B: MMN; C, D: ASSR in HS and SZ patients. Patients and HS (n's=42/group) were tested after 1 pill of either PBO or MEM (10 or 20 mg po; 20 mg shown here) in a double-blind, balanced cross-over design. Tests were 7d apart. Compared to HS, patients had deficits in MMN (B) and ASSR (C,D). MEM (20 mg) significantly enhanced PPI (A; p<0.04 for 10-120 ms; p<0.01 for 60 ms), MMN (B; p<0.014; stimulus "mismatch": Duration; Pitch; Combined) and ASSR (C: evoked power, 40 Hz *p<0.025; D: gamma phase locking, *p<0.002).

Using structural equation modeling in 1415 SZ patients, we[60] reported that measures of EAIP, including MMN, had a direct effect on cognition (p<0.001), that cognition had a direct effect on negative symptoms (p<0.001), and that both cognition (p<0.001) and negative symptoms (p<0.001) had direct effects on functional outcome. Thus, EAIP had a fully mediated effect on functional outcome, engaging general rather than modality-specific cognition. Explicitly, this model predicts that a 1 μV change in EAIP (including MMN) will result in improvements of d=0.78 for cognition and d>0.28 for psychosocial functioning. While the time-course for such cognitive and functional changes is not known, these findings nonetheless suggest that interventions that reliably enhance measures of EAIP in SZ patients would be rational targets for therapeutic development. Our other findings indicate that EAIP (MMN, ASSR) also predict the amount of auditory learning by patients within a TCT session [64,65]; these findings specifically suggest that interventions that enhance EAIP would be rational targets for accelerating or increasing learning, and hence the gains from TCT, within a "PACT" design.

Measuring Target Engagement: Auditory Processing Speed (APS) Learning is the target to be engaged in this application. APS is tested via the TCT "Sound Sweeps" training exercise (Posit Science; brainhq.com): a frequency discrimination time-order judgment task [66,67]. Subjects hear pairs of frequency-modulated sound sweeps and indicate whether they perceive each sweep as becoming higher or lower in pitch (Fig. 3). Sweep duration, frequency range and interstimulus interval get shorter after correct responses, but longer after incorrect responses. APS is calculated based on the shortest duration of stimuli that subjects discriminate correctly. Before and after each training session, subjects complete an APS assessment to quantify simple auditory learning **(APS pre- minus post-training)**[29]. This "auditory learning" is believed to be a critical component of TCT-based clinical and neurocognitive gains and **serves as our operational measure of target engagement**[26,30,68].

Memantine acutely enhances APS learning: Our preliminary findings provide evidence of target engagement for this PACT model: compared to PBO, one dose of MEM (20 mg) was associated with greater APS "learning" across 1-h of a "Sound Sweeps" training session of TCT. Consistent with our previous report of amphetamine (10 mg), MEM did not alter APS, but it significantly enhanced auditory learning over 1-h of training in both HS and SZ patients, and these gains in learning were not state-dependent and lasted at least 1 week[43].

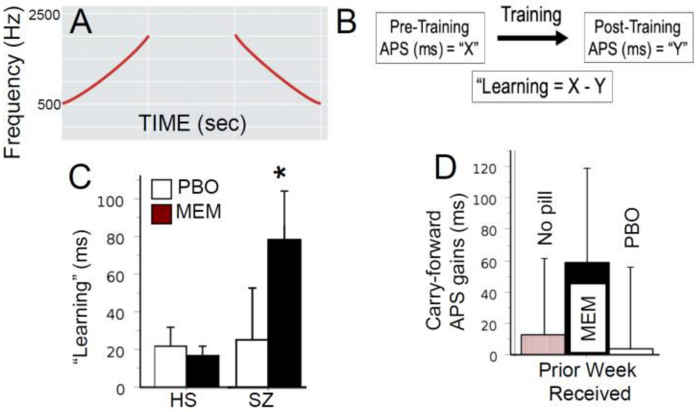

Fig. 3. MEM effects on Sound Sweeps learning. **A**. Subjects identify "sweep direction" of frequency modulated tones; adaptive trials increase in difficulty as subject learns. **B**. Auditory Processing Speed (APS, in ms; smaller # = better discrimination) is calculated both pre- and post-training. APS "learning" is defined as "APS pre minus APS post". **C**. In a within-subject design, 20 mg MEM significantly enhanced APS learning in SZ patients (diagnosis by drug: F=5.0, p<0.035). **D.** MEM-induced gains in APS learning "carried forward" to pre-testing 1 wk later; this did not happen if either no pill or PBO was received the previous week (Order: F<1.0; drug: F=4.95, p<0.04); order by drug (F<1.0). Thus, MEM-enhanced learning is not "state-dependent".

Biomarkers are critical to a PACT strategy: An explicit feature of a PACT intervention [40-42] is that it incorporates one or more biomarkers that distinguish subgroups of individuals whose biology is more vs. less sensitive to the ability of a drug to magnify, accelerate or make more durable the clinical gains from cognitive therapy. Indeed, we predict that not every patient will gain from either TCT or the addition of MEM to TCT, and thus a critical

feature of both PACT and this application is the development of reliable, quantitative predictive measures. Our group has written elsewhere about the optimal features of such predictive measures[51,69,70]; our preliminary studies identified strong candidate biomarkers: 1) that predict the amount of APS learning in the initial hour of Sound Sweeps exposure demonstrating plasticity in the cortical source contributions following 1-h Sounds Sweeps[65,71]; and 2) an individual's sensitivity to neurocognitive and clinical gains from TCT[31,32]. We have previously reported EAIP biomarkers, particularly mismatch negativity (MMN), are robust predictors of APS learning in the first hour of Sound Sweeps training[65,71], as shown in Fig. 4. This is one of several findings from our group, suggesting a strong connection between measures of early auditory information processing, and learning mechanisms engaged via auditory TCT.

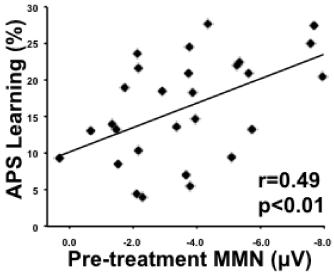

Fig. 4. Mismatch Negativity (MMN) prior to Sound Sweeps predicted APS learning (here as % baseline gains) in 31 SZ patients

Predictors of gains from 30-h of TCT: Neurocognitive (primarily but not exclusively verbal learning) and/or clinical gains after 30 sessions of TCT were predicted by both baseline levels and changes in specific EEG measures after 1 hour of Sound Sweeps. First, baseline (pre-TCT) levels of the Auditory Steady State Response (gamma power) and theta activity both predicted post-TCT gains in neurocognition[32]. Second, gains in P3a amplitude over the course of the first hour of Sound Sweeps significantly predicted both gains in verbal learning ($r=0.58$, $p<0.025$) and reduced hallucinations (SAPS; $r=-0.54$, $p<0.04$) after 30-h of TCT[31].

PACT predictors? Compared to biomarker predictors of a TCT response, or a drug response, predictors of a PACT response – i.e. the ability of a drug to augment the therapeutic effects of TCT - might reflect more complex mechanisms, e.g. related to: 1) an individual's sensitivity to either pro-cognitive or adverse effects of the drug; 2) the specific "match" between the pro-cognitive mechanisms of a drug and an individual's cognitive profile/deficits or capacity for TCT-based learning/neuroplasticity; 3) disease-related factors influencing capacity for clinical change over 30-h of TCT, or to some combination thereof. This application (Aim 3) will pilot the use of several specific biomarkers and outcome measures to identify patients most sensitive to the ability of MEM to augment TCT benefits; most promising measures will be applied in a subsequent "Confirmatory Efficacy trial". Biomarker findings will also inform models for system-level mechanisms whereby: 1) basic information processing capacity moderates the impact of drug-enhanced procedural learning, and 2) enhanced "bottom-up" sensory learning promotes higher order changes in neurocognition, symptoms and function.

B. Innovation: While neither the proposed use of pro-cognitive drugs, nor TCT, are independently innovative in the treatment of SZ, combining these treatments within a PACT design to augment the gains from TCT - as described in this application - is highly innovative and responds to the FOA's goal to support *"the development… of novel… interventions… as augmentations to a standard treatment."* This application must be limited to the use of one pharmacologic agent, and the Investigators have carefully selected MEM based on our meticulous "foundational" parametric dose-response and time-course studies in healthy subjects and SZ patients, that included several potential neurophysiological biomarkers[47,49-51,72-75]. Ongoing mechanistic studies of MEM effects in SZ patients, supported by the final year of MH94320, have detected MEM-induced gains in auditory processing fidelity that might serve as future predictive biomarkers for MEM sensitivity[76]. Nonetheless, it is anticipated that the PACT design tested in this application will be applied to other classes medications in addition to MEM, and the Investigators have studies in progress to establish parametric and PACT trial data for other classes of putative pro-cognitive agents, including stimulants. Thus, it is anticipated that **findings from this application will advance an innovative treatment strategy that will have implications well beyond its focused scope**.

A major challenge to the PACT approach is the availability of drugs with evidence-based pro-cognitive effects in SZ patients; in fact, trials of potential pro-cognitive agents in SZ have largely yielded negative results[77-82]. However, these "negative" studies generally share two important weaknesses. First, they were not conducted in the context of cognitive therapy, and *drugs that enhance specific domains of perceptual functioning, e.g. auditory processing speed and learning, might not yield clinical benefits unless paired with interventions that access those components, i.e. utilize/place demands on auditory processing.* An analogy is seen in the effects of anabolic steroids, which produce minimal gains in muscle mass unless *paired with an exercise* that puts "demands" on that muscle. In many "negative" studies, the candidate pro-cognitive agent, including previous studies of memantine described below, was simply added to a *passive* daily medication regimen, without any new cognitive "load"[79-81]. A "proof of concept" for the PACT approach of pairing a drug that has a target pro-cognitive mechanism with a therapy that demands that mechanism is seen in the use of pro-extinction drugs to selectively enhance the therapeutic impact of exposure therapy for anxiety disorders[83,84].

A second weakness of most "negative" studies of pro-cognitive agents for SZ is that these trials suffer from the lack of biomarkers that identify sensitive "enriched" clinical subgroups of patients[85-87]. Given the range of neurocognitive and symptom profiles across chronic psychotic disorders, reflecting the heterogeneous

neurobiology of these disorders, it is not surprising that a drug acting through one mechanism to enhance one domain of functioning might not generate significant improvement in a non-stratified patient cohort.

The PACT approach in general, and in this application specifically, diverges from past "negative" pro-cognitive trials in these two critical ways. First, PACTs pair a putative pro-cognitive agent with a cognitive "load" that puts demand on cognitive resources that are augmented by that drug. For example, as described below, this application proposes to combine a drug that enhances early auditory information processing with a TCT task that specifically targets auditory information processing. By pharmacologically enhancing low-level sensory information processing, we found (Fig. 3) that we can enhance TCT learning in an optimized laboratory setting; if this finding is replicated in Aim 1, we will test the hypothesis that enhanced learning generates greater clinical gains from a full course of TCT (Aim 2). Second, PACTs incorporate biomarkers to identify individuals who are most likely to benefit from the pairing of drug and cognitive therapy. In the PACT design described herein, potential biomarkers range from neurophysiological measures of forebrain mechanisms that regulate early auditory information processing, to performance markers of sensitivity to a "test dose" of a drug+TCT pairing.

The proposed use of EEG and objective behavioral biomarkers will produce system-level mechanistic insights, e.g. regarding the relationship of early sensory processing, auditory discrimination, and target engagement to clinical and neurocognitive benefits of pharmacologically augmented TCT. This approach is consistent with the NIMH goal to *"identify reliable and stable biomarkers that… are correlated with individual treatment response, or identify subjects that would most benefit from the intervention"* (NOT-MH-14-007). In a future, larger trial, these biomarkers may be implicated as mediators of PACT clinical gains[60].

"Does this application challenge... current research/clinical practice?" As noted above, the PACT strategy in this application fundamentally challenges current treatment models for SZ. In a successful use of PACT, there is a specific pairing of drug and cognitive "load": a drug that enhances specific neurocognitive domains is paired with a cognitive therapy that utilizes/places demands on those domains. Ultimately, treatment choice will be guided by biomarkers that might include laboratory-based measures, e.g. a patient's neurophysiological and TCT performance response to a challenge-dose of a pro-cognitive agent. A "deliverable" innovation of this application will be a novel PACT "prototype": a validated paradigm through which MEM will enhance the therapeutic benefits of TCT in biomarker-identified SZ patients.

Another **innovative feature** in this application fundamentally challenges existing paradigms by suggesting that treatment should be aimed at ***spared, healthy circuitry*** **in SZ patients** - identified in laboratory studies - rather than at pathological circuitry per se (which is both widely distributed and highly variable among SZ patients [41,88]). The goal of enhancing "spared" function departs from a prevailing failed strategy of trying to use drugs to "undo" decades of miswired neuropathology of SZ[41]; a focus on intact physiological mechanisms (e.g. auditory processing) and normal brain circuit function rather than disorder-specific pathology and symptoms is also *consistent with an RDoC framework.* We propose that evidence for the requisite "spared" neural circuitry in patients, and hence a target for MEM action, is provided by gains in auditory processing in response to a MEM "challenge". This approach parallels the use of a test-dose to predict clinical benefits from treatments ranging from hormones[89] to anti-Parkinsonian drugs[90] to bronchodilators[91]. Evidence for enhanced EAIP in response to MEM challenge suggests that neural circuits relevant to auditory processing are spared, plastic and hence viable targets for pharmacotherapy. Ultimately, gains in EAIP and/or higher auditory processing after a "test-dose" of MEM could be used as a predictive biomarker for clinical MEM sensitivity. This "personalized medicine" approach has been used in other fields[92-94], and attempted for some psychotherapeutics[61], but not yet for SZ.

C. Approach: This application uses an **experimental therapeutics approach** in an "efficient pilot test" of the hypothesis that MEM will augment TCT-induced learning, and thereby clinical, neurocognitive and functional gains, in antipsychotic-medicated SZ patients. In so doing, this application will advance a PACT model for TCT in two specific ways: 1) building on strong preliminary findings, this application will confirm target engagement and identify biomarkers that predict the effectiveness of MEM-enhanced TCT learning, and 2) this application will provide the first randomized controlled trial to assess the ability of medication-enhanced TCT learning (target engagement) to produce significant and lasting clinical, neurocognitive and functional gains in biomarker-characterized SZ patients. In this way, **this application responds to RFA-MH-18-705**, "Development of Psychosocial Therapeutic and Preventive Interventions for Mental Disorders", which supports *"efficient pilot testing of novel psychosocial therapeutic and preventive interventions for mental disorders… using an experimental therapeutics approach*." Moreover, in this application, "*results, whether positive or negative, will provide information of high scientific utility and… support 'go/no-go' decisions about further development or testing of the intervention.*" The application uses a "*novel intervention strategy"* - the PACT model - in a design that will "*replicate target engagement and relate change in the… target/mechanism to clinical benefit."* Findings from this pilot study will **inform a future, fully-powered Confirmatory Efficacy trial.**

This application will confirm target engagement by demonstrating that MEM (20 mg po) enhances APS learning in a Sound Sweeps session in biomarker-characterized AP-medicated SZ patients; these patients will then be randomized to a 30-session RCT of TCT+MEM (n=27) vs. TCT+PBO (n=27). Findings will determine whether MEM augments the known neurocognitive and clinical benefits of TCT, and whether APS "MEM sensitivity" - based on APS gains in 2 test sessions - predicts the therapeutic impact of MEM over a 30-session TCT regimen. Other predictive biomarkers will be explored, including baseline neurocognitive (MCCB scores), ERP (MMN, P3a) / EEG (ASSR, evoked theta band power) measures, and MEM effects on auditory discrimination and on ERP changes across the first Sound Sweeps sessions.

General Methods: Environment: Subjects are screened, tested and undergo TCT at the UCSD Medical Center. Participants are 18-55 y.o. AP-medicated (stable regimen ≥1 month, in active outpatient treatment) patients with a primary diagnosis of SZ or schizoaffective disorder (depressed type), recruited and consented at UCSD as per[49,59,74]. Study inclusion/exclusion criteria are detailed in **"Protection of Human Subjects"**. Screening includes a confirmatory diagnostic assessment (M.I.N.I. 6.0), a general medical, psychiatric and substance history, physical exam by an M.D., EKG, vision and hearing tests, urine toxicology and pregnancy test (UTox/P). All medications and changes are recorded (AP doses in chlorpromazine equivalents; quantifying anticholinergic burden[95] as in [33,48,49]). Medication patterns of our last ≈ 100 SZ subjects are published elsewhere [49,74,96].

Design (Fig. 5). Enrolled patients complete clinical, neurocognitive and functional measures and candidate biomarkers (EEG, QuickSIN, WIN) tested in Sound Sweeps at baseline, and then assigned to PBO vs. MEM arms (n=27/arm) using stratified random sampling (over sex, age and high/low Test 1 (baseline) APS learning) blind to arm identity. Stratifying for baseline APS learning should increase the sensitivity of the primary target engagement metric. Test 2 follows ≈ 5-7 days later, after either PBO or MEM (20 mg po). Tests 1-2 are used to assess target engagement (Aim 1: MEM-enhanced APS learning) as well as MEM effects on ERP/EEG, auditory discrimination (QuickSIN, WIN) and neurocognitive measures (Aim 3: Biomarkers). Our design allows for 20% attrition from enrollment (n=69) to completion of target engagement testing (n=54).

For Aim 2, all participants will be titrated up to 20 mg MEM or Placebo over a 3-week period and concurrently begin TCT 3 d/week (M-W-F) for 1-h/d (recognizing the need for flexibility, T-Th are "make-up days"), and continues until a subject completes 30-h (≈ 10-12 weeks). TCT is delivered by trained staff (see below). Patients and staff are blind to study arm; staff are blind to patients' baseline, interval, and post-intervention assessments.

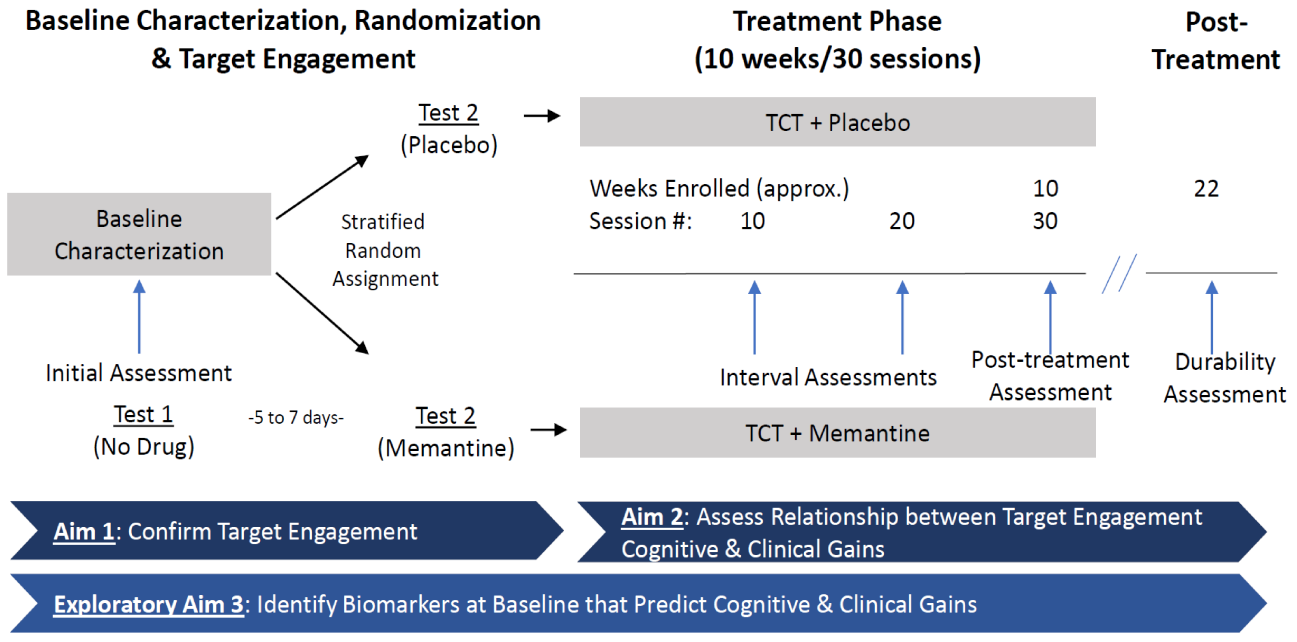

Fig. 5. Study Phases: Baseline & Randomization includes 2 Sound Sweeps tests: Test 1 with PBO and ≈ 5-7 days later Test 2 with either MEM or PBO. Treatment Phase includes 30 TCT sessions with outcome measures after 10, 20 and 30 sessions. Post-treatment tests assess both immediate biomarker changes and 12-week durability of outcome gains.

Assessments: 30 sessions of TCT will be completed, based on our findings (Fig. 1) and evidence that TCT efficacy is dose-dependent[25,68,97]. Assessment dates could be based on either: 1. session # (10, 20 and 30 and 12 weeks later); or 2. Time (days) since starting TCT. In our experience[2,31,34] TCT rates closely match 3/week (30 sessions mean = 65 days; 90% of subjects completed 30 TCT sessions within 11 weeks). Primary analyses will be based on session #, but session dates are recorded and confirmatory analyses will define assessment points based on time passage. Candidate predictive biomarkers are re-tested after session 30.

General Clinical Monitoring: In addition to their established ongoing care, patients are carefully monitored at each session as per our well-established procedures, and through to the week-22 follow-up, for any clinical changes. Each week, a member of our research staff checks vital signs and weight, does a detailed assessment of symptom changes, focusing on psychosis, depression, suicidality and abnormal movements, and tests UTox and pregnancy. A designated unblinded staff member processes UTox results. In the event that a subject tests positive for recreational drugs or a positive pregnancy test, it is promptly reviewed with the on-site Psychiatrist; any "yes" on the Columbia Suicide Severity Rating Scale[98] (C-SSRS) "Since Last Visit", or an exacerbation of psychotic symptoms, prompts detailed review, with appropriate clinical interventions. Patients testing positive for recreational drugs will be retested in 1 week; patients with 3 positive tests no longer continue in the study. **Data from subjects who, for this or other reasons, do not complete the full study are carried forward in an "intent to treat" design, and their data are analyzed using linear mixed-effects models.**

Outcome and "Go Decisions". In addition to confirming "target engagement", this application tests the hypothesis that SZ patients given MEM +TCT will exhibit greater, faster and/or more durable improvements (change from baseline) in symptoms, neurocognition and/or function compared to patients given PBO+TCT, and that this enhanced clinical response will be associated with greater MEM-enhanced TCT learning.

Table 1. "Go" criteria**:** #1, plus at least one of #'s 2, 3 & 4 (d>0.5)
1. **Target engagement:** APS learning, MEM > PBO, p<0.05
2. **Greater** clinical, neurocognitive **or** functional gains:
   - ↓PANSSt, ↑MCCB-C **or** ↑Fnxn: MEM+TCT > PBO+TCT
3. **Faster** clinical, neurocognitive **or** functional gains:
   - drug group x session interaction (p<0.05) followed by MEM+TCT > PBO+TCT at specific session # or combination;
4. **More durable** clinical, neurocognitive **or** functional gains:
   - MEM+TCT > PBO+TCT at post-TCT week 12.

PANSSt: Positive & Negative Symptom Scale total; MCCB-C: MATRICS Composite; Fnxn: Function (assessed via the World Health Organization Disability Schedule 2.0 (WHODAS)

The small "n" necessitated by the 3-year timeline and demanding treatment schedule greatly constrains the total number of outcome measures. Moreover, the plan to assess not only magnitude but also rate and durability of PACT gains triples the number of primary analyses. Thus, we selected only one **primary** clinical, neurocognitive and functional outcome measure (Table 1) – recognizing that they are relatively "blunt" metrics - but will track several other, more focused, measures as well as inter-measure correlations in exploratory analyses to inform future studies.

A "Go" decision requires: 1) confirmation of target engagement (APS learning, MEM>PBO, p<0.05); 2) evidence of gains from PACT ($d \geq 0.5$ (MEM > PBO)) in primary clinical, neurocognitive and/or functional measures (Table 1), and 3) the absence of adverse effects of MEM that are significantly greater than PBO levels. The decision to not use traditional statistical significance as "Go" criteria for Aim 2 reflects this RFA's call for "Pilot" studies, the need to identify candidate treatments for a subsequent larger "n" Confirmatory Efficacy R01 study, and the limits in sample size resulting from the 3-year R33 duration. Cohen defined a medium effect size (d=0.5) as one that is "visible to the naked eye," and this amount of change is viewed as clinically meaningful in trials targeting negative symptoms.[99-101] This d=0.5 "Go" threshold for Aim 2 corresponds to a "number needed to treat" (NNT) value ≈ 3.62. While a "Go" decision does not require identification of a biomarker predicting greater PACT sensitivity (Aim 3), the identification of one or more meaningful predictors that could be incorporated into a Confirmatory Efficacy trial would favorably impact a "Go" decision.

Staff assessing outcome measures are blind to treatment arm, and for some measures receive training using videotaped and practice interviews until achieving high (ICC≥.80) reliability based on 20 assessments by 2 raters. We achieve high inter-rater reliability (e.g., ICC>0.85 for PANSS total), and check for rater drift each 90d.

Specific Procedures: TCT is completed with a dedicated room and equipment. Staff trained with a UCSD-developed "TCT Clinician Competence Assessment" monitor TCT, clarify instructions and provide encouragement as needed. **In the initial "biomarker testing" session (Test 1),** subjects complete 1-h of Sound Sweeps to generate a "Pre-Training APS score" as described previously (Fig. 3) under identical circumstances and timelines as for the Test 2 session described below. Treatment group assignment (PBO vs. MEM) is then made using stratified random sampling (above) and 5-7 days later patients ingest either PBO (n=27) or 20 mg MEM (n=27) 240 min before completing the Sound Sweeps session ("Test 2"). MEM effects on APS learning are assessed both within-subject (APS (Test 1 - Test 2) for MEM group subjects: a key measure of **MEM sensitivity**) and between subjects (APS Test 2, PBO vs. MEM groups). After Test 2, all subjects are prescribed daily PBO or MEM using a standard titration schedule (MEM 5 mg at hs→ 5 mg bid→ 5 mg am/10 mg hs→ 10 mg bid over 3 weeks). Participants return to the laboratory to complete 1h TCT per our established procedures[2,34], which includes 5 modules of auditory/verbal processing exercises. Each module increases in difficulty as performance improves: 1) Sound Sweeps; 2) Syllable Identification: Patients distinguish between 2 similar phonemes (e.g., "ba" vs. "da"), differing only in their voice onset time; 3) Word Matching: Subjects match short similar-sounding consonant-vowel-consonant words (e.g., bad, dad); 4) Listen & Do: Subjects reconstruct a spoken series of instructions and use a computer mouse to click icons on the screen in a specified order. This exercise is designed to stimulate verbal WM processes supported by the lateral PFC as well as premotor and motor processes. 5)

Rhythm Recall: Subjects listen to and replicate a short rhythmic sequence. This task is designed to stimulate both basic auditory processes supported by primary auditory cortex, and non-verbal auditory WM.

Symptoms are assessed to track clinical status and TCT response. TCT reduces positive symptoms of psychosis, and we predict that MEM will augment these effects via enhanced TCT learning (i.e. not via a lasting direct pharmacological effect of MEM), detected by the Positive & Negative Syndrome Scale. For this reason, and based on the need to limit total outcome measures, **PANSS Total Score** is the primary clinical outcome measure. Positive and negative symptom subscales, as well as PANSS factor-derived measures, will be assessed in exploratory analyses. Other secondary clinical measures will include: 1) Psychotic Symptom Rating Scales (PSRS; assesses auditory hallucinations); 2) Young Mania Rating Scale; 3) Patient Health Questionnaire-9 (PHQ-9; current depressive symptoms); 4) Abnormal Involuntary Movement Scale (AIMS); 5) C-SSRS; "Lifetime/Recent version" at screen; "Since Last Visit" at follow-up visits). The C-SSRS is a reliable, valid and sensitive measure of suicidal ideation[98]; *any "yes" in C-SSRS prompts an immediate full evaluation/action plan by a study M.D.; and* 7) MEM Cessation Symptom Assessment (ACSA).

Neurocognition: Change in MCCB Composite performance from baseline is one outcome measure. The MCCB measures 7 cognitive domains: speed of processing, attention/vigilance, working memory (verbal and nonverbal), verbal learning, visual learning, reasoning/ problem solving and social cognition. Details are found in[36,102,103]; past studies of drug effects on MCCB performance include[48,104]. Since the MCCB is assessed multiple times, alternate forms of the HVLT-R and BVMT-R are used in counterbalanced order. MCCB Global Composite T-score (MCCB-C) is the primary neurocognitive outcome measure. Individual MCCB domain T-scores are used in secondary analyses to determine whether an overall effect is driven by specific aspects of neurocognition; TCT effects on verbal learning performance are known to be robust[24], and should be augmented by MEM. Baseline and MEM-enhanced (Test 2 vs. 1) MCCB performance (A/V subscale) will also be tested as potential biomarkers/moderators of MEM PACT efficacy.

Function is assessed via the World Health Organization Disability Schedule 2.0 (WHODAS)[105] at baseline, after sessions 10, 20 and 30, and week 12 post-TCT. WHODAS 2.0 is a 12-item patient-rated measure (< 15 min) using a 5-point Likert scale focusing on cognition, mobility, self-care, getting along with people, life activities and participating in society; it was selected by the DSM-5 Task Force committee to replace the global assess-ment of functioning scale[106]. NIH "PhenX Toolkit" scales of Impairment, QOL and Social Isolation will also be used. After session #30, the assessment will include a 14-item 7-point Likert scale of treatment satisfaction.

Biomarkers from Tests 1-2 will be assessed as predictors of target engagement (Aim 1) and PACT sensitivity (Aim 2); in some cases, these measures will also inform mechanistic models for PACT: 1. MEM-enhanced APS learning is both an outcome measure (Aim 1, "target engagement") and candidate predictor of clinical outcome (Aim 2); 2. Baseline and MEM-enhanced MCCB A/V scores are associated with APS MEM sensitivity[73], and thus may predict clinical outcome (Aim 2); 3. EEG measures of early auditory information processing variably predict response to early APS learning[87], MEM-enhanced APS learning[74], and TCT outcomes[31]. EEG/ERPs are acquired via 64-channel recording systems and assessed in the same order, as per[49,107]: MMN/P3a/Theta power/Phase locking (25 min; Oddball Paradigm), ASSR (6 min). 4. Auditory discrimination: Words-In-Noise (WIN; NIH Toolbox) and Speech-In-Noise (Quick-SIN; Etymotic Research) are acquired at Tests 1-2, TCT hour 30 and week 22. Both measures assess the ability to recognize speech over background noise, mimicking a conversation in a noisy environment, and are thought to index peripheral (WIN) vs. more central (QuickSIN) auditory processing. MEM-enhanced APS learning may predict PACT outcome (Aim 2); gains in auditory discrimination over 30 hours of TCT, and their augmentation by MEM, will be tracked to inform evolving models of PACT mechanisms. **Predictions:** First and foremost, target engagement (MEM-enhanced APS learning) will predict MEM-enhanced TCT outcomes (Aim 2) as described below. Exploratory analyses will test secondary predictions: for example, high vs. low baseline P3a amplitude and faster P3a latency will predict greater target engagement (Aim 1), and greater MEM-enhanced TCT outcome (Aim 2). MEM-enhanced (Test 2 vs. 1) ERPs, auditory discrimination and neurocognitive (A/V) measures will also be explored as outcome (Aim 2) predictors; significant pre- vs. post-TCT changes in ERP source dynamics will also be examined for mechanistic characterization[71]. Robust biomarker predictors will be examined as moderators of both indirect and direct paths between treatment and outcome in a future Confirmatory Efficacy trial.

Post-treatment protocol: On completion of 30 TCT sessions, participants will be offered the option of maintaining their assigned drug (PBO or MEM) through the 22-week durability assessments. Those who opt to discontinue the study drug will titrate off, over a 3-week period. After week 22, study blind will be broken, and subjects who continued to receive MEM will again be offered the option of titrating off of MEM vs. maintaining MEM treatment coordinated with, and under the supervision of, their primary provider.

Data Analysis: "Go/No Go" decisions are based on traditional statistical significance (Aim 1) and effect sizes (Aim 2). Formal statistical testing is conducted using linear mixed-effects (LME) models; hypothesis tests are 2-sided (α=.05). Model parameters are estimated via the R lme4 package and Cohen's d is estimated via the

EMAtools package. Type I errors are minimized by constraining the number of primary analyses; secondary analyses use false discovery rate corrections. Expected attrition rates are described above. Because outcome measures involve a "change from baseline", baseline metrics are compared between PBO and MEM arms, and any baseline differences are addressed in the outcome analyses. Differential drop-out rates from PBO vs. MEM arms are monitored. Data are assumed to be missing at random; full information maximum likelihood estimators are used, and all data/cases are included in analyses. Clinical follow-up is pursued with all patients exiting the study through post-TCT week 12. Cohort differences: For between-subject contrasts (main effect of drug), differences in age, antipsychotic and anticholinergic loads are tested and used as covariates[2,33,95]. Sex differences are tested for all measures. NIH Data Harmonization: 3 "PhenX Toolkit" (NOT-MH-15-009) measures of function are used; see above and "Resource Sharing Plan".

Aim 1 – Confirm target engagement: The primary dependent measure of target engagement is MEM-enhanced APS learning. APS learning is defined as the reduction in Sound Sweeps APS threshold (ms) Test 1 to Test 2 assessment. Target engagement was demonstrated in Fig. 3 [76], and will be confirmed if APS learning is increased by MEM (note that MEM and PBO groups are matched by Test 1 PBO performance via stratified randomized assignment). As appropriate, effect sizes and inferential statistics are based on standard regression or LME models with random intercepts. A significant interaction between contrast coded drug (PBO vs. MEM) and Test (Test 1 vs. Test 2) predictors on APS learning is a "Go" criteria and confirms target engagement.

Aim 2 – Test clinical, neurocognitive and functional impact of MEM on TCT, and its relationship to target engagement: We will determine if MEM+TCT produces greater, faster or more durable clinical, neurocognitive or functional gains from baseline, compared to PBO+TCT, over a 30-session course of TCT. A Cohen's $d \geq 0.5$ for the main effect of drug (PBO vs. MEM) or drug x session (#10, 20, 30) interaction in an LME model is the primary "Go" criteria. The effect of drug assesses "greater" gains. Reverse Helmert contrasts for session assesses "faster" gains. A separate model comparing differences in outcomes at 12-weeks post-TCT assesses "more durable" gains. Secondary analyses will examine whether change in MEM-enhanced APS learning ("within-subject" target engagement; Aim 1) predicts gains in Aim 2 outcome measures.

Aim 3 – Biomarkers of MEM-induced gains: We will determine if baseline levels of specific neurocognitive or EEG-based measures, or changes in specific measures with initial MEM exposure (Test 2 vs. 1), predict greater sensitivity to MEM-enhanced neurocognitive, clinical or functional gains over 30-h of TCT. Regression analyses will assess whether specific candidate biomarkers predict MEM-enhanced gains in outcome measures.

Subject "throughput": We will enroll and screen 23 subjects/yr, test target engagement in 18 subjects/yr, and (conservatively) complete TCT training in 12 subjects/yr. This will yield 2 "target engagement" Aim 1 arms of n=27, and (conservatively) 2 TCT completion Aim 2 arms of n=18. Training capacity (2 TCT stations with morning and afternoon sessions on M, W and F, with T and Th "make-up" days for missed appointments, 48 weeks/year) allows for a "maximum throughput" of 19.2 subjects/yr. To reach the target of **12 TCT-completing subjects /year**, we would need to maintain training rates of **62.5% capacity**. This is well within our historical testing levels.

Power analyses: Consistent with the FOA, this study will adequately power tests of target engagement (Aim 1) for traditional significance, while the "Go" signal for therapeutic impact (Aim 2) is based on effect size (d=0.5). To detect target engagement for an LME model with d=0.5, 80% power, alpha=0.05, test-retest correlation=0.8, and a linear treatment effect, n=27 randomized participants are required per Hedeker et al (1999). This is a conservative estimate of d for target engagement, since empirically, d for target engagement with 20 mg MEM was 0.85 (Fig. 3). A future Confirmatory Efficacy trial will be powered to apply more robust approaches to Aim 2, e.g. Path Analysis[60] to study moderating effects of APS MEM sensitivity and biomarkers on both indirect (moderated-mediation) and direct paths between treatment and outcome.

Innovative ideas raise questions. Here are some answers:

1. We don't know how MEM works. MEM is described as an uncompetitive low-affinity NMDA receptor antagonist with rapid blocking and unblocking ability. It is widely used to treat Alzheimer's Disease[44,45], but the cellular mechanisms responsible for MEM's clinical effects are a source of controversy, and the present application is fully agnostic to such mechanisms. One relevant clinical effect of MEM is to enhance verbal communication in Alzheimer's patients[108,109], which might reflect gains in auditory discrimination. It also has positive activity in cognitive measures in both healthy animals and a range of human and animal models for dementia, depression, ischemia and neuroinflammation[110,111]. Thus, consistent with an RDoC framework, the impact of MEM on disease states is defined more by a dimension of cognition, than by a diagnostic entity. MEM enhances hippocampal long-term potentiation (LTP), and reverses an experimentally-induced loss of LTP[111]; it also alters excitation/inhibition (E/I) dynamics in frontal circuitry implicated in models of SZ neuropathology[112] and associated with MMN and cognitive deficits in SZ[113]. We have also recently shown that acute 20mg of MEM normalizes novel measures of E/I balance in SZ patients[63]. **What we do know about MEM**, however, is that it acutely increases EAIP (Fig. 2) and Sound Sweeps APS learning in SZ patients (Fig. 3); here, we ask whether

such gains accompany daily MEM use and translate into an augmentation of TCT's clinical benefits.

2. MEM is not ketamine (or PCP, or MK-801). Unlike other NMDA antagonists, MEM has little impact on basal NMDA transmission[114]. In rats, PCP, ketamine and MK801 exhibit inverted-U dose-functions on spontaneous gamma power, and adversely impact other frequency bands; in contrast, gamma-enhancing effects of MEM follow a linear, positive dose-function and are selective for the gamma band[61]. In some behavioral measures, MEM is similar to ketamine but not PCP[115,116], while in others, MEM actually reverses the effects of ketamine[43]. In measures of glutamate-dependent synaptic plasticity, MEM effects are opposite to those of ketamine and MK-801[117]. In HS, ketamine reduces MMN[118], but MEM increases MMN *(d=0.87)*[62]. More generally, prevailing models suggest that SZ symptoms reflect NMDA hypoactivity. While these models *may* be valid at a level of cortical microcircuitry, trying to reconcile them with complex effects of oral drugs with differential effects at the NMDA receptor complex may not be useful. A cogent critique of current biological models of SZ as they relate to future therapeutics is found in, "Are we studying or treating schizophrenia correctly?"[41]

3. MEM is safe. The best evidence that MEM is very distinct from the PCP/ketamine class of NMDA antagonists is that MEM has been studied in >185 NIH trials, and used clinically by large numbers of elderly, frail dementia patients for years, without serious adverse events (SAEs). Unlike ketamine, which has psychotogenic effects in SZ patients[119], studies of MEM effects in SZ patients (Table 2) have consistently reported no increased SAEs; most report beneficial effects on symptoms and cognition (including 3 meta-analyses):

Table 2. Citation	**Study Design**	**N**	**mg/d**	**Wks**	**Δ Sx?**	**Δ Cog?**	**↑SAEs?**
Lieberman et al.[120]	D-B RCT + SGAPs	70	20	8	Ø	Ø	**no**
Lee et al.[121]	D-B RCT + APs	26	20	12	Ø	Ø	**no**
Omranifard et al.[122]	D-B RCT + APs	64	20	12	↑ GAF, QLS	NA	**no**
Veerman et al.[123]	D-B RCT + clozapine	52	20	12	↓ neg	↑	**no**
Veerman et al.[124]	1-y extension of above	24	20	52	↓ pos/neg	Ø	**no**
De Lucena et al.[125]	D-B RCT + clozapine	21	20	12	↓ pos/neg	↑ MMSE	**no**
Krivoy et al.[126]	Open label	7	5-20	6	↓ pos/neg	Ø	**no**
Rezaei et al.[127]	D-B RCT + risperidone	40	20	8	↓ neg	NA	**no**
Mazinani et al.[128]	D-B RCT + risperidone	22	20	12	↓ neg	↑ MMSE	**no**
John et al.[129]	Chart-based retrospective	26	10-20	8-100	↓ pos/neg	↑	**no**
Fakhri et al.[130]	D-B RCT added to olanz.	30	20	6	↓ pos/neg	NA	**no**
Zheng et al.[131]	Meta-analysis	452	--	--	↓ neg	↑ MMSE	**no**
Kishi et al.[132-134]	Meta-analysis	226	--	--	↓ neg	↑ MMSE	**no**
Zheng et al. [46]	Meta-analysis	512	--	--	↓ neg	↑ MMSE	**no**

D-B: Double-blind; RCT: randomized controlled trial; +: added on; QLS: Quality of life scale; GAF: global assessment of functioning; MMSE: Mini-mental State Exam; Sx: Symptoms; SAEs: serious adverse events; pos/neg: symptoms.

In addition to Table 2, many other studies and case reports describe clinical and functional gains after MEM in SZ patients[135-138]. As noted above, *none of the studies in Table 2 utilized MEM in concert with a systematic cognitive intervention*, nor were any specific biomarkers used to predict sensitive patient subgroups. Both cognitive intervention and predictive biomarkers[139] are part of the treatment model that will ultimately be driven by the present application.

4. MEM is pretty weak, but... We fully acknowledge that, even in its primary clinical use (Alzheimer's Disease), MEM has relatively modest clinical impact. Importantly, MH94320 identified something that MEM is really good at: **increasing EAIP in HS and SZ patients**[47,50,90,107]. ***No other drug is known to do this***, and this ability of MEM, along with its ability to enhance APS learning (Fig. 3) – provides the basis for the key hypotheses in this application: that MEM will enhance the clinical impact of TCT, and that an individual's sensitivity to this effect will be predicted by EAIP-based biomarkers.

As it relates to MEM's potency in this PACT model, it is important to acknowledge that the dose selected for the Aim 1 "Test 2 MEM challenge" (20 mg; Fig. 5) is based on empirical evidence that this dose – but not 10 mg MEM – enhances EAIP in HS and SZ patients, while 30 mg MEM (not tested in SZ patients) failed to enhance some EAIP biomarkers (but DID produce some adverse effects)[47]. In other words, MEM appears to have "inverted-U dose-effects" on at least some EAIP measures, and 20 mg produces the most consistent, robust effects[47,49,50,63,76], in addition to its ability to enhance APS learning in SZ patients (Fig. 3). Also importantly, the dose of MEM selected for the 10-week treatment phase of this study (20 mg daily in divided doses) is based on the known safety and tolerability of this dose, which is the one most commonly used to target neurocognitive deficits in SZ (and all other) patients (Table 2).

5. MEM offers advantages over other drugs in a PACT "Confirmatory Efficacy" study. Our group has studied several potential pro-cognitive drugs for use in a PACT model, including MEM, tolcapone, modafinil and amphetamine; based on positive results, we are separately pursuing R33 support to pilot amphetamine/PACT studies (see Budget Justification). **However**, MEM offers some advantages over amphetamine in the PACT model, particularly in relation to its "scalability" for larger clinical trials: 1) amphetamine is administered by a staff member prior to each TCT session, while MEM is taken daily as part of the patient's routine regimen; 2) after amphetamine administration, subjects remain "on-site" for safety monitoring during training, which is not needed with MEM; 3) the literature documenting MEM safety in SZ patients, as it will be used in the present application (10 mg bid; Table 2) is substantial; and 4) positive UTox results for amphetamine are not easily interpreted during an amphetamine trial. More generally, we anticipate that there will be variable sensitivity to PACT drugs, that no single drug will effectively enhance TCT effects in all patients, and that - guided by predictive biomarkers - it will be important to have alternative treatment options for use in this model.

6. Target Engagement. APS learning is the target to be engaged in this application. We hypothesize that this learning is critical for the therapeutic benefits of TCT; by enhancing APS learning, patients will benefit more from TCT. Consistent with the experimental therapeutics model supported by this RFA, TCT "learning", as detected by gains in APS, is the *"factor that an intervention intends to modify, based on a hypothesis that [its] modification will result in improvement of symptom, behavior, or functional outcomes."* This choice of targets is consistent with this RFA instruction, *"Targets might include…potentially modifiable…cognitive processes…"* We hypothesize and will test in this application that MEM-enhanced learning during TCT – **of which APS learning is an accessible, quantitative metric** - will amplify, accelerate or make more durable the clinical, neurocognitive and functional gains after a 30-h course of TCT, i.e. *"test the hypothesis that the target is relevant to the clinical problem under study."* In addition to TCT Sound Sweeps used in target engagement, clinical delivery of TCT involves training modules that engage auditory sensory processing in ways that are distinct from Sound Sweeps. Nonetheless, we predict that MEM-enhanced gains in APS learning reflect processes that will generalize to MEM-enhanced gains in the therapeutic impact of these other auditory sensory-based tasks. Other potential measures of target engagement, including "APS plateau"[68] will also be explored as secondary outcomes.

Based on this model, we acknowledge that – in addition to gains in APS learning - "target engagement" could be demonstrated either via gains in EAIP or other behavioral measures of auditory discrimination (QuickSIN score), but feel that the target that is most proximal to the therapeutic impact of TCT in this model is learning, i.e. gains in APS. Because of its "proximity" to the therapeutic impact of PACT, APS learning should be a target that provides a robust engagement signal and that strongly predicts the clinical consequences of the PACT intervention. Also, compared to EEG, measures of APS learning are more "scalable" for use in future "Confirmatory Efficacy" trials.

7. This approach might not work: Some evidence suggests that drug-enhanced APS learning, symptom reduction and neurocognitive gains from TCT may be dissociable, i.e. MEM might acutely enhance APS learning and EAIP biomarkers but have no impact on TCT-induced neurocognitive gains. Cain et al.[92] reported that SZ patients taking the pro-extinction NMDA agonist, D-cycloserine (DCS) – but not those taking PBO - exhibited significant gains in APS learning and negative symptom reduction during an 8-week trial of TCT; DCS-enhanced APS learning was evident at the first time point (1 week). Interestingly, PBO- but not DCS-treated patients exhibited significant gains in MCCB performance. Thus, enhanced APS learning was associated with symptom reduction but not neurocognitive gains. This R33 application differs from the Cain et al.[92] study in several specific ways, including: 1) this R33 uses a detailed conceptual framework for PACT design and implementation; 2) it benefits from previous dose/time optimization studies to maximize drug effects on APS learning; and 3) it uses a suite of hypothesis-driven biomarkers to identify potentially sensitive patient subgroups. Nonetheless, it is clear that MEM-enhanced TCT gains might be most evident in some but not all metrics (symptoms, neurocognition, function), even in the face of robust target engagement. With this awareness, we will carefully track and dissect these distinct outcome measures over the course of the R33, as we have in our published studies[2,31,33,34,65,71].

8. Is this PACT "scalable"? This R33 design is optimized: a) patients are carefully characterized as part of a clinical trial; b) MEM dose and timing are optimized based on our previous studies; c) potential logistical hurdles that might cause subject attrition (transportation, food, reimbursement) are minimized; and d) biomarker assessment uses high fidelity lab-based measures that are not readily available in community-based settings for mental healthcare delivery. While this optimized design is feasible in our on-site laboratory (see below, "Feasibility"), a robust PACT design will face challenges in implementation in the "real world". Importantly, we are studying different PACT delivery models, and are in discussions with a local Intensive Outpatient Program (IOP) (comparable to "Day Treatment"). The IOP model has been particularly successful in San Diego, and could be adapted to incorporate PACT into an integrated treatment, along with standardized biomarkers for personalized regimens, careful monitoring of clinical state and antipsychotic adherence, and individual and group supportive therapies. If this and a Confirmatory application support PACT efficacy, it will be "scalable."

9. No control groups. Adding control "computer game" groups might reveal if PACT benefits reflect MEM use independent of TCT, computer use independent of TCT, and/or interactions of MEM+computer use. These are 3 very-low probability outcomes. We opted against this since: 1) efficacy of TCT is already known, so our primary aim is to assess the impact of added MEM vs. PBO; 2) recruitment demands for 4 groups will not yield "n's" needed to detect meaningful Go/No-Go criteria; and 3) requiring patients to complete 30-h of non-therapeutic games for 10 weeks, or exposing patients to MEM under conditions that are unlikely to have lasting clinical value, is not ethically justified. Fisher et al.[37] found that patients randomized to computer game groups exhibited significant declines in verbal memory. Healthy subjects are not studied since no likely PACT effects on such subjects would alter the clinical implications of either positive or negative findings in SZ patients.

10. There is still "room to move": TCT effects on neurocognitive and clinical metrics might be "at ceiling", and thus insensitive to further gains from MEM. Compared to treatment as usual, 30-h of TCT in our studies resulted in medium-to-large effect size gains in Verbal Learning (d=0.65) and auditory discrimination ("Words-in-Noise"; d=0.67), and in reductions of auditory hallucinations (d=-0.64). However, TCT did not produce gains in other MCCB domains (e.g. Cohen's d's for processing speed, attention/vigilance and working memory were -0.1, -0.3 and -0.29, respectively), or in broader symptom profiles (e.g. SANS). Thus, despite robust gains in some areas after TCT, there is clearly "room to move"[51] for added PACT-induced gains. Furthermore, while past studies assessed the magnitude of TCT effects at a single point (after 30 sessions), the proposed study will assess both the onset and durability of PACT effects. It is possible that MEM will accelerate and/or prolong the gains from TCT, in ways that were not assessed in our previous studies, but which are certainly of clinical importance. The small sample size of this "pilot" study will not easily detect traditional ($p<0.05$) statistically significant group differences, but "Go/No-Go" decisions will be based on "clinically meaningful" Cohen's d=0.5.

11. "Negative results… [will] guide further intervention development"? This R33 has clear *'Go/No-Go'* criteria. If target engagement is not confirmed, we will conclude that this design setting is unsuitable for detecting robust MEM group effects on APS learning. Based on the data (e.g. evidence of MEM bioactivity), we will develop testable hypotheses (e.g. cohort differences) that might account for differential outcomes in the present application. Even if there are not robust MEM effects on APS learning (target engagement), it is possible that heterogeneity in MEM APS sensitivity might reflect meaningful subgroups of patients (e.g. low vs. high attention/vigilance), and that APS MEM sensitivity might still predict (correlate with) MEM enhancement of TCT therapeutic effects. In other words, a lack of a group difference (placebo vs. MEM) would not preclude the utility of MEM APS sensitivity as a predictive biomarker. Conversely, even if target engagement is confirmed, it is possible that group differences (placebo vs. MEM) in therapeutic gains might not be detected. A "valid" negative outcome (e.g. lack of positive MEM effects on outcome measures despite evidence of target engagement and adequate TCT exposure) will indicate that MEM-enhanced APS learning is not sufficient to enhance TCT effects in a clinically-defined SZ cohort. Analyses will then determine if MEM enhances TCT effects in biomarker-defined SZ subgroups. A failure to detect such subgroups would suggest a dissociation between target engagement in the first Sound Sweeps session and therapeutic benefit after 30-h of TCT.

12. This work is feasible. Our study team includes 2 Board Certified Psychiatrists (NS, YJ) with SZ clinical trial experience, a licensed Clinical Psychologist/Neurophysiologist and PI of a BBRF-funded trial of biomarker predictors of TCT in SZ patients (GL), an expert statistician with a career focus on analytic pathways from biomarkers to clinical function in SZ (MT), a UCSD SZ Research Program with a >30 y history of recruiting and testing thousands of psychosis patients in studies with ERPs and other biomarkers proposed in this R33 (published retest stability with 1-year follow-up[140]), as well as double-blind, PBO-controlled trials of putative pro-cognitive agents (including MEM) in SZ patients and HS[48,49,74,104]. We completed and published extensively on a 30-session clinical trial of TCT in SZ patients and with many of the same biomarkers proposed herein.

13. Why an R33 and not a full Confirmatory Efficacy Trial? While this work provides a strong empirical justification for MEM target engagement, this evidence is insufficient to justify moving to a full, large scale, Confirmatory Efficacy trial with PACT at this time. We acknowledge that MEM may enhance TCT-induced APS learning in a single session, but still not enhance, accelerate, or make more durable TCT-induced clinical, neurocognitive or functional gains following a full course of TCT. Even with evidence of target engagement, and its replication and optimization, it is still only a *hypothesis* that enhanced learning during TCT will augment its clinical benefits. In fact, MEM has different neurobiological effects after one (as shown in our preliminary results, Figs. 2-3) vs. multiple doses[141,142] (as proposed in this application); in some models, it is effective after acute but not chronic dosing[141]. *Consistent with this RFA-MH-18705* ("*replicate target engagement and relate change in the… target/mechanism to clinical benefit"),* **testing this hypothesis, with specific "Go/No Go" criteria, will be the most critical development of the present application.** Beyond this critical step, this application will leverage a substantial foundation of findings to explore (i.e. "learn/confirm") mature hypotheses related to mechanisms and predictors of pro-cognitive therapeutics for SZ.

Section 4 - Protocol Synopsis (Study 1)

4.1. Brief Summary

Current therapies for chronic psychotic disorders such as schizophrenia include antipsychotic medications, which do not significantly improve function or correct cognitive deficits in this disorder, and cognitive therapies, which produce only modest benefits to most patients. We hypothesize that medications that specifically target neurocognitive processes like attention and vigilance will significantly augment the clinical benefits of cognitive therapies in schizophrenia. Here, we will confirm that the pro-cognitive medication, memantine, enhances learning of an auditory processing task in a computerized targeted cognitive training (TCT) program in antipsychotic-medicated schizophrenia patients, and complete a randomized, double-blind clinical trial in antipsychotic-medicated schizophrenia patients, comparing TCT (30 sessions) plus memantine (MEM) vs. TCT (30 sessions) plus placebo (PBO).

4.2. Study Design

4.2.a. Narrative Study Description

Screened, eligible patients complete clinical, neurocognitive and functional measures and candidate biomarkers (EEG, QuickSIN, WIN). Aim 1 is completed in 2 tests, approximately 1 week apart. In Test 1, Sound Sweeps are tested after PBO for all subjects, who are then randomized to TCT+PBO vs. TCT+MEM arms (n=27/arm) using stratified random sampling (over sex, age and high/low Test 1 APS learning) blind to arm identity, similar to our previous studies. Test 2 follows approximately 5-7 d later; this test is identical to Test 1, except that subjects receive their assigned study drug (PBO vs. MEM (20 mg po)) 1 hour prior to Sound Sweeps. Tests 1 and 2 are used to assess target engagement (Aim 1: MEM-enhanced APS learning) and MEM effects on auditory discrimination (QuickSIN, WIN). Our design allows for 20% attrition from enrollment (n=69) to completion of target engagement testing (n=54). For Aim 2, TCT in these same subjects is scheduled 3 d/week (M-W-F) for 1-h/d, recognizing the need for flexibility (T-Th are “make-up days”), and continues until a subject completes 30-h (approximately 10-12 weeks). TCT is delivered by trained staff. 60 min prior to each TCT session, patients take either PBO or 20 mg MEM, as per arm assignment. Patients and staff are blind to study arm; staff are blind to patients’ baseline or post-intervention assessments. Outcome metrics assess symptom, neurocognitive and functional changes after 10, 20 and 30 TCT sessions, and 12 weeks post-TCT. Candidate biomarkers are re-tested after completion of 30 sessions of TCT.

4.2.b. Primary Purpose: Treatment

4.2.c. Interventions

Type	Name	Description
Behavioral (e.g., Psychotherapy, Lifestyle Counseling)	Targeted Cognitive Training (TCT)	Thirty, one-hour sessions over approximately 10 weeks, of computerized cognitive training modules.
Drug (including placebo)	memantine	The pro-cognitive medication, memantine (placebo vs. 20 mg) will be administered orally, 1 hour prior to targeted cognitive training, in a double-blind, randomized design.

4.2.d. Study Phase: Phase 2/3

Is this an NIH-defined Phase III Clinical Trial? ❍ Yes ● No

4.2.e. Intervention Model: Parallel

4.2.f. Masking: ● Yes ❍ No

☑ Participant ☑ Care Provider ☑ Investigator ☑ Outcomes Assessor

4.2.g. Allocation: Randomized

4.3. Outcome Measures

Type	Name	Time Frame	Brief Description
Primary	Auditory Processing Speed (APS) learning	Test 1 and Test 2, one week apart	APS is measured before and after the TCT "Sound Sweeps" frequency modulation session, to determine the amount of "learning", based on the reduction in detection gap (ms). More learning (greater gain in processing speed) with memantine vs. placebo confirms target engagement, which is the primary outcome of Aim 1.
Primary	MCCB Composite	baseline vs. post-TCT session 10, 20 and 30 (approximately 10 weeks), and 12 weeks post-TCT	Change in MCCB Composite performance from baseline is one outcome measure. The MCCB measures 7 cognitive domains: speed of processing, attention/vigilance, working memory (verbal and nonverbal), verbal learning, visual learning, reasoning/ problem solving and social cognition. Details are found in[25, 89, 90]; past studies of drug effects on MCCB performance include[37, 91]. Since the MCCB is assessed multiple times, alternate forms of the HVLT-R and BVMT-R are used in counterbalanced order. MCCB Global Composite T-score (MCCB-C) is the primary neurocognitive outcome measure. Individual MCCB domain T-scores are used in secondary analyses to determine whether an overall effect is driven by specific aspects of neurocognition; TCT effects on verbal learning performance are known to be robust[13], and should be augmented by MEM. Baseline and MEM-enhanced (Test 2 vs. 1) MCCB performance (A/V subscale) will also be tested as potential biomarkers/moderators of MEM PACT efficacy.
Primary	PANSSt: Positive & Negative Symptom Scale total	baseline vs. post-TCT session 10, 20 and 30 (approximately 10 weeks), and 12 weeks post-TCT	TCT reduces both positive and negative symptoms of psychosis; we predict that memantine will augment these effects via enhanced TCT learning, detected by the PANSS. For this reason, and based on the need to limit total outcome measures, PANSS Total Score is the primary clinical outcome measure. Positive and negative symptom subscales, as well as PANSS factor-derived measures, will be assessed in exploratory analyses. Other secondary clinical measures will include: 1) Psychotic Symptom Rating Scales (assesses auditory hallucinations); 2) Young Mania Rating Scale; 3) Patient Health Questionnaire-9 (current depressive symptoms); 4) Abnormal Involuntary Movement Scale; 5) Columbia Suicide Severity Rating (C-SSRS); “Lifetime/Recent version” at screen; “Since Last Visit” at follow-up visits). The C-SSRS is a sensitive measure of suicidal ideation; any “yes” in C-SSRS prompts an immediate full evaluation/ action plan by a study M.D.

Primary	World Health Organization Disability Schedule 2.0 (WHODAS)	baseline, after sessions 10, 20 and 30, and 12 weeks post-TCT	Function is assessed via the World Health Organization Disability Schedule 2.0 (WHODAS) at baseline, after sessions 10, 20 and 30, and week 12 post-TCT. WHODAS 2.0 is a 12-item patient-rated measure (< 15 min) using a 5-point Likert scale focusing on cognition, mobility, self-care, getting along with people, life activities and participating in society; it was selected by the DSM-5 Task Force committee to replace the global assess-ment of functioning scale. NIH "PhenX Toolkit" scales of Impairment, QOL and Social Isolation will also be used. After session #30, the assessment will include a 14-item 7-point Likert scale of treatment satisfaction.
Primary	EEG biomarkers	baseline,visit 2 and after session 30	Biomarkers from Tests 1-2 will be assessed as predictors of target engagement (Aim 1) and PACT sensitivity (Aim 2).EEG measures of early auditory information processing variably predict response to early APS learning[74], MEM-enhanced APS learning[62], and TCT outcomes[18]. EEG/ERPs are acquired via 64-channel recording systems and assessed in the same order, as per[38, 94]: MMN/P3a/Theta power/Phase locking (25 min; Oddball Paradigm), ASSR (6 min). Exploratory analyses will test secondary predictions: for example, high vs. low baseline P3a amplitude and faster P3a latency will predict greater target engagement (Aim 1), and greater MEM-enhanced TCT outcome (Aim 2). MEM-enhanced (Test 2 vs. 1) ERPs, auditory discrimination and neurocognitive (A/V) measures will also be explored as outcome (Aim 2) predictors; significant pre- vs. post-TCT changes in ERP source dynamics will also be examined for mechanistic characterization[20]. Robust biomarker predictors will be examined as moderator

4.4. Statistical Design and Power — 4.4_Statistical_Design_and_Power.pdf

4.5. Subject Participation Duration — approximately 24 weeks

4.6. Will the study use an FDA-regulated intervention? — ❍ Yes ● No

4.6.a. If yes, describe the availability of Investigational Product (IP) and Investigational New Drug (IND)/ Investigational Device Exemption (IDE) status

4.7. Dissemination Plan — 4.7_Dissemination_Resource_Sharing.pdf

4.4 Statistical Design & Power

Design (see "Research Strategy", Figure 7): Screened, eligible patients complete clinical, neurocognitive and functional measures and candidate biomarkers. In Test 1, all subjects are tested in Sound Sweeps after PBO, and then assigned to TCT+PBO vs. TCT+MEM arms (n=27/arm) using stratified random sampling (over sex, age and high/low Test 1 (baseline) APS learning) blind to arm identity, similar to[107]. Stratifying for baseline APS learning should increase the sensitivity of the primary target engagement metric. An example of a table for arm assignment is seen here:

Gender	Baseline	Age	Group	Group	Group	Group	Group	Group	Group	Group
Male	High	< 39	PBO	PBO	MEM	MEM	MEM	MEM	PBO	PBO
	High	≥ 39	PBO	MEM	PBO	MEM	MEM	PBO	MEM	PBO
	Low	< 39	MEM	MEM	PBO	PBO	PBO	PBO	MEM	MEM
	Low	≥ 39	MEM	PBO	MEM	PBO	PBO	MEM	PBO	MEM
Female	High	< 39	PBO	PBO	MEM	MEM	MEM	MEM	PBO	PBO
	High	≥ 39	PBO	MEM	PBO	MEM	MEM	PBO	MEM	PBO
	Low	< 39	MEM	PBO	MEM	PBO	PBO	MEM	PBO	MEM
	Low	≥ 39	MEM	MEM	PBO	PBO	PBO	PBO	MEM	MEM

Example of table for stratified random sampling, by gender, baseline APS learning (post-PBO) and age (based on approximate mean age (39 yo) of last 100 SZ patients tested in our program)

Test 2 follows approximately 5-7 days later. Tests 1 and 2 are used to assess target engagement (Aim 1: MEM-enhanced APS learning) and MEM effects on auditory discrimination. Our design allows for 20% attrition from enrollment (n=69) to completion of target engagement testing (n=54).

For Aim 2, TCT is scheduled 3 d/week and continues until a subject completes 30-h (approximately 10-12 weeks). 60 min prior to each TCT session, patients take either PBO or 20 mg MEM, as per arm assignment. Patients and staff are blind to study arm; staff are blind to patients' baseline or post-intervention assessments. Outcome measures are assesed after sessions 10, 20 and 30, and 12 weeks post-TCT. See "Research Strategy" for details.

General Statistical Approach: Hypothesis tests are 2-sided (α=.05). Type I error is controlled by selecting optimal *a priori* targets for primary analyses; secondary analyses use false discovery rate corrections. When appropriate, missing data are handled via multiple imputation or full information maximum likelihood estimation. Expected drop-out rates and ways to minimize them are described in the parent application.

Differential drop-out rates from PBO vs. MEM arms are monitored. Clinical follow-up is pursued with all patients exiting the study through post-TCT week 12.

Cohort differences: For between-subject contrasts (drug), baseline demographic, clinical, antipsychotic/anticholinergic load and performance differences are tested and used as covariates if indicated. Sex differences are tested for all measures.

Procedures for Alternative or Unexpected Results: Linear mixed-effects models allow for the analysis of all available data using an intent-to-treat approach. This approach, however, assumes that data are missing at random. A potential concern is nonrandom dropout, particularly when dropout is related to the (missing) outcomes; that is, not missing at random. To make the missing at random assumption more plausible, we will explore the use of auxiliary variables. Auxiliary variables are covariates that predict both dropout and outcomes. By adding

auxiliary variables to our models, nonrandom dropout is corrected by conditioning treatment effects on the predictors of dropout.

Primary Efficacy Endpoint(s): Aim 1: Target engagement – APS learning will be significantly greater under MEM vs. PBO conditions. Aim 2: Pilot trial - Compared to PBO arm patients, MEM arm patients will exhibit greater clinical benefit, as indicated by one or more of the following: greater, faster or more durable improvements in PANSSt (reduced scores; see below, "Go/No-Go"), MCCBc (increased scores) or WHODAS (reduced scores).

Secondary Efficacy Endpoint(s): Aim 3: Biomarkers - baseline levels of specific neurocognitive or EEG-based measures, or changes in specific measures with initial MEM exposure, predict greater sensitivity to MEM-enhanced neurocognitive, clinical or functional gains over 30 sessions of TCT.

Analyses testing primary and secondary hypotheses will include all subjects' clinical, neurocognitive and functional assessments. Data from subjects who do not complete the full study are carried forward in an "intent to treat" design, and their data are analyzed using linear mixed models. Separate analyses will compare completion / drop-out rates across arms, as well as a 14-item 7-point Likert scale assessing treatment satisfaction.

Go/No-Go decision and primary and secondary endpoints

"Go/No-Go" decisions are based on traditional statistical significance (Aim 1) and effect sizes (Aim 2). Formal statistical testing is conducted using linear mixed-effects (LME) models; hypothesis tests are 2-sided (α=.05). Model parameters are estimated via the R lme4 package and Cohen's d is estimated via the EMAtools package. Type I errors are minimized by constraining the number of primary analyses; secondary analyses use false discovery rate corrections.

PANSSt scores will be assessed at specific intervals. Because TCT is associated with improvements in both positive and negative symptoms, our *a priori* hypothesis is that a PACT effect will be evident in both positive and negative symptoms. Hence, PANSSt is used as the primary metric of symptomatic gains. A greater reduction in PANSSt score will be detected using data from baseline through TCT 30h, by a significant main effect of group (arm) or a significant interaction of group x session, with post-hoc contrasts revealing significant group differences at TCT 30h. A faster reduction in PANSSt scores will be detected using these same data by a significant interaction of group x session and a significant group difference at a point prior to TCT 30h. A more durable reduction in PANSSt scores will be detected using data from TCT 30h through 12 weeks post-TCT, by a significant main effect of arm or a significant interaction of group x post-TCT day, with post-hoc contrasts revealing significant group differences 12 weeks post-TCT.

Comparable analytic approaches will be used for neurocognition (MCCBc) and function (WHODAS) as described in "Research Strategy". Data will be described as means with SEM. Tests for normalcy will be applied to all data and non-parametric comparisons will be used if normalcy is not demonstrated. The Analysis Set for these data will be all subjects who have completed 30h of TCT and post-TCT clinical, neurocognitive and function assessments. When appropriate, missing data are handled via multiple imputation or full information maximum likelihood estimation.

Secondary regression analyses assess the predictive value of putative biomarkers on clinical, neurocognitive or functional gains from the addition of MEM to TCT. Future studies with larger samples will assess potentially more informative models, e.g. Path Analysis to study moderating effects of APS MEM sensitivity and biomarkers on both indirect (moderated-mediation) and direct paths between treatment and outcome.

Power analyses

Consistent with the FOA, this study will adequately power tests of target engagement (Aim 1) for traditional significance, while the “Go” signal for therapeutic impact (Aim 2) is based on effect size (d=0.5). To detect target engagement with d=0.5, 80% power, alpha=0.05, test-retest correlation =0.8, and a linear treatment effect, n=27 randomized participants are required per group. This is a very conservative estimate of d for target engagement, since empirically, d for target engagement with 5 mg MEMetamine was 0.85 (Fig. 3A). A future Confirmatory Efficacy trial will be powered to apply more robust approaches to Aim 2, e.g. Path Analysis[108] to study moderating effects of APS MEM sensitivity and biomarkers on both indirect (moderated-mediation) and direct paths between treatment and outcome.