# Biomarker Predictors of Memantine Sensitivity in Patients With Alzheimer's Disease

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Study Protocol and Statistical Analysis Plan

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**Abstract**

This application responds to **PAR-16-365:** “Pilot Clinical Trials for the Spectrum of Alzheimer’s Disease ...”, and its calls for “Studies to define and refine the target population” and “address heterogeneity of response… identification of specific individuals… more [vs.] less likely to benefit from the intervention(s).”

Alzheimer’s Disease (AD) is a severe neurodegenerative brain disorder, with limited therapeutic options. The NMDA receptor antagonist, memantine (MEM) is approved for treatment of moderate-to-severe AD; its mechanisms are not well understood, but may include a blunting of glutamate-mediated neurotoxicity. While meta-analyses confirm MEM’s effectiveness in delaying the progression of cognitive and behavioral disturbances in AD, the clinical response to MEM is modest, short-lived and highly heterogeneous, with many AD patients showing no gains even with an extended MEM trial. The utility of MEM in AD might be greatly enhanced if patients could be identified as “MEM-sensitive” vs. “MEM-insensitive” in advance of a therapeutic trial, using a **predictive biomarker**.

For the past decade, the PI has studied the acute neurophysiological effects of MEM in laboratory animals, healthy human subjects (HS) and schizophrenia (SZ) patients. These studies demonstrated that a single “challenge dose” of MEM (20 mg) significantly enhanced specific laboratory measures of early auditory information processing (EAIP) in HS and SZ patients: prepulse inhibition (PPI), mismatch negativity (MMN) and gamma band auditory steady-state response (ASSR; including gamma power and synchronization). **This application will determine whether the EAIP response to an acute MEM-challenge can be used to predict a positive therapeutic response to MEM** in patients with mild-to-moderate AD, over a 24 week trial.

Aim 1 tests the hypothesis (H1) that a single dose of MEM (20 mg vs. placebo (PBO)) will significantly enhance EAIP measures in patients with mild-to-moderate severity AD (n=88). Assessed across the full cohort of patients, PPI, MMN and ASSR should be significantly greater after MEM vs. PBO. However, the magnitude of this “MEM effect” (MEM minus PBO) will vary across measures and patients. Aim 2 will leverage this response heterogeneity to test the hypothesis (H2) that patients exhibiting a larger “MEM effect” on EAIP will experience a significantly greater clinical response to MEM, compared to patients with a smaller “MEM effect” on EAIP. After Aim 1 testing, MEM treatment will be initiated and titrated to 10 mg bid in all patients. Clinical outcome measures will be assessed at baseline, 8, 16 and 24 weeks. Analyses will determine whether MEM effects on EAIP measures (individually, and in composite scores) predict the magnitude of the clinical response to MEM in these patients. Moderating factors will be tested, including specific single nucleotide polymorphisms known to moderate MEM sensitivity. This application leverages a unique set of empirical laboratory findings with MEM to develop a novel biomarker predicting sensitivity to MEM’s therapeutic impact in patients with AD.

**Specific Aims:** This application responds to **PAR-16-365:** "Pilot Clinical Trials for the Spectrum of Alzheimer's Disease...", and its calls for "Studies to define and refine the target population" and "address heterogeneity of response… identification of specific individuals… more [vs.] less likely to benefit from the intervention(s)." Specifically, this application develops a **laboratory-based biomarker that predicts clinical sensitivity to the NMDA receptor antagonist, memantine, in patients with mild-to-moderate severity Alzheimer's Disease.**

Alzheimer's Disease (AD) is a severe neurodegenerative brain disorder, with limited therapeutic options. The low-affinity, voltage-dependent, uncompetitive NMDA receptor antagonist, memantine (MEM) is FDA-approved for treatment of moderate-to-severe AD; its mechanisms are not well understood, but may include a blunting of glutamate-mediated neurotoxicity. While meta-analyses confirm MEM's effectiveness in delaying the progression of cognitive and behavioral disturbances in AD, the clinical response to MEM is modest, short-lived and highly heterogeneous, with many AD patients showing no gains even with an extended MEM trial. The utility of MEM in AD might be greatly enhanced if patients could be identified as "MEM-sensitive" vs. "MEM-insensitive" in advance of a therapeutic trial, using a **predictive biomarker**.

For the past decade, the PI has studied the acute neurophysiological effects of MEM in laboratory animals, healthy human subjects and schizophrenia patients. These studies demonstrated that a single "challenge dose" of MEM (20 mg) significantly enhanced laboratory measures of early auditory information processing (EAIP) in healthy subjects and schizophrenia patients: prepulse inhibition (PPI), mismatch negativity (MMN) and gamma band auditory steady-state response (ASSR; gamma power and synchronization). MEM-induced increases in these EAIP measures after one pill provides evidence that MEM is bioactive in cognition-relevant brain circuitry; *conceivably, such a brain "signal" might also identify individuals most likely to benefit from MEM's therapeutic effects.* **This application tests whether the EAIP response to an acute MEM-challenge can predict a therapeutic response to MEM** in patients with mild-to-moderate AD, over a 24-week trial.

This application has two phases: 1. Biomarker assessment; and 2. Therapeutic trial. Phase 1 will test the acute effects of MEM (20 mg po) vs. placebo (PBO) on EAIP measures in 88 carefully characterized patients with mild-to-moderate severity AD who are not currently taking AD medications. From this "challenge" test, a set of "EAIP MEM sensitivity" measures will be derived for each patient. In Phase 2, all patients will begin an open-label trial of MEM monotherapy, titrated to 10 mg bid, with outcome measures collected after 8, 16 and 24 weeks of treatment. Medication adjustments are not restricted, and response heterogeneity is anticipated.

Phase 1 and Phase 2 measures will be used to achieve **2 Specific Aims:**

**Aim 1.** *To assess the acute effects of MEM (0 vs. 20 mg) on measures of early auditory information processing (EAIP) in patients with mild-to-moderate severity AD.* Measures of PPI, MMN and ASSR will be assessed in 88 individuals with mild-to-moderate severity AD after placebo (PBO) and 20 mg MEM po, in a double-blind, randomized order cross-over design. **Hypothesis 1**: Assessed across the full cohort of patients, PPI, MMN and ASSR will be significantly greater after MEM vs. PBO. However, the response to MEM will be heterogeneous: the magnitude of this "MEM effect" (MEM minus PBO) will vary across the dependent measures, and across patients, with some patients demonstrating robust increases in EAIP measures after MEM, and other demonstrating little response or reduced EAIP performance.

**Aim 2.** *To determine the ability of MEM-induced alterations in EAIP in AD patients to predict their clinical response to MEM.* The magnitude of the "MEM effect" will be calculated for each patient and each EAIP measure. MEM treatment will be initiated and titrated to 10 mg bid in all patients. Clinical severity will be assessed at baseline, 8, 16 and 24 weeks using the ADAS-cog as a primary measure. Analyses will determine whether the MEM effect on some or all EAIP measures moderates the magnitude of the clinical response to MEM. **Hypothesis 2***:* Patients exhibiting a larger "MEM effect" on EAIP measures will exhibit a more robust positive clinical response to MEM, compared to patients exhibiting a smaller MEM effect on EAIP measures. This hypothesis will be tested empirically for each of the primary EAIP measures, and for regression-derived composite scores from multiple EAIP measures.

Analyses (linear mixed-effects model) will test the hypothesis (H2) that EAIP MEM sensitivity (in Phase 1) predicts the clinical response to MEM (in Phase 2). Moderating roles of specific variables on MEM sensitivity will be assessed, including demographic/clinical variables, baseline neuropsychological measures and genotypes associated with MEM sensitivity or AD. Source localization will identify anatomical substrates associated with MEM effects on EAIPs, and enable us to test structure- and circuit-based mediation models.

**In total**, this application: 1. identifies measures that detect changes in cognition-relevant circuitry after a single pill of MEM in AD patients, and 2. determines whether patients most sensitive to such acute MEM effects are ultimately most sensitive to MEM's therapeutic effects, including its ability to delay cognitive decline and behavioral disturbances in AD. Developing an acute "challenge test" to identify MEM-sensitive patients could be an important step towards a personalized medicine approach for AD.

**AChE-I**: acetylcholinesterase inhibitor; **AD**: Alzheimer's Disease; **ADAS**: Alzheimer's Disease Assessment Scale; **AP**: antipsychotic; **ASSR:** auditory steady state response; **EAIP**: early auditory information processing; **GBS:** gamma band synchronization; **HS**: healthy subjects; **MCI**: mild cognitive impairment; **MEM**: memantine; **MMN**: mismatch negativity; **PBO**: placebo; **PPI**: prepulse inhibition; **RON**: reorienting negativity; **SZ**: schizophrenia

**Research Strategy. A. Significance:** Alzheimer's Disease (AD) is a severe neurodegenerative brain disorder affecting about 50 million people worldwide. Its cost to society is well documented (2), as are stories of suffering among AD patients and their families. Despite significant advances in the identification and characterization of mild cognitive impairment (MCI) and "pre-MCI" risk factors, many millions of individuals will continue to manifest the cognitive and behavioral symptoms of AD for the foreseeable future. Even as scientific efforts focus increasingly on preventative strategies, both the prevalence of the AD and its negative impact on society are expected to rise. ***Thus, identifying effective treatments for the cognitive and behavioral disturbances of AD is – and will continue to be - of paramount importance.***

**Enhancing treatment effectiveness by using predictive biomarkers:** Four medications are approved for the treatment of AD worldwide: three cholinesterase inhibitors (AChE-I's) and the noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, memantine (MEM) (as well as a combination of an AChE-I + memantine). MEM is approved for treatment of moderate-to-severe AD, though it is frequently used in earlier stages of AD and even in Mild Cognitive Impairment (MCI) (86). A retrospective analysis of 5200 patients with newly diagnosed AD started on monotherapy, MEM was initiated in roughly 16% of these patients, and 1-year discontinuation rates and subsequent non-adherence rates were the lowest among all treatments (including 3 AChE-I's) (4). MEM's mechanisms of action are not well understood, but may include its ability to reduce glutamate-mediated neurotoxicity (66). While meta-analyses have confirmed that MEM monotherapy reduces cognitive impairment (3,14,93,119) and behavioral disturbances (40-1) in AD, these salutary effects are generally modest is magnitude and duration. Moreover, the clinical response to MEM is highly heterogeneous, with many AD patients showing little or no gains even with an extended MEM trial (14).

Both AD and MCI are heterogeneous in terms of presumed etiologies and symptom profiles (9,11). One way to enhance treatment efficacy is to narrow the target population from a broader, heterogeneous patient group to more selective "sensitive" clinical subgroups, identified through the use of laboratory-based biomarkers. In this approach to "precision medicine", biomarkers are used to stratify patients into subgroups for whom a medication would be more vs. less likely to be effective. Conceivably, a biomarker predicting MEM sensitivity could identify AD patients for whom MEM would be most protective against the progression of cognitive and behavioral disturbances; conversely, patients lacking a positive biomarker response might be counseled towards other treatments, to avoid the loss of time and resources at a critical phase of illness progression.

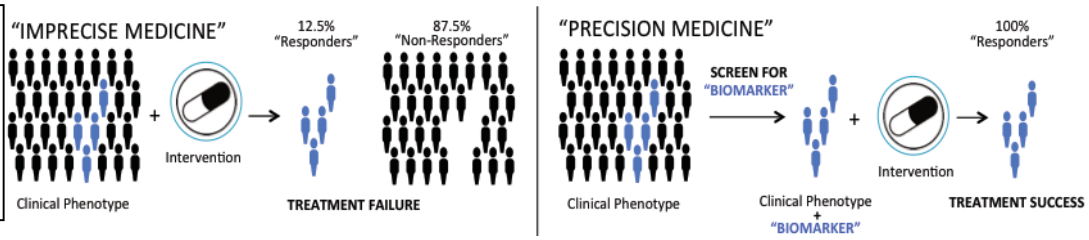



**Fig. 1.** Schematic showing the idealized impact of adding a biomarker to a heterogeneous population defined by a clinical phenotype to enhance the likelihood of treatment response. = treatment-sensitive patient.

This application responds to **PAR-16-365**: "Pilot Clinical Trials for the Spectrum of Alzheimer's Disease and Age-related Cognitive Decline", and to this FOA's specific calls for **"Studies to define and refine the target population" and "Studies that address heterogeneity of response… identification of specific individuals… [who] are more likely or less likely to benefit from the intervention(s)."**

For the past decade, the PI has assessed the acute neurophysiological effects of MEM, first in laboratory animals (107), and then in healthy human subjects (HS) and schizophrenia (SZ) patients (5,65,99,107), with the goal of using MEM or related drugs as pro-cognitive interventions in SZ. [Note: no investigators in this application have any financial relationship with the commercialization of MEM.] In the past 4 years, supported by the NIMH, these studies assessed the effects of acute MEM challenge (placebo (PBO), 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) are reliable, objective and quantitative; 3) consistently detect deficits in neuropsychiatric populations; 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, and 6) are each regulated by NMDA activity, with at least some evidence for enhanced performance associated with MEM (29,44,107), including the PI's studies demonstra-

ting MEM-enhanced PPI in HS (107), and those of others reporting MEM-enhanced MMN (44) in HS.

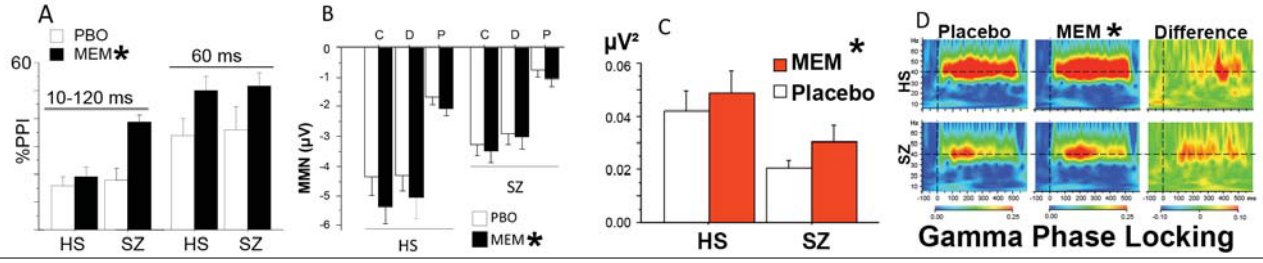



**Fig. 2. Effects of MEM on A: PPI; B: MMN; C, D: ASSR** in HS and SZ patients. Patients and HS (n's=42 & 42) were tested after 1 pill of either PBO or MEM (10 or 20 mg po; 20 mg shown here) in a double-blind, balanced cross-over design. Tests were 7d apart. Compared to HS, patients had deficits in MMN (B) and ASSR (C,D). MEM (20 mg) significantly enhanced PPI (99) (A; p<0.04 for 10-120 ms; p<0.01 for 60 ms), MMN (99) (B; p<0.014; stimulus "mismatch": Duration; Pitch; Combined) and ASSR (65) (C: evoked power, 40 Hz *p<0.025; D: gamma phase locking, *p<0.002). *An identical test design is proposed for the present study, to identify "MEM-sensitive" patients with AD.*

Via a placebo (PBO)-controlled within-subject cross-over design, we detected statistically significant effects of MEM (20 mg) on PPI, MMN and ASSR (65,99) (Fig. 2A-D). In each case, **one pill** of MEM significantly enhanced EAIP. These changes were not explained on the basis of AP medication interactions, or other factors related to treatment or illness chronicity, since similar changes **were detected in both patients and HS**, as we had previously reported in HS with PPI (107), and **as others had previously reported in HS with MMN** (44).

This is the 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 of MEM-enhanced EAIP are novel and important, as they demonstrate significant plasticity in brain mechanisms that are thought to contribute to "core" neurocognitive deficits in SZ. Because EAIP are known to mediate neurocognition and function in these patients (111), in a separate application, we hope to use these findings to identify novel therapeutic strategies for SZ and related disorders.

Ironically, this approach has faced some headwinds, based on a concern that use of an NMDA antagonist in SZ patients might lead to symptom exacerbation; this concern persists in peer review despite numerous controlled trials and meta-analyses of the use of MEM as an adjunctive therapy in SZ, all showing that it is well-tolerated, and most demonstrating clinical benefits (cf. 127). Clearly, this pattern of safety and tolerability parallels the clinical experience with MEM in AD populations (37-8).

**Of greater relevance to the development of AD therapeutics**, our findings show that it is possible to detect robust neurophysiological effects of MEM after a single 20 mg pill. These neurophysiological "signals" reflect the fact that MEM is reaching the brain and acting on neural mechanisms that we know are highly relevant to neurocognition (111). Importantly, as with clinical responses to MEM, while the groups show statistically significant increases in EAIP after MEM, there is **heterogeneity in this MEM response**, with some HS and patients exhibiting robust EAIP increases after MEM, and others showing little change, or even small EAIP reductions (Fig. 3). This heterogeneity is critically important to our ability to identify individuals who are more vs. less sensitive to MEM effects on EAIP, and by extension, to ultimately using biomarkers to stratify patients into subgroups for assessing a differential clinical response to MEM.

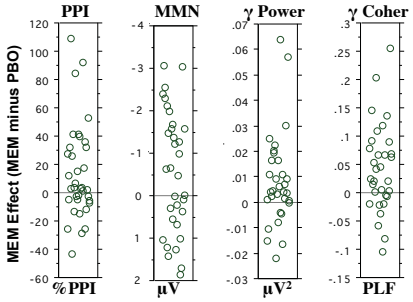



**Fig. 3.** Distributions of "MEM effect" (MEM (20 mg) minus PBO) on EAIP performance, for (L to R): PPI (60 ms), MMN, gamma power and gamma coherence, in HS and SZ patients (pooled, since there were no group differences in MEM effects). This application tests the hypothesis that AD patients defined as "MEM-sensitive" based on these "MEM effects" will exhibit a superior clinical response to MEM in a 24 week trial. Different criteria for "MEM-sensitivity" will be empirically assessed, based on discrete distributional criteria (eg. median split) and continuous (linear mixed-effects model) approaches, and using individual EAIPs vs. composite (weighted) indices (see text).

Conceivably, **AD patients in whom MEM most readily accesses the brain, and positively impacts cognition-relevant brain circuitry as detected by enhanced EAIP, will ultimately be most sensitive to its therapeutic effects,** including its ability to slow cognitive decline and behavioral disturbances in AD. We propose to test this hypothesis in the present application.

We hypothesize that evidence for "MEM sensitivity" that identifies an optimal candidate for MEM treatment will be provided by gains in EAIP in response to a MEM "challenge". This "personalized medicine" approach

parallels the use of a test-dose to predict clinical benefits from treatments ranging from hormones (7) to anti-Parkinsonian drugs (34) to bronchodilators (20). Evidence for enhanced EAIP in response to MEM challenge suggests that MEM is accessing neural circuitry relevant to neurocognition, and furthermore, that such circuitry has yet been spared, and remains plastic and hence a viable target for pharmacotherapy. Conceivably, via this approach, in the span of two office visits separated by one week (see "Methods") subgroups of AD patients will be stratified as "MEM-sensitive" vs. "MEM-insensitive", based on their physiological responses (eg. gain in EAIP) to an acute MEM challenge-dose. This "personalized medicine" approach has been used in other fields (7,20,34) and attempted for some psychotherapeutics (cf. 19,22), but has not yet been developed for AD.

**B. Innovation:** The primary innovation in this application is the *development of a clinically feasible test to predict sensitivity to the therapeutic response to MEM in AD patients based on the "biomarker" of MEM-enhanced EAIP*. As described in the PAR, "mediators of the therapeutic intervention" will also be identified.

**Specifically, we hypothesize** and will test in Aims 1-2 that in AD patients, evidence of MEM-enhanced EAIP will predict a superior clinical response to MEM. Prior to starting a standardized MEM treatment regimen, subjects not currently receiving AD medications will be tested to their EAIP response to placebo or a single pill of MEM (20 mg). They will then be followed clinically during MEM monotherapy, consisting of a titration to a daily dose of 10 mg bid for 24 weeks. Clinical indices will be assessed at baseline, weeks 8, 16 and 24. Clinical responses will then be compared across groups stratified by the magnitude of MEM-enhanced EAIP (Fig. 3), and the relationships of MEM-enhanced EAIP measures to the magnitude of clinical gains will be tested using both discrete (ANOVA) and continuous (linear mixed-effects model) approaches. Secondary analyses will be used to optimize criteria for EAIP "MEM sensitivity", to determine whether EAIP MEM sensitivity also predicts the rapidity and durability (in addition to the magnitude) of MEM's clinical benefits, and whether specific demographic, clinical and genetic factors moderate the predictive strength of MEM EAIP sensitivity. If our hypothesis is supported by these findings, we plan to expand our tests in future applications to include combination therapies (MEM+AChE-I) vs. AChE-I monotherapy, and MCI / early AD patients, with the prediction that combined regimens (but not AChE-I monotherapy) will also produce superior gains among subgroups exhibiting the greatest MEM-enhanced EAIP response. Positive findings in this application may also justify tests with other AD therapeutics for which an acute EAIP-based laboratory biomarker is available.

**Summary**: Current therapeutics for AD are suboptimal. In particular, clinical response to MEM in AD is variable and modest. One strategy to enhance treatment efficacy is to prospectively identify "drug-sensitive" subgroups, based on the presence of predictive biomarkers for drug response (Fig. 1). MEM enhances neurophysiological measures of EAIP in healthy subjects as well as patients with SZ (Fig. 2). We now propose to determine if greater sensitivity to MEM-enhanced EAIP (Fig. 3) predicts a superior therapeutic impact of MEM in AD patients. This application is thus responsive to **PAR-16-365**, and its specific calls for **"Studies to define and refine the target population…" and "Studies that address heterogeneity of response."**

**Measures of EAIP:** The 3 measures of EAIP to be used in this application are reviewed below. The clear experimental rationale for their use in this present application is that they are known to be sensitive to an acute 20 mg dose of MEM in both patients and HS, independent of the presence of a disease-related EAIP deficiency (Fig. 2 and citations below) or the concomitant use of psychotropic medications. Furthermore, inspection of Figure 3 reveals that the distributions of "MEM effects" on these EAIP measures (MEM minus PBO) show inter-individual heterogeneity, which is important if these measures are to serve as biomarkers predicting a heterogeneous clinical response to MEM. Measuring these EAIP **will enable us to test the primary hypothesis** of this application. In brief, these measures are:

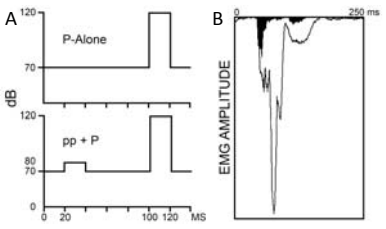



**Fig. 4.** PPI is the automatic inhibition of a startle response to an intense, abrupt stimulus or "pulse" (A. "P-Alone", top), when this startling stimulus is preceded by a weaker "prepulse" (A. "pp+P", bottom). In our studies, PPI is assessed using EMG of orbicularis oculi (blink response). B. At right are two superimposed EMG responses to either a P-Alone (solid black) or pp+P (open, white area). The % reduction in EMG amplitude produced by the prepulse is the operational measure of sensorimotor gating, and is regulated by well-studied neural circuitry (101,108).

1. **PPI:** PPI is an operational measure of sensorimotor gating (108). In PPI, a weak lead stimulus inhibits the magnitude of a startle response to an intense, abrupt stimulus occurring 30-120 ms later (Fig. 4). On average, the amount of reflex inhibition caused by the prepulse is reduced in patients with one of several brain disorders (108); of the 3 reports testing PPI in MCI and AD samples, however, two detected no deficits, and one reported mixed results (26,78,89,115). The PI of this application has been instrumental in understanding the neurobiology and clinical relevance of PPI, starting with his first contribution to this literature in 1982 (95), and continuing in roughly 200 peer-reviewed data papers on these topics.

PPI is generally viewed as a measure of automatic, preattentional inhibition (17). It is regulated by circuits connecting portions of the prefrontal and mesial temporal cortex, with subcortical systems extending from the basal ganglia to the pons (101). Drugs acting at prominent nodes in this circuitry have potent effects on PPI (108). For example, PPI is **increased** in HS by the NMDA antagonists ketamine (1,15), amantadine (105) and MEM (107). We first reported the PPI-enhancing effects of MEM (20 mg po) in HS (107); these findings were extended as seen in Fig. 2. MEM (20 mg) significantly increased PPI in SZ patients (Fig. 2A), across 10-120 ms prepulse intervals; comparable effects were detected in HS at 60 ms intervals, which is a critical "sensitive" temporal window for therapeutic drug effects (103). Interestingly, these potent MEM effects in SZ patients were evident despite the fact that, as a group, these patients were not deficient in PPI, consistent with the fact that 90% were taking PPI-normalizing second generation antipsychotics (SGAPs) (48,103,123).

MEM effects on PPI are moderated by age and baseline PPI level, but not by AP load, anticholinergic load or demographic or clinical variables (5,99). These findings support the strong prediction that PPI will be a potent signal of MEM "bioactivity" in this application – a quantifiable measure of MEM's activity within the brain, and specifically within PPI-regulatory circuitry that includes elements relevant to the pathophysiology of AD - and might conceivably serve as a "biomarker" predicting therapeutic response to MEM among AD patients.

2. **MMN** is an auditory event-related potential (ERP) component 50-150 ms after a deviant, "oddball" stimulus is presented within a sequence of repetitive standard sounds (Fig. 5: shown, from mild-to-moderate AD patients, n=20 (G. Light)); it reflects the properties of automatic, memory-based comparison processes (cf. 74). It is rapidly assessed, and while it is highly stable in normal and impaired patient populations across the lifespan (63), studies of this form of MMN in AD patients have reported inconsistent results, with many studies reporting no differences vs. age-matched HS (21,76-7). MMN is largely automatic and preattentional, yet it is strongly associated with neurocognition and psychosocial function, even in HS (64).
MMN is regulated by forebrain circuitry, and is sensitive to NMDA activity: it is disrupted in infrahuman primates by PCP (cf. 36), and in HS by ketamine (23), but is **increased in HS by an acute 30 mg dose of *MEM (d=0.87)*** (44). The PI and Co-PI (Dr. Light) have published extensively on MMN and its deficits in patient vs. HS cohorts (57-8,63-4,84).

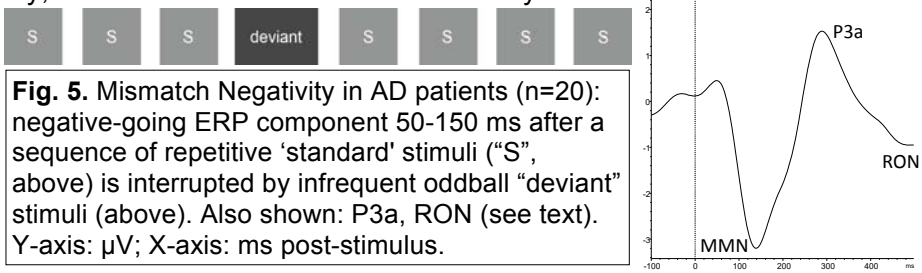


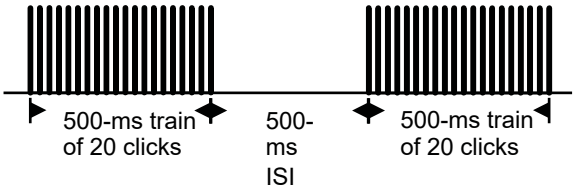

**Fig. 5.** Mismatch Negativity in AD patients (n=20): negative-going ERP component 50-150 ms after a sequence of repetitive 'standard' stimuli ("S", above) is interrupted by infrequent oddball "deviant" stimuli (above). Also shown: P3a, RON (see text). Y-axis: μV; X-axis: ms post-stimulus.

We reported significant MEM-induced increases in MMN in both HS and SZ patients after a single oral dose of 20 mg (99). **As seen in Fig. 2B,** compared to HS, SZ patients exhibited MMN deficits ($p<0.007$). MEM (20 mg) significantly increased MMN across both groups ($p<0.014$); this effect was highly consistent within subjects, and across each of 3 different deviant conditions (27). Others report MEM-enhanced MMN in rodent models (112), and in HS after 30 mg po (44).

**Exploratory measures of EAIP** in this application include P3a and reorienting negativity (RON) (84-5), which were root causes of neurocognition and functional outcome in our study of 1415 SZ patients (111). MMN, P3a and RON are automatically elicited as a 3-peak waveform complex in response to unattended oddball stimuli (Fig. 5). MMN is the primary dependent variable based on evidence that it is MEM-sensitive (27,44,112); P3a and RON will also be carefully assessed as their sensitivity to MEM has not been previously studied. The P3a ERP is thought to reflect the transition from perception and sensory registration to focal attention or orienting to the stimulus; RON is thought to reflect reorienting of attention after distraction. Together with MMN, these components reflect processes underlying auditory perception, auditory learning and memory and other complex cognitive functions (84). MMN, P3a and RON arise from broadly distributed patterns of neural activation (85) and are regulated by NMDA activity (88). Thus, it is conceivable that these measures will be sensitive to MEM, and that this sensitivity may serve as a biomarker for clinical response to MEM, either as stand-alone measures, or in regression-derived composite measures (below). In addition to assessing the **magnitude** of these potential biomarkers, source localization (12-13,109,124) will be used to **identify anatomical substrates** associated with MEM-induced changes in EAIP in AD patients, as a first step towards understanding the **neural mechanisms** underlying the predictive value of these potential "biomarkers". Using these same source localization strategies, we previously reported that deficits in different ERP components (eg. MMN vs. P3a) map onto distinct frontal and temporal regions (39,85); here, we propose to use similar strategies to localize sources of MEM-induced changes that best predict clinical response.

3. **ASSR:** Synchronous neural oscillations centered at 40-Hz appear to reflect brain resonance that is critical for cortico-cortical communication and large-scale integration of distributed neural functions (18,49,71,96,116). The automatic entrainment of gamma band oscillations to 40-Hz auditory steady-state stimulation (ASSR) is

deficient in SZ (49), even at first episode (96); in contrast, the literature on ASSR in AD patients is inconsistent, reflecting a lack of standardized paradigms for eliciting ASSR across these studies (eg. 75,116,118). Gamma oscillations are enhanced by MEM in rodent models (29,67) and by ketamine in both rodents and HS (31,33,50). Our recent studies (Fig. 2) detected deficits in ASSR gamma evoked power and phase locking in SZ patients, that were significantly corrected by MEM. However, MEM-enhanced gamma power and phase locking was also detected in HS (Fig. 2C-D) (65). These findings support the strong prediction that ASSR metrics will be a potent signal of MEM-sensitivity in this application. The PI and Co-PI (Dr. Light) have published extensively on the characterization of the ASSR and its deficits in patients vs. HS (39,59-62).



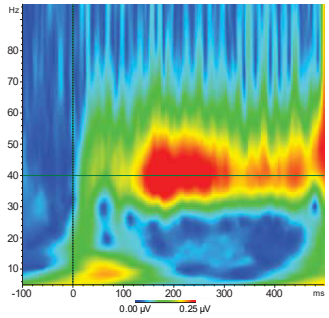




**Fig. 6**. ASSR in AD patients (n=20): (γ power, phase locking (PL)): 40 Hz clicks/ tones (left) drive γ power and PL in the EEG; this may reflect capacity of the auditory system to generate oscillations in synchrony with the stimuli. At right, γ PL time-frequency plot: x-axis: time in ms; y-axis: frequency. Color = PL factors.

**The extant literature does not make a strong prediction regarding levels of baseline EAIP performance in AD patients.** It is thus important to emphasize that the prediction of MEM-enhancement of these EAIPs in AD patients is independent of the presence of any AD-related EAIP deficiency. For example, MEM enhances PPI, MMN and ASSR in HS (65,99,101) (who by definition do not have EAIP deficits), and MEM enhances PPI in SZ patients whose PPI has been "normalized" by SGAPs (103,123). Furthermore, MEM-enhanced EAIP was detected in patients despite the presence of a variety of psychotropic medications (including SGAPs) that potentially will also be used by the AD patients in this application. *Thus, the ability to detect the proposed biomarker for MEM-sensitivity in this application should not be impeded by the presence (or absence) of abnormalities in baseline EAIP levels or the use of psychotropic medications in the AD patient group.*

**High Risk / Low Cost: Genetic biomarker(s)?** Cross-species studies report a connection between MEM effects and genetic variations in glutamate ionotropic receptor subtypes (GRIN2A, GRIN2D); these variations are associated with sensitivity to anticonvulsant effects of MEM (51-2,80). While no SNPs in GRIN2A or 2D were tested in our previous studies with MEM, of the 4 SNPs tested, one in GRIN3A (rs1337697) moderated MEM effects on both neurocognition and PPI (n=19) (5). Interestingly, other SNPs in GRIN2B (rs10845840) and 3A (rs3739722) are reported to be associated with AD (54,97). Based on these findings with GRIN2A 2D and 3A (51,52,54,80,126), this application will test the potential contribution to MEM sensitivity of functional SNPs in GRIN2A, 2B, 2D and 3A (MAF's=0.32-0.43), as a high-risk / low-cost exploratory aim. *Assessing genetic predictors of a therapeutic MEM response in AD patients responds to PAR-16-365's request to identify "specific individuals according to genetic profiles… more likely or less likely to benefit from the intervention(s)."*

Though not part of the primary or exploratory aims, we will also assess APOE genotype (rs7412, rs429358) to further characterize MEM-sensitive vs. -insensitive patients. Subjects will be divided in two groups: carrier subjects (ε4+) with one or two ε4 alleles, and non-carrier subjects (ε4−) without any ε4 allele. Because we have no *a priori* predictions that these biomarkers will identify MEM-sensitive patients, or moderate the impact of EAIP sensitivity on MEM clinical response (but see (53)), analyses assessing such relationships will be undertaken only on a post-hoc, exploratory basis. As recommended in this FOA, this application will "store blood… for future genomic and other… analyses aimed at interrogating treatment responsiveness..."

**C. Approach:** This application will test the acute effects of 20 mg MEM (po) on EAIP in patients with mild-to-moderate severity AD (**Mini-Mental State Examination score between 10-22 at screening**), and determine whether sensitivity to MEM-induced changes in primary EAIP measures predicts the clinical response to MEM in these patients over a 24-week trial. The primary clinical outcome measure will be the Alzheimer's Disease Assessment Scale (ADAS-cog) (87), as described below. *Secondary analyses will assess alternate potential biomarkers, alternate outcome measures, and moderating roles of specific patient characteristics (including genotype), as well as the anatomical localization of the MEM-induced changes in EAIP measures.*

**Aim 1.** *To assess the acute effects of MEM (0 vs. 20 mg) on measures of early auditory information processing (EAIP) in patients with mild-to-moderate severity AD.* Measures of PPI, MMN and ASSR will be assessed in 88 individuals with mild-to-moderate severity AD after placebo (PBO) and 20 mg MEM po, in a double-blind, randomized order cross-over design. **Hypothesis 1**: Assessed across the full cohort of patients, PPI, MMN and ASSR will be significantly greater after MEM vs. PBO. However, the response to MEM will be heterogeneous: the magnitude of this "MEM effect" (MEM minus PBO) will vary across the dependent measures, and across patients, with some patients demonstrating robust increases in EAIP measures after MEM, and other demonstrating little response or reduced EAIP performance.

**Aim 2.** *To determine the ability of MEM-induced alterations in EAIP in AD patients to predict their clinical response to MEM.* The magnitude of the "MEM effect" will be calculated for each patient and each EAIP measure. MEM treatment will be initiated and titrated to 10 mg bid in all patients. Clinical severity will be assessed at baseline, 8, 16 and 24 weeks using the ADAS-cog as a primary measure. Analyses will determine whether the MEM effect on some or all EAIP measures moderates the magnitude of the clinical response to MEM. **Hypothesis 2***:* Patients exhibiting a larger "MEM effect" on EAIP measures will exhibit a more robust positive clinical response to MEM, compared to patients exhibiting a smaller MEM effect on EAIP measures. This hypothesis will be tested empirically for each of the EAIP measures, and for regression-derived composite scores from multiple EAIP measures.

**Design Overview:** The general study design is modeled after that employed by Peskind et al. (79) and Pomara et al. (81). These authors conducted a 24-week, multi-site placebo-controlled randomized trial of MEM monotherapy (10 mg bid) in mild-to-moderate AD, demonstrating significant gains in cognitive, global and behavioral measures. The present study will differ from that of Peskind et al. (79) in 3 major ways: 1. Patients will be characterized at baseline to assess their EAIP response to a MEM "challenge dose" (20 mg); 2. This is a single site study; 3. All patients will receive MEM treatment; to test the key hypothesis (H2), analyses will compare groups stratified by the magnitude of their EAIP response to MEM. Other minor methodological differences will be described below.

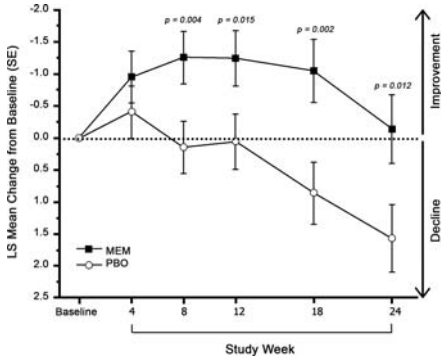



Fig. 7. From Pomara et al. (81). MEM (10 mg bid) significantly improved ADAS-cog scores after 8-24 weeks in patients with mild-to-moderate AD. LS = least squares; SE = standard error. Total n = 394.

88 AD patients who consent to participation undergo a full neurocognitive characterization and medical evaluation. Patients also receive baseline ADAS-cog assessment and secondary outcome measures (described below) by Dr. Lisa Delano-Wood (Neuropsychologist; Clinic Director: UCSD Memory, Aging and Resilience Clinic [MARC]; Co-I).

Patients will then be tested in a PBO-controlled double-blind within-subject cross-over study of the effects of 20 mg MEM po on EAIP measures (PPI, MMN, ASSR). This proposed "N" is adequate to be stratified into "low" vs. "high" MEM sensitivity using discrete criteria, eg. median split, for categorical analyses (eg. ANOVA) and to detect medium effect size (d=0.5) differences in outcome between MEM-sensitive vs. -insensitive patients (see "Power Considerations"). Inclusion and exclusion criteria are seen in Table 2.

**Table 1. Inclusion and exclusion criteria**

**Inclusion:** 1. ADRC-confirmed diagnosis of AD; 2. **MMSE score 10-22**; 3. Age 50-70 y; 4. Knowledgeable caregiver; 5. Ambulatory; 6. Medically stable; 7. Audiometric testing (detection ≤ 40 db(A) at 1000 Hz); 8. Informed consent.
**Exclusion:** 1. Active systemic illness (eg. heart disease, liver or renal failure, cancer, HIV, tuberculosis, Hepatitis C); 2. Current psychiatric or neurologic illness other than AD; 3. History of vascular disease, MI, CVA, TIA, seizure, head injury with loss of consciousness; substance dependence (including EtOH and Opioid); 4. Past treatment with MEM; 5. AChE-I or investigational drug treatment within 30 d of screening; 6. Current medication: amantadine, riluzole, other pro-cognitive medication, opioids; 7. Positive urine toxicology for non-prescribed psychoactive substance; 8. Actively enrolled in cognitive remediation therapy.

**Clinical Evaluations:** Based on the above inclusion / exclusion criteria, patients will be recruited through: 1) the UCSD MARC and Medicine for Seniors Clinic, under the supervision of Lisa Delano-Wood, PhD and Jairo Romero, MD (Geriatric Internal Medicine physician; Co-I), (typical clinic flow: 8 new AD patients/week); 2) the UCSD ADRC (see below and accompanying letter; typical evaluation of new AD cases = 5/week); and 3) community providers within a network affiliated with the UCSD ADRC (eg., Glenner Center; Alzheimer's San Diego; Southern Caregivers Resource Center). Importantly, this target population is in relatively less research "demand", based on the current UCSD research focus on MCI and "pre-MCI."

Patients who agree to participate undergo an initial **screening session**, during which inclusion and exclusion criteria are confirmed, patients provide study consent (see "Human Subjects") and undergo a full clinical and neurocognitive characterization as well as baseline ADAS-cog and secondary outcome measures (described below). A structured history is obtained of the onset, course, and nature of cognitive and other symptoms, medical history (and review of medical records to investigators by the participant, including copies of clinical neuroimaging studies) and system review, family history, documentation of current and past medications.

After enrollment in the study, the ADAS-cog will be administered under the direction of Dr. David Salmon (clinical psychologist; Director of Neuropsychology, UCSD ADRC; see letter). The standard ADAS-cog scores range from 0-70; higher scores indicate greater impairment. The validity and factor-derived subscales of the ADAS-cog are described in (79). For this study, the ADAS-cog will also have an added delayed recall subtest, and will be administered using newly standardized instructions (per D. Salmon). This ADAS-cog administration

provides the primary baseline ("week 0") assessment metric; alternate list versions are used in subsequent (week 8-24) tests. Secondary assessment measures are behavioral symptoms documented by the Neuropsychiatric Inventory (NPI-Q) and the Geriatric Depression Scale. An RA administers the Clinical Dementia Rating (CDR), Montreal Cognitive Assessment (MoCA), Functional Activity Questionnaire (an IADL scale), Health & Safety and Managing Money subscales of the Independent Living Scales, and the modified Lawton physical self-maintenance schedule (a basic ADL scale). Blood is collected at baseline for exploratory genetic biomarkers (described above). A **physical examination** (≈ 40 min) is performed by a study physician, including a neurological examination under Dr. Galasko's direction, and an electrocardiogram (EKG). Unless already performed, blood tests will rule out other causes of cognitive impairment (eg., vitamin / metabolic deficiencies; endocrine disorders). Audiometric testing ensures that patients detect 40 dB(A) tones at 1000 Hz.

A **comprehensive neuropsychological evaluation** is conducted under the supervision of Dr. Lisa Delano-Wood (≈ 2h; see "Human Subjects"). This test battery is used to characterize "MEM-sensitive" vs. "-insensitive" subgroups, and includes sensitive measures of memory (California Verbal Learning Test-II, WMS-IV Visual Reproduction Test with delayed recall and recognition, WMS-IV Logical Memory with delayed recall and recognition), attention (WAIS-R Digit Span Forward and Backward), language (Boston Naming Test; Letter and Category Fluency Tests), problem solving and executive function (modified Wisconsin Card Sort Test, Digit Symbol Substitution Test; Trailmaking, Fluency Switching, and Color-Word Interference subtests of the Delis-Kaplan Executive Functioning System [DKEFS]); constructional and visuospatial abilities (WASI-II Block Design Test, Clock Drawing Test, D-KEFS Visual Scanning), and global cognition (Mattis Dementia Rating Scale-2). Appropriate normative data are used for all tests (eg., Mayo Older American Normative Studies; 98). The number of prior cognitive assessments will be recorded for each subject to ensure that experimen-tal groups (MEM-sensitive vs. MEM-insensitive) do not differ in the number of prior assessment exposures. A follow-up visit with the primary provider is scheduled for approximately 2-3 weeks after screening. All subjects are paid after screening, test days and 8, 16 and 24-week follow-ups (see "Human Subjects").

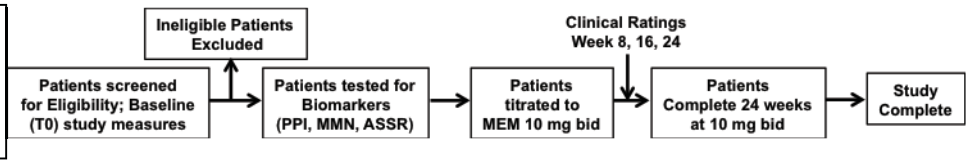
**Test Days:** Subjects who complete the **screening session** are scheduled for **EAIP Test Days** within 7 days. Transportation is provided. Urine toxicology is repeated on each test day. Test Days last from 0830–1600, much of which is spent relaxing, watching television, reading or eating. In brief, EAIP are assessed as in (59,99), 210 (PPI) and 345 min (MMN, ASSR) after administration of placebo or MEM (20 mg po), in a randomized order double-blind design. Vital signs (VS) and visual analog scale (VAS) measures of subjective self-ratings (99) are taken throughout the day. MEM pharmacokinetics are linear for single oral doses of 5-40 mg; $T_{max}$ for a single 20 mg dose is 6.89 h; elimination $T_{(1/2)}$ is 60-80 h (94). Test days are ≈ 1 week apart.

| A. | ≈Day | B. Screen Day | C. Test Days a.m. | D. Test Days p.m. |
|---|---|---|---|---|
| Screen Day | 1 | Consent, Structured history | 830: Brkfst, vital signs, UTox | VS, VAS cont'd |
| Test Day 1 | 8 | Baseline clinical metrics | 900: Pill (PBO or MEM) | 1230: PPI (30 min) |
| Test Day 2 | 15 | Physical exam, EKG, audiometry | VS q 30 min, VAS q 60 min | Break |
| | | Neuropsychological testing | 1130: Lunch | 1445: EEG (50 min) |

**Fig. 8. Schedule**: A. 3 sessions over ≈2 weeks. B. Screen Day: C-D. Test Days: Total test time ≈80 min; vital signs (VS) and VAS ratings taken all day, and parameters are set to notify the on-site study MD.

**Intervention:** Within 1 week of completing EAIP testing, subjects are seen by their primary provider, for initiation of MEM therapy. Many but not all patients will be treated through the UCSD MARC and Medicine for Seniors Clinics, where they were recruited. MEM is initiated at 5 mg/d and titrated with 5 mg weekly increments. During this time, subjects / caregivers are contacted weekly by study staff to assess adherence. Intervention Week 1 will begin when dosing reaches 10 mg bid.

**Fig. 9.** Study flow chart. Screening, testing and dose titration take 5 weeks. Starting from full dose (10 mg bid), ratings are completed at weeks 8, 16 & 24. Final n = 88.

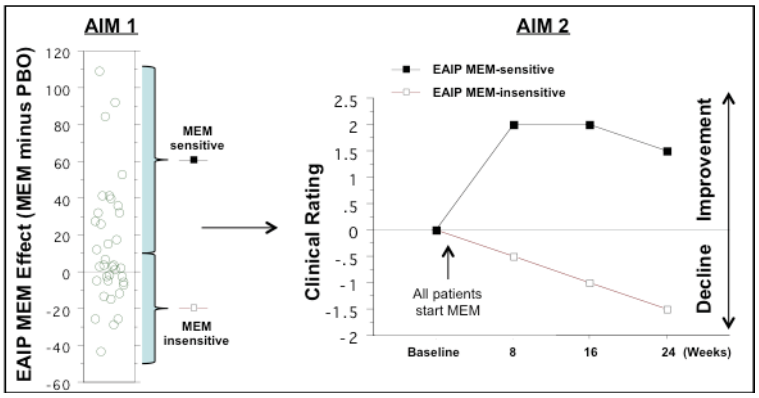



**Outcome measures:** At the end of Intervention Weeks 8, 16 and 24, subjects will be evaluated with the ADAS-cog, the NPI-Q and Geriatric Depression Scale.

**Analytic strategy:** This study is not designed to test whether MEM monotherapy leads to clinical gains in mild-to-moderate severity AD: **this has already been studied** in large, multi-site trials (3,14,79,81). Rather, this study will determine whether laboratory-based biomarkers **predict which patients will benefit most from a "therapeutic" trial of MEM.** This hypothesis can only be tested using patients who complete a nominally "therapeutic" trial. Based on previous findings (Fig. 7), we plan to include in our full analyses all subjects who complete at least 8 weeks of MEM monotherapy (10 mg bid). Dose adjustments are permitted during weeks 3-8, but subjects unable to tolerate 10 mg bid by the end of week 7 will be characterized as "incomplete

treatment" in the final analyses. Importantly, separate analyses will consider "incomplete treatment" patients, those who discontinue MEM use or add an AChE-I in testing H2, as described below.

**Fig. 10. Schematized overview of study design.** Aim 1 tests EAIP MEM sensitivity in patients with mild-to-moderate severity AD, in a double blind, within-subject cross-over design of MEM (20 mg po) vs. PBO. The primary result of Aim 1 is a biomarker character-ization of "EAIP MEM-sensitivity" for each subject. Patients are also screened for baseline outcome and neurocognitive measures. In Aim 2, all patients titrate to 10 mg MEM bid, and outcome measures are collected at 8, 16 and 24 weeks. Analytic strategies are then used to test the hypothesis (H2) that greater EAIP MEM sensitivity predicts greater clinical MEM sensitivity.




**Detailed Methods:**

**Stimulus parameters: The rationale for startle and ERP stimuli** reflects both **empirical** and **theoretical** considerations, as discussed in our published reports (99,103). Empirically, these stimuli and session designs detect consistent MEM effects on PPI, MMN and ASSR (Fig. 2), and thus afford us the strongest predictions in terms of MEM effects in this application. There are also pragmatic considerations specific to each measure; for example, weaker pulse intensities generate smaller startle responses, and with the habituation resulting from repeated testing (2 test days), this results in an unacceptable number of startle "non-responders". Space does not allow a full description of the many such decision points with each measure, which are supported by a rich literature and the PI's and Co-PI's > 30 years of published experience.

**Startle and PPI** are assessed as in (99), with 42 trials, of 6 types: a 118 dB(A) 40 ms noise (pulse), and pulse preceded 10, 20, 30, 60, or 120 ms by a prepulse 16 dB over a 70 dB(A) background. Primary measure: **MEM effect (MEM minus PBO) on mean %PPI with 60 ms prepulse intervals**. Total time: 30 min, with clean-up.

**EEG** data are continuously recorded from 64 channels using a BioSemi ActiveTwo system with a sampling rate of 1 kHz. Subjects are assessed on EEG biomarkers in the same order: MMN/P3a/RON (20 min), ASSR (5 min). Auditory stimuli are presented at 85 dB SPL via insert earphones. All setup and data acquisition take < 60 min with breaks. Stimulation, recording and analysis techniques for MMN are as in (57,99), using binaural stimulation (1 kHz square wave stimuli, 5 ms rise/fall) with fixed stimulus onset-to-onset asynchrony of 500 ms. A pseudorandom sequence of tones, of which 82% are standards (50 ms, 1000 Hz) and 18% are deviants (6% per deviant type): duration deviants are 100 ms, 1000 Hz; frequency deviants are 50 ms, 1500 Hz; double-oddball deviants are 100 ms, 1500 Hz. **MMN, P3a** and **RON** metrics are extracted as per (84,99) (**primary measures: $\mu$V at Fz, 135-205 ms, 250-300 ms and 350-450 ms, respectively**). For **ASSR**, gamma evoked power and phase locking are assessed in response to 250 1-ms clicks presented in 500-ms trains at a frequency of 40Hz, played with an inter-train interval of 1-s, as per (39,49,59). Evoked power (**primary measure: mean $\mu V^2$ at Fz (0-500 ms)**) and phase locking (**primary measure: mean PLF (0-500 ms)**) are calculated using wavelet transformation of the segmented data across the 30-50 Hz frequency layer, as per (59). Exact Low Resolution Electromagnetic Tomography Analyses (eLORETA) uses voxel-by-voxel comparisons to localize significant drug effects on ERP neural sources, as per (109,124).

**Exploratory genetic analyses:** Alleles in specific GRIN-2A, -2B, -2D and -3A SNPs are identified in DNA isolated from venous blood via RFLP after PCR amplification. Allele frequencies will be compared with published reports (eg. 126), including our own study (5). The rationale for studying these genes is described above, based on their potential impact on MEM sensitivity in humans and animal models (5,52,80), and/or with AD (54,97). In addition to GRIN2B (rs10845840) and 3A (rs1337697, rs3739722), specific GRIN2A and 2D SNPs will be selected based on available functional and MAF data (eg. 51,52,80,126) and guidance from B. Rana, and tested to determine if they moderate either MEM effects on EAIP, or the impact of the "MEM-sensitive" EAIP phenotype on clinical measures (below). We recognize that glutamate-related genotypes are implicated across normal and aberrant cognitive phenotypes; the rationale for interrogating these genes is based **specifically** on **empirical findings** described above, and otherwise is viewed as "**agnostic**" to mechanisms of MEM action or the role of glutamate in the pathology of AD. If data support a moderating role for any SNP(s), this information will be viewed in the broader context of potential neurochemical interactions, and of glutamate as a moderator of neurocognitive function and dysfunction.

**Statistical Analyses - General Approach:** All analyses are conducted in the general linear model and mixed model frameworks (32). Consistent with standard recommendations (10), violations of statistical assumptions will be identified using statistical tests and diagnostic plots. When appropriate, robust standard errors will be

used to derive test statistics. Outliers will be detected using Cook's distance. Missing data over time will be handled by analyzing all available data (assuming data are missing at random) and by using full information maximum likelihood estimation, or through multiple imputation of data. Analyses are conducted using R.

**Statistical tests for each hypothesis:**

**Aim 1:** Repeated measure (Rm)-ANOVAs will determine whether PPI, MMN and ASSR significantly differ after MEM vs. PBO (Hypothesis 1). Primary variables for each EAIP, as well as exploratory "secondary" measures, are identified above. Based on a recruited sample of 88 total participants, we will have 80% power to detect effect sizes of d = 0.35 (small) using a Bonferroni corrected α = .05/3 (2-tail). Heterogeneity of response will be characterized using variance components expressed as intraclass correlation coefficients (ICCs).

**Aim 2:** Linear mixed-effects models regressing change in ADAS-cog outcome scores (at 8, 16, and 24 weeks vs. baseline) onto a fixed effect of group defined by MEM sensitivity will test the hypothesis that patients exhibiting a larger "MEM effect" on EAIP measures will exhibit a larger positive clinical response to MEM. Groups will be defined by a median split of "MEM effect" (MEM minus PBO) on EAIP. Power was estimated using methods described by Hedeker et al. (25) solving for sample size with α = .05 (2-tail), test-retest correlations of .50, and 5% attrition at each time point. A sample of 88 total participants provides us with 80% power to detect a medium effect of d = 0.50. The primary outcome will be chosen based on effect sizes obtained as part of Aim 1. In exploratory analyses, we will also examine group by time interactions using orthogonal polynomial contrasts. We will also predict change in clinical outcomes as a function of all four candidate biomarkers in order to create an optimal prediction equation. We will perform ROC curve analyses using EAIP MEM sensitivity values as continuous variables and predict clinical improvement after MEM as a discrete outcome (responders vs. non-responders) in order to determine the clinical utility of this approach defined in terms of sensitivity and specificity. Secondary analyses will also be conducted with biomarker groups defined as "MEM-sensitive vs. –insensitive" via specific thresholds for EAIP MEM sensitivity.

**Multiple analytic strategies:** The critical test of H2 will utilize both continuous and discrete analyses. Treating EAIP MEM sensitivity as a **continuous variable** will enable us to identify moderating factors (i.e. factors moderating the relationship between EAIP MEM sensitivity and clinical MEM sensitivity) with greater power. By identifying these moderators, and their relative contributions to the biomarker-clinical response relationship, *this continuous approach will potentially be more informative regarding the mechanisms by which EAIP MEM sensitivity is related to MEM's clinical impact.* Compared to ANOVA, a linear mixed-effects model is also relatively less impacted by missing data.

Treating MEM sensitivity as a **discrete variable** will enable us to divide the patient sample via a specific threshold, eg. median split, based on EAIP MEM sensitivity (Fig. 3). Exploratory analyses will test more or less stringent thresholds, aiming to optimize the positive and negative predictive value of this discrete threshold. *This approach has the potential practical value of establishing preliminary criteria for distinct MEM-sensitive and MEM-insensitive clinical subgroups.*

**Secondary / Exploratory Analyses:** To test H2, we will examine multiple metrics of **EAIP MEM sensitivity**:
1. **primary** measures from each individual EAIP measure (described above);
2. **secondary** composite variable based on these 4 primary measures, generated via multiple regression;
3. **exploratory** measures of EAIP: P3a amplitude and RON (described above).

An underlying premise of H2 is that the neurobiological bases of MEM effects on the Aim 1 EAIP measures share some amount of variance, and hence positive and negative predictive value, with the clinical therapeutic response to MEM in AD. However, MEM effects on these 4 EAIP primary measures likely reflects its actions on (overlapping but) non-identical neural substrates; this is consistent with findings that MEM sensitivities on these measures are positively correlated for some (MEM effects on MMN and ASSR (65)) but not other measures (MEM effects on PPI and MMN (99)). *Which of these primary measures, or composite measure, provides the best prediction of MEM effects on AD-relevant neural substrates, and hence provides the best prediction of MEM's therapeutic sensitivity, is an important empirical question that we will address in Aim 2.*

Analyses to test H2 will also utilize primary, secondary and exploratory metrics of **MEM's clinical effects**:
1. **primary** outcome measure: ADAS-cog, change from baseline;
2. **secondary** outcome measures: NPI-Q and Geriatric Depression Scale (change from baseline);
3. **exploratory** outcome measures: ADAS-cog factor-derived subscales (change from baseline) (eg. 81).

Factors that moderate the relationship of the primary biomarkers (EAIP MEM sensitivity) and clinical outcome will also be explored, including: 1. Genetic markers (GRIN, APOE, described above); and 2. Patient characteristics (eg. age; past AD medications; baseline (T0) neuropsychological and outcome measures).

Lastly, we will probe the specific anatomical basis of MEM effects on MMN and ASSR using **source locali-**

**zation techniques** (109,124); conceivably, these analyses will provide information more generally relevant to the anatomical basis for MEM's therapeutic actions, i.e. the "mediators of the therapeutic intervention", as described in this PAR. A simple model for such mediation might be: "MEM action on 'structure X' ➔ EAIP change ➔ enhanced therapeutic sensitivity," but more complex, circuit-based models will also be explored.

For simplicity, this application associates greater "EAIP MEM sensitivity" with an EAIP-enhancing response to MEM challenge (eg. EAIP (MEM minus PBO) > median value); this approach is parsimonious because arithmetically larger EAIP values are generally associated with positive neurocognitive and functional change (21,56,58,64,111). However, one might rationally argue that *any significant change in response to MEM challenge reflects "MEM sensitivity", and thus directionality of the MEM response should not be assumed.* Thus, while we represent arithmetically larger MEM responses as "MEM-sensitive" in this application, Aim 2 analyses (linear mixed-effects model) make no assumptions of directionality; exploratory categorical analyses will also define "MEM sensitivity" based on the absolute change in EAIP amplitude (i.e., positive or negative).

**Important questions related to experimental design and analysis:**

**Does Aim 2 depend on Aim 1?** *What if MEM does not significantly enhance EAIPs in the inclusive group of AD patients?* While our strong *a priori* hypothesis is that MEM will significantly increase PPI, MMN and ASSR in AD patients, this prediction is based on findings in HS and SZ patients (29,44,65,99,107), and secondarily, from findings in rats (107). Conceivably, brain circuitries that mediate the EAIP response to MEM might be damaged in AD, and hence MEM effects on EAIP measures may be blunted and not reach statistical significance across the inclusive group of AD patients. Importantly, for testing H2, the key outcome of Aim 1 will be *a heterogeneous EAIP response to MEM*, since the ability to detect significant moderating effects of MEM sensitivity on clinical response in Aim 2 relies on the *shared variance* between EAIP MEM sensitivity on the one hand, and clinical MEM sensitivity on the other. Thus, neither the ability to test H2, nor its importance, is diminished by an absence of a statistically significant effect of MEM in AD patients in Aim 1. *In fact, a lack of MEM response in Aim 1 might be a particularly important (negative) predictor of MEM clinical sensitivity, precisely because it reflects AD-related damage to brain substrates required for MEM's therapeutic effects.*

**Why test only 20 mg MEM vs. EAIP?** The PI has tested the effects of 10, 20 and 30 mg MEM po in HS, and 10 and 20 mg MEM po in SZ patients. Based on these studies, the 20 mg dose was selected for this application, because it produced significant increases in PPI (107) and MMN (44) in HS, and in PPI, MMN and ASSR in both HS and SZ patients (65,99) (Fig. 2). A 10 mg dose of MEM was **inactive** in all 3 measures of EAIP (65,99) and 30 mg was associated with dizziness in HS (107). Thus, there is a strong prediction that a 20 mg "challenge dose" will generate a significant effect of MEM on EAIP performance, and thereby confirm that the intervention is "bioactive". We expect to find that 20 mg MEM (vs. PBO) produces EAIP-enhancing effects in AD patients this application; such a "replication" of our previous findings in HS and SZ patients will allow us to most easily interpret the relationship between MEM-induced changes in EAIP and clinical response to MEM in AD patients. *However, even if no significant effect of MEM on EAIP performance is detected across the full AD cohort, it is possible that EAIP MEM sensitivity would still be predictive of a clinical response to MEM in a subgroup of patients (eg. those with "MEM effects" greater than a specific threshold), and that mixed effect models would still detect meaningful moderators of MEM clinical sensitivity.*

We will re-evaluate the choice of the 20 mg dose of MEM based on our experience with **the first 6 test subjects**. For example, despite its benign effects in HS and SZ patients (eg. no effect on vital signs; (99)) and other patient populations up to a 40 mg po "challenge dose" (24), it is conceivable that a single 20 mg pill of MEM might produce different effects (eg. sedation) in our AD cohort. If this occurs, after discussion with, and approval of, our NIA Program Officer, we may reduce the challenge dose to 10 mg MEM: this dose reduc-tion would be defensible since the effects of a "challenge dose" of MEM on EAIP might predict an individual patient's clinical response to MEM, even if that dose does not significantly enhance EAIP performance across an inclusive group of patients. **Body weight** will also be used to calculate a precise **mg/kg dose** for each subject, for post-hoc correlations with the magnitude of MEM effects on each measure. *Ultimately, to make this biomarker "scalable" to clinical practice, it will be important to streamline the "challenge dose" assessment, and to minimize dose requirements; such "fine tuning" would be the goal of a future application.*

**Why test only 20 mg daily as a therapeutic dose?** This dose is the one most commonly studied in AD trials (40); it has been shown to enhance, and/or slow the decline of, cognition in mild-to-moderate severity AD, using the primary outcome measure and treatment duration proposed for this study (3,14,79,81). This dose of MEM is generally well-tolerated, and while we anticipate non-serious adverse events (AEs), several large studies document that the rate of AEs at this MEM dose is not different from the placebo rate (3,14,40,82).

**Why MEM monotherapy?** MEM is often used clinically in combination with an AChE-I (eg. donepezil). This study excludes patients at study entry who are already taking an AChE-I for 3 reasons: 1. We have no evidence that the critical biomarkers – MEM-enhanced EAIP responses – will be present in patients who are

taking an AChE-I; this would significantly complicate interpreting a negative finding in Aim 1. In fact, studies in laboratory animals and HS report complex and drug-dependent effects of AChE-I on each of the Aim 1 EAIP measures (27-9,42); 2. MEM added to an AChE-I in mild-to-moderate severity AD has failed to produce clinical gains (82); without evidence of positive therapeutic impact in the absence of biomarker stratification, it will be difficult to interpret a negative findings in the present study; 3. The mechanistic interpretation of any positive findings in the present study will be much more complex if patients are using a combination regimen. Nonetheless, we recognize that patients may opt to add an AChE-I to their MEM regimen in the course of this study. If this occurs, and patients also remain adherent to MEM, outcome measures will be collected analyzed with a grouping structure that distinguishes them from patients who remain on MEM monotherapy.

Parenthetically, MEM significantly enhanced EAIP measures in SZ patients, despite their use of complex medication regimens, including antipsychotics, antihypertensives and anticholinergics (99). This suggests that complex medication regimens in AD patients should not obscure the primary "biomarker", with the possible exception of AChE-I's, which are thus an exclusion criterion.

**Why 24 weeks of treatment?** In several studies, MEM benefits vs. placebo in mild, moderate and severe AD first become evident by week 8; by week 24, clinical symptoms in MEM-treated patients have typically returned to pre-MEM baseline, though their progression is effectively delayed by 24 months, compared to placebo-group patients (eg. 81). Thus, while our primary analyses will assess whether the clinical response to MEM is **larger** in EAIP-defined "MEM sensitive" vs. "MEM-insensitive" patients, a 24-week study should also enable us pursue secondary analyses to determine whether "MEM-sensitive" individuals experience clinical gains from MEM that are **more rapid** or **more durable**, compared to "MEM-insensitive" individuals.

**How will we address "incomplete" (MEM dose < 10 bid) or discontinued subjects?** It is possible that the proposed biomarkers (eg. "MEM-insensitive" EAIP) will predict the likelihood to *discontinue* MEM treatment. All subjects who complete T0 outcome measures will be considered "valid". Based on published data (Fig. 7; (81)), subjects who complete at least 8 weeks (10 mg bid) will be considered "complete", and included in the primary Aim 2 analyses. Data from all "valid" subjects will be used in separate analyses to understand how MEM EAIP sensitivity impacts the likelihood to "complete" the MEM trial, vs. remain at doses < 10 mg bid, discontinue MEM or add an AChE-I. MEM costs are budgeted through week 24 for all subjects.

**Why Mild-to-Moderate AD?** This application is designed to test the hypothesis that, nested within a heterogeneous cohort is a subgroup that will be identified via the proposed EAIP measures as "MEM-sensitive", and that this "enriched" subgroup will exhibit a strong positive clinical response to MEM (Fig. 1). MEM is FDA-approved for use in patients with moderate-to-severe AD; the efficacy of MEM at these middle and late stages of AD has been demonstrated in several studies, without the use of predictive biomarkers. *Testing our hypothesis (H2) will be most feasible in an AD population that is NOT maximally sensitive to MEM.* At the other extreme, patients with MCI do not appear to benefit from MEM (186); it is likely that in the present study design, a low baseline level of symptoms in MCI could obscure a positive clinical response, even among a biomarker-defined "sensitive" subgroup. While MEM is commonly prescribed to patients with Mild-to-Moderate AD, its benefits appear to be modest among cohorts that are not stratified by predictive biomarkers (eg. Cohen's d in mild-to-moderate AD at 8 weeks = 0.3 (81)). We are thus proposing to test patients with mild-to-moderate AD, based on the expectation that such a cohort will include a subgroup of MEM-sensitive individuals, and that detecting a clinical response in these patients will be facilitated by the fact that baseline symptoms are within a dynamic range, i.e. neither at extreme "floor or ceiling" levels. Importantly, MEM is FDA-approved for use only in moderate-to-severe AD based on documented efficacy **(rather than any increased risk of adverse events in mild-to-moderate severity patients)**. Thus, we anticipate that MEM use in Aim 2 will not incur any added risks to the well-being of the study participants.

**Why no "control" group?** Two potential "control" groups are not included in this design. First, no parallel HS group is included to confirm the predicted effect of MEM on EAIP measures during laboratory testing. Because such findings in HS have already been reported by our group, and extended in various forms by other groups (discussed above), we view a re-replication as unjustifiable in terms of effort or cost, particularly because the outcome of HS testing would not directly alter the interpretation of the key results (prediction of clinical response in AD patients). Second, no parallel AD treatment group is proposed. Thus, it is conceivable that the EAIP response to MEM might reflect a degree of neuroplasticity or other biological characteristic within AD-relevant brain substrates, that is a *general predictor* of a positive clinical response to other drugs, eg. AChE-I's, rather than a *specific predictor* of a therapeutic response to MEM. While we considered this possibility, we felt that the most cost-effective and biologically parsimonious approach was to test whether EAIP MEM sensitivity predicts clinical MEM sensitivity; a positive finding might justify studies in a future application, testing similar predictive value in combined regimens (MEM+AChE-I) as well as AChE-I monotherapy.

**FOA-specific information:** The use of MEM in AD has been the focus of many large multi-site studies; this application will not broadly address MEM's therapeutic role, i.e. its mechanism of action, the role of NMDA in

AD pathogenesis, or the predicted optimal disease stage for MEM use. The focus of this application is to test the predictive value of EAIP MEM sensitivity on its therapeutic impact in AD. Justification for experimental parameters is provided above, including treatment dose, duration and target population; we do not propose fundamental studies of MEM's clinical effects, eg. pharmacodynamics, because these are well established, though we plan to store blood for future tests of potential predictive biomarkers. Our plans for future clinical development are described briefly above, and include: 1. Tests that include patients using combination regimens (MEM + AChE-I); 2. Tests with patients at earlier stages of illness; 3. Tests with other AD therapeutics for which an acute EAIP-based laboratory biomarker is available.

**Sex as a biological variable:** Sex will be included as a grouping factor in all major analyses; groups will be sex balanced as described in "Human Subjects". The PI was the first to report sex differences and menstrual cyclicity of PPI in HS (102), and some EAIP sex differences may persist into late life (48). No sex differences in **MEM effects** on PPI, MMN (99) or ASSR (65) were noted in our studies (Fig. 2).

**Timeline** (Table 2)**:** Screening 114 subjects/57 mo. (2 subjects / mo.) is well within the capacity of our referral sources in the UCSD Memory and Geriatric Medicine Clinics, ADRC and affiliated community network (cumulative flow >>15 new AD patients/wk). Proposed EAIP testing rate (< 4 tests / mo.) is well within our capacity (typical rate 16 tests / mo.). Thus, subject recruitment and test rate should meet the demands of the proposed "N". EAIP methods are currently in use in our lab, so no new training, equipment or infrastructure is needed. At a rate of ≈1.5 completed subjects/mo., we will reach our target in 57 months, leaving 3 months for genotyping, data analysis and writing. Interim analyses at ≈6 months will confirm the fidelity of all measures and potentially adjust MEM test dose (see above), and at 36 months will guide a future NIA application.

**Key benchmarks of success will include:** 1. Recruitment and clinical and neurocognitive assessment of 88 individuals with Mild-to-Moderate AD (M:F ≈ 44:44). 2. Completion of two days of EAIP testing in study subjects, one week apart, in a double-blind, cross-over design using PBO vs. 20 mg MEM. 3. Titration of MEM to target dose (10 mg bid). 4. Repeated clinical and neurocognitive assessment after 8, 16 and 24 weeks of MEM treatment. 5. Completion of proposed analyses of EAIP and clinical data to test hypotheses, H1 and H2.

**Potential Limitations:**

**Table 2. Projected screening and completion "n"**

| Study Month | 12 | 24 | 36 | 48 | 57 |
|---|---|---|---|---|---|
| Screen | 24 | 48 | 72 | 96 | 114 |
| EAIP Tests | 21 | 43 | 64 | 86 | 103 |
| Wk 8 measures | 20 | 40 | 60 | 81 | 98 |
| Wk 16 measures | 19 | 38 | 57 | 77 | 93 |
| Wk 24 measures | 18 | 36 | 54 | 73 | 88 |

**1. Recruitment:** One concern is the possible difficulty recruiting sufficient numbers of individuals with mild-to-moderate severity AD. A strength of this application is its intimate ties to active AD clinics and ongoing studies with large samples of well-characterized older adults, many of who have been diagnosed with AD, through collaborations with the UCSD Memory Clinic and Geriatric Medicine Outpatient Clinic, the UCSD ADRC and its affiliated networks throughout San Diego. Recruitment needs for this application are ≈ 2 new subjects / mo. (see "Timeline" and Table 2), with a projected 10% attrition at screening, and 5% attrition at each of the subsequent points of study contact (EAIP testing, weeks 8, 16 and 24), based on past experience and ongoing studies. The target population (mild-to-moderate AD) is not a focus of most ongoing UCSD studies, which prioritize MCI and Pre-MCI subjects; thus, availability of AD patients for this application will support much higher recruitment needs if attrition exceeds projected rates.

**2. EAIP measures in AD patients:** Our published reports of MEM effects on EAIP measures involved HS and SZ patients, ages 18-55 (65,99). The present application proposes such measures in patients with mild-to-moderate severity AD, ages 50-70 y; thus, both diagnosis and age of the proposed cohort differ from our past experience, and may present unexpected challenges. Importantly, our laboratory group has published extensively on EAIP measures across the full lifespan (eg. ages 18-88: (16)), and in populations with severe dementia and behavioral disturbances (eg. Huntington's Disease (104)), and recently completed a study of EAIP measures in 20 patients with mild-to-moderate severity AD (G. Light, PI). Thus, neither the clinical symptoms nor age of the proposed cohort should limit the acquisition or interpretation of EAIP measures.

**3. Expertise:** The PI was Co-Director of the UCSD GHPP Clinic for 10 years (serving patients with Huntington's Disease (HD) and Hereditary Ataxias), and has been a clinically active Board Certified Psychiatrist for 24 years. While he has never had a primary role in AD clinical research, the PI brings to Aim 1 over 30 years of studies of EAIP measures, including studies of MEM effects on EAIP measures in rodents, HS and patients with SZ, and EAIP studies in patients with HD, Tourette Syndrome and other brain disorders (100,104). He has published extensively on the development, validation, interpretation and conceptual implications of EAIP measures as biomarkers in HS and patient populations (cf. 60-3,108). Importantly, the PI has assembled a team with expertise in AD, its assessment and treatment, and the conduct of clinical trials in AD. This team includes active Co-I's of Drs. Greg Light, Michael Thomas, Lisa Delano-Wood and Jairo Romero, who collectively have significant expertise in these areas (see Biosketches) as well as the active collaboration of the leadership of the UCSD ADRC (Drs. Galasko and Salmon) and ADCS (Drs. Oltersdorf and Huisa-Garate) (see Letters).

**Protection of Human Subjects**

(1) The final subject population for the present application will include approx. 88 adults, ages 50-70, carrying a diagnosis of AD, mild-to-moderate severity (based on a Mini-Mental Status Examination (MMSE) score of 10-22. Rationale for selection of this level of AD severity is discussed in the Research Strategy. The representation of males and females will be roughly equal; sample size is based on power analyses described above. We anticipate that, in order to complete testing in 88 subjects, we will need to "consent" and screen approximately 114 subjects; the remaining subjects will be excluded based on specific screening results, or self-initiated study withdrawal.

Subjects are recruited through: 1) the UCSD MARC and Medicine for Seniors Clinic, under the supervision of Lisa Delano-Wood, PhD and Jairo Romero, MD (Geriatric Internal Medicine physician; Co-I), (typical clinic flow: 8 new AD patients/week); 2) the UCSD ADRC (see below and accompanying letter; typical evaluation of new AD cases = 5/week); and 3) community providers within a network affiliated with the UCSD ADRC (e.g., Glenner Center; Alzheimer's San Diego; Southern Caregivers Resource Center). Subjects are only approached for study participation if they express an interest in initiating MEM therapy to treat their symptoms of AD, they have not previously been treated with MEM, and they are not currently taking another medication for that purpose.

In essence, for a patient who has decided to initiate a trial of MEM for treatment of their AD symptoms, this study involves the following added steps of: 1) a thorough screening evaluation; 2) Test days 1 and 2, for EAIP measures; 3) weekly calls by the Study Coordinator once MEM treatment is initiated, to assess adherence and off-target effects, and 4) three 2-hour symptom assessments (weeks 8, 16 and 24). Otherwise, once MEM titration has begun, all decisions regarding MEM use are made solely to address the clinical needs of the patient, based on the assessment of the treating physician. *This use of flexible, individualized dosing and "real life conditions" to gauge MEM effects emulates features of a pragmatic clinical trial (PCT).*

Screening assessments will exclude subjects for current substance abuse or history of other significant medical illness (e.g. cancer, diabetes, heart disease, HIV, seizures), open head injury or closed head injury with loss of consciousness > 1 min, or hearing impairment. A screening physical examination (with laboratory measures as needed) is conducted by a licensed physician to rule out significant medical illness, including EKG abnormalities and positive urine toxicology findings of recreational drugs. Audiometric testing is used to carefully assess hearing in all subjects, with strict exclusion criteria. These rigorous screening measures have been applied towards the testing of AD patients through the UCSD ADRC for decades. Appropriate subjects are scheduled to come to the UCSD Medical Center, Clinical Teaching Facility (CTF); once at the CTF, the study is described again. Females of childbearing potential will undergo a urine pregnancy test to rule out pregnancy; urine toxicology, and pregnancy testing in females, will be repeated in each subsequent visit for these patients. Payment is $15/hour: $60 for the Screen Day (approx. 4 hours), $120 for Test Day 1 (approx. 8 h) and $120 for Test Day 2 (approx. 8 h), and $30 for clinical assessments (weeks 8, 16, 24: approx. 2 hours). This payment is required to maintain subject flow and thereby avoid cost-ineffective "down time". Study medications are provided through 24 weeks at the maximum proposed dose (10 mg bid). Subjects and their caregiver will be driven to and from Test days 1 and 2 at the UCSD Medical Center by an established patient transportation service, at no cost.

(2) Records and data will be rigorously protected, as described below. Historical and questionnaire data and EAIP measures will be obtained; urine will be obtained for toxicological analysis as part of the subject exclusion process. Blood will be obtained by venopuncture for determination of the subjects' GRIN2A, 2B, 2D and 3A polymorphisms, and genotypes of APOE. Each subject is assigned a unique identification code and all data are entered into our database under this code number in order to protect privacy. The hard copy of the data (i.e., forms and source documents) is kept in a locked file in a secure room with access limited to study personnel. Medical information is made available to the subject's physician upon written request for release of information.

(3) Subject recruitment and informed consent procedures follow the PI's established methods, approved by the UCSD Human Subjects IRB. In all cases, fully informed consent is obtained by a trained technician or the PI or Co-PI's (Drs. Romero or Delano-Wood), and the PI will be directly available to clarify questions. Signed and witnessed consents are kept on file with other subject data. The UCSD IRB has authorized no waivers or modifications of normal procedures.

(4) Potential risks are minimal. **Methods for MEM administration for EAIP testing have already been vetted and approved by an NIH clinical trials review committee (ITVA) for use in PI's studies in HS and SZ patients from MH093453 and MH94320.** The rating scales and questionnaires are innocuous. Neuropsychological testing is standard for these research clinics and is not burdensome to patients or caregivers; it includes: the Clinical Dementia Rating (CDR), the Montreal Cognitive Assessment (MoCA), Functional Activity Questionnaire (an IADL scale), the Health & Safety and Managing Money subscales of the Independent Living Scales, and the modified Lawton physical self-maintenance schedule (a basic ADL scale).

Startle and electroencephalographic testing exposes subjects to the application of skin tape electrodes and to brief loud sounds, which in >25 years of testing have caused no side-effects. Alternative measures have been considered; the selection of these specific EAIP measures reflects the significant advantages of applying towards these studies the substantial body of information generated by > 25 years of systematic studies of the startle reflex, MMN and ASSR in humans and laboratory animals. The drug (MEM) at the single dose (20 mg) in this proposal for EAIP testing carries a very small risk of toxicity or adverse effects. Based on our substantial experience with this and higher doses (170,178), and experience of others using 40 mg as a "challenge dose" (24), adverse reactions are not likely; such effects should be identified in initial tests, permitting dose reductions for subsequent tests. The only subjective effect associated with the 20 mg dose in healthy subjects (178) was a small increase in self-rated "happiness"; a similar trend was detected in SZ patients (170), with no other subjective effects detected. No significant effects of MEM (20 mg) were detected on heart rate or blood pressure in our past studies with HS or SZ patients (170); this includes SZ patients who were in their early 50's, with complex medication regimens (typically 4-5 different medications, including 2-3 psychotropic meds, antihypertensives, a diuretic and an oral hypoglycemic), and the typical stigmata of the illness that include metabolic syndrome, hypertension, tobacco dependence and its accompanying medical sequelae. Alternative drugs have been considered, and the proposed drug was selected, based on: 1) existing data with this drug, dose and measures; 2) its effects on specific neurochemical substrates being investigated; 3) its lower likelihood of producing toxicity or significant side-effects, compared to other drugs with related pharmacological properties.

The target MEM dose (10 mg bid) for Aim 2 is standard in terms of clinical use (see "Research Strategy"). MEM has been carefully studied in >120 NIH clinical trials (ClinicalTrials.gov). Its cumulative clinical sales exceed 1 billion dollars, with over 1 million prescriptions written, with an outstanding safety record (66,69).

(5) The overall risks of this proposal are small. MEM will not be administered to subjects in whom it is contraindicated. Aim 1 studies will be conducted at the UCSD Medical Center, under the direct supervision of a licensed physician. To address the potential for MEM to produce changes in heart rate or blood pressure, all subjects' vital signs will be carefully monitored, and subjects will not be released from the laboratory until their vital signs are within normal limits. To address the possible side effect of somnolence, subjects will arrange for their transportation from the hospital on test days. All women of child-bearing potential will 1) be tested in early follicular phase; 2) have negative urine pregnancy tests at each visit, and 3) agree to utilize double-barrier contraception throughout the duration of study participation. If a female of childbearing potential becomes pregnant during the study, the subject will be exited from the study. The investigator will determine whether the subject was exposed to active study drug or placebo, and notify the subject of this information (see also "Inclusion of Women and Minorities"). Drugs will be dispensed by a licensed physician or pharmacist, and a licensed physician will be present on the premises at all times during testing to assess and address potential adverse drug effects, and if needed, to administer medical assistance to the test

subject. MEM effects on PPI, MMN and ASSR performance have been studied in > 120 HS and patients in the PI's laboratory without a single adverse event. Protection of subject confidentiality and privacy will be rigorously guarded by the assignment of coded numbers to each file in the computer analysis and database. The PI's UCSD psychophysiology research group has studied over 5000 HS and patients without any problems with confidentiality.

The dose selected for use in these Aim 2 studies is the most common therapeutic dose for MEM when used clinically, and the proposed titration in 5 mg increments is also standard practice with MEM. Clinical care will be managed by the patients' usual physician; medication adjustments – including dose of MEM, and addition of other medications – will not be altered by study participation. MEM is FDA-approved for use only in moderate-to-severe AD based on documented efficacy **(rather than any increased risk of AEs in the mild-to-moderate severity patients)**, and its use in mild-to-moderate severity AD is standard practice in the community. Thus, we anticipate that MEM use in Aim 2 will not incur any added risks to the well-being of the study participants.

(6) It is anticipated that the proposed studies will yield important, new information about a new clinical intervention of direct relevance to several neuropsychiatric disorders. Thus, these studies have a significant potential for providing major gains in our understanding of the basis and optimal treatment strategies for a disorder (AD) that is severe and common. Because subjects in these studies will not be treated differently on the basis of their Aim 1-identified EAIP biomarkers, this information will not be of direct benefit to them. Nonetheless, due to the prevalence of AD in our community, it is anticipated that AD subjects will benefit indirectly - through a cascade of familial and societal benefits - from the information generated by these studies. Furthermore, based on their awareness of the potential importance of this work to their community, all subjects will benefit in areas of self-esteem and self-understanding. Information from physical examinations, and screening measures, may also be of direct benefit to test subjects. The overall risks of the proposed studies are low, as described above. Thus, on balance, the risk/benefit ratio of the proposed studies is very low.

**Data and Safety Monitoring Plan and Board:**

**1. The Data Safety and Monitoring Plan (DSMP)** for this application is commensurate with the **low risks and complexity of this study.**

It is important to note that the **"pragmatic"** treatment phase of the proposed study includes the following features, which in some cases obviate the typical role of a DSMB:

1. **All subjects receive treatment with the same FDA-approved treatment** for AD (memantine (MEM)) using a dose titration schedule that is consistent with common clinical practice. Thus, the **treatment phase is not blinded**, and thus there is no opportunity to utilize a DSMB for purposes related to study blinding or unblinding;
2. All adjustments to MEM dose and other medications are done **by the treating physician, based on the clinical needs of the patient**, and are **not constrained by experimental design**;
3. **No placebo control or other patient comparison group** is utilized: analyses rely on groups distinguished by biomarkers identified prior to initiation of MEM therapy;
4. The study is **not designed to test the therapeutic benefits of MEM in AD per se**, as this issue has already been repeatedly addressed in large, multi-site clinical trials.

While the proposed therapeutic intervention is not experimental, and has had longstanding FDA approval in AD, this application nonetheless meets NIH criteria for a "Clinical Trial", defined in "Notice of Revised NIH Definition of 'Clinical Trial". Thus, a DSMP is described below, and a DSMB is available through faculty within the UCSD ADCS. Plans for implementing this DSMB, should this application be funded, are described below and in a supportive letter from the ADCS faculty (attached).

**Potential Risks**

The potential risks to study participants include:

1. Venopuncture: During the initial assessment phase, blood will be collected for assessing patient's health status, and for study-related genetic markers. With any venopuncture procedure, there may be temporary slight discoloration of the skin.

2. Aim 1 "test dose" of placebo or MEM, 20 mg po: The drug (MEM) at the single dose (20 mg) in this proposal for EAIP testing carries a very small risk of toxicity or adverse effects. Based on our substantial experience with this and higher doses (99,107), adverse reactions are not likely; such effects should be identified in initial tests, permitting dose reductions for subsequent tests. The only subjective effect associated with the 20 mg dose in healthy subjects (107) was a small increase in self-rated "happiness"; a similar trend was detected in SZ patients (age range: 18-55) (99), with no other subjective effects detected. No significant effects of MEM (20 mg) were detected on heart rate or blood pressure in our past studies with HS or patients (99). Significantly higher "challenge" doses of MEM have been studied in other patient populations without adverse effects (eg. 40 mg po (24)). Alternative drugs have been considered, and the proposed drug was selected, based on existing data with this drug, dose and measures, including its effects on specific biomarkers being investigated. Subjects will be driven to and from test days by an established patient transport service, at no cost.

3. Aim 1 electroencephalographic (EEG) and electromyographic (EMG) measures: EEG and EMG testing exposes subjects to the application of skin tape electrodes and to brief loud sounds, which in >25 years of testing have caused no side-effects. Alternative measures have been considered; the selection of these specific EAIP measures reflects the significant advantages of applying towards these studies the substantial body of information generated by > 25 years of systematic studies of the startle reflex, MMN and ASSR in humans and laboratory animals.

4. Aim 2 titration and ongoing treatment with MEM: The dose selected for use in these Aim 2 studies is the most common therapeutic dose for MEM when used clinically, and the proposed titration in 5 mg increments is also standard practice with MEM. Importantly, this dose and titration schedule is not associated with adverse event rates in excess of placebo rates in several large AD studies (eg. 3). Clinical care will be managed by the patients' usual physician, with medication adjustments –

including dose of MEM, and addition of other medications – unaffected by study participation. MEM is FDA-approved for use in moderate-to-severe AD, and its use in mild-to-moderate severity AD is standard practice in the community.

**Potential Benefits**

The potential benefits to study participants include:

1. A thorough diagnostic assessment to identify alternative, potentially treatable etiologies of cognitive decline.
2. Careful symptom monitoring at weeks 8, 16 and 24 of MEM treatment might identify clinically relevant changes in symptoms.
3. Because subjects in these studies will not be treated differently on the basis of their Aim 1-identified biomarkers, this information will not be of direct benefit to them. Nonetheless, due to the prevalence of AD in our community, it is anticipated that AD subjects will benefit indirectly - through a cascade of familial and societal benefits - from the information generated by these studies. Furthermore, based on their awareness of the potential importance of this work to their community, all subjects will benefit in areas of self-esteem and self-understanding. Information from physical examinations, and screening measures, may also be of direct benefit to test subjects.

**Adverse Event (AE) and Serious Adverse Event (SAE) Monitoring and Reporting**

AE and SAE Monitoring:

Aim 1: For EAIP testing, subjects are monitored during their study sessions at the UCSD Medical Center, as described in “Research Strategy”. While on the premises during the remainder of the screen day and both testing days, subjects are accompanied by a trained laboratory assistant, who regularly inquires about their subjective well-being and is trained to observe subjects for any changes in behavior; the protocol calls for the on-site covering MD to be notified if the subject either complains of discomfort or is noted to have a change in behavior. Both vital signs (heart rate, blood pressure) and subjective ratings (visual analog scale) are obtained at regular intervals on test days; the protocol calls for the covering MD to be notified if vital signs fall outside of established parameters. Subject are not discharged from the test days until their vital signs are within parameters established within the IRB-approved protocol.

In tests using this identical design with over 100 subjects to date in our laboratory, including this drug and dose (or higher dose), there has not been any adverse changes in behavior or vital signs in any test subject.

Aim 2: Primary and secondary outcome measures will be assessed at study weeks 8, 16 and 24 at clinic visits, including the ADAS-cog, the Neuropsychiatric Inventory (NPI-Q) and the Geriatric Depression Scale.

2. Frequency of monitoring, including any plans for interim analysis and stopping rules (if applicable).

Aim 1: Monitoring can theoretically occur at different frequencies, based on circumstances. Vital signs and visual analog scales (VAS) data of subjects’ subjective states are assessed approximately hourly throughout the test day. In the event that the covering MD is contacted by the study technician and deems it necessary, the subject will be examined immediately. A protocol is established for contacting the study pharmacy and unblinding the MD to the study drug if viewed as necessary by the study MD. In the absence of an AE, the results of each test are monitored by the PI within one week of testing, during weekly laboratory meetings. More generally, as described in the “Research Strategy”, interim analyses of study results are planned as described below.

Aim 2: Subjects will be treated by their primary physician, who will make all medication adjustments according to the clinical needs of the patient. Patients will be contacted weekly by the Study Coordinator to confirm and record medication adherence. Scheduled monitoring for primary outcome measures will be conducted by the Study Coordinator / RA at 8, 16 and 24 weeks; at that time, the Study Coordinator will also record any changes in MEM dose or addition of other medications, and the reasons for such changes.

3. The process by which Adverse Events (AEs), including Serious Adverse Events (SAEs) will be managed and reported as required:

Aim 1: In the acute presentation, any AE or SAE is managed first by the study technician, per our existing IRB-approved protocol. The on-site study MD is contacted immediately, and personally assesses the subject to determine the appropriate management; because the laboratory is located at the UCSD Medical Center, approximately 50 yards from the UCSD Medical Center Emergency Room, subjects can be escorted to this facility for evaluation and management in the event of a more serious AE.

Aim 2: During the treatment phase, patients will be monitored primarily by their treating physician and will be instructed to follow all recommendations of the primary physician in the context of clinical deterioration, including AEs and SAEs. In addition, patients will be contacted weekly by the Study Coordinator as described above, and any concerns related to the patient well-being – and specifically any increased symptoms of depression or suicidality - will be reported to the PI, who will contact the patient, caregiver, and if warranted, the treating physician.

In the aftermath of an AE or SAE (or any other unexpected event), the PI will contact the UCSD IRB by phone, and (in the event of a non-acute event) via submitting a written report to our eIRB service. The UCSD IRB and Research Compliance Program “decision tree” for reporting procedures is found at: https://irb.ucsd.edu/Decision_tree_UPRs.pdf

Reporting of AE or SAE to other agencies, including the OBA and FDA, is accomplished as directed by the UCSD IRB, and described in “UCSD Human Research Protections Program IRB Standard Operating Policies and Procedures Reporting Adverse Events and Unanticipated Problems. Section 3.13 ; Version Date: 3/21/2017. Specifically,

> “The Director, Human Research Protections Program, is responsible for reporting unanticipated problems involving serious risks to subjects, instances of serious or continuing noncompliance with regulations or committee requirements, and any suspension or termination or committee approval, to the US Food and Drug Administration, OHRP and appropriate institutional officials, in compliance with guidance provided by federal regulations and University policy.”

4. The individual(s) or group that will be responsible for trial monitoring and advising the appointing entity.

Given the low risk and complexity of this trial, the default approach is that the PI will responsible for trial monitoring and advising the appointing entity. **This DSMP is consistent with our existing approved omnibus UCSD IRB protocol for human psychophysiological studies, and will be confirmed with our UCSD IRB in advance of enrolling any R01 study subjects for this renewed application.**

However, as noted below, Drs. Tilman Oltersdorf and Branko Huisa of the UCSD ADCS are available to serve as DSMB Safety Specialist and Medical Monitor, respectively, for this study, should NIA program determine that a DSMB is required for this project.

**Protection Against Study Risks**

All subjects are carefully screened to rule out significant medical illness, and MEM is not administered to any individual for whom it is contraindicated. In Aim 1, subjects receive a single pill of MEM (20 mg) prior to laboratory testing, and all dependent measures are experimental rather than health-related per se. As described in “Protection of Human Subjects”, “The MEM dose (20 mg) is a standard daily dose for clinical use. MEM has been carefully studied in >120 NIH clinical trials (ClinicalTrials.gov). Its cumulative clinical sales exceed 1 billion dollars, with over 1 million prescriptions written, with an outstanding safety record (35). In clinical trials in AD patients, with a daily dose of 20 mg, MEM is not associated with increased risk for serious adverse events; non-serious adverse event rates do not generally exceed placebo levels, and are described in “Protection of Human Subjects”. Based on our substantial experience in acute “drug challenge” studies with 20-30 mg of MEM (99,107), adverse

reactions are not likely; such effects should be identified in initial tests, permitting dose reductions for subsequent tests. The only subjective effect associated with the 20 mg dose in healthy subjects (107) was a small increase in self-rated “happiness”; a similar trend was detected in schizophrenia patients (up to age 55) (99), with no other subjective effects detected. No significant effects of MEM (20 mg) were detected on heart rate or blood pressure in HS or patients (99). Higher (40 mg) oral doses produced no adverse effects in other reports (24).

Also as described in “Protection of Human Subjects”, “Startle and electroencephalographic testing exposes subjects to the application of skin tape electrodes and to brief loud sounds, which in >25 years of testing have caused no side-effects.”

Importantly, all study-related assessment and testing is conducted in a safe, hygienic environment by experienced clinicians and laboratory staff. Supervising investigators and staff have many years, and in some cases decades, of experience with the proposed measures in clinical populations.

Aim 2 involves treatment of all subjects with MEM monotherapy, in a manner (titration rate, final dose) that is common practice in clinical settings. This medication regimen has had longstanding FDA approval for use in moderate-to-severe AD; its use in mild-to-moderate severity AD as proposed here is both common community practice, and has been the focus on many large, multi-site studies (eg. 3,14,40-1,79,81,86). **There is no placebo control group or blinding during this phase, and no restrictions on medication adjustments based on clinical need, and thus none of the procedures are viewed to carry any added experimental risk beyond that encountered in standard community clinical practice.** Events that would preclude a participant from continuing with the intervention are based entirely on the clinical judgment of the patient’s physician in consultation with the patient. There are no constraints on this process imposed by the study design. Subjects who finish baseline (T0) and biomarker (Aim 1) testing will be considered “valid”, and subjects who finish 8 weeks of MEM treatment and week 8 outcome measures will be considered “complete”.

Informed Consent Process: Informed Consent is conducted according to standard ADRC protocol, supervised by Dr. Delano-Wood. The consent process informs a subject about the study, indicates the participation is voluntary and he/she has the right to stop at any time. Risks are enumerated in the informed consent form and described orally during the consent process. The PI has successfully conducted Informed Consent with study subjects for over 25 consecutive years of NIH-funded studies.

## INTERIM ANALYSIS

Interim analyses after 6 subjects will assess visual analog scales for subjective drug effects during Aim 1 and potentially adjust MEM test dose as described in the Research Strategy should unanticipated effects (e.g. sedation) be evident. Interim analyses of Aim 1 biomarker data at ≈ 6 months (expected n=18) will confirm the fidelity of all EAIP measures, and at ≈ 36 months (expected n=54) will guide a future NIA application. **Because the critical test of H2 does not involve an absolute magnitude of clinical change in the subject group, or even a statistically significant group change, there are no plans to stop the study based on an interim analysis demonstrating either outcome**.

## DATA AND SAFETY MONITORING

The Principal Investigator (PI) will be responsible for ensuring participants’ safety on a daily basis. As a single-site, minimal risk clinical trial, data and safety monitoring will be accomplished by a Safety Officer (SO): either Tilman Oltersdorf MD, Director, Medical and Safety Core of the UCSD ADCS, or Branko Huisa MD, ADCS Medical Monitor, unless instructed otherwise by NIA Program (see letter attached). The SO will act in an advisory capacity to the NIA Director to monitor participant safety, evaluate the progress of the study, to review procedures for maintaining the confidentiality of data, the quality of data collection, management, and analyses. To accomplish this, the SO will conduct annual reviews of all safety reports, study procedures, and test results as summarized in annual NIA Progress Reports.

**Frequency of Data and Safety Monitoring**

The PI will be informed of serious adverse events as soon as they occur and will notify the NIA and Safety Officer within 24 hours of notification. The PI and Safety Officer (SO) will meet annually, either in-person or by teleconference call to review study progress, data quality, and participants safety. Safety reports are sent to the SO annually and will include a detailed analysis of study progress, data and safety issues.

**Content of Data and Safety Monitoring Report**

The content of the data and safety monitoring report will include: study status, recruitment records, participant descriptive information, safety information, study data.

Proposed Safety Officer (see letter attached): Tilman Oltersdorf MD, Director, Medical and Safety Core of the UCSD ADCS, or Branko Huisa MD, ADCS Medical Monitor.

**Conflict of Interest for SO**

The SO has no direct involvement with the study investigators or intervention. The SO will sign a Conflict of Interest Statement which includes current affiliations, if any, with pharmaceutical and biotechnology companies (e.g., stockholder, consultant), and any other relationship that could be perceived as a conflict of interest related to the study and / or associated with commercial interests pertinent to study objectives.

**Protection of Confidentiality**

Data will be presented in an anonymized manner in meetings with the SO and in reports to the SO; data and discussion are confidential. Participant identities will not be known to the SO.

**The Data and Safety Monitoring Board (DSMB)** is an independent group of UCSD experts established through our UCSD ADCS, based on a Data and Safety Monitoring Plan (DSMP) specific to a clinical trial application. The DSMB will operate under a charter, according to NIA guidelines (https://www.nia.nih.gov/research/dgcg/clinical-research-study-investigators-toolbox/data-and-safety-monitoring).

Aim 1 of this application is an **experimental medicine study** rather than a phase-associated clinical trial per se, and while it meets the NIH definition of “clinical trial” via the involvement of humans, the study endpoints are experimental rather than health-related measures. Aim 2 of this application involves an open trial of an FDA-approved medication, in a manner that is common practice in the community; as noted above, there is no proposed placebo control group or blinding during this phase, and no restrictions on medication adjustments based on clinical need, and thus none of the procedures are viewed to carry any added risk beyond that encountered in standard community clinical practice.

If convened, the DSMB will consist of 2-3 ADCS multidisciplinary leaders, free from any conflicts of interest or direct involvement with the academic or other credit resulting from this study, who advise both the PI and the NIA, based on their expertise. The primary responsibilities of the DSMB are enumerated below; they include to periodically review and evaluate the accumulated study data for participant safety, study conduct and progress, and efficacy, and to make recommendations to the NIA concerning the continuation, modification, or termination of the R01 protocol. The DSMB considers study-specific data as well as relevant background knowledge about the treatments (memantine) and patient population under study (mild-to-moderate severity AD).

The DSMB is also responsible for maintaining the confidentiality of its internal discussions and activities as well as the contents of reports provided to it. During this application, the DSMB will review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial. Our approved IRB protocol describes timely reporting requirements by the PI related to any adverse events (AEs), serious adverse events (SAEs) or unanticipated problems (UPs). DSMB members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the nature of the event and the safety and welfare of the study

participants. The DSMB will also assess the performance of overall study operations and any other relevant issues, as necessary. **A letter of support from Tilman Oltersdorf MD, Director, Medical and Safety Core of the UCSD ADCS, and Branko Huisa MD, ADCS Medical Monitor is attached, indicating their readiness to convene and oversee a DSMB for this study.** Nominally, the "primary responsibilities" of this DSMB include:

1. Conducting an initial review of the proposed research protocol, informed consent documents and plans for data safety and monitoring, to assure quality study conduct;
2. Reviewing study procedures to assure quality of study conduct, including SOPs for data management and quality control procedures, and when satisfied, recommend subject recruitment be initiated;
3. Evaluating the quality of the ongoing study conduct by performing quarterly evaluations of the study accrual, compliance with eligibility, participant adherence to study requirements, and accuracy and completeness of data;
4. Considering factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study;
5. Protect the safety of the study participants: recommending appropriate study review or temporary halting for any SAE, and termination due to the occurrence of 3 SAEs (e.g. symptom exacerbation or suicidality associated specifically with MEM treatment), or inability of PI to provide necessary assessment information or to answer study questions;
6. Recommending continuation of ongoing studies at quarterly reviews;
7. Ensure the confidentiality of the study data and the results of monitoring; and,
8. Assist the NIA by commenting on any problems with study conduct, enrollment, sample size, and/or data collection.
9. Considering the overall picture, including need to pursue both primary and appropriate secondary analysis, and making these recommendations during the data analysis phase of the project;
10. Reviewing final results, via DSMB report by PI within 90 days of completion of final study subject.

**Inclusion of Women and Minorities**

**Inclusion of women**: Women will be included in this study. One key dependent measure (PPI) exhibits clear sexual dimorphism; previous studies of memantine effects detected **no interactions of sex with drug or diagnosis** for any of the proposed measures of early auditory information processing (PPI, MMN or ASSR). Importantly, it is possible that a significant effect of memantine on auditory processing measures, or an interaction between memantine sensitivity and potential moderating factors (e.g. age, baseline performance, single nucleotide polymorphisms) will be evident in only one or the other sex, or that relationships among specific measures might be sex-specific. Thus, there is a strong rationale for assessing the predicted drug effects in both men and women, to test specific predictions of drug-behavior relationships.

To avoid exposure of any fetus to memantine, subject recruitment is limited to individuals $\geq$ 50 years of age; subjects will be excluded if they are pregnant, nursing, imminently planning pregnancy or have a positive urine pregnancy test on screen day or any test day will be excluded from this study. Second, pre-menopausal women will be tested only in the early follicular phase of their menstrual cycle. Third, female subjects of childbearing potential (defined here as any female who has not had a total hysterectomy and/or bilateral oophorectomy, or who has had a menstrual cycle within the past year) will have urine pregnancy tests on each visit. The pregnancy test is able to detect the pregnancy hormone hCG (Human Chorionic Gonadotropin) in urine. HCG will be measured by a urine "dip-stick" ("Surecheck Early Pregnancy Test"). This test is a midstream format test for the detection of the pregnancy hormone in urine. Fourth, in all subjects of childbearing potential, drug testing will occur within days 1 - 10 of menses onset. Fifth, to participate in this study, females of childbearing potential must agree to use a double-barrier method of contraception when participating in sexual intercourse (regardless of other methods of contraception). If a female of childbearing potential becomes pregnant during the study, the subject will be exited from the study. The investigator will determine whether the subject was exposed to active study drug or placebo, and notify the subject of this information.

Typically, the gender distribution for our studies is roughly equal for men and women. Regarding our most recent San Diego county US Census Bureau statistics, 49.8% of San Diego county's population are women. Within the ADRC, the gender distribution is 55% women.

**Inclusion of minorities:** Ethnic representation is based on recruitment response, with every effort made to include minority groups. Exclusion criteria are not based on ethnicity. Based on our experience, the following ethnic percentages are expected in the AD patient study sample, based on patient demographics at the UCSD ADRC: Caucasian = 74%; Black, not of Hispanic origin = 3%; Latino = 13%; "Other" (Asian American, Native American) = 10%. Thus, prospective recruitment efforts for this renewal period will attempt to buttress those percentages to be more comparable with the US Census Bureau's percentages for San Diego county (approximately 65% White, 5% African American, 11% Asian American or Pacific Islander, 1% Native American, and 32% reporting of Hispanic or Latino origin; 5% reporting two or more races). This recruitment goal of increasing racial and ethnic minority representation will initially be accomplished through a campaign of advertisements in various media sources (i.e., local newsletters, newspapers, periodicals, and radio) and lectures by the candidate and/or co-investigators to local groups and independent living residential facilities as well as with other outreach efforts targeting racial and ethnic minority senior groups (eg., local chapters of the Advisory Committee on Minority Veterans, California Black Health Network, UCSD ADRC Hispanic Program, etc). Our targeted/planned enrollment table numbers reflect this goal of increasing racial and ethnic minority representation to be more comparable to the UCSD ADRC as well as with the US Census Bureau's statistics for San Diego county.

Any ethnic differences in "MEM-sensitive" vs. "MEM-insensitive" groups recruitment will be explored in post-hoc analyses. The source of recruitment information is recorded for each subject, providing us with ethnic "success rates" for particular means of recruitment. Ethnic representation is then reviewed with annual progress reports. Recruitment efforts are adjusted, based on "success rates" of specific recruitment means, to respond to any significant deviation from the projected ethnic recruitment rates. The UC San Diego Health System is an equal opportunity employer; pursuant to state legislation, the University of California no longer applies affirmative action criteria in the student application process.

## Resource Sharing

All biomarker data will be shared with the National Database for Clinical Trials (NDCT) related to Mental Illness, with descriptive and raw data submitted semi-annually, and complete data and analyses within 6 months of study closure, via the Global Unique Identifier (GUID) and Data Dictionary technology. Language will be included in all study consent forms to address this plan. **Budgeting for data management support will account for this activity in the final months of PY5, when experimental activity will be completed (month 57), and effort from research staff will be shifted to support these data sharing requirements at the time when they will be most critical** (see “Justification”, Ms. Talledo).

Results will also be shared via presentations to the relevant scientific community. The PI and Co-PI’s are active members of many professional organizations where the proposed science will hold great interest, including the American College of Neuropsychopharmacology, Society of Biological Psychiatry and Society for Neuroscience, and will present data from these studies at the meetings for those societies. When data are ready for peer review, they will be disseminated to the scientific community via publications in high impact journals.

It is anticipated that this application will generate at least five distinct types of “resources”:

1. Raw data, both descriptive and quantitative, will be disseminated as described above;
2. Processed and analyzed data will be disseminated via presentations and data-based publications, as described above;
3. Conclusions will be reached from the above data regarding a range of topics, from new biomarkers to opportunities for new therapeutic interventions in our field, and will be disseminated as #2, above, in addition to higher profile symposia;
4. Conceptual ideas related to “bigger picture” issues, from study design to novel ways to understand treatment algorithms, will be reported in review articles and book chapters;
5. With patient release, all genomic information will be shared with NIH via the procedures and timelines described in the **“NIH Genomic Data Sharing Policy” (NOT-OD-14-124).**