Official Title: Determination of the Circadian Resetting Effects of Escitalopram and Testing for Correlations Between Circadian Resetting and Antidepressant Effects

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CLINICAL AND TRANSLATIONAL RESEARCH CENTER
Oregon Health & Science University
RESEARCH PROTOCOL

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Protocol Title: Circadian Effects of Escitalopram

Investigators:

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* Required by NIH if the PI is not a licensed MD with clinical privileges. Will be considered a co-investigator for OCTRI reporting purposes.

RCR Training: Have all study personnel completed RCR training? [X] Yes [ ] Pending
(Must be complete before OCTRI approval will be given.)

Structured Scientific Abstract (one page):

Background: The human biological clock (circadian pacemaker) has long been thought to play a role in non-seasonal depression. A connection is suggested by the demonstration of 24-hour rhythms in mood, subjective and objective changes in sleep with depression, and reports of changes in the timing and amplitude of biological rhythms in depression. Furthermore, it is known that the neurotransmitter serotonin has a significant role in regulating biological rhythms and that drugs that act on serotonin (such as some antidepressants) are able to reset the biological clock in animals.

Objective: The aim of the study is to obtain **preliminary data** that will test whether the antidepressant medication escitalopram has a resetting effect on the human biological clock and whether the improvement in depression symptoms with escitalopram correlates with the degree to which the timing of the biological clock is realigned with the timing of sleep.

Design: 14-16-week, fixed dose (after titration), open label trial.

Setting and Subjects: 15 individuals with unipolar, non-seasonal depression will be studied over 1 year.

Intervention: Subjects will first complete a one week, single-blind placebo lead-in phase. Subjects will then receive escitalopram for 8 weeks (10 mg/day for the first 2 weeks of treatment and then 20mg/day for the

remaining 6 weeks of treatment).

Measurements: Subjects will keep a sleep diary and wear a wrist activity monitor throughout the study to document the timing and quality of sleep. On two occasions (end of placebo week and end of last treatment week) blood and/or saliva will be sampled every 30 minutes for 7 hours and the resulting samples will be assayed for melatonin. The onset of melatonin secretion (dim light melatonin onset or **DLMO**) will be used to mark the timing of the biological clock (circadian phase). Circadian misalignment will be measured using the time interval between the DLMO and the average midsleep of the prior week (phase angle difference or **PAD**). Mood will be assessed throughout the study using the Hamilton Depression Rating Scale (HAM-D) as well as the Beck Depression Inventory-II (BDI-II) and Beck Anxiety Inventory (BAI).

Analysis: A paired t-test will be used to determine whether there is a difference before and after treatment in the average time of the DLMO. Linear regression analysis will be used to determine if there is a significant correlation between depression symptom severity (HAM-D as well as the BDI-II and BAI score) and PAD.

a. Specific Aims:

1. Primary Aims and Hypotheses:

   Primary Aim # 1: To determine whether the antidepressant medication escitalopram has a resetting effect on the human biological clock (circadian pacemaker).

   Hypotheses for Primary Aim #1:

   We hypothesize that escitalopram causes a shift in the timing of the biological clock (circadian phase) to either an earlier or later time.

   Primary Aim # 2: To demonstrate that there is a correlation between improvement in symptoms of depression with escitalopram and the degree of realignment between the timing of sleep and the timing of the biological clock (circadian phase).

   Hypotheses for Primary Aim #2

   We hypothesize that the degree of circadian realignment from escitalopram correlates with improvement in depression symptoms in subjects with non-seasonal major depressive disorder.

b. Background and Significance:

1. Introduction to Circadian Physiology

The biological clock (circadian pacemaker) is located in the suprachiasmatic nuclei (**SCN**) of the hypothalamus and is responsible for regulating the near 24-hour rhythms in a wide range of physiological variables such as the timing of hormone secretion, oscillations in core body temperature, and alertness.[1] The molecular "gears" of the clock consist of transcription-translation feedback loops in SCN neurons.[1] The clock is synchronized (or entrained) to the 24-hour day by external time cues (**zeitgebers**). Although various stimuli, such as physical activity, can reset the biological clock, the primary zeitgeber in humans is the external light/dark cycle.[2] A subset of retinal ganglion cells containing melanopsin are responsible for transmitting light information to the SCN via the retinohypothalamic tract .[3] The SCN also receives input from the intergeniculate leaflet (**IGL**) of the thalamus and the median raphe nuclei (MRN) of the midbrain both of which play a role in modulating the effects of light as well as weaker non-photic zeitgebers on the biological clock. Light in the

morning generally causes a shift in the timing of the clock (**circadian phase**) to an earlier hour (**phase advance**) while evening light causes a shift to a later hour (**phase delay**).[2]

An important principle in circadian biology is that all of the various parameters under the control of the central pacemaker are yoked together. For example, it has been shown that the rhythms in core body temperature, cortisol, and melatonin all shift in concert in response to light exposure.[4] Although the timing of some events are under volitional control in humans (such as the timing of sleep) the endogenous circadian drives for sleep and wakefulness remain coupled to the other rhythms under the control of the central pacemaker.

Table 1. Definitions

Circadian Term	Definition
Phase	The timing of the biological clock. A specific point within a circadian cycle (e.g. the onset of melatonin secretion).
Phase Delay	Shift in the biological clock to a later time
Phase Advance	Shift in the biological clock to an earlier time
Zeitgeber	Stimuli able to reset the biological clock (e.g. light or exogenous melatonin)
Phase angle difference (PAD)	Time difference between the dim light melatonin onset (a measure of circadian phase) and midsleep. A measure of circadian misalignment.

The onset of melatonin secretion in dim light (the dim light melatonin onset or **DLMO**) has been demonstrated to be a precise and accurate marker of circadian phase in humans.[5, 6] The DLMO can be thought of as the "hands of the clock" that accurately reflect the workings of the underlying molecular "gears of the clock."

2. Serotonin and the Circadian System

Serotonin (5-HT) is an important neurotransmitter for the circadian system. Serotonergic input to the pacemaker occurs via both median raphe nucleus (MRN) projections to the SCN as well as dorsal raphe nucleus (DRN) projections to the IGL.[7] Destruction of serotonin neurons increases the sensitivity of the circadian pacemaker to the resetting effects of light and can change the period of the circadian pacemaker.[8] Electrical stimulation of both the MRN and DRN has been shown to reset the circadian pacemaker and it has also been demonstrated that 5-HT agonists are capable of resetting the circadian pacemaker both in vitro and in vivo.[7] Administration of 5-HT agonists to SCN slice preparations have been shown to reset the timing of SCN neuronal firing (both phase advances and phase delays) while both systemic and intracerebral administration of 5-HT agonists have been shown to reset the timing of behavioral rhythms (primarily phase advances).[8] Furthermore, the 5-HT receptor subtypes involved in circadian resetting have begun to be elucidated. Although experimental and species differences complicate the picture, 5-HT_{1A}, 5-HT_{1B}, and 5-HT_7 receptors appear to play a role in mediating circadian effects. The phase-resetting effects of 5-HT agonists can be blocked by pindolol (in vivo) and ritanserin (in vitro) which are selective 5-HT_{1A}, and 5-HT_7 antagonists, respectively.[8] Stimulation of 5-HT_{1B}, and 5-HT_7 receptors block the resetting effects of light on the circadian system.[8, 9] Taken together, it appears that one role of 5-HT in the circadian system is to modulate the effects of light and specifically to dampen the circadian effects of light.

Finally, various lines of evidence indicate that antidepressant drugs that influence the serotonin system have an effect on the circadian pacemaker. The monoamine oxidase inhibitor (MAO-I) clorgyline has been shown to increase circadian period and cause a phase delay in entrained rhythms in the hamster while fluoxetine administration in the rat has also been shown to lengthen circadian period. Fluoxetine has been shown to induce a downregulation of the 5-HT_7 receptors in the SCN and administration of citalopram reduces the effects of 5-HT_7 receptor stimulation in the hippocampus.[10] In humans, light treatment (which resets the circadian pacemaker)[11] has been shown to be synergistic with citalopram treatment.[12]

3. Circadian System and Depression

The circadian system has long been speculated to play a role in non-seasonal major depressive disorder.[13] In addition to circadian rhythmicity in mood [14], other evidence suggestive of a relationship includes symptoms of insomnia, hypersomnia and diurnal mood variation; polysomnographic findings of shortened REM latency, increased sleep latency and increased wakefulness during sleep [15]; differences in the amplitude of circadian rhythms and in timing of the biological clock [13]; the therapeutic effects of manipulations of sleep (sleep deprivation or advancement of sleep timing) [16, 17]; and therapeutic effects of light therapy.[18, 19]

One of the first circadian theories for non-seasonal major depressive disorder, the internal coincidence model [20], proposed that the biological clock is set abnormally early relative to the timing of sleep in depression and that sleep is depressant when it occurs at certain biological times.[16] In other words, the misalignment between the timing of sleep and the timing of the circadian pacemaker contributes to depression symptoms. Consistent with this hypothesis, advancing the timing of sleep has been found to be antidepressant in some studies.[20]

Although neither an advanced or delayed circadian phase position has consistently been found in individuals with depression compared to healthy controls [15, 17], it nonetheless remains possible that a *misalignment* between the timing of sleep and the circadian pacemaker in either direction may be depressant in vulnerable individuals.[21]

c. Preliminary Studies / Progress Report:

Our recent findings in winter depression [seasonal affective disorder (SAD)] have shown that the degree of misalignment between the timing of sleep and the timing of the pacemaker correlates with symptom severity.[21] We measured symptoms using the 28-item Hamilton Depression Rating Scale while the degree of circadian misalignment was measured using the interval in time (phase angle difference or PAD) between the DLMO and the timing of midsleep. Although average PAD was no different from our historical controls, PAD correlated with symptom severity in these depressed individuals (Figure 1).[21]

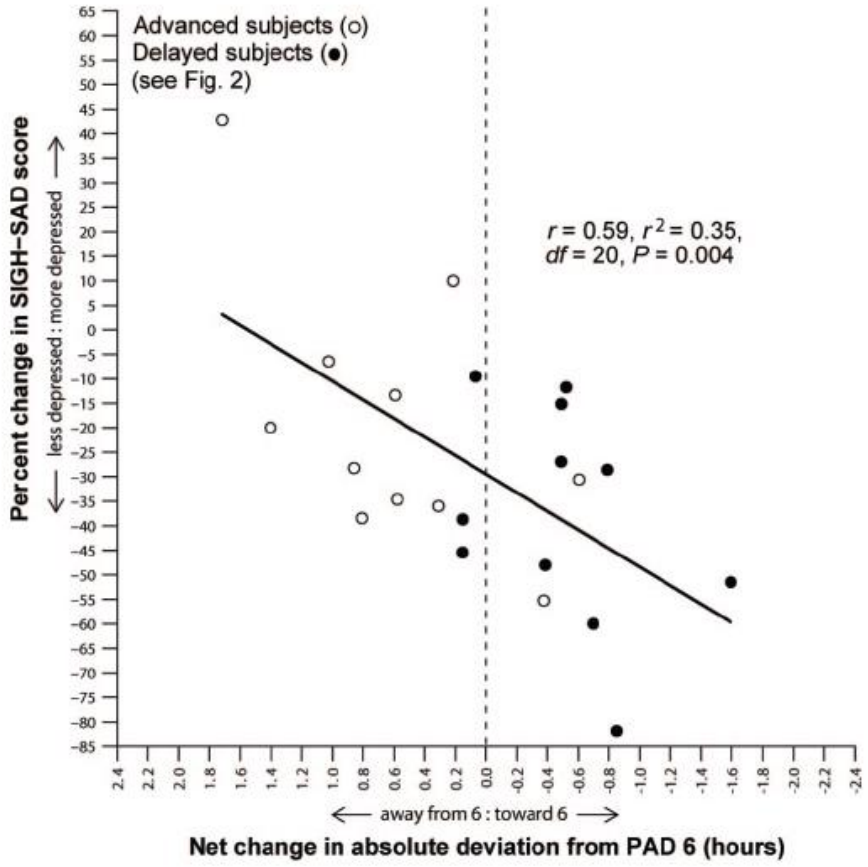

Figure 1. Correlation of symptom improvement with change in PAD in seasonally depressed patients treated with afternoon melatonin. Symptoms were measured with the 28-item HAM-D (SIGH-SAD). Change in the PAD toward the normal value of 6 hours correlated with a reduction in depression symptoms. From reference 21

d. Research Design and Methods

1. Experimental Design:

The study is a 14-16 week, fixed dose (after titration), open label trial of the circadian effects of escitalopram in 15 individuals with unipolar, non-seasonal depression.

Description of subjects

1.1a. Screening

Subjects will be interviewed by a study physician, the protocol will be explained and questions will be answered. A complete medical, psychiatric, and social history will be obtained by a study physician with special emphasis on sleep history; a physical exam and a structured psychiatric interview will be performed (Structured Clinical Interview for DSM-IV Axis I Disorders, SCID-I, modules A through F); and laboratory screening tests including CBC, Complete Metabolic Set, TSH, EKG,

urinalysis and pregnancy test (if appropriate) will be done. Subjects will also be interviewed by a trained rater using the 21-Item Hamilton Depression Rating Scale (HAM-D) and will complete the Horne-Ostberg Morningness-Eveningness questionnaire which is a rating of subjective morning or evening preference.

1.1b. Inclusion and Exclusion Criteria (evaluated during the screening visit)

All subjects enrolled in the protocol must meet the following inclusion and exclusion criteria. Inclusion criteria are as follows: (1) age 18-65 years old; (2)able to comply with the requirements of the experimental protocol; (3) competent to sign informed consent; (4) have mild to severe major depressive disorder (without psychotic features and without a seasonal pattern) according to DSM-IV-TR criteria as determined by a psychiatric examination and currently be under the care of a licensed mental health care provider (PhD or masters level psychologist, licensed social worker, licensed professional counselor, psychiatric nurse practioner, or psychiatrist) or primary care physician (to help exclude potential SAD subjects we will require individuals with their first episode of major depression to either have had the onset of symptoms before the Fall equinox or to have had symptoms persist after the Spring equinox). All subjects (even if they are not taking any psychiatric medications) are required have a provider with prescribing authority (primary care provider, psychiatrist, or psychiatric nurse practitioner); (5) score $\geq$ 7 when interviewed by a trained rater using the 21-Item Hamilton Depression Scale (HAM-D) [22]; (6) be in good physical health (no clinically significant abnormalities) as determined by physical examination and laboratory screening tests (including CBC, Complete Metabolic Panel, TSH, EKG, and urinalysis); (7) not be suicidal; (8) not be taking any other antidepressant medications besides escitalopram during the study; (9) be free of antidepressant medications for 2-4 weeks prior to beginning the study; (10) not have a history of transmeridian travel or shift work in the past 2 months and have no plans for transmeridian travel or shift work for the duration of the study; (11) be able to maintain a regular sleep wake schedule for the weeks one and nine of study (bedtimes and waketimes $\pm$ ½ an hour) and (12) women of childbearing potential must have a negative pregnancy test and practice an acceptable method of birth control. Acceptable methods are birth control pills, Depo-Provera, an IUD, a diaphragm or condom with spermicide, or abstinence. Norplant is no longer on the market and thus, will not be listed as a possible contraceptive.

Exclusion criteria are as follows: (1) abnormal heart, liver, or kidney function; (2) significant laboratory abnormalities on CBC, Complete Metabolic Set, TSH, EKG, & urinalysis; (3) shift work or transmeridian travel in the last 2 months; (4) current use of melatonin; or (5) evidence of a primary sleep disorder by history. Women who are pregnant or lactating are not eligible. Throughout participation, potential subjects cannot be taking medication(s) with known sedative or stimulating effects or that would interfere with production of melatonin, e.g. benzodiazepines or psychostimulants, antipsychotics, hypnotics, anxiolytics, anticonvulsants, opiates, beta adrenergic antagonists, alpha-2 agonists, corticosteroids, antihistamines (H_1 antagonists), theophylline, anti-parkinsonian dopaminergic medications, melatonin or over-the-counter cold preparations containing pseudoephedrine. Only clinically relevant abnormalities on screening laboratories will result in subject exclusion. During the study subjects will be required to limit caffeine intake to a maximum of two cups of coffee (or caffeine equivalent) before noon, limit alcohol to one alcoholic drink per day,

and limit naps to 30 minutes per day but not six hours prior to bedtime.

Description of study procedures

Subjects will have a total of 12 visits to the OCTRI over the 14-16 weeks of study. Subjects will first undergo an initial screening visit as described above. Subjects who meet criteria for study and agree to participate will then have a study initiation/materials visit followed by 9 visits during treatment with placebo or escitalopram. A final post-study follow-up safety visit will be scheduled at the end of treatment (Table 2).

1.2a. Washout

Subjects who meet criteria for the study and who agree to participate, will initially enroll into the washout phase of the study. Subjects will discontinue any current antidepressant medications for a period of 2-4 weeks. Most subjects will enroll in a 2-week washout, as research has indicated the body clock needs about 2 weeks to reset itself.[23, 24] However, subjects taking fluoxetine (Prozac) will need an additional 2 weeks of washout because fluoxetine has a longer half-life (it stays in the body longer). The study schedule will be extended two weeks (to 14 weeks) for those taking fluoxetine.

During the washout phase, the study doctor will conduct weekly mood assessments over the phone and study staff will administer the HAM-D, BDI and BAI over the phone to ensure subject safety throughout this period when the subjects are not taking any antidepressant medications.

1.2b. Treatment

Subjects will first complete a one week, single-blind placebo lead-in phase. Subjects will then receive escitalopram for 8 weeks. Subjects will receive 10 mg/day for the first 2 weeks of active treatment and then 20 mg/day for the remaining 6 weeks of treatment. Medication will be dispensed on a weekly basis (see Table 2). Forest Laboratories will provide escitalopram in 10 mg strength tablets and placebo tablets. Compliance will be assessed using the Trackcaps MEMS6® system (AARDEX Ltd, Union City, California). The system is comprised of two parts, a standard plastic container with a threaded opening and a cap that contains a micro-electronic circuit which registers time when the capsule container is opened and when it is closed. Time-stamped medication events stored in the MEMS6® can be transferred at any time through the MEMS6® Communicator to a Windows-based computer. AARDEX® software analyzes and displays the computed parameters of the patient's compliance. At the end of every treatment week any adverse experiences will be documented and vitals signs (temperature, blood pressure, heart rate and respiratory rate) recorded.

1.2c. Assessment of Sleep

Subjects will keep sleep diary during the placebo/treatment phase of the study and will also wear a wrist actigraph to further document sleep/wake times, total sleep time, sleep quality and compliance. At the end of every study week actiwatch data will be downloaded by study staff, sleep diaries collected, and any discrepancies resolved by reviewing the data with the subjects. Subjects will also be asked to maintain a consistent sleep/wake schedule (8 hour sleep episodes with bedtimes and wake-times ±0.5 h) of their choosing during the placebo week and the last week of treatment (each of the weeks prior to the inpatient assessment of circadian phase, see below). Subjective sleep quality will be assessed using the Pittsburgh Sleep Quality Index (PSQI) questionnaire (which asks about sleep over the past month)

at each inpatient phase assessment.

1.2d. Assessment of Mood

Mood will be assessed by study staff (trained raters) using the 21-Item Hamilton Depression Rating Scale (HAM-D), Beck Depression Inventory-II (BDI-II) and Beck Anxiety Inventory (BAI) in a weekly phone interview during the washout period and **in person**, biweekly, starting Visit 3 (end of the placebo lead-in week, through treatment).

1.2e. Inpatient Assessment of Circadian Phase

Subjects will have two assessments of the timing of their biological clock (**circadian phase**) via serial blood and/or saliva sampling both before and after treatment with escitalopram. For each assessment, subjects will be admitted to the OHSU Oregon Clinical and Translational Research Center (OCTRI) for 8 hours (1 hour before their 7-hr sampling period begins). An intravenous catheter will be inserted into a forearm vein and, under dim light (~10 lux), blood samples will be drawn and saliva samples collected every 30 minutes for 7 hours (from about 6 hours before habitual bedtime to 1 hour after habitual bedtime), for a total of 15 draws/collections. Five milliliters of blood will be drawn for each sample. The amount of blood drawn during each visit will be 75 ml (about 5 tablespoonfuls) and the total amount of blood drawn will be 150 ml (about 10 tablespoonfuls). A small salivette is used for saliva collection. Saliva collection will allow us to study individuals unable to tolerate an intravenous catheter, those with failure of the intravenous catheter, or those with mild anemia. The blood and saliva samples will be analyzed for melatonin so that the onset of secretion in the evening (an indication of the timing of the body clock) can be determined (see below). All interview and questionnaire based assessments of mood and sleep scheduled during these visits will be conducted prior to insertion of the intravenous catheter and subsequent blood collection.

During sampling, subjects will be asked to refrain from cranberry juice, chocolate, bananas, caffeinated and decaffeinated coffee, tea, and soda (naturally caffeine free items such as Sprite are ok), avoid wearing lipstick (though standard lip balm is ok), and avoid the use of toothpaste. We also ask subjects avoid the use of Aspirin, Ibuprofen, and other non-steroidal anti-inflammatories during sampling.

Subjects will also be asked not eat or drink anything (including water) in the 10 minutes prior to each sample If a subject has accidentally had something to eat or drink in that 10 minutes, the nurses will be instructed to simply wait to administer the sample until a full 10 minutes has passed.

If a subject has difficulty saturating the salivette, we will suggest they drink plenty of fluids, sit up a few minutes before taking the sample and chew on the salivette for longer (a few minutes, if needed).

1.2f. Assessment of Vital Signs and Adverse Events

At the end of every week of treatment subjects will come to the OHSU outpatient OCTRI for a check of vital signs (temperature, blood pressure, heart rate, and respiratory rate) and any adverse events or side effects will be noted.

1.2.g. Stopping Escitalopram

At the end of the last week of study medication (end of week 11 or 13) a study physician will contact the subject and their prescriber and inform them both of the current research medication regime

(escitalopram 20 mg po qd) and their response to treatment in comparison to their prior therapy. The study physician will be available to both the subject and their prescriber for consultation regarding whether the prescriber should maintain the subject on escitalopram or taper them off escitalopram and restart their previous medication(s). If the subject and their prescriber elect to continue escitalopram, the prescriber will be responsible for providing this. If subjects choose to discontinue the escitalopram the study doctor will provide 2 weeks of escitalopram at a dose of 10 mg by mouth per day to complete a taper of the medication. The safety follow-up visit (see below) is timed to occur one week after the end of this taper.

During the taper, the study doctor will conduct weekly mood assessments over the phone and study staff will administer the HAM-D, BDI and BAI over the phone to ensure subject safety throughout this period.

1.2h. Safety Follow-up Visit

Three weeks after finishing the study medication (one week after completing the possible escitalopram taper) (end of week 14 or 16) , subjects will come to the outpatient OCTRI for a repeat physical and psychiatric exam conducted by a study physician as well as an electrocardiogram (EKG) and laboratory screening tests (the same tests done during the initial screening visit).

Table 2. Study Schedule (assuming a 2- or 4-week washout)

Study Phase:	Consent / Screening	Washout	Materials	Placebo	Lexapro 10 mg qd		Lexapro 20 mg qd						Possible taper: Lexapro 10 mg qd		Follow up
Visit #:	1		2	3	4	5	6	7	8	9	10	11			12
Week of Study (2-week Washout):	0	1 and 2	2	3	4	5	6	7	8	9	10	11	12	13	14
Week of Study (4-week Washout):	0	1 to 4	4	5	6	7	8	9	10	11	12	13	14	15	16
Consent, screening, and Psychiatric Exam	X														
Physical Exam and labs	X														X
Mood Assessment: HAM-D, BDI-II & BAI	X	X (weekly)		X		X		X		X		X			
Sleep assessment: PSQI				X								X			
Circadian Assessment: 7 hours of blood sampling				X								X			
Receive actiwatch & sleep diary			X	X	X	X	X	X	X	X	X				
Dispense Medication or Placebo			X	X	X	X	X	X	X	X	X				
Adjust Dose						X									
Check Vital Signs	X			X	X	X	X	X	X	X	X	X			X
Check Adverse Events/Side Effects				X	X	X	X	X	X	X	X	X			

Description of analytic (laboratory) methods

1.3a. Wrist Actigraphy

Wrist actigraphy has been demonstrated to be a reliable way of assessing sleep/wake schedules and sleep quality.[25] We will use the AW-64 Actiwatch ® with 64K memory and event marker made by Mini-Mitter Co., Bend, OR. Actigraphy data will be collected for study weeks 1 through 9. Total sleep time, sleep latency, wake after sleep onset and sleep efficiency (percentage of time in bed that equals sleep) will be calculated from the actigraphy data.

1.3b. Melatonin Assays

All melatonin assays will be performed in the OCTRI Core Laboratory. The samples will be assayed by RIA based on the assay developed by Kennaway [26, 27] and reagents will be supplied by ALPCO Ltd., Windham, NH. The lower limit of sensitivity is 0.25 pg/ml; the coefficient of variation is 10.2% for concentrations of 15 pg/ml and 20% for concentrations of 0.5 pg/ml. This assay has been validated by GCMS.[28]

1.3c. Circadian Phase

Circadian phase will be estimated using the plasma or salivary dim light melatonin onset (DLMO) which is the time at which the melatonin concentration rises above a threshold of 10 pg/ml and 3 pg/ml, respectively. These markers are equivalent and have been demonstrated to be reliable and highly accurate markers of circadian phase.[21, 27, 28] Phase will be determined by calculating a linear regression between the last melatonin data point below threshold and the first data point greater than threshold. Resolving the regression equation for the time at which the concentration is either 10 pg/ml or 3 pg/ml yields the DLMO. Under normal entrained conditions the 10 pg/ml plasma DLMO and 3 pg/ml salivary DLMO typically occur 3 hours before sleep onset. Although posture is not fixed during the two 24-hour assessments, there doesn't appear to be an influence of posture on the DLMO.[29]

2. Statistical considerations:

2.1 **Primary Endpoints (Primary Aim # 1)**: **To determine whether the antidepressant medication escitalopram has a resetting effect on the human biological clock (circadian pacemaker).**

2.2 **Secondary Endpoints (Primary Aim # 2)**: **To demonstrate that there is a correlation between improvement in symptoms of depression with escitalopram and the degree of realignment between the timing of sleep and the timing of the biological clock (circadian phase).**

2.3 Covariates/Confounders (Describe measures for covariates/confounders):

This is a single blind, open label trial and therefore researcher bias may be a factor. Also, there may be an order effect with the placebo control comparisons. Each subject will serve as their own control which will minimize confounders to some extent. However, **the goal of the study is to gather**

preliminary data that we may use to propose a larger double blind, placebo controlled cross-over study to fully test these same hypotheses.

2.4 Analysis for primary outcomes (Describe the analysis for *each* of the primary outcomes):

Primary Aim # 1: The timing of the biological clock (circadian phase) will be estimated using the plasma or salivary dim light melatonin onset (DLMO). A paired t-test will be used to determine whether there is a difference before and after treatment (weeks 1 and 9, respectively) in the average time of the DLMO.

Primary Aim # 2: Circadian misalignment will be measured using the time interval between the DLMO and average midsleep during the prior week (phase angle difference or **PAD**).[21] Linear regression analysis will be used to determine if there is a significant correlation between depression symptom severity (HAM-D as well as the BDI-II and BAI score) and PAD.

2.5. Sample Size Justification (Include sufficient detail so that all calculations can be verified.):
A paired t-test will be used to determine whether there is a difference before and after treatment in the average time of the DLMO. For a paired-t test, the equation for sample size is [30]:

$$N = \frac{4\sigma^2 (z_\alpha + z_\beta)^2}{D^2}$$

D is the expected difference in DLMO and σ is the expected standard deviation of the difference. We have a limited data set from which to estimate σ for the change in DLMO timing in non-seasonal depressives. However, using our data set of winter depressives whose circadian pacemaker was reset using oral melatonin administered in the evening, σ is estimated to be 0.55 h for the average change in DLMO. When $\alpha = 0.05$ ($z_\alpha = 1.96$), $\beta = 0.20$ ($z_\beta = 0.842$), and N =15 solving for D gives 0.80 h (48 minutes). This difference is similar to the change in DLMO (0.89 h) we found in patients with winter depression in whom changes in PAD correlated with symptom improvement (Figure 1).

Linear regression analysis will be used to determine if there is a significant correlation between symptom severity of depression and PAD. The R^2 statistic and corresponding p-value will be the measures used to determine significance ($p \leq 0.05$). Given that there will be 15 patients, using Russ Lenth's online sample size calculator[31] for linear regression this sample size will allow us to find a $R^2 \geq 0.426$ (with $\beta = 0.20$, $\alpha = 0.05$). Current data on non-seasonally depressed patients (n=10, unpublished data) have shown that there is a linear correlation between PAD and depression symptom severity (HAM-D) with R^2 values of 0.4008. Therefore, this sample size will afford us a minimum power of ~0.80.

e. Human Subjects

1. Risks to Subjects
   a) Human Subjects Involvement and Characteristics
      1) Involvement of human subjects:

      We intend to study 15 individuals with unipolar, non-seasonal depression in a 14-16-week open label trial of the circadian effects of the antidepressant escitalopram over the course of one year. As described below depressed subjects must have moderate to severe major depression but be otherwise healthy.

2) Subject population:
All subjects enrolled in the protocol must meet the following inclusion and exclusion criteria.

Inclusion criteria are as follows: (1) age 18-65 years old; (2) able to comply with the requirements of the experimental protocol; (3) competent to sign informed consent; (4) have mild to severe major depressive disorder (without psychotic features and without a seasonal pattern) according to DSM-IV-TR criteria as determined by a psychiatric examination and currently be under the care of a licensed mental health care provider (PhD or masters level psychologist, licensed social worker, licensed professional counselor, psychiatric nurse practitioner, or psychiatrist) or primary care physician (to help exclude potential SAD subjects we will require individuals with their first episode of major depression to either have had the onset of symptoms before the Fall equinox or to have had symptoms persist after the Spring equinox). All subjects (even if they are not taking any psychiatric medications) are required have a provider with prescribing authority (primary care provider, psychiatrist, or psychiatric nurse practitioner); (5) score $\geq$ 7 when interviewed by a trained rater using the 21-Item Hamilton Depression Scale (HAM-D) [22]; (6) be in good physical health (no clinically significant abnormalities) as determined by physical examination and laboratory screening tests (including CBC, Complete Metabolic Panel, TSH, EKG, and urinalysis); (7) not be actively suicidal; (8) not be taking any other antidepressant medications besides escitalopram during the study; (9) be free of psychotropic medications for 2 weeks prior to beginning the study; (10) not have a history of transmeridian travel or shift work in the past 2 months and have no plans for transmeridian travel or shift work for the duration of the study; (11) be able to maintain a regular sleep wake schedule for the weeks one and nine of study (bedtimes and waketimes ± ½ an hour) and (12) women of childbearing potential must have a negative pregnancy test and practice an acceptable method of birth control. Acceptable methods are birth control pills, Depo-Provera, an IUD, a diaphragm or condom with spermicide, or abstinence. Norplant is no longer on the market and thus, will not be listed as a possible contraceptive.

Exclusion criteria are as follows: (1) abnormal heart, liver, or kidney function; (2) significant laboratory abnormalities on CBC, Complete Metabolic Set, TSH, EKG, & urinalysis; (3) shift work or transmeridian travel in the last 2 months; (4) current use of melatonin; or (5) evidence of a primary sleep disorder by history. Women who are pregnant or lactating are not eligible. Potential subjects cannot be taking medication(s) that would interfere with production of melatonin, e.g. beta adrenergic antagonists, or melatonin. Only clinically relevant abnormalities on screening laboratories will result in subject exclusion. During the study subjects will be required to limit caffeine intake to a maximum of two cups of coffee (or caffeine equivalent) before noon, limit alcohol to one alcoholic drink per day, and limit naps to 30 minutes per day but not six hours prior to bedtime.

3) Rationale for inclusion of vulnerable populations: n/a

b) Sources of Materials:
Measures of mood and sleep will be made using the Hamilton Depression Scale (HAM-D) structured interview administered by a trained rater, the Beck Depression Inventory-II (BDI-II, a

self-rating scale for depression), the Horne-Ostberg Questionnaire (a measure of morning versus evening preference), and the Pittsburgh sleep quality index (PSQI, a self-rating of sleep) at various points during the study (see Table 2 and section d.1.2). Subjects will complete a sleep diary daily throughout the experiment. Subjects will wear activity monitors 24 hours per day to document sleep times and quality. Medical histories obtained from records may also form part of the database. Subjects will have blood and/or saliva specimens collected on the inpatient OCTRI on two occasions. The data collected from these sources will be strictly confidential and used only for research purposes.

c) Potential Risks:

i. Discontinuing Medications. During the 2- to 4-week washout period, when the subject is not taking any antidepressant medications, there is a chance that their depression symptoms could get worse or that they might experience other mood symptoms.

ii. Antidepressant Medication. The most common side effects with Escitalopram are nausea, insomnia, difficulty with ejaculation, diarrhea, dry mouth, sleepiness, fatigue, dizziness, increased sweating, and decreased libido.

iii. Blood drawing. The indwelling catheter may cause a local infection with swelling, redness and pain, bleeding where the tube is placed, and bleeding under the skin forming a bruise. Rarely, there can be a severe infection of the blood stream or the heart valves or the formation of a blood clot that could go to the lungs. Complications are unlikely, but treatment would require hospital care. During the two evening admissions the catheter will be in place for up to seven hours. The total amount of blood drawn is 150 milliliters.

iv. Saliva sampling. Some dry mouth may be experienced, as well as potential gingival irritation from the dental sponge.

v. Activity monitor. There is risk of inconvenience and mild skin irritation

vi. Psychological interview and questionnaires. The psychological interviews may be stressful for the subjects due to the very personal or potentially embarrassing nature of the questions.

vii. Discontinuation of escitalopram. If the subject elects to discontinue escitalopram at the end of the study, they will be tapered off the medication over the course of two weeks (see section d.1.2.g, above). There is a risk of antidepressant discontinuation symptoms including: insomnia, agitation, anxiety, irritability, dysphoric mood, nausea, diarrhea, muscle aches, malaise, dizziness, vertigo, and vivid dreams.

2. Adequacy of Protection Against Risks

a) Recruitment and Informed Consent:

Subjects will be recruited through newspaper advertisements, the OHSU Study Participation Opportunities web page, physician or mental health provider referral and by word of mouth.

Candidates will be interviewed by a study physician. Candidates who are considered appropriate for the study and interested in participating will be asked to sign an IRB approved consent form. A general medical, psychiatric, developmental and social history will be obtained.

Candidates who are considered appropriate for the study (see above) and who are interested in participation will be provided with a written copy of the consent form. All subjects must be able to provide informed consent, as judged by the study doctor.

b) Protection against risk:

i. Discontinuing Antidepressant Medications.

To monitor for a change in mood during the washout period, the study doctor will conduct a psychiatric assessment weekly via telephone and a trained rater will also evaluate mood via telephone using the 21-Item Hamilton Depression Rating Scale (HAM-D), Beck Depression Inventory-II (BDI-II) and Beck Anxiety Inventory (BAI). If serious side effects occur, such as if the subject's mood worsens to the degree that in the clinical judgment of the study physicians they are no longer able to participate in the study (e.g. are actively suicidal or have an increase in their HAM-D rating by > 50%), they will be discontinued from the study. The study doctor will be available to evaluate the patient and, in consultation with their prescribing physician, to recommend a course of treatment (e.g., restart their prior antidepressant medications and increase visit frequency for psychotherapy) for any serious side effects that are experienced.

ii. Antidepressant Medication.

As noted above in section 1.2f., adverse events, side effects and vital signs (body temperature, blood pressure, heart rate and respiratory rate) will be documented and monitored at the end of every week of taking the study drug. And as noted above in section 1.2d., subjects' mood will be evaluated at the end of the placebo lead-in week and every two weeks thereafter throughout treatment. Finally, during a safety follow-up visit at the end of treatment, subjects will have a repeat physical exam as well as an electrocardiogram (EKG) and laboratory screening tests (the same tests done during the initial screening visit).

iii. Blood sampling.

Blood draws will be done with good sampling technique and hemoglobins and hematocrits will be done before the study as part of the screening to assess for the possibility of anemia. If a subject is tending towards anemia prior to blood sampling, we will limit the number of blood draws, substitute salivary collections for plasma collections or drop the subject from the study.

iv. Saliva sampling. Drinking water will be available to alleviate dry mouth. If there is gingival irritation and discomfort from the dental sponge the procedure will be stopped.

v. Activity monitor. Skin irritation will be minimized by fabric or ultrasuede casings for monitors.

vi. Psychological interview and questionnaires. Subjects will be told that they may refuse to answer any questions they do not wish to answer. If the questions are very upsetting to the subjects, the

subjects will be referred to their licensed mental health practitioner or primary care physician.

vii. Discontinuation of escitalopram. If the subject elects to discontinue escitalopram at the end of the study they will be tapered off the medication over the course of two weeks. During the taper, the study doctor will conduct weekly mood assessments over the phone and study staff will administer the HAM-D, BDI and BAI over the phone to ensure subject safety throughout this period.

One week after they have completed the taper, they will come to OHSU for a safety follow-up visit which includes a repeat physical and psychiatric exam conducted by a study physician as well as an electrocardiogram (EKG) and laboratory screening tests (the same tests done during the initial screening visit, see section d.1.2.h, above).

3. Potential Benefits of the Proposed Research to the Subjects and Others:

Subjects are free to withdraw from the study at any time. Subjects may contribute new information that may benefit others in the future. Results of this investigation may have implications for individuals suffering from depression.

Subjects will receive $80 for each of the two evening admissions to the Oregon Clinical and Translational Research Center at OHSU with blood drawing and/or saliva sampling and $40 for 9 weeks of wearing the actiwatch and completing the sleep diaries as well as completing the questionnaires and interviews during the assessment visits. The total compensation will be $200.

4. Importance of Knowledge to be Gained:

This study may allow us to devise better treatments for individuals who suffer from non-seasonal major depression. It may allow us to find ways to improve both mood and sleep in individuals with major depression. It may also allow us to predict which individuals will respond to circadian resetting treatments for depression.

f. Future Plans

We intend to use the pilot data from this study for a randomized, placebo controlled, cross-over study of the effects of escitalopram and circadian phase and circadian alignment. An equivalent duration of time on placebo and escitalopram and the elimination of any order effects would be only some of the improvements on the current study design. This future application would either be another industry supported, investigator initiated study or an NIH sponsored study. Because the work would remain somewhat exploratory in nature we anticipate submitting an R-21 application should funding be sought from the public health service.

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