Study Protocol and Statistical Analysis Plan

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Treatment of Fragile-X Associated Tremor/Ataxia Syndrome (FXTAS) With Allopregnanolone

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Protocol Title: Treatment of Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) with Allopregnanolone

1) Author of Protocol

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2) IRB Review History

None

3) Objectives

The overall purpose of this study is to examine the safety and efficacy of Allopregnanolone as a possible treatment for FXTAS.

Aim 1. To evaluate the effect of allopregnanolone for improving cognitive deficits, specifically memory problems, seen in premutation carriers aged 50-85 years who have neurological problems and FXTAS.

We hypothesize that:

A. Cognitive measures of working memory will improve with allopregnanolone treatment.
B. Hippocampal activation during an associative memory recall task will increase on allopregnanolone.
C. ERP word repetition effect amplitudes, which measure verbal learning and memory processes, will improve with allopregnanolone treatment.

Aim 2. To evaluate the benefits of allopregnanolone on neurological symptoms, specifically on scores of tremor and ataxia (CATSYS tremor and sway measures) in premutation carriers with FXTAS.

We hypothesize that neurological symptoms, specifically tremor and ataxia as measured by the CATSYS, will improve after 12 weeks of treatment.

Aim 3. To assess changes in structural MRI variables that may reflect neurogenesis and neuroprotection in those treated with allopregnanolone.

We hypothesize that improvements will be seen in structural brain measurements with allopregnanolone treatment, including increased hippocampal volume due to increased neurogenesis; better white matter integrity evidenced by higher fractional anisotropy (FA), mean length, and tract volume; and lower mean diffusivity (MD), particularly in limbic and motor-related fiber tracts, which have shown a correlation with FXTAS severity (cerebellar peduncles, cingulum and corpus callosum).

4) Background

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-adult–onset neurodegenerative disorder that affects, with age- and gender-specific penetrance,

carriers of premutation alleles (55-200 CGG repeats) of the fragile X mental retardation 1 (*FMR1*) gene. Defining clinical features of FXTAS include progressive kinetic tremor, gait ataxia, executive function and memory deficits, peripheral neuropathy, and parkinsonism; with associated, variable features, such as cognitive decline into dementia, and psychiatric problems, including depression, and dysautonomia (Berry-Kravis et al., 2007; Hagerman et al., 2001; Jacquemont et al., 2003; Tassone and Berry-Kravis, 2010).

For many individuals with the premutation, neurological and memory problems, executive function deficits, and neuropathy and psychiatric symptoms, particularly anxiety, can develop well before the onset of tremor and ataxia, the cardinal features of FXTAS (Bourgeois et al., 2009; Bourgeois et al., 2011; Coffey et al., 2008; Grigsby et al., 2008; Hagerman et al., 2007; Soontarapornchai et al., 2008). Neuroimaging in FXTAS reveals white matter disease of both the cerebellum and cerebrum (Brunberg et al., 2002; Cohen et al., 2006; Hashimoto et al., 2011; Loesch et al., 2008); atrophy of the brain stem, cerebellum and cerebrum (Adams et al., 2007; Adams et al., 2010; Hashimoto et al., 2011); spongiosis in the white matter; and intranuclear inclusions in neurons and astrocytes throughout the CNS and PNS (Greco et al., 2006; Hashimoto et al., 2011; Hunsaker et al., 2011).

This grant proposes to assess the safety and efficacy of allopregnanolone as a new treatment for adults with both the premutation and neurological problems, including FXTAS. Allopregnanolone is a naturally occurring neurosteroid and positive modulator of $GABA_A$ receptors that can stimulate neurogenesis in the hippocampus (Brinton, 2013), reverse hippocampal-dependent learning and memory problems (Singh et al., 2011), and confer neuroprotection by both reducing the expression of the proapoptotic protein caspase 3 (Djebaili et al., 2005; Djebaili et al., 2004) and inhibiting the mitochondrial permeability transition pore, a key process in the intrinsic pathway of apoptosis-induced loss of neurons (Sayeed et al., 2009). Our preliminary data demonstrate abnormal network bursting activity in hippocampal neurons cultured from premutation mice. Such persistent bursting is a plausible mechanism for the neurodegeneration that leads to the neurological symptoms in FXTAS. We have further demonstrated that allopregnanolone, by potentiating $GABA_A$-receptor signaling, eliminates the abnormal bursting, providing a mechanism for neuroprotection (Palop et al., 2007). Given that allopregnanolone can prevent neuronal loss and also stimulate the generation of new neurons from neural stem and progenitor cells, allopregnanolone may counteract the development of neurological disability in FXTAS. Allopregnanolone is an endogenous metabolite of progesterone that lacks hormonal activity and is expected to be safe for chronic treatment; this drug is being studied in Alzheimer's disease by Dr. Roberta Brinton at the University of Southern California (USC). Allopregnanolone has also demonstrated increased neurogenesis in animal studies after a single administration, however; greater improvements were found after weekly administration (As reviewed by Irwin and Brinton, 2014). Considering that pulse administration of allopregnanolone correlated with increased neurogenesis, these researchers hypothesize that this may have resulted due to the neurobiological system being allowed to return to homeostasis. *We therefore hypothesize that through stimulation of neurogenesis, which confers neuroprotection, and $GABA_A$-receptor–positive modulatory effects, weekly administration of allopregnanolone to premutation*

carriers with early FXTAS will lead to improvement in working memory deficits and other neurological symptoms. In addition, we hypothesize that there will be a reduction in functional and volumetric-imaging changes

5) Inclusion and Exclusion Criteria:

Inclusion Criteria

- premutation carrier status (55 to 200 CGG repeats in *FMR1*),
- men and women≥50 and <85 years of age
- FXTAS including an intention tremor and/or ataxia and/or deficits on the BDS-2 demonstrating executive function deficits. The absolute presence of clinical tremor and ataxia is not essential because subclinical tremor or ataxia can be detected on the CATSYS, as described below, in carriers.

Exclusion Criteria

- other genetic problems in addition to the premutation
- a history of significant brain trauma
- significant substance abuse
- inability to follow the protocol
- liver or kidney disease
- heart failure
- active cancer
- other serious systemic disease
- current use of phenytoin.

6) Number of Subjects: We will enroll 15 subjects

7) Recruitment Methods:

Subjects will be recruited from Dr. Hagerman's research database from FXTAS participants we have seen in previous research.

We are requesting waiver of HIPAA for recruitment purposes only. The research team will identify potential subjects and access medical records to verify eligibility prior to the treating physician and research coordinator approaching the potential subject. Consent to participate and HIPAA Authorization to access additional information in the medical records will be obtained prior to enrollment into the study. The protected health information will not be reused or disclosed to outside persons or entities.

The review of subjects' medical records is for limited information and only to determine eligibility. The data are derived from clinically indicated procedures and there is minimal risk to the subjects' status, employment, or insurability. Only research personnel will access medical records via EMR, and all personnel are required to use the "Quick Disclosure" function in EMR to document review. Without an initial review of the medical

record for screening purposes, it would not be possible to identify potential subjects and confirm their applicability for study participation.

Once eligibility is confirmed, subjects will be approached to obtain their authorization to access and use their health information for the research.

8) Compensation to the Subjects: None

9) Study Timelines

Subjects will participate in –14 visits over 4 months. It is estimated that enrollment will occur over the course of one year.

10) Study Endpoints

Primary endpoints

Our primary endpoint includes cognitive measures of working memory, specifically the CVLT2 (California Verbal Learning Test2), at the end of 12 weeks compared with baseline. We expect to see a 20% improvement on this primary endpoint by 12 weeks.

Secondary endpoints

Cognitive Measures: Improvements of 20% on the Behavioral Dyscontrol Scale (BDS-2); Integrated Visual and Auditory Continuous Performance Test (IVA CPT); CANTAB Battery; COWAT; Frontal Systems Behavioral Task.

Electrophysiological Measures: Improvement of 20% on the word learning N400 amplitude and P300 amplitude in the oddball paradigm on the ERP and eye-tracking measures.

Emotional Battery: Improvement of 20% in the scores on the SCL90 and Beck Anxiety Inventory. Resolution of depression or anxiety on the SCID.

MRI measures: Improvement of the size of the hippocampal volume due to increased neurogenesis will be assessed. Improvements of 5% of total volume would be considered significant. Improvement in the white matter integrity with higher fractional anisotropy (FA), mean length, and tract volume, and lower mean diffusivity (MD), particularly in limbic and motor-related fiber tracts (cerebellar peduncles, cingulum and corpus callosum), will be assessed after 12 weeks.

Motor/Balance Measures: Improvement of 15% on scores of tremor and/or balance as measured by the CATSYS tremor and sway assessments and the FXTAS rating scale (video protocol).

11) Procedures Involved

If exclusion/inclusion criteria are met then the subject will complete the following measures:

Medical History, Physical, and Neurological Exam – Our medical history protocol, which we have used in our previous papers, is extensive and includes birth and development information, school history, health history, review of all systems, and

medication history(Riddle, Cheema et al. 1998, Leehey, Berry-Kravis et al. 2007, Coffey, Cook et al. 2008). We have added a detailed section regarding any neurological or related medical problems. Such components include: sensory deficits (numbness, tingling or pain in extremities, hearing loss), balance problems/ataxia, tremor of any kind, muscle pain, weakness, loss of stamina, any autonomic problems (orthostatic hypotension, hypertension, cardiac arrhythmias, pacemaker placement, impotence, urine or stool problems), visual changes, vertigo, reproductive problems (age of menarche, perimenopausal symptoms, age of menopause, PMS symptoms, regularity of periods), other endocrine problems (diabetes, thyroid dysfunction), autoimmune problems (lupus, arthritis, thyroid disease), and any previous medical or neurological diagnoses. We also take a detailed psychiatric history and document any use of medications, including psychotropic medications.

All study subjects will receive a physical examination, which includes our assessment of typical fragile X features which are sometimes seen in carriers, as previously described in Riddle et al. (1998); growth parameters (height, weight and head circumference); heart rate and blood pressure; and a limited number of anthropometric features that are significantly influenced by FMRP in the pedigree analysis (Loesch, Huggins et al. 2003, Loesch, Huggins et al. 2004). We will also screen for hearing problems. Drs. Hagerman and/or Olichney will perform a detailed neurological examination that emphasizes the major motor features of FXTAS: tremor, cerebellar ataxia, neuropathy, and parkinsonism.

Structured Video Protocol: Standardized testing for the neurological signs is videotaped, and the presence and severity of these signs are quantified with use of the FXTAS Rating Scale (Leehey, Berry-Kravis et al. 2011). This scale is a combination of items from three commonly used scales: the International Cooperative Ataxia Rating Scale (Trouillas, Takayanagi et al. 1997), the Unified Parkinson's Disease Rating Scale (Fahn, Elton et al. 1987), and the Clinical Rating Scale for Tremor (CRST, Fahn, Tolosa et al. 1998). In addition, the tandem test from the Unified Huntington's Disease Rating Scale (Huntington Study Group 1996) is included for quantitative tandem gait assessment. These assessments are needed to document the presence and severity of FXTAS symptoms.

Eye-Tracking – With the Tobii eyetracker, we will document changes in pupillary diameter with exposure to light and dark stimuli, and we will document their eye movements in saccade, smooth pursuit, and anti-saccadic tasks.

Saccade task: Saccades are fast, ballistic movements with both eyes towards a target. The participants are asked to follow a target with their eyes. The target jumps in a sequence of 10, 30 and 60 steps in the horizontal and vertical plane.

Smooth pursuit task: The participants are asked to follow a moving target with their eyes. The target moves horizontally and vertically in a sinusoidal waveform with maximum amplitude of 20cm, at a frequency of 0.2 and 0.4 Hz.

Anti-saccade task: The anti-saccade task requires the subjects to make a saccadic eye movement away from a target, rather than towards it. It is widely used in neuroscience research to prove insight into executive control in monkeys and

humans. The anti-saccade task requires the top-down inhibition of automatic pro-saccade responses and the generation of voluntary anti-saccades.

Laboratory Studies –Safety labs include the comprehensive metabolic panel (Chemistry 12 panel) and a complete blood count with differential.

Molecular *FMR1* testing will also be carried out with the initial blood draw. CGG-repeat size and methylation status will be determined by both Southern blot and PCR analysis, as previously described (Tassone, Pan et al. 2008, Filipovic-Sadic, Sah et al. 2010). *FMR1*-mRNA levels will also be measured by RT-PCR, as described by Tassone et al. (2000).

Molecular outcome measures to be carried out by Dr. Paul Hagerman (Med: Biochemistry and Molecular Medicine, 4303 Tupper Hall, One Shields Ave, Davis, CA 95616). We propose to follow at least three measures of cellular dysfunction as indicators both of the degree of dysfunction and of a therapeutic response. Specifically, we will measure levels of p53, phospho (g) H2AX, and protein nitrosylation (Ross-Inta, Omanska-Klusek et al. 2010, Napoli, Ross-Inta et al. 2011), since each of these three measures will reflect a global degree of dysfunction. We have implemented a time-resolved (tr) FRET-based assay (Schutzius, Bleckmann et al. 2013) capability in the lab, which has replaced our ELISA method for quantifying FMRP. In each case, one of the antibodies directed against the protein of interest (e.g., p53) will be labeled with the Lanthanide donor, and a second antibody labeled with an allophycocyanin (APC) acceptor fluorophore (Perkin-Elmer). In the course of these measurements, which will be performed in 96 or 384-well format, we will also compare the same proteins in peripheral blood lymphocytes as well as fibroblasts, since the former represents a more convenient source for serial measurements throughout the treatment process.

We will also utilize a number of outcome measures of mitochondrial function, including changes in ATP beta subunit (ATPB), the mitochondrial (Mn) superoxide dismutase (MnSOD), and nitrotyrosine load in ATPB as a measure of reactive nitrosyl species (a measure of oxidative stress) and they were abnormal in FXTAS so they will be utilized here as an outcome measure from blood. We also plan to utilize fluorescence-activated cell sorting (FacScan, MoFlo) using a range of fluorescent dyes that are specifically designed to measure mitochondrial membrane potential, a sensitive indicator of mitochondrial dysfunction. Our initial studies would involve measuring reduced mitochondrial membrane potential, using preparative flow cytometry (MoFlo) and fluorescent probes for reduction in mitochondrial membrane potential [e.g., JC-1, $DilC_1(5)$, or MitoTracker RedCMXRos] (Perry, Norman et al. 2011). We also plan to utilize the new Mito-ID® Oxygen and pH Sensors (Enzo) as an additional outcome measure. The primary outcome is a measure of mitochondrial function following the addition of allopregnanolone.

Motor/Gait Measures –
CATSYS Protocol

Our CATSYS protocol includes all four standardized measures of the neuromotor control (Despres, Lamoureux et al. 2000), amended protocols for the postural sway, and protocols for intention tremor as outlined in our previous publications (Aguilar, Sigford et al. 2008, Narcisa, Aguilar et al. 2011). Subjects are asked to hold the Tremor Pen as they would hold an ordinary pen, with the elbow joint bent at a right angle and free of body contact, and the pen positioned approximately 4 inches from the navel. For measuring resting tremor, subjects hold the pen for 24.6 seconds in each hand. For measuring intention tremor, subjects hold the Tremor Pen and tap the center of two circular stickers, approximately ½ inch in diameter, placed on opposite ends of the bottom portion of the computer monitor. The subjects are also asked to trace a line across the table using the Tremor pen. For measuring postural sway, subjects are asked to stand on a force plate with their feet standing approximately 3 inches apart, first with their eyes open and then with their eyes closed, for 60 s, and also for 32.5 s. From our preliminary data, we found that some of our older subjects were not able to stand on the force plate for the standardized 60 s, so we chose to also use the 32.5 s protocol that R. Letz employed in neurotoxicology studies (Gerr, Letz et al. 2000, Gerr, Letz et al. 2000, Letz and Gerr 2000, Frumkin, Letz et al. 2001, de la Paz, Philen et al. 2003).

Cognitive Ability

Wechsler Scales of Intelligence: We will use the Wechsler Adult Intelligence Scale–Fourth Edition (WAIS-IV; Wechsler 2009) for cognitive testing.

Executive Function and Attention Battery

Behavioral Dyscontrol Scale-2 (BDS): The BDS is a 9-item, 19-point scale adapted from the work of A.R. Luria (1966, 1980). The BDS has been validated as a measure of the ability to regulate purposeful, goal-directed activity and to engage in activities of daily living.(Kaye, Grigsby et al. 1990, Grigsby, Kaye et al. 1998, Grigsby, Kaye et al. 2000, Grigsby, Kaye et al. 2002). In addition, the scale has been sensitive to involvement in premutation carriers (Loesch, Bui et al. 2003, Grigsby, Brega et al. 2006). Although the BDS consists primarily of motor items, the nature of the items and the manner in which they are scored minimizes the effect of tremor on performance.

Integrated Visual and Auditory Continuous Performance Test (IVA CPT): The IVA CPT (Sandford, Fine et al. 1995, Turner and Sandford 1995) is a computerized measure that assesses impulsivity, inattention, and hyperactivity. It is an integrated 13-minute auditory and visual continuous performance test designed to assess two major factors: response control and attention control. The normative data consists of 1700 typical individuals, ages 5 to 96 years. This test is sensitive to premutation involvement, and it reflects executive function involvement and clinical problems with attention and concentration.

Letter Number Sequencing Test: This subtest of the WAIS-IV (Wechsler 2009) and WISC-IV (Wechsler 2003) was developed as a measure of working memory. Subjects are presented with mixed sequences of letters and numbers (e.g., 3-e-7-9-p) and are required to separate letters and numbers and repeat them in the proper sequence. The test has been shown to be a reliable and valid instrument (Wechsler 2009), which we have found sensitive to premutation involvement.

Controlled Oral Word Association Test (COWAT): The COWAT is a subtest of the Neurosensory Center Comprehensive Examination for Aphasia. Most commonly

described as a measure of verbal fluency or cognitive flexibility, this test has been selected because it provides useful information concerning the ability to think actively in generating information, and it is strongly correlated with other measures of executive functioning. This test is sensitive to involvement in premutation carriers (Spreen and Strauss 1998).

Frontal Systems Behavioral Scale (FrSBe): The FrSbe is a brief questionnaire with versions completed by both the subject and the spouse or caregiver. It is a good measure of executive function change over time following an illness or injury (Grace and Malloy 2001).

Memory Function

Wechsler Memory Scale – Fourth Edition (WMS-IV): The WMS-IV (Wechsler 2009) is an individually administered battery that focuses on domains of immediate, delayed, and working memory. Each of these domains is tested across two modalities, auditory and visual, and in two task formats, recall and recognition. The WMS-IV has been normed on individuals aged 16 to 90 years. This measure is very sensitive to FXTAS involvement and to premutation involvement in those without FXTAS, particularly in the phonological loop scores including the Digit Span, Letter-Number Sequencing, and the Verbal Paired Associates tasks.

The Cambridge Neuropsychological Test Automated Battery (CANTAB): The CANTAB consists of a series of interrelated computerized non-verbal tests of memory, attention, and executive function. Especially for the assessment of cognitive dysfunction, the use of a computerized battery presents some methodological and practical advantages over traditional neuropsychological tests. The CANTAB has been standardized on a large population, aged 4 to 90 years old, in various research studies, and its validity has been established in a wide variety of clinical populations (Fray and Robbins 1996). Measures from the CANTAB are as follows:

Motor Screening Test (MOT) is a short task designed to familiarize the subject with the touch-screen computer. This measure can identify any problems in vision, movement, or comprehension that could affect performance on the subsequent CANTAB subtests.

Paired Associates Learning (PAL) assesses visual memory and new learning by using patterns that are difficult to verbalize. Performance on the PAL is dependent on the functional integrity of the temporal lobe, particularly the entorhinal cortex (Owen, Downes et al. 1990). The CANTAB PAL performance of patients with mild AD was impaired relative to both demographically-matched healthy controls (Sahakian, Morris et al. 1988) and to individuals with frontal variant fronto-temporal dementia (Lee, Rahman et al. 2003). Importantly, PAL was found to be relatively insensitive to major unipolar depression (only 7 percent of scores of patients with depression and AD fell within an overlapping range)(Swainson, Hodges et al. 2001), thus the assessment won't be biased by an underlying mood disorder.

One Touch Stockings of Cambridge (OTS) is a spatial planning test that gives a measure of frontal-lobe function. Performance on this subtest targets activation of a neural network known as the dorsolateral prefrontal cortex (Baker, Rogers et al. 1996), which is impaired in patients with frontal-lobe damage (Owen, Downes et al. 1990).

Spatial Working Memory (SWM) measures the ability to retain spatial information and manipulate it in working memory. It is a self-ordered task that also assesses heuristic strategy. Performance on this task is impaired by damage to the prefrontal cortex (Owen, Downes et al. 1990, Manes, Sahakian et al. 2002). In neuroimaging studies, SWM performance is associated with activations in the dorsolateral and mid-ventrolateral prefrontal cortex (Owen, Doyon et al. 1996).

Reaction Time (RT) is designed to assess vigilance and impulsivity. It measures motor- and mental-response speeds, as well as accuracy and tendency to respond prematurely. It also allows for a measure of anticipatory responding, preservative responding, and inter-trial variability in responding. Frontal and parietal lobe are measured by this subtest. Looking at both the movement time and the reaction time allows us to differentiate speeding or slowing of motor function from any speeding or slowing of cognitive function.

Stop Signal Test (SST) assesses motor inhibition and impulsivity. Performance on this measure is associated with the integrity of the right inferior frontal gyrus (Aron, Dowson et al. 2003).

Rapid Visual Processing (RVP) is a test of sustained attention. This task is similar to traditional continuous performance tasks. Performance on this measure has been shown to be associated with activation in a network brain structure, including the parietal and frontal lobes (Coull, Frith et al. 1996, Coull, Sahakian et al. 1996).

Delayed Matching to Sample (DMS) assesses forced, choice-recognition memory for non-verbal patterns by testing both simultaneous matching and short-term visual memory. This test is primarily sensitive to damage in the medial temporal lobe area, with some input from the frontal lobes.

Electrophysiological measures (ERP/EEG)

EEG and ERPs consist primarily of summed post-synaptic excitatory and inhibitory neuronal potentials (Nunez and Srinivasan 2006), and provide a non-invasive and time-sensitive measure of synaptic dysfunction in neurological disorders (e.g., Luck, Mathalon et al. 2011, Olichney, Yang et al. 2011). Our prior ERP study in FXTAS found marked attenuation of the N400 repetition effect (Olichney, Chan et al. 2010) – an established electrophysiological index of semantic priming and other verbal memory processes sensitive to amnestic mild cognitive impairment (MCI) and early AD (Olichney, Iragui et al. 2006, Olichney, Taylor et al. 2008, Kutas and Federmeier 2011).

For the EEG/ERP, the subject wears a cap that has multiple sensors, which are attached to the scalp of the subject. Each scalp site is cleaned thoroughly, and then the sensor, a small sticker with some gel on it, is attached to the scalp.

Our ERP word repetition paradigm, which assesses language and memory, utilizes 216 auditory category statements (e.g. a breakfast food); each statement is followed by a visual target word that either (50% probability) fits the category (e.g. pancake) or does not (e.g. a military title- 'quarter'; 50% probability of semantically incongruous targets). Subjects perform a simple semantic judgment task, in which they indicate by a verbal yes/no response if the target word fits the preceding category or not. No demands are made on declarative memory during the ERP recordings, and subjects with mild to

moderate dementia can generally perform the task well. These category-target pairs are repeated in a pseudo-random fashion that provides electrophysiological measures of implicit and explicit memory processes. One-third of the statements are presented only once. One-third of the statements are repeated twice, each repetition with a long lag (~120 s later), and one-third of the statements are repeated once with a short lag (1-4 trials or ~10-40 s later). The experiment takes approximately 70 minutes.

To assess attention and working memory, we propose to use a classic auditory P300 "oddball" paradigm, which utilizes either high (200 Hz) or low (133 Hz) auditory tones, with a dual response task. Our prior published studies of the P300 in FXTAS have shown the frontal P300 amplitude is reduced, and the degree of this reduction correlates well with executive dysfunction (Yang, Chan et al. 2013, Yang, Simon et al. 2013). The subject indicates by button press (and reaction time data recorded) if the stimulus is the less frequent (probability = 25% of tones) *target* tone (i.e. specific 'rare' tone, low or high, which alternates across blocks), and also keeps a mental count of the number of target tones within each block. The auditory P300 experiment takes about 25 minutes to complete (6 blocks lasting ~4 minutes each).

Anxiety and Psychopathology

Symptom Checklist-90–Revised (SCL-90-R): We will continue to use the SCL-90-R (Derogatis 1994) in adults, which has been productive in demonstrating emotional problems in carriers, both with and without FXTAS, that relate to molecular measures, particularly the *FMR1* mRNA levels (Hessl, Tassone et al. 2005). The SCL-90-R is a standardized self-report measure of psychological symptoms (Hessl, Tassone et al. 2005). There are 90 items with a 5-point rating scale, which has been normed on individuals aged 13 years and older.

Beck Anxiety Inventory: To obtain a more detailed measure of anxiety, we will use the Beck Anxiety Inventory (Beck, Epstein et al. 1988) in all adults with the premutation and with controls. The Beck Anxiety Inventory is a 21-item self-report questionnaire that includes typical symptoms of anxiety during the past week, such as nervousness, inability to relax, and heart pounding or racing. It has high internal consistency (Cronbach's alphas ranging from 0.90 to 0.94) and test-retest reliability over a one-week interval (0.67 to 0.93). It has demonstrated good convergence with other measures of anxiety in adults in both psychiatric and general populations. Moore et al. (Guy 1976, National Institute of Mental Health 1985) found that males with the premutation reported higher rates of anxiety on this measures than controls.

Structure Clinical Interview for DSM-IV Axis 1 Disorders (SCID): The SCID (First, Spitzer et al. 1997) is a clinician administered, semi-structured interview covering a broad range of psychiatric diagnoses according to DSM-IV criteria. We and others have demonstrated significant emotional problems in carriers with and without FXTAS utilizing the SCID (Bourgeois, Coffey et al. 2009, Roberts, Bailey et al. 2009, Bourgeois, Seritan et al. 2011). We will use this measure to document anxiety and other psychiatric disorders in our subjects at baseline.

Columbia-Suicide Severity Rating Scale (C-SSRS): The C-SSRS was designed to distinguish the domains of suicidal ideation and suicidal behavior. Four constructs are measured. The first is the severity of ideation, which is rated on a 5-point ordinal scale in which 1=wish to be dead, 2=nonspecific active suicidal thoughts, 3=suicidal thoughts with methods, 4=suicidal intent, and 5=suicidal intent with plan. The second is the

intensity of ideation subscale which comprises 5 items, each rated on a 5-point ordinal scale: frequency, duration, controllability, deterrents, and reason for ideation. The third is the behavior subscale, which is rated on a nominal scale that includes actual, aborted, and interrupted attempts; preparatory behavior; and nonsuicidal self-injurious behavior. And the fourth is the lethality subscale, which assesses actual attempts; actual lethality is rated on a 6-point ordinal scale, and if actual lethality is zero, potential lethality of attempts is rated on a 3-point ordinal scale. This scale will be carried out at baseline and at 3 months, 6 months. For the 5 who continue for one year this will be carried out at 3 month intervals until the end of the study. In 2011 Posner and colleagues demonstrated that the C-SSRS had good convergent and divergent validity with other multi-informant suicidal ideation and behavioral scales and it is an optimal scale to use in clinical trials because it can document change in ideation and behavior over time.

***MRI Protocol*:** MR images will be acquired from a Siemens Trio 3T MRI scanner with a 32-channel head coil (Siemens Medical Solutions, Erlangen, Germany). DTI images with 30 gradient directions will be obtained using parallel imaging to reduce localized signal loss and shorten the length of image acquisition. The images will be acquired twice to increase signal-to-noise ratio and will be zero-filled to 256 × 256 pixels in plane by the scanner reconstruction software to increase image resolution. The DTI images will be acquired parallel to the AC-PC line in 68 axial slices of 2 mm slice thickness without gap. The GRAPPA acceleration factor will be set to 3. The FOV will be 240 mm, matrix 128x128, TR 7100ms, TE 72 ms, and flip angle 90°. The diffusion-weighted images will be obtained at b-value of 800 s/mm² in 30 directions uniformly distributed in space along with four additional images with minimum diffusion weighting. The high-resolution, T1-weighted, 3D magnetization-prepared rapid gradient-echo (MPRAGE) images will be acquired for volumetric analysis along with other standardized structural images, including fluid-attenuated inversion recovery (FLAIR) MRI for lesion detection, and T2-weighted gradient-echo sequence (GRE) for hemorrhage. For the MPRAGE, the participants will be scanned in 208 sagittal slices of 0.95 mm thickness (no gap) with FOV 243 mm, 256 × 256 matrix interpolated to 512 × 512, TR of 2,500 ms, TE of 4.33 ms, and 7° flip angle. The FLAIR images will be acquired in 104 sagittal slices of 1.9 mm thickness (no gap) with FOV 243 mm, 512 × 512 matrix, TR of 5,000 ms, TE of 455 ms, and inversion time 1,700 ms. The GRE sequence will be acquired in 48 axial slices of 3 mm thickness (no gap) with FOV 240 mm, 384 × 384 matrix, TR of 4,000 ms, TE of 80 ms, and NEX 2.

***DTI measurements*:** We have previously shown that DTI measurements show high sensitivity to structural changes associated with the premutation (Wang, Johnston et al. 2005, Hashimoto, Srivastava et al. 2011); we will thus continue using these methods to evaluate the improvement in white matter integrity as a consequence of treatment with allopregnanolone. The analysis will include all major tracts that can be reliably reconstructed under current image resolution, with a particular emphasis on limbic and motor-related fiber tracts (cingulum, middle and superior cerebellar peduncles, and the body of the corpus callosum; see **Figure**). From the reconstructed fiber tracts, tract-based measurements will be obtained for quantifying the extent of change from baseline

to follow-up at one year. Measurements include the structural integrity measurements averaged over the extent of the tract, such as fractional anisotropy for measuring diffusion directionality, axial diffusivity for the amount of water diffusion along the predominant diffusion direction, radial diffusivity for the amount of diffusion perpendicular to the predominant diffusion direction, and volume-related measurements, such as mean length (i.e. the average length of the DTI streamlines reconstructed for a specific fiber tract) and tract volume (i.e. the number of voxels occupied by the reconstructed DTI streamlines). To detect more localized directionality and diffusivity changes not detectable by DTI tractography, a voxel-based analysis, tract-based spatial statistics (TBSS) will be performed at core white matter. The combination of these two leading DTI methods will maximize the chance of detecting potential drug-related structural changes in premutation carriers.

***Hippocampal measurements*:** Hippocampal volumes will be quantified by operator-guided manual segmentation using Mayo BIR's Analyze 8.5 and 9.0 (Robb 2001). These guidelines were developed from the anatomical analysis of post-mortem human brains using histological sections of tissue cut perpendicular to the hippocampal axis; they have been used previously by our group in this patient population (Koldewyn, Hessl et al. 2008). For a detailed description of this protocol see Schumann et al. (2004). Hippocampal volumes will also be corrected for total cerebral volume to account for overall individual differences in brain volume.

Medication trial:

If subjects qualify after screening measures, they will be invited to participate in the open trial of Allopregnanolone. The first 3 infusions and follow up observation will be performed at the UC Davis CTSC Clinical Research Center (CCRC). Infusion personnel will use the Richmond Agitation Sedation Scale (RASS) (Sessler et al., 2002; Ely et al., 2003) to assess subjects before and during infusion. Subjects must obtain a RASS score of 0 (alert and calm) prior to infusion and the medication will be stopped if their RASS score falls below -1 (Drowsy) during infusion. Subjects will all begin with 2.0 mg dosage. If tolerated, the next infusion will be 4.0 mg, and if that is tolerated, the next infusion will be 6.0 mg. Subject infusions will remain stable at the highest dosage tolerated for the remainder of the study. This means that study participants may be on different doses of the medication for the remainder of the trial. These 9 (stable dose) infusions may also be administered at the Mount Sinai Beth Israel Phillips Ambulatory Infusion Center, as described below in item 19.

The 2.0 mg, 4.0 mg, and 6.0 mg aliquots of the 0.5 mg/ml allopregnanolone in 6% sulfobutylether-β-cyclodextrin with 0.9% sodium chloride injection solution will be dispensed by the UC Davis Investigational Drug Pharmacy, or the Mount Sinai Beth Israel Phillips Research Pharmacy in single-use syringes. Syringes will be attached to the infusion pump for slow intravenous administration. Subjects will be observed for 2 hours after infusions at the center to assess side effects and tolerance. During this time, if the participant's RASS score falls below -1, the dosage during the next visit will be lowered and the observation period may be extended as needed until the subject returns to their

baseline RASS score. Infusion personnel will adhere to the CTSC guidelines, or the Mount Sinai Beth Israel Phillips Ambulatory Infusion Center procedure manual, for the treatment of sedation. In addition, subjects must be accompanied by someone who can drive them home from the CRCC infusion center or the Mount Sinai Beth Israel Phillips Ambulatory Infusion Center, and remain with them for at least 20 hours to monitor the subject for any adverse effects of the medication.

To ensure consistency between sites, the UC Davis Investigational Drug Pharmacy will directly educate personnel at the Mount Sanai Beth Israel Research Pharmacy regarding the dispensing and storage of the study drug.

The infusion personnel at the UC Davis CCRC infusion center, with Dr. Rogawski, will educate the infusion personnel at the Mount Sanai Beth Israel Phillips Ambulatory Infusion Center regarding the study procedures and the use of the RASS.

Dr. Randi Hagerman will have monthly phone calls with Dr. Naomi Lubarr ensure procedures are consistent. In addition, Dr. Hagerman is available to Dr. Lubarr in the event of questions or concerns about the study.

12) Data and Specimen Banking

Each sample will be coded by a unique numerical identifier.
There will be a up to a total of five blood draws throughout the study. The first blood draw will have a total volume of up to 45ml. The final blood draw will have a total volume of up to 30ml. Safety labs drawn before every escalating dose will have up to 8 ml. Safety labs will be processed through the UCD Davis Pathology Laboratory. Remaining samples will be delivered to Dr. Tassone's lab at the UC Davis MIND Institute's Wet Lab for processing and storage. Samples will be kept until the end of the study, at which point they will be destroyed. Alternatively, on the study consent form, participants may indicate permission to allow for samples to be kept for a period of 15 years after the end of the study.

13) Data Management and Confidentiality

The clinical study data will be entered into and stored in REDCap, which is managed by the Biomedical Informatics Program of the UC Davis Clinical and Translational Science Center. REDCap (Research Electronic Data Capture) data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the Biomedical Informatics Program. The iterative development and testing process results in a well-planned data collection strategy for individual studies. The REDCap system provides secure, web-based applications that are flexible enough to be used for a variety of types of research, provide an intuitive interface for users to enter data and have real time validation rules (with automated data type and range checks) at the time of entry. These

systems offer easy data manipulation with audit trails for reporting, monitoring and querying patient records, and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). REDCap servers are housed in a local data center at UC Davis Health System and all web-based information transmission is encrypted. REDCap was developed specifically around HIPAA-Security guidelines. For example, upon login, an approved username and password is required. REDCap has been disseminated for use locally at other institutions and currently supports 240+ academic/non-profit consortium partners on six continents and over 26,000 research end-users (www.project-redcap.org). All research personnel on this IRB protocol will have some type of access to this database.

14) Provisions to Monitor the Data to Ensure the Safety of Subjects

A Data Safety Monitoring Board (DSMB) will be established that includes two neurologists familiar with FXTAS, in addition to a statistician from UC Davis. All of the DSMB members will be independent of this study. The board will meet within the first 3 months of initiating this study (after the first patient is dosed), and subsequently every 6 months; it is also available to meet on an emergency basis as needed. Our statistician, Dr. Danh Nguyen, will prepare the data so that the DSMB can review the ongoing adverse events (AEs) of the study on allopregnanolone to assess if any significant problems are occurring with allopregnanolone, particularly serious adverse events (SAEs) which are also reported immediately to our IRB, NIH, and the FDA. The DSMB will send a summary report to Randi Hagerman (PI) and the sub-investigators Dr. Rogawski and Olichney. As the study continues there will also be outcome data that will be available from those who have completed the 12 weeks of the trial. The DSMB will make recommendations to the PI regarding whether the study should continue or stop depending on the AEs, including SAEs and the beneficial effects of the outcome data. The PI will abide by the recommendation of the DSMB regarding stopping the study.

15) Withdrawal of Subjects

If sedation occurs at the lowest dose of 2.0, then their dose will be cut in half at the subsequent visit. If this dose is not tolerated, then they will be removed from the study with safety labs obtained at the end of study visit.

If a patient misses more than 3 doses they will be removed from the study, unless illness or an appropriate excuse is known. These subjects, as well as subjects who want to discontinue the study before visit 14, will be asked to come in for an end of study visit that will include safety labs and any of the outcome measures that are tolerated by the patient. If sedation develops in visits after the highest tolerated dose, then the dose can be de-escalated and they will move back to the previous dose that did not cause sedation.

16) Risks to Subjects

This is a very comprehensive study, and subjects are asked to undergo a large number of tests and procedures. There are side effects from all procedures as listed below.

The amount of time dedicated to testing may make subjects tired. We make an effort to spread the testing out over two days in order to make this easier, and subject are offered multiple breaks throughout the course of the testing day.

Sometimes people feel nervous while taking the cognitive tests, or they get tired during the testing. We take several breaks during the testing to help with fatigue. The test administrators do what they can to reassure the subjects.

Event Related Potentials may cause mild skin irritation at the sites where electrodes have been placed but these symptoms have always resolved within a few days.

The blood draws are mildly painful but in our experience this has not been a problem in adults particularly aging adults who have experienced many blood tests prior to this research. We will clean the skin with alcohol before the blood draw and then apply pressure after the blood draw with a subsequent pressure bandage. We have never had problems with infection from our blood draws. Similarly, IV placement can be mildly painful, and there is a small risk of infection at the site.

There are no known health risks associated with MRI. There is risk of injury for subjects with metal in or on the body; but we will carefully screen subjects as part of the standard protocol at the imaging center. It is also possible that individuals may experience claustrophobia during the MRI studies from being in the small tube. Subjects with a history of claustrophobia or significant anxiety can be given a mild sedative prior to the MRI to reduce these symptoms.

Previous and currently ongoing clinical trials of allopregnanolone have not identified any significant risks. A clinical efficacy trial of allopregnanolone is being assessed in individuals with moderate to severe traumatic brain injury (TBI) under the direction of Dr. Michael Rogawski. The TBI study has enrolled 12 subjects, 6 of which have received allopregnanolone. In these subjects, no adverse events attributable to the drug product were reported. An additional 4 subjects received allopregnanolone intravenous solution in the treatment of status epilepticus under Emergency Use INDs. No adverse events attributable to the drug product were reported in these subjects. There are four other published reports of studies where allopregnanolone had been administered to groups of human subjects (As reviewed by Irwin and Brinton, 2014). In these reports, treatment emergent adverse events were mild and transient, and included nausea, mild sedation, fatigue, flushing, and anxiety. Given existing safety data in humans, we anticipate that mild, transitory sedation may be produced at the high dose levels of allopregnanolone in this protocol; some subjects may experience mild nausea, but we do not expect other adverse events. If nausea persists, we will go to the lower dose for the weekly infusions.

All adverse events will be reported to the IRB annually along with the annual report and renewal request. If the event is serious, it will be reported immediately, as is customary for all clinical trials.

17) Potential Benefits to Subjects

Allopregnanolone has the potential to improve symptoms of FXTAS, such as executive function deficits and psychiatric symptoms, particularly the anxiety that is common in affected individuals. With the data in AD demonstrating benefit in Dr. Brinton's studies, including neurogenesis in the mouse model, and because many with FXTAS also have AD, there may also be additional benefits for neurogenesis in the hippocampus in FXTAS. We hypothesize that FXTAS subjects treated with allopregnanolone will show slower neurological deterioration, improvements in memory and executive function, and improvements in brain processing on electrophysiological measures (event related potentials: ERP).

18) Vulnerable Populations

Not applicable. All subjects will be adults who are competent to consent.

19) Multi-Site Research:

After the stable dose has been reached over the first 3 infusions here at UC Davis, under the supervision of Dr Randi Hagerman MD, subjects may receive the remaining 9 infusions under the supervision of Naomi Lubarr, MD at the Mount SInai Beth Israel Phillips Ambulatory Care Center. This is an outpatient clinical facility located at 10 Union Square East, NY, NY, 10003. The Mount Sinai Beth Israel Phillips Ambulatory Infusion Center is on the 4th floor of this building. It has 17 infusion chairs. The infusion center nurses are trained and experienced in giving chemotherapy, monoclonal antibodies, transfusions, iron infusions, etc., and are well able to manage the procedural tasks required for infusions as well as infusion-related reactions. The center has a policy and procedure manuals in place that address ambulatory infusions, hypersensitivity reactions, and anaphylactic reactions.

Dr Lubarr's outpatient office is located in the same building on the floor above the Infusion Center, in the Department of Neurology Suite.

20) The Phillips Ambulatory Care Center is located a short distance away from the inpatient hospital of Mount Sinai Beth Israel Medical Center, which is an 856-bed teaching hospital with an inpatient neurology floor, 2 designated neuro-critical care specialists, a neurology residency with 24 hour in-house neurology coverage, and a movement disorders fellowship. Community-Based Participatory Research: Not Applicable

21) Sharing of Results with Subjects

Experimental results will not routinely be shared with participants. If requested, an informal feedback session can be scheduled during the visit to provide this information.

22) Setting

Participants will be seen at the MIND Institute for screening, mid-point and end-point visits. Medication infusions will be at the CTSC Clinical Research Center (CCRC). This is a highly specialized patient unit that provides medical scientists with opportunities for careful study of disease.

23) Resources Available

We have the appropriate resources and personnel to conduct this research. Dr. Hagerman employs several full-time research personnel, including research coordinators and several faculty members have roles on this project, including psychologists, psychiatrists, and neurologists. We have completed many years of this project at UC Davis since 2001.

24) Prior Approvals:

This study was approved by the UC Davis Institutional Review Board on May 7, 2015. A modification to the protocol was approved on June 16, 2015.

25) Provisions to Protect the Privacy Interests of Subjects

All records associated with this study will be kept confidential and will be coded so that the subjects the data was obtained from are not directly identifiable with the data collected. The information obtained during this study will only be accessible by the researchers involved in this study. All records will be kept in a locked filling cabinet in a secured room.

26) Compensation for Research-Related Injury

It is important that you promptly tell the person in charge of the research if you believe that you have been injured because of taking part in this study. If you are injured as a result of being in this study, the University of California will provide necessary medical treatment. Depending on the circumstances, the costs of the treatment may be covered by University or the study sponsor or may be billed to

your insurance company just like other medical costs. The University and the study sponsor do not normally provide any other form of compensation for injury. For more information about compensation, you may call the IRB Administration at (916) 703-9151 or email at IRBAdmin@ucdmc.ucdavis.

27) Economic Burden to Subjects

Subjects will only be economically responsible for the costs due to traveling to and from the center, and other transportation costs while here.

28) Consent Process

The consent will conform to HRP-090.

29) Process to Document Consent in Writing

Written documentation of consent will follow guidelines as outlined in SOP, HRP-091

30) Drugs or Devices

A stock solution of allopregnanolone containing 2 mg/mL allopregnanolone in 24% sulfobutylether-β-cyclodextrin is stored frozen at the UC Davis Good Manufacturing Practices Laboratory (2921 Stockton Blvd, Sacramento CA 95817). The GMP facility will formulate the injection solution by dilution of the stock solution to 0.5 mg/ml allopregnanolone in 6% sulfobutylether-β-cyclodextrin with 0.9% sodium chloride injection, USP. The stock and final solutions will be subjected to sterility and pyrogenicity testing, and testing for pH, osmolality, and particulates. The final product in 50 ml aliquots will be stored at 4 °C until used, but for no longer than one month, which is the current assured stability limit. The injection solution will be stored at and dispensed by the UC Davis Investigational Drug Pharmacy (2315 Stockton Blvd, Rm DT0762, Sacramento CA 95817). The aliquots of 2.0 mg, 4.0 mg, and 6.0 mg will be dispensed in a labeled syringe. The drug administration will be done by an infusion pump over the period of 30 minutes.

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Statistical Analysis Plan

Treatment of Fragile-X Associated Tremor/Ataxia Syndrome (FXTAS) With Allopregnanolone

NCT02603926

Prespecified Primary Outcome Analysis:

The prespecified primary outcome in this study is the California Verbal Learning Test II (CVLT2) Trial 1-5 Free Recall Total Raw Score. Mean and standard deviation for raw score at baseline/pre-treatment and at 14 weeks/post-treatment will be calculated, as well as the percentage of patients whose primary outcome measure showed improvement from baseline to follow-up.

Secondary Outcomes Analyses:

The prespecified secondary outcomes in this study include the Behavioral Dyscontrol Scale - 2 (BDS-2) Total Score, the CATSYS Dot-to-Dot Tremor Intensity (CATSYS DTD TI), and the Hippocampal Volume as measured by structural MRI. Mean and standard deviation at baseline/pre-treatment and at 14 weeks/post-treatment will be calculated for all secondary outcome measures, as well as the percentage of patients who showed improvement from baseline to follow-up on each measure.

Statistical Analysis:

All statistical analyses will be conducted in R 3.2.5 language and environment. One-sided paired t tests and Pearson correlation coefficients will also be utilized to assess for significance of changes in outcome measures, both primary and secondary. The threshold for statistical significance will be set at 0.05.