Clinical Study Protocol

Does Xyrem® Influence Brain Dopamine in Patients with Narcolepsy? A PET Imaging Investigation

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Investigators
PI Stephen Kish, Human Brain Lab, Research Imaging Centre, CAMH
QI Gerald O'Leary, UHN – TGH, Clinical appointment at CAMH

Institution / Sponsor
Centre for Addiction and Mental Health (CAMH)
Research Imaging Centre
250 College Street
Toronto, ON M5T 1R8

The signature below denotes confirmation that this research study will be conducted according to all stipulations of the protocol, and according to local legal and regulatory requirements and ICH GCP guidelines.

Signature

9/25/2017
Date

PI: Stephen Kish, PhD
Research Imaging Centre
Human Brain Lab
CAMH

Protocol Version	Date Issued	Summary of Revisions
V 1.0	24-Feb-2014	- original REB submission
V 2.0	08-Apr-2014	- removal of stimulant using group and all related text (including associated consent form) - removal of antidepressants as an exclusion - revision of negative drug test requirement on scan days - expansion of precautions after taking Xyrem® - minor corrections as necessary
V 3.0	09-Dec-2014	- change in title
V 4.0	22-Jun-2015	- change to Dr. Shapiro's clinic location/contact information - change to study start/finish dates - addition of Data Management and Regulatory Issues sections - corrections and reformatting as necessary
V 5.0	05-Jun-2017	- addition of J. Warsh as Co-Investigator, I. Vitcu as monitor - addition of healthy control group - request for permission to access participants' GP and/or medical records for medical screening (optional) - additional blood sample taken at 0.5 hours post Xyrem® - corrections and reformatting as necessary
V 6.0	13-Sep-2017	- removal of Dr. Shapiro and TWH-UHN as a study Co-investigator / site with subsequent changes to: Site Number; Recruitment and Screening procedures - slight modification to inclusion criteria indicating that all narcolepsy participants will have a confirmed diagnosis, as determined by a sleep specialist

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1. LIST OF INVESTIGATORS

Principal Investigator
Stephen Kish, Ph.D.
Research Imaging Centre - Human Brain Lab
Centre for Addiction and Mental Health
250 College Street
Toronto, ON
M5T 1R8
Phone: 416-535-8501 x 36256
Fax: 416-979-6871
Email: Stephen.kish@camh.ca

Qualified Investigator
Gerald O'Leary, MD
Anesthesiologist-in-Chief
University Health Network – Toronto General Hospital
200 Elizabeth Street, EN3-460
Toronto, ON
M5G 2C4
Phone: 416-340-4359
Email: Gerald.O'Leary@uhn.on.ca

Co-Investigator
Isabelle Boileau, Ph.D.
Research Imaging Centre - Addiction Imaging Section
Centre for Addiction and Mental Health
250 College Street
Toronto, ON
M5T 1R8
Phone: 416-535-8501 x 34918
Email: Isabelle.boileau@camh.ca

Co-Investigator
Jerry Warsh, MD
Laboratory of Cellular and Molecular Pathophysiology
Centre for Addiction and Mental Health
250 College Street
Toronto, ON
M5T 1R8
Phone: 416-535-8501 x 34279
Email: Jerry.warsh@camh.ca

Monitor
Irina Vitcu
PET Centre, Research Imaging Centre
Centre for Addiction and Mental Health
250 College Street
Toronto, ON
M5T 1R8,
Phone: 416-535-8501 x 36491

Clinical laboratory involved in the trial
CAMH Clinical Laboratory
Queen Street Site
100 Stokes Street - Bell Gateway Building
Toronto, ON
M6J 1H4
Phone: 416-535-8501 x 32052
Fax: 416-583-4605

2. BACKGROUND AND RATIONALE

Animal data suggest that the sedative drug Xyrem® (Sodium Oxybate, Gamma-hydroxybutyrate (GHB)), used in treatment of patients with narcolepsy, might act on the brain dopamine system, but human findings are lacking.

GHB is a short chain endogenous fatty acid and GABA metabolite that has sleep-inducing CNS depressant effects. Sodium oxybate, the sodium salt of GHB is approved in Canada (as "Xyrem®") for the (night-time) treatment of cataplexy in patients diagnosed as having narcolepsy with cataplexy (hereafter referred to as "narcolepsy"), a sleep disorder characterized in part by excessive daytime sleepiness. GHB has also been proposed for use as a "neuroprotective" agent in brain disorders such as Alzheimer's disease and major depressive illness, and is also an illicit recreational, abused drug (for reviews see [1] and [2]).

Because Xyrem® is presently approved for clinical use in Canada, and is likely to be used in future for other conditions (e.g., see ongoing clinical trials in "clinicaltrials.gov"), information on the mechanism of action of Xyrem® in subjects having medical conditions which might benefit from its use, might help in understanding the actions and unwanted side effects of this clinically used drug. At present, information on the possible mechanism of action of this drug is limited to animal studies of uncertain relevance to the human and to our preliminary findings in normal subjects.

As described below, the **overall aim** of this investigation is to establish whether an action of Xyrem® on the brain dopamine system in patients with narcolepsy, and in a comparison control group, might explain part of the anti-narcoleptic effect of the drug.

Xyrem®, taken at night, is thought to act by providing continuous "refreshing" sleep to patients with narcolepsy resulting in less periods of daytime sleepiness. The mechanisms of this action are unknown; however, the manufacturer suggests that, based on animal literature, a key action might be a reduction in brain dopaminergic activity during sedation ([3] ; see also below). In this regard, some animal data suggest that an acute dose of Xyrem® depresses activity of brain dopamine neurones and, as a result, dopamine is not released from the nerve ending (see below). On the other hand, investigators leading a study of Xyrem® in Parkinson's disease patients who have sleep problems suggest a different mechanism: that Xyrem®-induced improvement of daytime sleepiness might be caused by above-normal release of dopamine (acting as a modest dopamine-delivering stimulant) during drug withdrawal (which would occur at daytime), as the dopamine built up in the neurone during sleep/sedation is released during the day [4].

Animal data (since 1966 [5]), which could address the above questions are still contradictory and sparse on the nature of GHB-dopamine interactions [6-10] (e.g., review "Does GHB inhibit *or* stimulate central DA release?")[11]. Cruz and colleagues, on the basis of some electrophysiological findings speculate that "recreational" doses of GHB could *stimulate* dopamine neurones, but that at "higher" concentrations dopamine release might be inhibited [12]. Missing from experimental animal studies are data convincingly showing effects of GHB "withdrawal" on dopamine (i.e., is withdrawal associated with a potentially beneficial dopaminergic hyperactivity?).

Animal findings, however, can only suggest what might occur in the human and need to be confirmed in the clinically relevant human.

In light of the speculations above, the available animal findings, and our preliminary brain imaging findings in normal subjects described below, we wish to determine in a brain imaging study whether Xyrem® might produce a concentration and time-dependent biphasic change in dopamine activity in

(Xyrem®-naïve) patients with narcolepsy. We will also include a group of control subjects in order to determine whether this proposed mechanism of action of Xyrem® might or might not be dependent on disease state.

How can brain dopamine be measured in living humans?
Extracellular dopamine levels cannot be directly measured in living human brain and **can only be inferred** by conducting a brain imaging (e.g., positron emission tomography; PET) study in which binding of a short-lived radiolabelled probe to components of the brain that are **competitively acted upon by dopamine** is measured.

The radiolabelled probes that we have selected are ^{11}C-raclopride and ^{11}C-dihydrotetrabenazine (^{11}C-DTBZ). ^{11}C-raclopride, has low affinity for the D2 dopamine receptor in the dopamine-rich striatum (caudate, putamen), and its binding can be influenced by changes in the concentration of extracellular dopamine. Thus, in imaging studies, administration of a stimulant drug (e.g., amphetamine) that promotes neuronal release of dopamine, and consequently increased extracellular dopamine levels, will reduce the specific binding of ^{11}C-raclopride in striatum (see [13] and references therein). ^{11}C-DTBZ binds to the striatal vesicular monoamine transporter ($VMAT_2$) and is sensitive to changes in vesicular neuronal stores of dopamine. This tracer can be used to establish whether vesicular (intraneuronal) dopamine levels are altered during Xyrem® exposure (see [14, 15]).

Should Xyrem® initially <u>decrease</u> activity of brain dopamine neurones and dopamine release during the sedated state, one would expect <u>increased</u> binding of ^{11}C-raclopride to the D2 dopamine receptor, localized *outside* the dopamine neurone, because of decreased competition from dopamine at the dopamine receptor. We would expect a decrease in ^{11}C-DTBZ binding to $VMAT_2$ due to attenuated dopamine release, accelerated dopamine biosynthesis, and resultant increase of dopamine in vesicular stores competing with ^{11}C-DTBZ for binding at $VMAT_2$.

In contrast, during withdrawal from Xyrem®, a time at which some animal data suggest that dopamine release might be <u>increased</u> (as part of a "rebound" phenomenon) we would expect binding of ^{11}C-raclopride to be <u>decreased</u>, a result of increased competition from dopamine. As described below, our preliminary data in normal subjects support these predictions.

Preliminary Study supports possibility that Xyrem® induces biphasic dopamine changes in normal human brain.
As part of a Health Canada-approved study (CTA 139361, CTA-A1 154385, CTA-A2 157188) of the influence of Xyrem® on brain dopamine measures we have obtained pilot PET data suggesting that Xyrem® changes <u>both</u> intra- and extra-neuronal dopamine levels in brain, initially depressing dopaminergic activity during sedation but later causing a slight, and potentially beneficial dopaminergic hyperactivity.

<u>^{11}C-Raclopride scans</u>.
Two subjects received a <u>baseline</u> one hour ^{11}C-raclopride scan. On a separate day subjects received a single 4.5g Xyrem® oral dose followed by <u>three</u> ^{11}C-raclopride scans: <u>45 minutes</u>, <u>six hours</u>, and <u>24 hours</u> later. The 45-105 minute scan coincides with a time of peak sedation, whereas the six hour time represents washout/early withdrawal when most/all of the Xyrem® will have been metabolized. At 6 and 24 hours we investigated the question whether there might be increased dopamine release as a result of built-up dopamine.

^{11}C-Raclopride binding potential (BP_{ND}) in whole striatum showed almost identical changes in both subjects: BP_{ND} levels were modestly *above* baseline (by 6, 13%) at the 45 minute scan and *below* baseline (-7%, -6%) at six hours, when blood GHB levels were almost undetectable. BP_{ND} levels were still lower than baseline (by 6% and 8%) when scanned the next day following Xyrem®—a time (32 half-lives) well into Xyrem® withdrawal. Our findings, which go well beyond animal data [6-11], suggest that Xyrem® can, during initial sedation, decrease dopamine release in human brain, and increase dopamine release during drug withdrawal.

^{11}C-DTBZ scans. DTBZ findings were, as hypothesized, generally "opposite" to those of raclopride. We have promoted the novel use of ^{11}C-DTBZ binding to the intraneuronal vesicular monoamine transporter **($VMAT_2$)** in dopamine neurones as an index sensitive to intra-neuronal dopamine [14-16] (the "flip-side" of extra-neuronal raclopride binding). In this scenario, a hypothetical Xyrem®-induced buildup of intraneuronal striatal dopamine would be reflected by decreased striatal ^{11}C-DTBZ BP_{ND}, because of increased competition of dopamine at $VMAT_2$. Employing the same protocol as with raclopride, striatal ^{11}C-DTBZ BP_{ND}, unlike that of raclopride, was ***decreased*** at both 45 minutes (-21%) and 6 hours (-14%), and normal [-3%] at 24 hours. This suggests that Xyrem® does indeed cause build-up of dopamine inside its neurones.

Taken together, the preliminary raclopride and DTBZ findings, provide the first hint that Xyrem® might induce, in the normal human brain, initial buildup of intraneuronal dopamine and decreased release, followed hours later by above-normal dopamine release. We now aim to determine whether either of these changes occurs in brain of patients with narcolepsy, the indication for which Xyrem® is approved, following a single dose of the drug.

3. OBJECTIVE AND HYPOTHESES

Our **Trial Objective** is to establish, in Xyrem®-naïve patients with narcolepsy and in matched controls, whether a single dose of Xyrem® causes changes in striatal binding of ^{11}C-raclopride and ^{11}C-DTBZ that would suggest decreased activity of brain dopamine neurones during the initial sedation and increased dopaminergic activity during Xyrem® withdrawal.

Our **WORKING MODEL**, based on our preliminary human PET brain data, is that an initial action of Xyrem® is to cause, during sedation (which is very fast onset with Xyrem®), depressed activity of nigrostriatal dopamine neurones and dopamine release together with marked intraneuronal biosynthesis of dopamine. At this early time, intraneuronal concentrations of dopamine (competing with ^{11}C-DTBZ at $VMAT_2$) will be high whereas extraneuronal levels of dopamine (competing with ^{11}C-raclopride at the dopamine receptor) will be low because (we predict) Xyrem® causes accelerated synthesis of dopamine while depressing activity of dopamine neurones.

During drug withdrawal, dopamine that has been building up inside the neurone is released, leading to increased extraneuronal dopamine (accompanied by decreased raclopride binding at the dopamine receptor) and a normalization of dopamine levels inside the neurone. The modestly increased dopamine activity might persist somewhat in drug withdrawal and be beneficial to the patient.

Our **HYPOTHESES**, based on our pilot data and employing a five scan approach, are that, relative to baseline, striatal 1) **^{11}C-raclopride binding** will be increased one hour post-Xyrem® but decreased at seven hours, suggesting decreased and increased neuronal dopamine release, respectively, at these times; and 2) **^{11}C-DTBZ binding** will be decreased at five hours post-Xyrem®, suggestive of increased intraneuronal vesicular dopamine buildup during Xyrem® exposure. We predict that this biphasic change in dopamine will occur in both narcolepsy and in healthy control study groups.

4. TOTAL NUMBER OF SITES AND NUMBER OF CANADIAN SITES

There will be a single Canadian site:

1) Centre for Addiction and Mental Health (CAMH), Toronto, ON Canada

5. STUDY PARTICIPANTS

5.1. Sample Size

A maximum of 15 patients diagnosed with narcolepsy, who are not currently being treated with stimulant drugs (e.g. Alertec®, Ritalin®, or Dexedrine®) to manage their narcolepsy, and a maximum of 15 healthy controls, will complete all study procedures.

Monte Carlo simulations [17] (>10,000) were performed using BP estimates and standard deviations derived from our data in normal subjects and assumed mixed models analysis and constant standard deviations, with full maximum likelihood for evaluation and time treated as categorical variable to facilitate pre-planned comparisons of outcome values. Results indicate that a sample of 15 will provide sufficient power (>80%, 0.05 alpha) to detect changes (baseline vs. GHB day scans, within subject comparisons) of the magnitude observed in the small pilot study for both raclopride and DTBZ binding.

5.2. Patient Population

Subjects are Xyrem®-naïve adult males or females aged 19 or over.

5.3. Inclusion Criteria

General inclusion

- Able to sign and date informed consent
- Willing and able to complete trial as described in protocol
- Age 19 or over, both genders
- Willing to forgo alcohol and any other sedative-hypnotic drug 48 hours prior to and 24 hours after Xyrem® administration

Narcolepsy inclusion criteria (in addition to criteria listed above):

- Must fulfill the International Classification of Sleep Disorders classification for a clinical diagnosis of narcolepsy with cataplexy as determined by a physician specializing in sleep disorders.

5.4. Exclusion Criteria

General exclusion criteria

- Use of any sedative hypnotics, tranquilizers, anticonvulsants, antihistamines (except non-sedating), benzodiazepines, clonidine, or any medications known to modulate dopamine (e.g. dopamine agonists, antagonists, DAT inhibitors, MAO-A/B inhibitors) at start of baseline period
- Have taken any investigational drug within the past 30 days prior to consent interview
- Have sleep apnea, are shift workers, or have any disorder other than narcolepsy that might be considered to be a cause of excessive daytime sleepiness
- History or evidence of current or recent (within one year of consent) substance abuse or dependence (with exception of nicotine) per interview and urine toxicology; This will include especially any subject who is a heavy alcohol drinker or who cannot control their drinking behaviour

- Any clinically significant unstable or uncontrolled medical or psychiatric disease assessed at physical examination or medical history including but not limited to cardiovascular, hepatic, renal, GI, pulmonary, neurological, or other systemic disease that might interfere with participation in study
- Clinically significant abnormal clinical laboratory urinalysis/blood findings or pre-trial EKG results demonstrating clinically significant abnormality
- Clinically significant history of head trauma, past intracranial surgery, or seizure disorder
- Have ever taken Xyrem® / sodium oxybate / GHB at any time
- Subject is on sodium-restricted diet
- Pregnancy or lactation and women of childbearing potential not taking adequate contraceptive measures [menstrual cycle status on scan days will be documented]
- Hypersensitivity or other contraindications to sedatives
- Current, past, or anticipated exposure to radiation exceeding 20mSv in the last 12 months
- Metal implants or paramagnetic objects within the body which may interfere with MRI
- Claustrophobia
- Succinic semialdehyde dehydrogenase deficiency
- Subject has any other problem that in investigators' opinion would preclude participation in trial

6. STUDY DESIGN AND DURATION

6.1. Summary of Design

Those subjects meeting inclusion/exclusion (I/E) criteria will receive, on one day, a single ^{11}C-raclopride and a single ^{11}C-DTBZ PET brain scan to provide baseline measurement, along with a 3T MRI scan for PET co-registration. On a later date each subject will receive a single 3.0 g oral dose of Xyrem® followed by three scans: two ^{11}C-raclopride scans at approximately one and seven hours and one ^{11}C-DTBZ scan approximately five hours following Xyrem® administration. Subjects will participate in a final follow-up telephone call the day after taking Xyrem® for a brief interview.

The pilot study design required subjects to be scanned four times (e.g., baseline; one, 6 and 24 hours post- Xyrem®) with raclopride and four times with DTBZ, which would require one group of subjects receiving four raclopride scans, and a separate group of subjects receiving four DTBZ scans—for which cost would be high in terms of monies, number of subjects, and radiation exposure to humans. Further, subjects receiving raclopride scans would not be those receiving DTBZ scans and, importantly, it would not be possible to correlate in individual subjects the extent of dopamine buildup inside the dopamine neurone (with ^{11}C-DTBZ) with the extent of dopamine released by the neurone (with ^{11}C-raclopride).

We, and the external reviewers of our pilot data, feel that there is value in an approach that: 1) "measures" both intraneuronal (DTBZ) and extraneuronal (raclopride) dopamine in the same group of patients in order to assess presynaptic dopamine buildup as well as dopamine release; and 2) is cost effective in terms of number of scans and subjects. We will therefore follow a five scan approach: two baseline scans using both ^{11}C-raclopride and ^{11}C-DTBZ. On a separate day subjects will receive three scans after ingesting Xyrem®: ^{11}C-raclopride at approximately one and seven hours post; ^{11}C-DTBZ at approximately 5 hours post-Xyrem®.

The raclopride scan at one hour post- Xyrem® corresponds to the time we found the single dose of Xyrem® to be maximally sedating and for which we anticipate the maximal reduction of dopamine release. Based on our feasibility data, at five hours post-Xyrem®, dopamine should have been "well" accumulated within dopamine neurones and can be assessed by a DTBZ scan. The seven hour ^{11}C-raclopride scan is conducted during drug withdrawal (approximately 14 Xyrem® half-lives) and would

correspond to the time a subject participates in morning work duties following the typical second night-time Xyrem® dose. This subject therefore might benefit from modest dopamine hyperactivity. Thus, this scan time has been selected for both "clinical" relevance and for logistical considerations.

6.2. Duration

The **study duration** for each subject will involve approximately one-two days medical screening, two PET/MRI scan days (baseline and Xyrem® day), including one MRI session for co-registration of the PET scans, and a follow-up telephone call the day after taking Xyrem®. Unscheduled visits may occur if required (e.g. subject must return for: blood work/EKG if could not be scheduled on same day as screening visit, etc.). Subject participation will likely occur over a period not longer than 5 weeks. Anticipated study start and finishing dates are September 1, 2015 and May 1, 2019, respectively.

6.3. Details of Study Design

6.3.1. Recruitment

Control subjects will be recruited by print and online advertisement and invited to contact the Research Coordinator (RC – by phone or email), who will explain the study and answer questions posed. If narcolepsy patient groups are available, the PI will speak to such groups, describe the study, and provide flyers/brochures explaining the study which will include a contact number for the RC. If the subject is interested, RC will proceed with a telephone/email screen, asking general questions to try to determine whether the subject will meet I/E criteria. If subject passes this screen they will be asked to come in for the screening and baseline evaluation day with RC.

In addition, patients meeting criteria for a diagnosis of narcolepsy with cataplexy may be referred by a specialist at sleep/neurology clinics aware of the study. The patients will be informed (through conversation and using the provided flyer/brochure) that there is an opportunity to participate in a PET study looking at the effects of a drug used to treat narcolepsy (Xyrem®) on brain dopamine. All patients will be informed that their potential participation or refusal will not jeopardize their care or change their clinical management. The patient can then contact the RC for further information if interested, and proceed through telephone/email screen for eligibility.

6.3.2. Schedule of Activities

Activity	Screening Visit(s)	Baseline Scan Day	Xyrem® Scan Day	Follow-up call
Written Informed consent	RC			
Consent review		RC	RC	RC/PI
Demographics/Drug - Alcohol Use History	RC			
Psychiatric Interview	RC			
Routine Blood Work[1] & EKG	CL			
Urine collection/drug screen[2]	RC	RC	RC	
Medical History & Physical Exam	SP			
I/E determination	PI/QI			
Adverse Event review/log		RC/PI/QI	RC/PI/QI	RC/PI/QI
MRI Scan		RIC-MRI		
I.V. or catheter placement		RIC-PET	RIC-PET	
Record/monitor vitals			A/QI	
PET Scans		RIC-PET	RIC-PET	
Xyrem® administration			A/QI	
Blood draw for GHB level			RIC-PET	
Interview regarding lingering effects of Xyrem®				RC/PI

[1]Routine Blood Work (at screening) includes serum chemistry (sodium, potassium, chloride, urea (BUN), creatinine, total protein, albumin, total bilirubin, ALT, AST, ALP, GGT, LDH, calcium, magnesium, TSH, free T4), random glucose, CBC + differential, ESR,

PT/INR, and serum β-HCG for all women of childbearing potential. [2]Urine drug screen includes a rapid response urine test panel immediately read by RC and broad spectrum analysis done by CAMH Clinical Lab. A = Anesthesiologist, CL = Clinical Lab, PI = Principal Investigator, QI = Qualified Investigator, RC = Research Coordinator, RIC-MRI = Research Imaging Centre MRI group, RIC-PET = Research Imaging Centre PET group, SP = Study Physician.

6.3.3. Pre-Study Screening and Baseline Evaluation (Days 1 and 2)

Subjects will come to CAMH for a Screening day visit, during which they will meet with RC who, after further explanation of the study, will take informed consent. Each potentially eligible person who has signed informed consent will be assigned a Subject ID in consecutive order starting from Jazz-001. Should the subject meet all criteria and continue through the study, they shall keep the same ID throughout. For those subjects who signed consent and subsequently fail to meet eligibility requirements, a minimal data set will be maintained under their Subject ID (i.e. consent form, demographics, assessments used to determine eligibility).

The following procedures will be performed at the screening visit(s):

- Obtain written informed consent
- Structured clinical interview for demographics, drug use history (including alcohol and nicotine using AUDIT questionnaire and Fagerstrom Test)
- Psychiatric interview for DSM-IV (DSM-5) Axis I disorders using SCID-NP, SIGH-SAD (Ham-D)
- Urine specimen collection for:
    - Rapid Response urine test panel (results immediately assessed by RC)
    - Broad spectrum urine drug analysis (assessed by CAMH Clinical Lab)
- EKG with 12-lead electrocardiogram (assessed by cardiologist)
- Blood collection for:
    - Routine blood work (see schedule of events for detailed list)
    - For female subjects of childbearing age, serum pregnancy test (β-HCG)
- Medical history and physical exam with study physician
- For narcolepsy subjects, if consent is given, a member of the study team will collect required information from the subject's referring physician in order to characterize their condition.

This visit will take approximately four hours, and may be broken up over more than one day in order for the physician to have access to the lab results prior to performing the physical evaluation.

The study physician will review all lab results once they are available, and make a recommendation for suitability for the study. If permission given, the study physician may contact the subject's family physician and/or review their medical records for corollary information to help alleviate any concerns regarding medical history or lab results if necessary. All the above information is provided to the QI and PI for final eligibility determination. If the subject qualifies and is enrolled in the study, the QI will provide a prescription for 3.0 g Xyrem®. RC will give the prescription to CAMH Pharmacy at least one week prior to the subject's scheduled Xyrem® scan day.

Subjects will be informed of their status, and if they are to continue in the study, RC will schedule the *Baseline Scan Day* appointment prior to subject leaving the screening visit (if possible).

Subjects will be informed that transportation to and from CAMH for all subsequent visits will be provided for them (i.e., by taxi) as required.

6.3.4. Treatment/Assessment visits

6.3.4.1. Day 3: Baseline PET/MRI

During this visit the following tasks will occur:

- RC meets with the subject, reviews the tasks to be completed for the day, and confirms the subject is willing to continue
- Urine specimen collected for:
  - Rapid Response urine test panel (immediately assessed by RC, must be negative for contraindicated drugs)
  - Broad spectrum urine drug screen (assessed by CAMH Clinical Lab)
  - Pregnancy test in females of child bearing age (immediately assessed by RC, must be negative)
- Subject receives a 3T MRI scan for PET co-registration (see below for details)
- A single I.V. line or fine needle catheter (one per tracer) is placed in the subject's arm for tracer administration
- Subject receives a single ^{11}C-raclopride scan followed by a single ^{11}C-DTBZ PET scan at CAMH PET Centre (see below for details)
- RC enquires about any adverse events (AEs) that the subject may have suffered as a result of the days activities; these are recorded on the AE log
- Subject is dismissed upon completion of 2nd scan

This visit should take approximately seven hours. While in the PET Centre, subjects will be provided with a private room where they can rest/lay down/sleep between procedures as necessary.

6.3.4.2. Day 4: Xyrem® Administration Day

The Xyrem® scan day should take place within one week of the Baseline scan day.

During this visit the following tasks will occur:

- RC meets with the subject, reviews the tasks to be completed for the day, and confirms the subject is willing to continue
- RC enquires about any AEs since last visit; these are recorded on AE log
- Subject is interviewed by RC and by anesthesiologist re: physical status and recent use of any medication, food, or liquids. Subject must confirm no food (including milkshakes, smoothies or instant breakfast drinks) since previous night and no liquids for the past 2 hours. If this is not the case, subject <u>does not</u> take Xyrem®
- Urine specimen collection for:
  - Rapid Response urine test panel (immediately assessed by RC, must be negative for contraindicated drugs)
  - Broad spectrum urine drug screen (assessed by CAMH Clinical Lab)
  - Pregnancy test in females of child bearing age (immediately assessed by RC, must be negative)
- An I.V. line is placed in the subject's arm to allow for blood sampling and tracer injection
- Subject is connected to a physiological monitor (supplied by PET Centre): vitals (HR, BP, Respiration rate, O_2 saturation) are taken and recorded. Vitals must be within normal range (assessed by anesthesiologist) for subject to receive Xyrem®.
- A sample of venous blood (approximately 7 ml) is taken for baseline GHB measurement.
- 3.0 g liquid Xyrem® is prepared in CAMH Pharmacy, with sign-off by Pharmacist(s), RC and a 2nd member of study staff, confirming correct dosage. Each dosing cup will be labeled, sealed, and brought by Pharmacist together with RC or 2nd staff to PET scan room.

- Anesthesiologist confirms/signs that the drug has been delivered, the seal is intact, and the subject ingests the full dose; RC records the time dose was ingested.
- A sample of venous blood is taken approximately 0.5, 1, 3, 5 and 7 hours post-Xyrem® ingestion for GHB measurement
- Subject receives two ^{11}C-raclopride scans starting approximately one and 7 hours post-Xyrem® and one ^{11}C-DTBZ PET scan approximately five hours post-Xyrem®
- Anesthesiologist will monitor subject and record the time when level of consciousness and vital signs have returned to normal, indicating that it is safe for subject to leave any time after that point. RC will record the time that subject actually leaves the PET/MRI Centre (after the 7 hour PET scan); by taxi or with companion who will drive them home. Both RC and Anesthesiologist will sign-off on this discharge form.
- RC enquires about any AEs that the subject may have suffered as a result of the days activities
- After final scan and AE evaluation, subject is paid the honorarium for full completion of the study, reminded that he/she cannot drive or operate heavy machinery (including farm equipment), and should not engage in strenuous activities, consume alcohol or any sedatives, make important decisions or engage in any activity requiring mental alertness for the next 24 hours.
- Subject will be given a wallet card printed with study details (e.g., Xyrem® dose, date and time taken, precautions/warnings, contact information for study personnel) and told that this should be kept on them (in their wallet) as a reminder of the precautions necessary and also to provide emergency contact information
- Subject is then discharged

This visit will take approximately 10 hours.

6.3.4.3. Telephone Follow-up

Subject is contacted the following morning, by RC and/or PI. The purpose of the phone call will be to enquire about the subject's wellbeing since leaving the PET Centre.

6.3.5. Unscheduled Visits

Subjects may need to return to the site for an unscheduled visit (i.e. extra day for screening visit if time/schedule did not permit completion over 2 days), or receive a follow-up phone call for evaluation of conditions relating to their participation in the study or the study drug (i.e. ongoing AE that the QI has determined needs to be tracked). The date and reason for these visits/phone calls will be recorded.

6.4. PET and MRI scan procedure, data acquisition, and image analysis

PET measurement of ^{11}C-raclopride binding to the striatal brain dopamine receptor and ^{11}C-DTBZ binding to $VMAT_2$ are common, routine procedures used worldwide (e.g., see our publications ref [14, 18] and references therein). Briefly, PET images of ^{11}C-raclopride (approximately 60 minute) or ^{11}C-DTBZ (approximately 60 minute) scans will be acquired using CPS-HRRT neuro-PET camera system (Siemens Medical Imaging, Knoxville, TN). Head movement minimization is achieved with a head immobilization system (Tru Scan Imaging, Annapolis USA). Transmission scans will be obtained using a single-photon ^{137}Cesium ($E\gamma$ = 662 keV) point-source, and used to correct the emission scans for the attenuation of 511 keV photons through tissue and head support.

The PET scans will be initiated following a bolus injection of ^{11}C-raclopride or ^{11}C-DTBZ into an arm vein. Raw data are reconstructed by filtered-back projection [19].

MRI (up to approximately one hour): The structural MRI protocol includes the primary structural MRI scan to be used for PET coregistration. This standard spin-echo proton-density weighted protocol (MRI; TE = Min Full, TR = 6000, field of view = 22 cm 2D, slice thickness = 2mm, NEX= 1) will be obtained (Signa 3Tesla GE MRI scanner, (Discovery MR750) General Electric Medical Systems, Milwaukee, WI) for purpose of region of interest (ROI) delineation.

ROI delineation and analysis will be performed following standard operating procedure for PET ROI analysis. In brief, a standard brain template will be transformed to fit individual high-resolution MRI scans, creating ROIs. These will then be refined, aligned and resliced to match the dimension of the PET images. The whole striatum (the primary region of interest) as well as bilateral sub-compartments of the striatum including ventral striatum and pre and post commissural caudate and putamen (as described by Mawlawi [20]) as well as reference tissues (which provide the input function), namely, cerebellar cortex (excluding vermis) for ^{11}C-raclopride [18] and the occipital cortex for ^{11}C-DTBZ [21] are selected as ROIs. ^{11}C-Raclopride and ^{11}C-DTBZ time activity curves will be obtained from the dynamic data and specific binding (BP_{ND}) will be estimated in each ROI. This method has been validated to reliably estimate BP_{ND} for ^{11}C-raclopride (e.g., [22]) and ^{11}C-DTBZ[14].

We will ensure that scans are not corrupted by motion artefact by de-noising and visually inspecting all images. Should MRI data suggest changes in volume/shape of ROIs, partial volume effect (PVE) adjustment will be made. We will report both PVE corrected and uncorrected data.

7. EFFICACY VARIABLES AND ANALYSIS

The **primary endpoint** is the change in ^{11}C-raclopride or ^{11}C-DTBZ BP in whole striatum, caudate, putamen, and bilateral sub-compartments of the striatum, from baseline scan vs. that at one, 5 and 7 hour scans.

The **secondary endpoint** is the blood concentration of GHB (expressed as the area under the curve; AUC) at 0.5, 1, 3, 5 and 7 hours following ingestion of GHB.

8. STUDY DRUG

8.1. Drug Formulation

Each bottle contains 180 ml of Xyrem® at a concentration of 500 mg/ml. Xyrem® is supplied as a kit, containing a press-in-bottle adapter and a 10 ml oral measuring device (plastic syringe). The pharmacy will provide a 100 ml dosing bottle.

8.2. Dosage Regimen

Each subject will receive a single oral dose of 3.0 g (6 ml of a 500 mg/ml solution) diluted in 60 ml of water. Subjects will lie down immediately after dosing.

The pilot study 4.5 g dose was selected by Dr. Mortimer Mamelak, Toronto (a consultant who has extensive clinical experience in administration of Xyrem® for narcolepsy; [23-27]) as a commonly used therapeutic dose that is sufficient to cause sedation but can be safely administered to a Xyrem®-naïve subject under supervision. Both Dr. Mamelak and PI believe that sedation will be required for Xyrem® to exert an effect on the dopamine system that can be assessed using ^{11}C-raclopride or ^{11}C-DTBZ. Further, it is Dr. Mamelak's experience that many subjects receiving smaller doses similar to 2.25 g will not experience somnolence. Regarding safety, we note that in one published Xyrem® multicenter trial in narcolepsy [28] a group of drug-naïve subjects received *two* 4.5 g doses (spaced 2.5-4 hours apart) *at their homes* (the trial coordinator phoned the subjects after their first night and three times weekly),

and the drug was well-tolerated. In a second study also in narcolepsy patients, the patients received a single initial dose of 4.5 g Xyrem® in a sleep lab setting and the drug was also well-tolerated [29].

The Xyrem® dose we have selected (3.0 g) to carry forward is slightly less than that used in our original study (4.5 g). In the pilot study, using 4.5 g dose of Xyrem® we did encounter subjects who developed nausea, some with vomiting (while lying supine), after ingesting the drug. While listed as a known adverse reaction, this does prevent scanning of the individuals during the earlier time points as they are unable to lie on their back in the scanner. We have chosen to reduce the dose to 3.0 g, which is well within the range of doses used in treatment of narcolepsy as a first dose; and is less likely to induce nausea/vomiting in Xyrem®-naïve subjects than the 4.5 g dose.

8.3. Washout Period

Xyrem® is rapidly eliminated by metabolism with a plasma half-life of approximately 0.5 to 1 hour and time to peak plasma concentration of 0.5 to 1.25 hours (Xyrem® monograph).

The almost certain (>95%) consequence of a single 3.0 g dose of Xyrem® is reversible sedation, depressed consciousness, and possibly sleep lasting approximately 15 minutes to 3.5 hours. Vital signs (HR, BP, respiration rate, oxygen saturation) and level of consciousness will be regularly monitored immediately before (baseline measurement) and post-Xyrem® by an anesthesiologist, working under supervision of Dr. O'Leary, the QI. The anesthesiologist present for the Xyrem® dose will also establish and sign-off (with co-sign-off by RC or PI) when the subject can safely leave (by taxi or driven by companion) the PET Centre (estimated 6 hours post drug). Subject will be contacted by phone the following morning, by RC and/or PI for a brief interview to enquire about their wellbeing since leaving the PET Centre.

9. CONCOMITANT MEDICATION

Subjects will be carefully screened at the beginning of the study to ensure that they are healthy and safely able to take Xyrem®. If subjects are currently on medication (including antidepressants for cataplexy) to treat a medical or mental illness, this condition must be stable/controlled, and they must be on a stable dose of said medication. The QI will determine whether the subjects' medications would be contraindicated. On the Xyrem® Scan day, specifically disallowed drugs/medications are any sedative-hypnotics, or any other CNS depressants including alcohol.

10. RESCUE MEDICATION AND RISK MANAGEMENT

10.1. Risks from Xyrem®

Our primary goal is the prevention of the two main predictable possible adverse events (airway obstruction and/or central respiratory depression from oversedation, aspiration of gastric contents). To this end we first undertake to review carefully the reports of the candidates' history and physical examinations before they enter the study to try to identify co-morbidities that might put the subjects at increased risk. If needed, a joint discussion with the QI, Dr. O'Leary and the PI would ensue to resolve issues identified before enrollment would continue.

While sedation is anticipated, significant respiratory depression with the dose proposed is not. If present, the drug profile indicates that this would be self limiting (i.e. does not require an antidote) and of short duration (2-3 hours). In this regard, data from a recent investigation of GHB overdose cases suggest that most such subjects coming to the ER do not actually require intubation and are managed conservatively by lateral positioning, either oropharyngeal or nasopharyngeal airway adjuncts, and close physiological and neurological monitoring [30].

During the study when Xyrem® is administered the anesthesiologist will monitor the subject's level of consciousness and vital signs. The physiological monitor in this unit will allow continuous display of oxygen saturation, HR, respiratory rate and intermittent blood pressures. Based on Dr. Mamelak's experience with Xyrem®, we expect that no subject will require any intervention, including oxygen. However a variety of ancillary airway devices will be available including the ability to give oxygen by nasal prongs and mask, address airway obstruction (oral and nasal airways, laryngeal masks), manually ventilate (Laerdal bag and mask), intubate (suction, laryngoscope, endotracheal tubes), medications for sedation (midazolam, propofol), muscle relaxation (succinylcholine, rocuronium), inotropic support and emergency management (ephedrine, epinephrine, phenylephrine). While we do not expect that interventions other than simple airway management will be needed, the anesthesiologist will have the expertise, skill set and equipment to protect the airway and manage ventilation if called upon. The spectrum of interventions that will be available is large and will be dictated by the patient's condition.

Per CAMH policy, a code blue call to Switchboard must be made, for example, in cases of respiratory arrest or significant depression of respiration or aspiration of vomit causing choking. Again, the anesthesiologist has the expertise to manage these issues. In these scenarios Switchboard would not be instructed to call 911 at the time of the code blue call as the resources within the unit are expected to meet the clinical needs. However, the CAMH-College Street site Attending Physician/Duty Doctor/Medical Doctor, who proceeds to the scene, leads the code and will determine whether a 911 call needs to be made. An external 911 call will be made if the subject does not respond to the management provided by the anesthesiologist or is considered by either the anesthesiologist or attending medical staff to require outside intervention because of a critical condition. An example would be signs of cardiac arrest or instability. In the unlikely event that it was felt that a transfer to an emergency department was indicated this would be activated according to the priority of the clinical situation. The anesthesiologist would accompany the patient to the ER.

Based on measurement of vital signs (HR, BP, respiration rate, oxygen saturation) and level of consciousness, the anesthesiologist will establish and sign-off (with co-sign-off by RC or PI) when the subject can safely leave (by taxi) the PET Centre (estimated 6 hours post drug). Subject will be given a wallet card printed with study details (e.g., Xyrem® dose, date and time taken, precautions/warnings, contact information for study personnel) and told that this should be kept on them (in their wallet) as a reminder of the precautions necessary and also as emergency contact information. Subject will be contacted by phone by PI and/or RC the following morning to enquire about his/her wellbeing since leaving the PET Centre.

There are other risks associated with Xyrem® (estimated from clinical trial data employing a variety of doses taken chronically) including headache, unspecified pain, confusion, diarrhea, urinary incontinence, and insomnia. As GHB can be an abused drug, there is generic (we feel very low) risk that the subjects might "like" the GHB experience and wish to take the drug recreationally. This risk is minimized by selecting subjects who do not have a history of substance abuse.

10.2. Other risks that might result from study procedures

There is the unknown risk of radiation exposure as part of the PET scanning procedure. However, the radiation dose provided has not been found to be associated with any harm. Subjects exceeding the yearly radiation dose limit (20 mSv) will be excluded from study. There is the risk of bruising and discomfort from venipuncture. Some subjects might find some questions on the screening tests (e.g., SCID) uncomfortable.

11. PREMATURE WITHDRAWAL/DISCONTINUATION CRITERIA

Subjects are advised in the consent form that they are free to withdraw from the study at any time without prejudice, and may be withdrawn at the Investigators discretion.

Trial will also be terminated should subject develop or be found to have any condition that might compromise subject safety (e.g., unstable vitals, presence of abused drug in urine) or confound interpretation of primary PET outcome measure.

If the subject withdraws from the study at any time prior to Xyrem® ingestion, they will be paid a pro-rated amount, and are free to leave at any time. If they withdraw from the study after Xyrem® ingestion, the subject must remain in the PET Centre, monitored by the anesthesiologist, until such time that the anesthesiologist determines it is safe for the subject to leave. Subject will then be paid a pro-rated amount, and allowed to leave by taxi or with companion. All data collected up to the point that the subject withdraws will be used (if applicable).

12. SAFETY VARIABLES AND ANALYSIS

Prior to and following Xyrem® administration the anesthesiologist will monitor the subject's level of consciousness and vital signs. The physiological monitor in the PET Centre unit will allow continuous display of oxygen saturation, HR, respiratory rate and intermittent blood pressures (every 15 minutes or more frequent if indicated).

12.1. Adverse Events

Adverse events will be assessed at each study visit following administration of the first PET radiotracer (Baseline scan day). Study staff will question the subject directly asking: how they are feeling following the scans/Xyrem®; how have they been feeling since last visit. All AEs, whether reported by the subject or observed by study staff/investigators, will be recorded (as a diagnosis not per symptom: i.e. common cold vs. sore throat, sinus congestion, etc.) on the AE log along with a brief description, start date/resolution date and any action taken (medications, Dr. visit, etc.). The AE log will be initialed by the QI, who will make the determination on relationship of the AE to the investigational drug. All AEs determined by the QI to have a causal relationship with the investigational product or as a result of study procedures, will be reported to CAMH REB as part of the annual ethics approval renewal process. Examples of this would include, but not be limited to: claustrophobia from either PET or MRI scanner; nausea and/or vomiting, headache – known possible reactions to Xyrem® as per Xyrem® monograph (depending on severity as assessed by QI).

Final contact with the subjects occurs the day after Xyrem® administration, via phone call from RC and/or PI. During this call, RC and/or PI will enquire about any new AEs or unresolved AEs that have been recorded in the subjects file. If the AE is still unresolved, they will be marked as ongoing, and the QI will determine whether the AE requires any further follow-up. This will be documented on the log, with follow-up calls/visits to occur if/as necessary using the unscheduled visit forms.

12.2. Serious Adverse Events

As defined by Health Canada, **a serious adverse event (SAE)** is an adverse event that:

- requires in-patient hospitalization or prolongation of existing hospitalization
- causes congenital malformation
- results in persistent or significant disability or incapacity
- is life threatening
- results in death

Important medical events that may not result in death, be life-threatening or require hospitalization, may be considered an SAE when, based upon medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

A **Serious Unexpected Adverse Drug Reaction (SUDAR)** as defined by Health Canada is a serious adverse drug reaction that is not identified in nature, severity or frequency in the risk information set out in the investigator's brochure or on the label of the drug.

If any AEs recorded are determined by the QI to be SAEs or SUADRs, they must be reported, within a specified time frame to:

1) CAMH REB: QI must complete and sign the CAMH REB Serious Adverse Events form and submit to the REB within 48 hours of knowledge. The QI will include a description of the event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality.

2) Health Canada: As required under Food and Drug Regulations, Part C, Division 5:
During the course of a clinical trial, the sponsor/investigator shall inform the Minister of any serious unexpected adverse drug reaction in respect of the drug that has occurred inside or outside Canada as follows:
(*a*) if it is neither fatal nor life threatening, within 15 days after becoming aware of the information; and
(*b*) if it is fatal or life threatening, within seven days after becoming aware of the information.

The sponsor/investigator shall, within eight days after having informed the Minister, submit a complete report in respect of that information that includes an assessment of the importance and implication of any findings made.

3) Jazz Pharmaceuticals: The investigators will inform Jazz Pharmaceuticals of any SAEs occurring within the study subject population. Jazz will be copied (via AEReporting@jazzpharma.com) on the CAMH REB SAE report, any subsequent correspondence, and will be provided a copy of the CIOMS form I report.

For expedited reporting to Health Canada and Jazz Pharmaceuticals, the investigators will use the CIOMS form I.

13. STATISTICAL ANALYSIS

The Biostatistical Consulting Service at CAMH, who provided the power calculations, will continue to advise on all statistical analyses to be conducted. Prior to analysis, data will be screened to ensure that underlying assumptions for statistical procedures are met. Should any demographic characteristic be found to be associated with outcome measures, they will be included as covariates in subsequent analyses. Changes in raclopride binding in whole striatum will be tested using a mixed model, with raclopride binding the dependent variable and time (baseline vs. one hour post-Xyrem® and vs. 7 hours post- Xyrem®) as the categorical independent variable. An unstructured covariance structure will be assumed. Should a significant time effect be detected, a series of Bonferroni-adjusted pairwise comparisons will be carried out. Changes in DTBZ will be assessed using a paired t-test. Subcompartments of striatum will be examined in a separate exploratory analysis. An exploratory correlation (Pearson) will investigate relationship between DTBZ binding changes at five hours (dopamine buildup) vs. raclopride changes at 7 hours (release of dopamine that had been "built-up").

14. DATA MANAGEMENT AND RETENTION

A Case Report Form (CRF) will be completed for each subject enrolled in the study. Investigators will protect privacy by assigning an identification code to each (i.e. Jazz-001). A master list containing ID codes matched to the subject's complete name and contact information will be held in a secure file, separate from the CRF. A subject screening log, noting reasons for screen failure, where applicable, will be maintained for all.

Digital files of PET and MRI data will be considered source documents and will be stored on a password-locked computer. The CRF will note the day and time of the PET scan, its number in the PET Centre Database, the radiotracer information (injected radioactivity, specific activity, total mass, etc.) and a note to the effect that the scan was completed with no technical problems.

Study data and other essential documents will be retained in a secure setting by the investigators for a period of 25 years as required by federal regulations.

15. REGULATORY ISSUES

15.1. Ethics Approval

Prior to initiation, this study will have received approval from CAMH REB as well as Health Canada No Objection Letter (NOL). CAMH REB approval is subject to annual renewal. Any amendments to the study will require approval from the above agencies prior to implementation, except when necessary to eliminate immediate hazards and/or protect the safety, rights or welfare of subjects.

15.2. Consent

Consent to enter the study must be sought from each subject only after a full explanation has been given, an information sheet offered and time allowed for consideration. Signed informed consent will be obtained prior to conducting any study procedures (with the exception of the initial phone screen). The right of the subject to refuse to participate without giving reasons must be respected. All subjects are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

15.3. Confidentiality

All personal study subject data collected and processed for the purposes of this study will be managed by the investigators and their staff with adequate precautions to ensure the confidentiality of those data, and in accordance with applicable national and local laws and regulations on personal data protection. The ethics committees approving this study, CAMH Monitor(s), and Health Canada will be granted direct access to the study subjects' original study records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subjects, to the extent permitted by the law and regulations. In any presentations of the results of this study at meetings or in publications, the subjects' identity will remain confidential.

15.4. Sponsor

Research Imaging Centre, CAMH

15.5. Funding

This study is being funded through a grant from Jazz Pharmaceuticals Inc.

15.6. Audits

As part of the quality assurance review of this research project, this study may be subject to inspection, monitoring and/or audit by CAMH or Health Canada staff.

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