

[TITLE PAGE – REVISED IRB PROTOCOL]

PROTOCOL TITLE: A Randomized, Double-Blinded, Placebo-Controlled Study to Determine if Caffeine Citrate Accelerates Emergence from Anesthesia

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Reversing Anesthesia with Caffeine

Patients who are anesthetized, wake after their bodies clear the anesthetic. Our preliminary studies found that in rodents that caffeine could dramatically accelerate emergence from anesthesia. Preliminary data from a Pilot study in human subjects suggest that humans show a similar response to caffeine. The goal in this application is to determine the mechanism for the emergence and to test whether caffeine can accelerate emergence from anesthesia in human subjects.

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INTERVENTION: Study Drug Caffeine Citrate

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STUDY SUMMARY (DRUGS)

TITLE	A Randomized, Double-Blinded, Placebo-Controlled Study to Determine if Caffeine Citrate (caffeine) Accelerates Emergence from Anesthesia
OBJECTIVES	To determine if caffeine will accelerate emergence from anesthesia.
PRIMARY OBJECTIVE	Determine whether subjects wake more rapidly from anesthesia after administration of caffeine as measured by the time between terminating anesthetic and the reinstatement of the gag reflex.
SECONDARY OBJECTIVES	<p>Determine whether subjects wake more rapidly from anesthesia after administration of caffeine as measured by the time between terminating anesthetic and responding to simple commands (“squeeze my hand”).</p> <p>Normally patients show cognitive impairments for hours after waking from anesthesia. We wish to determine whether caffeine administration ameliorates the cognitive impairment.</p>
STUDY CENTER(S)	Single-center at University of Chicago
NUMBER OF SUBJECTS	Up to 8
DIAGNOSIS AND MAIN INCLUSION CRITERIA	Healthy male, ages 25-40 with no systematic diseases or conditions, metabolic equivalents of functional capacity, low risk for Obstructive Sleep Apnea, no history of arrhythmia, BMI < 30 kg/m ² , no personal or family history of malignant hyperthermia, no history of any mental illness, no history of drugs or alcohol abuse, no history of seizure disorders or head trauma
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for up to 10 weeks
INVESTIGATIONAL PRODUCT	Caffeine Citrate
DOSE, ROUTE, REGIMEN	15 mg/kg body weight, given intravenously 10 min before the end of 1 hour isoflurane anesthesia

DURATION OF ADMINISTRATION	Study drug given intravenously across a 10 min time window
STATISTICAL METHODOLOGY	In this crossover study volunteers will serve as their own controls. The data will be analyzed with a paired t-test.

LIST OF ABBREVIATIONS

EXAMPLES

<i>UCMC</i>	<i>UNIVERSITY OF CHICAGO MEDICAL CENTER</i>
<i>UC</i>	<i>UNIVERSITY OF CHICAGO</i>
<i>OCR</i>	<i>OFFICE OF CLINICAL RESEARCH</i>
<i>IRB</i>	<i>INSTITUTIONAL REVIEW BOARD</i>
<i>URA</i>	<i>UNIVERSITY RESEARCH ADMINISTRATION</i>
<i>ORS</i>	<i>OFFICE OF RESEARCH SERVICES</i>
<i>CRSO</i>	<i>CLINICAL RESEARCH SUPPORT OFFICE</i>
<i>UCCRC</i>	<i>UNIVERSITY OF CHICAGO CANCER CLINICAL RESEARCH CENTER</i>
<i>CRC</i>	<i>CLINICAL RESOURCE CENTER (FORMERLY GCRC)</i>
<i>DSMB</i>	<i>DATA SAFETY AND MONITORING BOARD</i>
<i>FDA</i>	<i>FOOD AND DRUG ADMINISTRATION</i>
<i>CDER</i>	<i>CENTER FOR DRUG EVALUATION AND RESEARCH</i>
<i>CBER</i>	<i>CENTER FOR BIOLOGICS EVALUATION AND RESEARCH</i>
<i>NIH</i>	<i>NATIONAL INSTITUTES OF HEALTH</i>
<i>NCI</i>	<i>NATIONAL CANCER INSTITUTE</i>
<i>CTSA</i>	<i>CLINICAL AND TRANSLATIONAL SCIENCE AWARD</i>
<i>ITM</i>	<i>INSTITUTE FOR TRANSLATIONAL MEDICINE</i>

1. INTRODUCTION

1.1 BACKGROUND AND RATIONALE

1.1.1 Background: Disease and current understanding of disease

Our lab has studied anesthetics for almost fifteen years. We, as well as other labs, found that anesthetics inhibit neurotransmitter release (Crowder *et al.*; van Swinderen *et al.*, 1999; Winegar & MacIver, 2006; Herring *et al.*, 2009; Herring *et al.*, 2011; Saifee *et al.*). We thought that if we could reverse this action that we would be able to reverse aspects of anesthesia. In our preliminary studies, several drugs were studied that normally facilitate neurotransmitter release. If inhibiting neurotransmitter release is part of the mechanism leading to anesthesia (Crowder *et al.*; van Swinderen *et al.*, 1999; Winegar & MacIver, 2006; Herring *et al.*, 2009; Herring *et al.*, 2011; Saifee *et al.*) then drugs that facilitate neurotransmitter release may aid in reversing anesthesia. Elevating intracellular cAMP, with drugs like forskolin or caffeine, is well known to facilitate neurotransmitter release (Byrne & Kandel, 1996; Trudeau *et al.*, 1996; Fujita-Yoshigaki, 1998; Fujita-Yoshigaki *et al.*, 1998; Lonart *et al.*, 1998; Trudeau *et al.*, 1998; Takahashi *et al.*, 1999; Trudeau *et al.*, 1999; Kuromi & Kidokoro, 2000; Hilfiker *et al.*, 2001; Machado *et al.*, 2001; Sakaba & Neher, 2001; Burgoyne & Morgan, 2003; Kuromi & Kidokoro, 2003; Lonart *et al.*, 2003; Sakaba & Neher, 2003; Seino & Shibasaki, 2005; Kasai *et al.*). We found that drugs that elevate intracellular cAMP levels reversed the inhibition of neurotransmitter release caused by anesthetics in *in vitro* studies. In addition, the cAMP elevating drugs caused rats to wake up very quickly from anesthesia.

Normally recovery from anesthesia is passive. Anesthetic is terminated and people wake up. Some do so slowly. Age, genetics, gender and other factors come into play with regards to waking time (Solt *et al.*, 2011). But currently there is nothing that a clinician can do to hurry the process. Additionally, there are cognitive impairments that last for hours following anesthesia (Nathanson *et al.*, 1995; Larsen *et al.*, 2000; Chen *et al.*, 2001). Reaction times and memory are impaired following anesthesia (Nathanson *et al.*, 1995; Larsen *et al.*, 2000; Chen *et al.*, 2001). People (outpatients) are being released from surgery/ anesthesia on an ever shortening time scale to save money. It would be significant if clinicians could wake the patients safely, rapidly and completely with no cognitive issues.

Anesthesia is thought to work similarly in humans and rodents. In our preliminary studies we found that drugs that elevate intracellular cAMP, like caffeine, dramatically accelerated recovery from anesthesia in rats. *The question that we hope to address is the following: Do drugs that elevate cAMP levels work the same way in humans? Do these drugs accelerate emergence from anesthesia?* Preliminary data from a Pilot study suggests that this is in fact the case.

1.1.2 Investigational Agent

The drug that will be used in this study is caffeine citrate.

In August of 2013 the FDA was sent an application for IND exemption for caffeine. The exemption was not granted. Our next step was to obtain NIH funding for the project, which we obtained. Next we sent off a formal IND application for caffeine citrate. That

IND was approved with (IND #127863). The University of Chicago IRB also approved our protocol (IRB 15-0897).

1.1.3 Preclinical data

Previously my lab has shown that clinically relevant concentrations of anesthetics inhibit neurotransmitter release from both secretory cells and neurons. We tested isoflurane, propofol, etomidate and ketamine. All four anesthetics efficiently inhibited neurotransmitter release (Herring BE, Xie Z, Marks J and Fox AP (2009); Herring BE, McMillan, K., Pike, C. Marks, J, Fox AP., Xie Z (2011); Xie Z, McMillan, K., Pike, CM., Cahill, AL., Herring BE., Wang, Q., & Fox AP., (2013)).

We hypothesized that *inhibition of neurotransmitter release plays a key role in how anesthetics produce anesthesia in animals and humans*. Furthermore we hypothesized *that drugs that reverse the inhibitory effects of anesthetics on the release machinery may help accelerate recovery from anesthesia*. Historically, cAMP signaling has been shown to play a key role in synaptic function and plasticity. Elevating cAMP facilitates neurotransmitter release (Byrne & Kandel, 1996; Trudeau *et al.*, 1996; Fujita-Yoshigaki, 1998; Fujita-Yoshigaki *et al.*, 1998; Lonart *et al.*, 1998; Trudeau *et al.*, 1998; Takahashi *et al.*, 1999; Trudeau *et al.*, 1999; Kuromi & Kidokoro, 2000; Hilfiker *et al.*, 2001; Machado *et al.*, 2001; Sakaba & Neher, 2001; Burgoyne & Morgan, 2003; Kuromi & Kidokoro, 2003; Lonart *et al.*, 2003; Sakaba & Neher, 2003; Seino & Shibasaki, 2005; Kasai *et al.*). We posited that elevating intracellular cAMP might alter anesthetic action by restoring neurotransmitter release. Drugs that elevate [cAMP]_i levels, were tested; these drugs were found to completely reverse the inhibitory effects of anesthetics on neurotransmitter release in *in vitro* studies. When tested, *these same cAMP elevating drugs dramatically accelerated recovery from anesthesia in rats*. So far we have tested three cAMP elevating drugs (forskolin, theophylline and caffeine) in rats. All accelerate recovery from anesthesia in rats. The Pilot study that was just completed suggests that caffeine appears to accelerate emergence from anesthesia in humans.

There are two studies that bear directly on the cAMP accelerating emergence from anesthesia hypothesis. In the 1970's Cohn et al showed that direct inject of cAMP into the brain reduced the duration of narcosis for a series of sedative, hypnotic, tranquilizer and anesthetic drug tested {Cohn, 1975 #152}. They tested 8 different drugs. It appears that cAMP effectively reduced the waking times for all 8 drugs. More recently, the Solt lab has shown that activation of D₁ dopamine receptors accelerates emergence from anesthesia {Taylor, 2013 #68}. D₁ receptors are always coupled to the cAMP machinery i.e. activation of this class of receptor always produces an elevation of cAMP in cells that express the receptor.

Some of the preliminary results are shown below:

Isoflurane Blocks Neurotransmitter Release in PC12 Cells: Forskolin, Theophylline and Caffeine Reverse this Block

Anesthetics modulate the activity of various channels and receptors, thereby altering neurotransmitter release. For this experiment, PC12 cells were permeabilized with digitonin, and then cells were stimulated by exposing them to Ca^{2+} . Permeabilization disrupts the cell's resting potential and equilibrates the intracellular and extracellular Ca^{2+} levels. Under these conditions, modulation of channels or receptors should have no effect on neurotransmitter release. In earlier studies, PC12 cells were also dialyzed with known Ca^{2+} concentrations, via a patch pipette from a constant holding potential of -65 mV; these studies validated the digitonin methodology. Exocytosis was elicited in the presence and absence of isoflurane (0.5 mM). Basal (Ca^{2+} -independent) neurotransmitter release is virtually non-existent in digitonin permeabilized PC12 cells in Ca^{2+} -free conditions, but

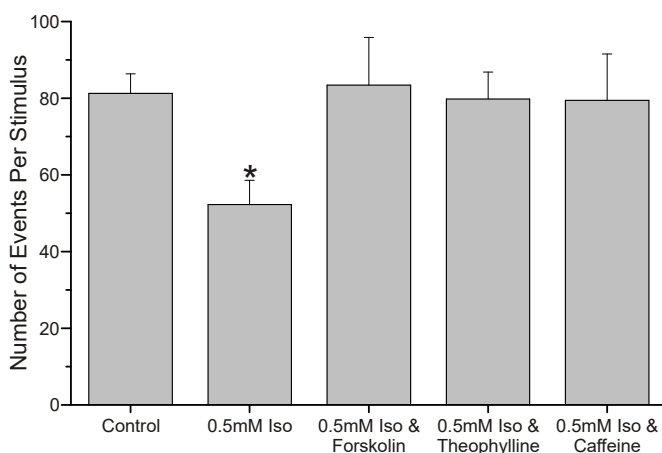


Fig. 1 – Forskolin, theophylline and caffeine reversed the inhibition of neurotransmitter release produced by isoflurane. PC12 cells were permeabilized with digitonin (20 μM) and then stimulated by exposing cells to a solution containing 100 μM Ca^{2+} . Neurotransmitter release was monitored with an amperometric electrode which gently touched the cell. Under these conditions, isoflurane (0.5 mM) significantly inhibited neurotransmitter release by ~36% ($p < 0.05$, relative to control and all other conditions). The number of events recorded from control PC12 cells were about the same as those from PC12 cells exposed to isoflurane + forskolin (5 μM) or isoflurane and theophylline (50 μM) or isoflurane and caffeine (50 μM) (Control 81.28 ± 5.1 ($n=36$), isoflurane 52.29 ± 6.31 ($n=17$), isoflurane + forskolin 83.4 ± 6.1 ($n=17$), isoflurane + theophylline 79.75 ± 7.07 ($n=12$), isoflurane + caffeine 79.43 ± 12.12 ($n=7$) events per stimulation).

robust release is observed upon exposing cells to Ca^{2+} -containing solutions. Physiologically, release is evoked by the activation of voltage-gated Ca^{2+} channels. The proximity of Ca^{2+} channels to synaptic release sites suggests that $[\text{Ca}^{2+}]_i$ may rise to levels above 100 μM at the vesicle. To mimic these levels in our experiments, evoked neurotransmitter release was elicited by exposing digitonin-permeabilized cells to 100 μM Ca^{2+} , for 2 min, in the absence or presence of isoflurane.

In previous studies we have shown that 0.5 mM (~1.5 MAC) isoflurane inhibits neurotransmitter release by ~39% (Herring *et al.*, 2009; Herring *et al.*, 2011). Fig 1 shows that isoflurane (0.5 mM) significantly inhibited neurotransmitter release by ~36% ($p < .05$) in this study.

Three drugs that elevate intracellular cAMP were then tested for their ability to reverse the inhibition produced by isoflurane. Fig. 1 shows that all three drugs, forskolin (5 μM) or theophylline (50 μM) or caffeine (50 μM), reversed the isoflurane-mediated inhibition of neurotransmitter release.

Caffeine Accelerates Recovery from Isoflurane Anesthesia

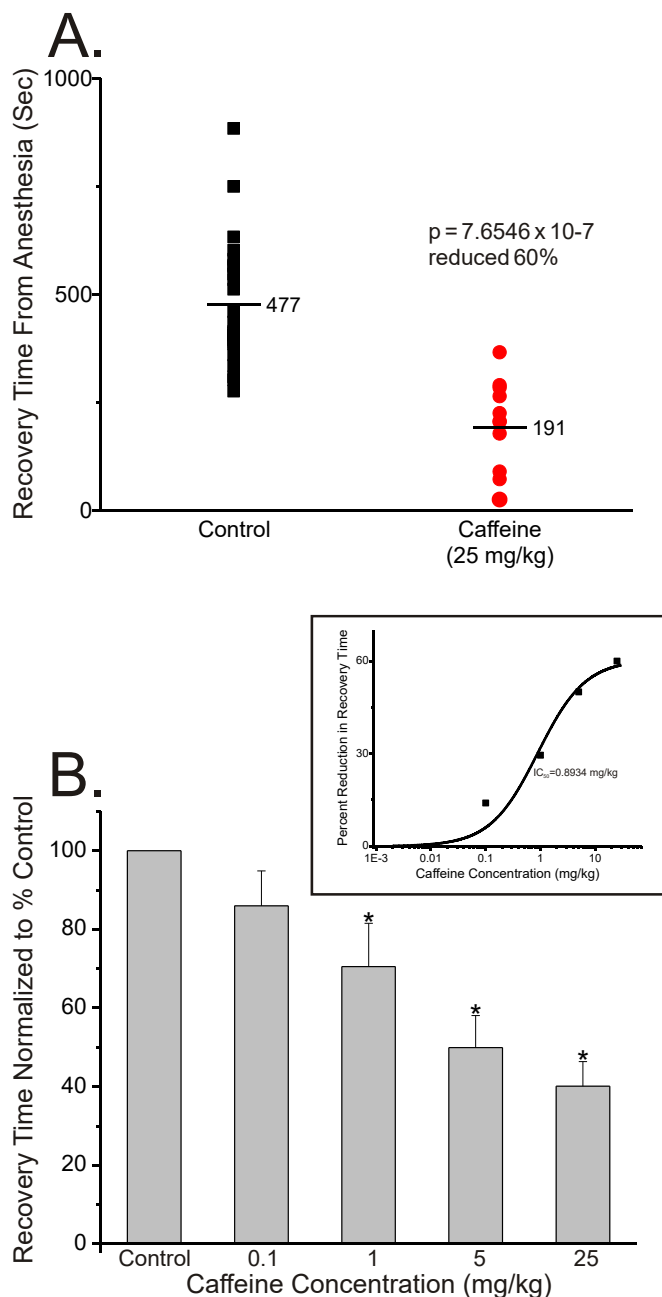


Fig. 2 - Caffeine accelerated recovery from isoflurane anesthesia. A, Adult rats were anesthetized as described. Five minutes prior to discontinuing the isoflurane the animals received an intravenous injection of either saline (control ■) or saline with 25 mg/kg caffeine (●). The isoflurane was terminated and the animals were allowed to recover. B, Average waking time normalized to the Control value, set to 100%, at different caffeine concentrations (n=13; control, n=8; 0.1 mg/kg, n=13; 1 mg/kg, n=12; 5 mg/kg, n=12; 25 mg/kg). Inset plots the percent reduction in waking time as a function of the caffeine concentration. The midpoint was at ~0.9 mg/ kg caffeine and the maximal reduction of waking time was ~60.5%.

with caffeine (●), 25 mg/kg, 5 minutes prior to discontinuing the isoflurane. Caffeine dramatically sped recovery from anesthesia. Fig. 2B shows the average recovery time at

The results in Fig. 1 suggest that elevating intracellular cAMP reverses the inhibition of neurotransmitter release produced by isoflurane *in vitro*. Does elevating cAMP alter recovery from anesthesia in animals? To address this question, we examined emergence from anesthesia in rats. First, we assessed waking from anesthesia in the absence of any other drug to assess population variability. Rats, weighing 420-510 gm, were placed in an anesthetizing apparatus consisting of a gas-tight box where they were exposed to 3% isoflurane (in 3 L/min O₂) for 8 minutes. Isoflurane levels were then decreased to 2% (in 2L/ min O₂) for an additional 45 minutes. The rats were then removed from the anesthetizing chamber and placed on their backs in the middle of a large table. Recovery time from anesthesia for rats is defined as the time from when the animals are removed from the anesthetizing chamber to when they stand upright with 4 paws on the table. Caffeine elevates intracellular cAMP by inhibiting phosphodiesterase. Caffeine is of particular practical interest as it is such a widely used and largely innocuous drug. Fig. 2A shows a graph of recovery times from isoflurane anesthesia for control rats (■) or for animals injected

different concentrations of caffeine. The inset shows a plot of the dose response curve fit to the data showing that the midpoint occurs at approximately 0.9 mg/kg. Of note, an average person consumes more than 0.9 mg/ kg of caffeine in a cup of coffee.

Methods: Adult rats were anesthetized with 3% isoflurane for 8 minutes and were then exposed to 2% isoflurane for 45 minutes. During the anesthesia an I-V was inserted into a tail vein, while anesthesia was maintained with a nose cone. Five minutes prior to discontinuing the anesthetic the animals received an I-V injection of either saline (control, ■) or saline with drug (●). The anesthetic was then terminated and the animals were allowed to wake up breathing room air. The rats were placed on their backs on a table. Waking time was defined as the time between terminating the anesthetic and the rats were standing with 4 paws on the table.

Caffeine Does not Alter Heart Rate, Blood Pressure or Breathing Rate

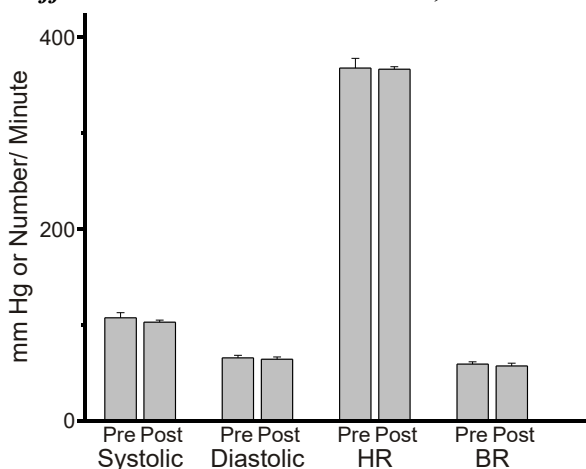


Fig. 3 plots vital signs immediately before or 10 minutes after injection of caffeine in isoflurane anesthetized rats. Anesthetic conditions were similar to those in figure 2. No significant changes in heart rate, blood pressure (BP) or breathing rate (BR) were observed as a result of caffeine application (25 mg/kg). In all cases O₂ blood saturation was at either 99% or 100%.

Fig. 3 - No significant change in blood pressure, heart rate or respiratory rate in rats exposed to drug. Rats were anesthetized as described in Fig. 2. Drug, 25 mg/ kg, was injected into each animal. Just prior to drug injection blood pressure, heart rate and respiratory rate was measured with an IITC Life science Multi Channel Blood Pressure system, using a tail cuff. 10 minutes after drug, the parameters were re-measured. HR= Heart Rate, BR = Breathing Rate. Pre = Pre drug application. Post = 10 minutes after drug application.

Caffeine Accelerates Recovery from Propofol Anesthesia

Fig. 4 plots recovery times from propofol anesthesia from control rats (■) or from rats injected with 25 mg/kg of the drug (●). Adult rats were anesthetized in the following manner. Rats were placed in a gas tight anesthesia box where they were exposed to 3% isoflurane for 8 minutes. IVs were inserted into a tail vein and the rats were allowed to wake. The rats then received a bolus inject of propofol (4 mg/kg) and either saline (Control) or drug in saline (25 mg/Kg). The rats were placed on their backs on a table

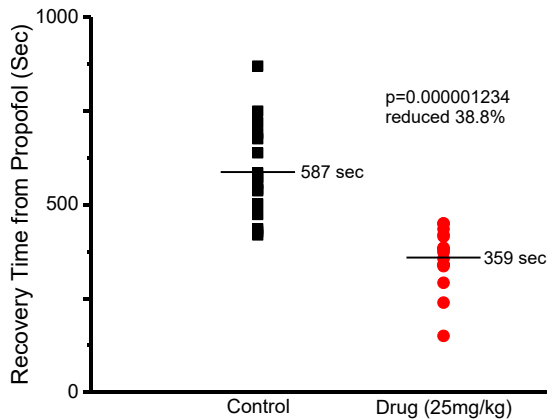


Fig. 4 - Drug accelerates recovery from propofol anesthesia. Adult rats were anesthetized with 3% isoflurane for 8 minutes at which time an I-V was inserted. The animals were allowed to wake and then a bolus of propofol (4 mg/ kg) was injected along with either saline (Control, black squares) or with 25 mg/kg drug in saline (red circles). The animals were allowed to wake. The same animals were used as controls and to test drug (n=16 per group).

and allowed to wake with room air. As before, waking was defined as 4 paws on the table. Note that the caffeine was applied at the same time as the propofol which may not have been optimal.

This last result opens the possibility that caffeine accelerates recovery from anesthesia for all anesthetics.

1.1.4 Clinical Data to Date

Work Done By Others:

There are two clinical trials that have tested caffeine in anesthetized patients. Both trials were carefully vetted. In a recent clinical trial in anesthetized children, caffeine was found to be safe (the trial was to determine whether caffeine could reduce the number of children who experience adverse post-extubation respiratory events (Khalil *et al.*, 2008) – caffeine helped significantly). Note that the concentration of

caffeine used in this study was quite high.

Caffeine has been shown safe to use in anesthetized adults. In a clinical trial, to determine whether caffeine ameliorated post anesthesia nausea and vomiting, patients were given 500 mg caffeine during anesthesia. This combination, anesthesia and caffeine, was found to be safe (Steinbrook *et al.*). Note that 500 mg in that trial is similar to the low end of the range that we propose for our trial.

Preliminary Data from Pilot Study:

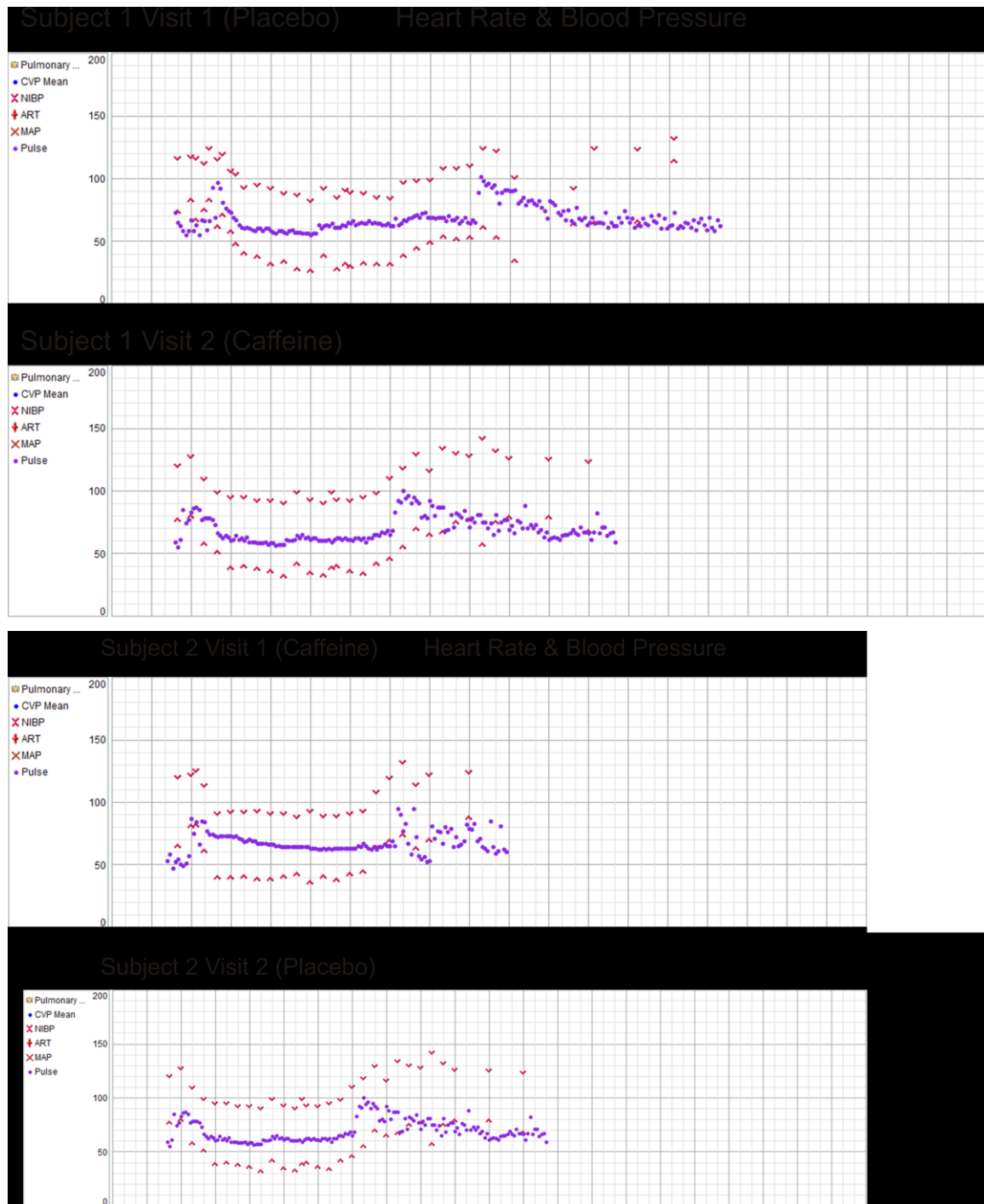
A: Safety of Administering Caffeine to Test Subjects Anesthetized with Isoflurane

Safety:

The good news, is that neither subject experienced any cardiac or respiratory effects due to caffeine. Their hemodynamic responses were unaffected during the trial. No differences in vital signs were observed between sessions.

Subject 1 and 2:

The graphs below plot heart rate and blood pressure throughout the anesthesia session. No significant difference was observed when comparing placebo and caffeine injections. There were also no difference in blood O₂ saturation (always close to 100%) or respiratory rates.



There were two minor medical incidents during the trial.

1. Subject #1 had a mild sore throat immediately following the anesthesia. The sore throat was resolved pretty quickly. It was gone soon after he had a chance to take a drink following the anesthesia procedure. It appeared to be mainly due to the dryness of his mouth during anesthesia. In any case it was very mild since it disappeared so quickly.

Sore throats are very common during anesthesia since the doctors insert a tube into the throat to ensure that the airway stays open. It is equivalent to having a sore arm after an injection. This one disappeared after a drink.

2. Subject #2 had a headache that started 8 hours after the caffeine was administered. He was fine at his departure from UC. The subject attempted to study in the afternoon. At approximately 5 pm (caffeine was given at 9 am) he started to have a headache. He went to bed and his headache resolved after a brief rest. The headache could have been due to the caffeine, but we are not sure. Caffeine has a half-life of ~5 hours in the body. This was 8 hours after he received the caffeine. Most of the caffeine should have been gone by that time. A more likely scenario is that he had studied very late the night before and had slept little. Then he started to study again. He had similar headaches before although not frequently. In any case, the headache disappeared after a brief rest.

Analysis of Efficacy.

Below is shown an analysis of 2 subjects that were anesthetized with and without caffeine (10 mg/ kg caffeine citrate with corresponds to 5 mg/ kg of pure caffeine). This analysis was done blind. The codes were broken after the data had been analyzed and plotted.

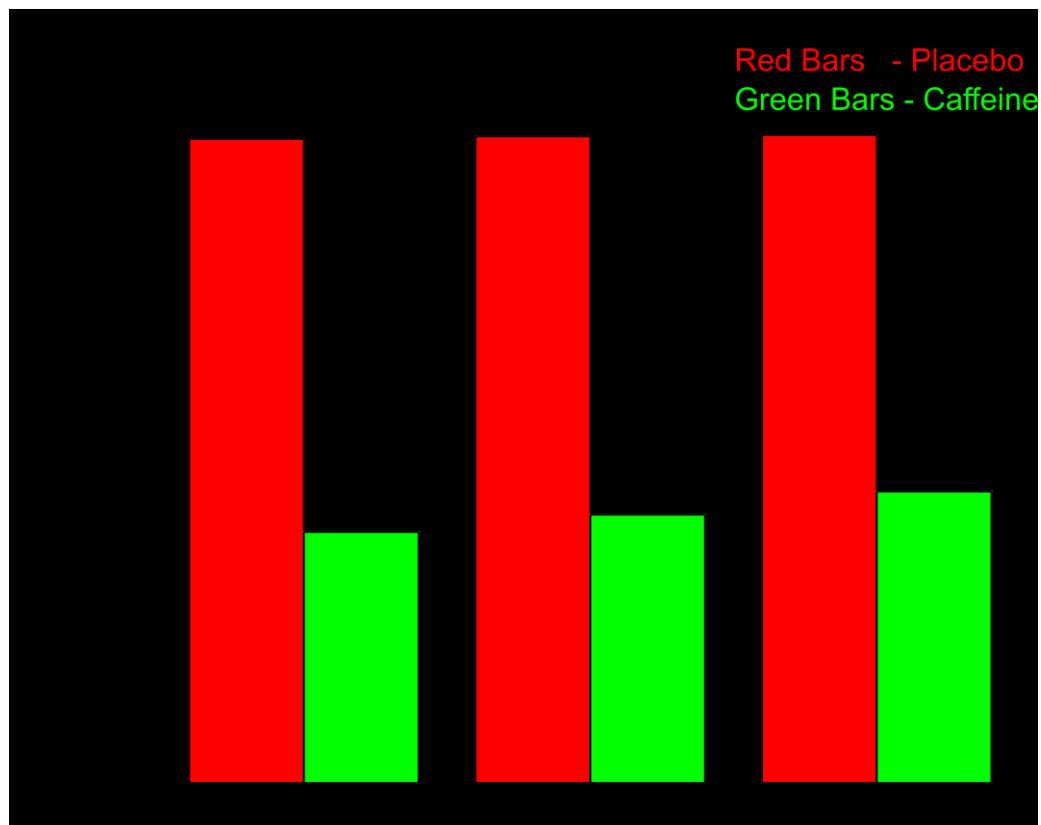
Section 1 - Waking Times

Section 2 - Estimate of isoflurane Concentration at waking

Section 3 - Memory Test

Section 4 - VAS Test

1 – **Waking Times** - It turns out that we made a mistake on visit 1 of subject 2. We forgot to change the oxygen flow rate after turning off the isoflurane at the end of the hour. Normally during the anesthesia itself 2 liters per minute of O₂ were used. At the end of the hour of anesthesia the isoflurane is turned off and the O₂ flow rate is turned to 8 L/ min. If you leave the anesthesia machine at 2 L/ min of O₂ the machine keeps recirculating the gases thereby giving recycled isoflurane to the test subject. So the isoflurane goes down much more slowly. When the isoflurane is stopped the 2L/ min of O₂ should be stopped as well. Instead 8 L/ min of O₂ needs to be given. On visit 1 of subject #2, this was not done. When the time course of isoflurane's drop in concentration in the lungs, after turning off the isoflurane, in anesthesia session #1 was compared with that on anesthesia session 2 (which had a correct procedure) they were very different. But they should have been similar. The difference is that for session #2 we used a flow rate of 8 L/ minute of O₂ while for session #1 we used 2 L/ minute of O₂, at least at first. So no good comparison between sessions is possible. Below is plotted the waking times for subject #1, where no mistakes were made. Three different parameters are plotted. Time to Gag, Time to Open Eyes and Time to Respond to Commands.

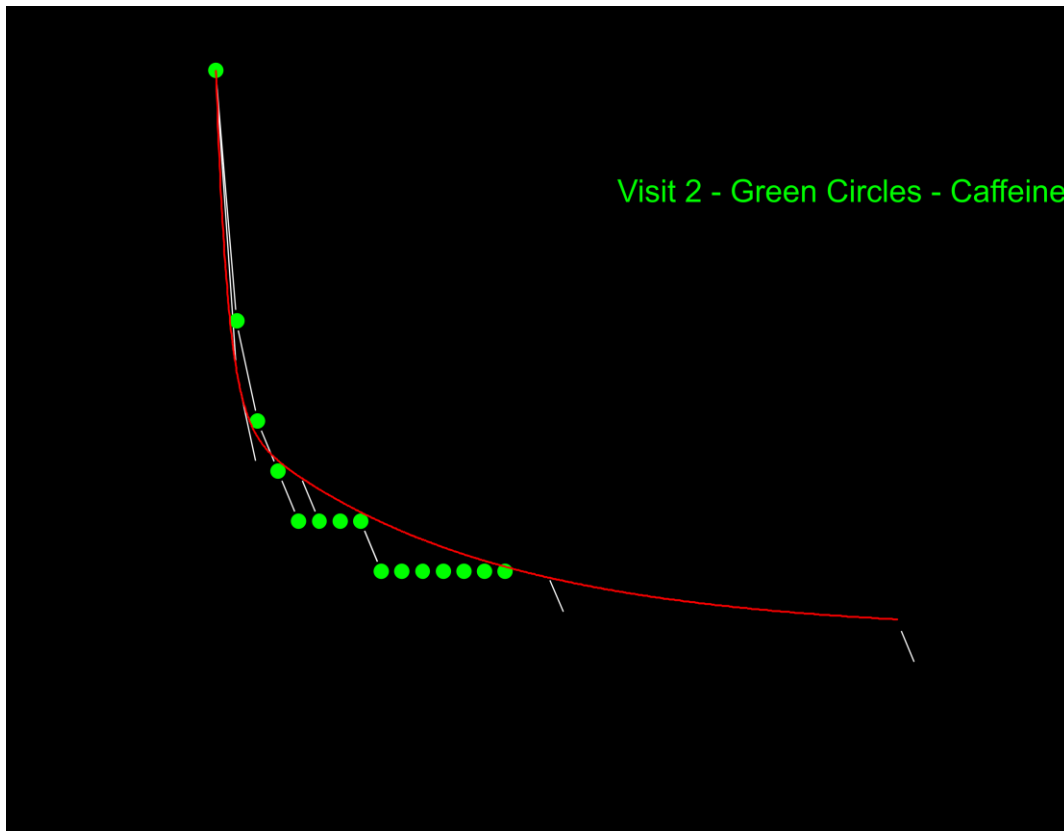


Best Estimate for Subject 2 – In Visit 1 he gagged in 14 minutes 18 seconds (with caffeine). In visit 2 he gagged at 14 minutes and 10 seconds (with placebo). The two waking times were very similar. But the O₂ flow rate of 2 L/ min O₂ was maintained for 2 to 3 minutes after the isoflurane was turned off. So a simple correction would be that subject 2 would have woken 2 to 3 minutes earlier if the flow rate had been administered correctly in visit 1. No adjustment is needed for visit 2. This is simply an estimate.

2 - Estimate of isoflurane concentration for subject 1 waking times:

Note that EPIC takes data once per minute and only stores a single digit of the isoflurane concentration. That limits our precision.

Subject 1 came in twice. Below is plotted the end tidal isoflurane concentration as a function of time. The first visit is plotted in black squares and the second visit in green circles. Note that the data from both visits fall along the same curve. We fit the data with a double exponential. It may be that we would have needed 3 exponentials to fit the data perfectly all the way to 0 % isoflurane, but for the range we explored, 2 exponentials was fine. Using the fit we can obtain an estimate of the isoflurane concentration during each visit. In visit 1 Subject 1 woke (gagged) in 34 minutes, 3 seconds (2043 sec). In visit 2 he woke (gagged) in 13 minutes, 17 seconds (797 sec). From the fit this corresponds to waking at **0.202% isoflurane** in visit 1 and **0.317% isoflurane** in visit 2. That's a pretty big difference.



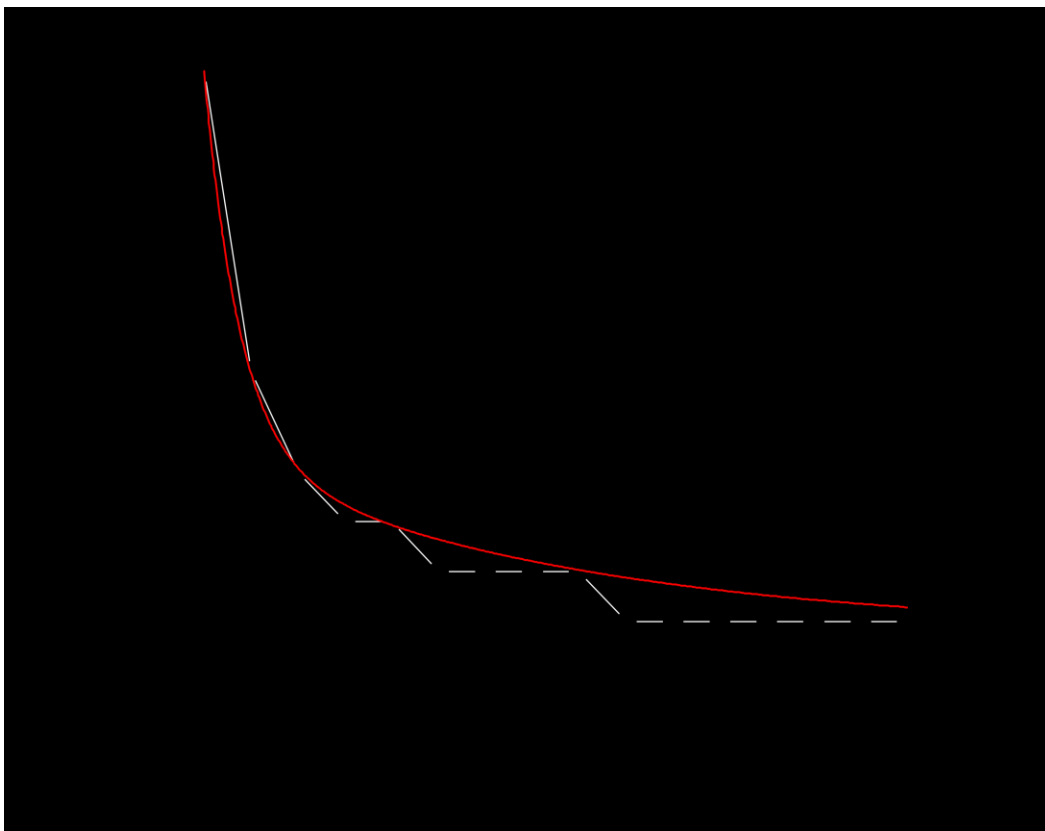
There are far fewer green circles because the subject emerged from anesthesia so much more rapidly when he received the caffeine injection. The isoflurane measuring probe is removed when the subjects wakes. Green data points that look square represent data point where the black squares and green circles overlay each other.

Estimate of isoflurane concentration for subject 2 waking time:

Note that EPIC takes data once per minute and only stores a single digit of the isoflurane concentration. That limits our precision.

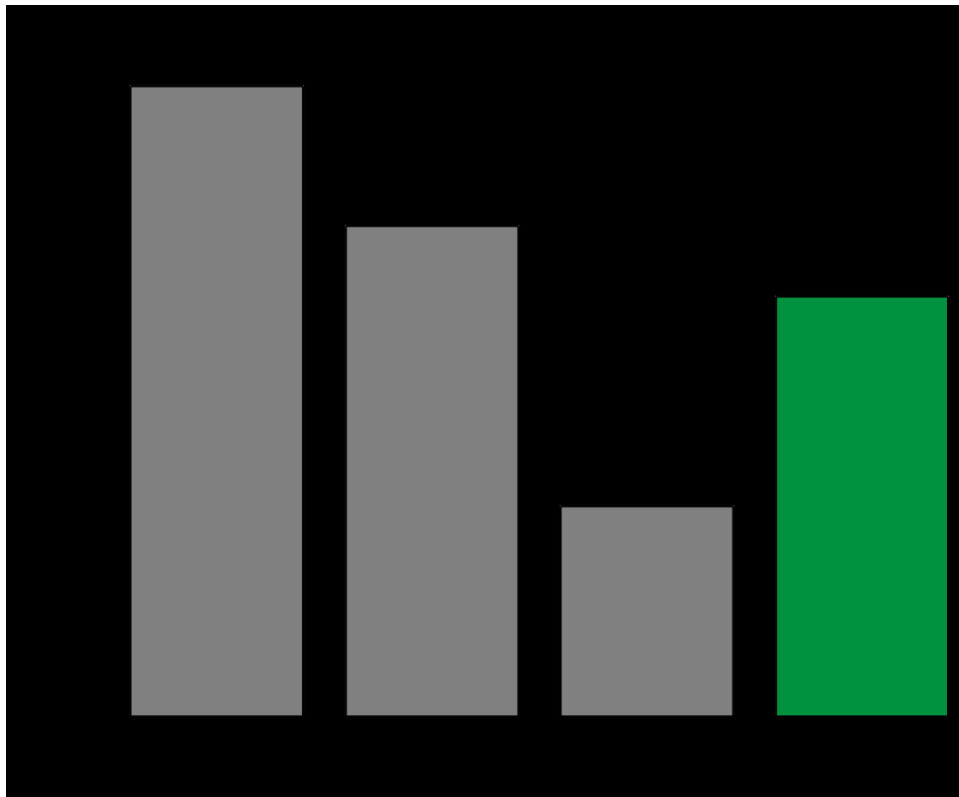
Subject 2 came in twice. Unfortunately in the first visit the O₂ levels were left at 2 L/ min for several minutes after the isoflurane was shut off. This means that the time course was distorted for visit 1. Below is plotted the end tidal isoflurane concentration as a function of time. The second visit is plotted in black squares and the first visit is not plotted. We fit the data with a double exponential. We are somewhat skeptical of the fit since there was not a lot of data to fit, since he woke quickly. We are pretty sure that the fit here is not accurate. But this is the best we could do. Using the fit we can obtain an estimate of the isoflurane concentration during visit 2. In visit 2 Subject 2 woke (gagged) in 14 minutes, 10 seconds (850 sec). From the fit this corresponds to waking at **0.22%**

isoflurane. Thus the isoflurane concentration that subject 2 woke at is similar to that of subject 1. Although we don't know the exact isoflurane concentration at waking for visit 1, it was ~0.3% (reading directly from EPIC). Again not so different than subject 1.

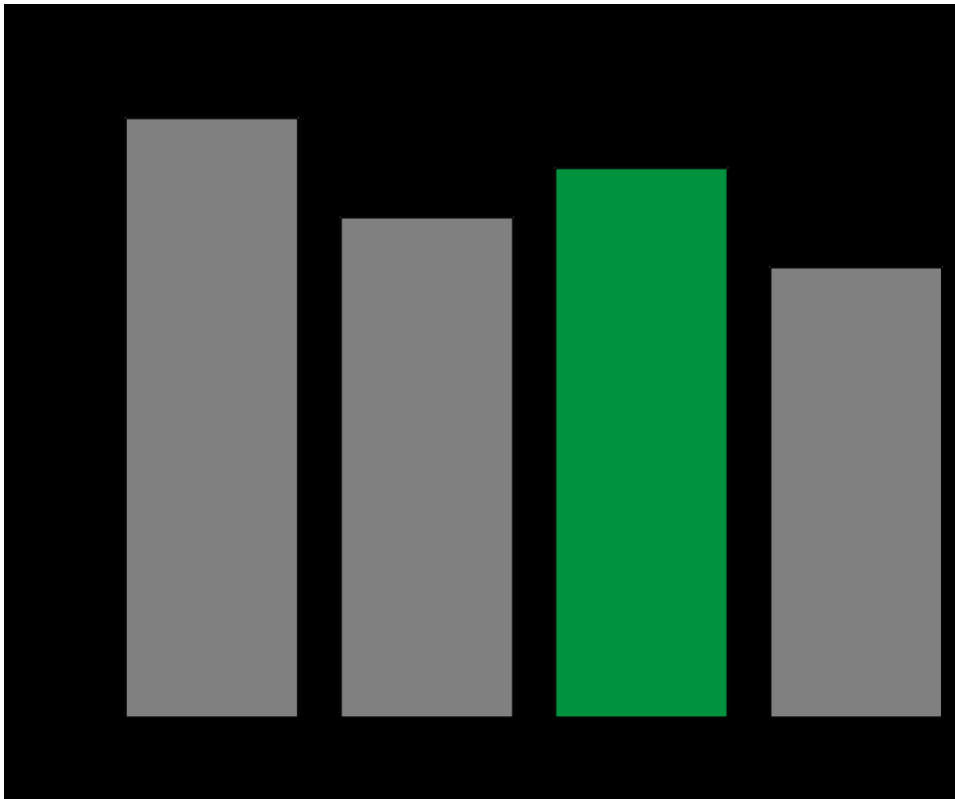


*Note that the **black squares** corresponds to the visit with the **placebo** injection. Data from the caffeine injection is not plotted.*

3 – **Memory Test** – Here we made a choice that was not optimal. Originally we had planned on giving the memory test at 30 minutes after turning off isoflurane. This original plan assumed that a typical person would wake from 1 hour of isoflurane anesthesia in about 15 minutes. But then reality stepped in. Subject 1 woke in visit 1 (placebo) at 34 minutes. In addition, he was not ready to complete the memory test at 45 minutes. He was still disoriented. So he took the memory test at 60 minutes and was clearly impaired. But we used an identical timing paradigm for subject #2. He took his memory test at 60 minutes as well. He woke at ~15 minutes and was mostly recovered at 60 minutes. Now we understand that we need multiple memory tests. The first memory test should take place soon after emerging from anesthesia and then additional tests should be spaced every 15 minutes afterwards.

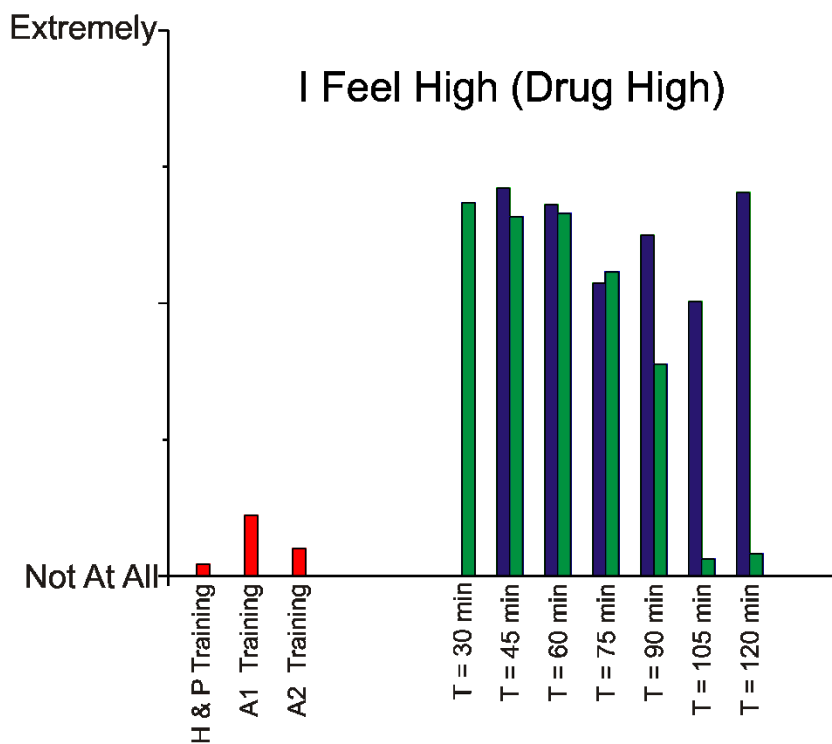
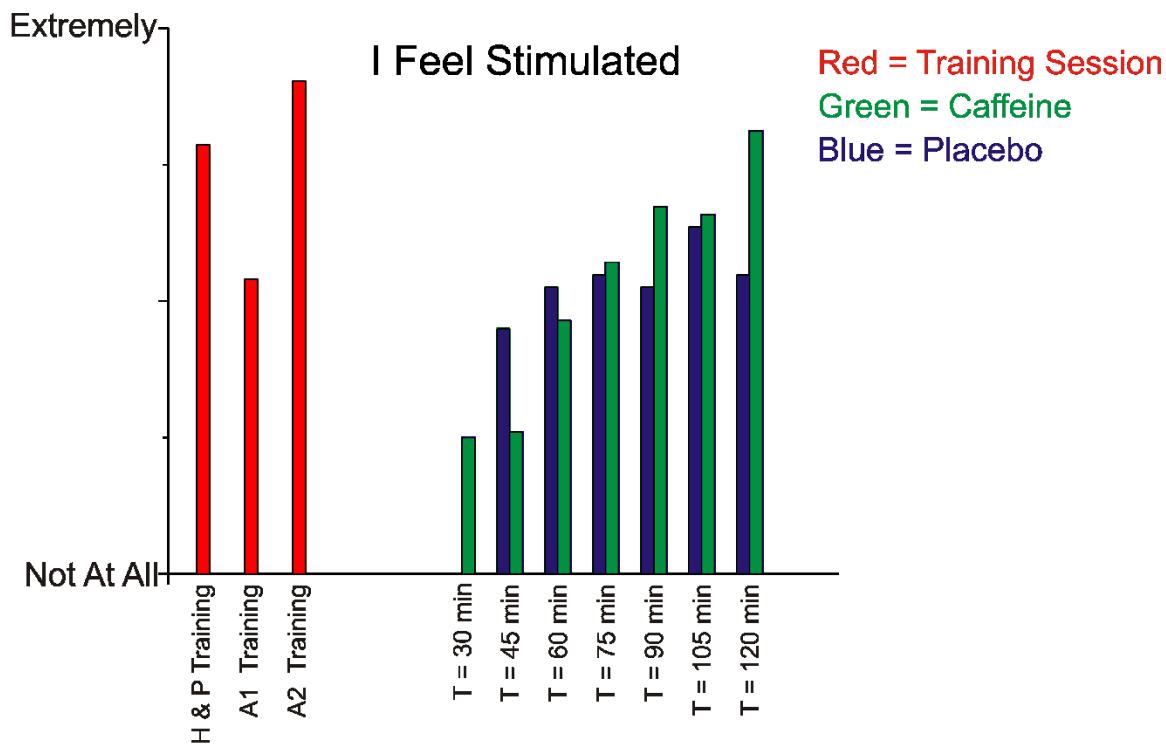


Memory Test – Anesthesia # 1 was with placebo, while anesthesia #2 was with caffeine.

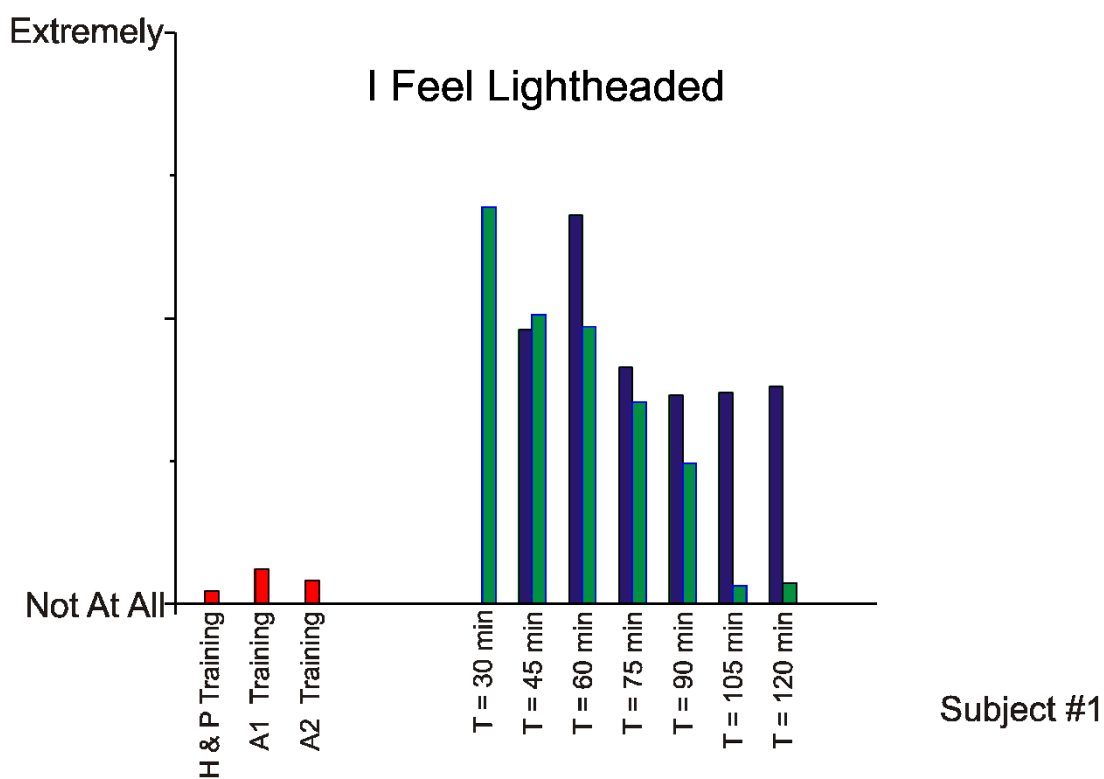
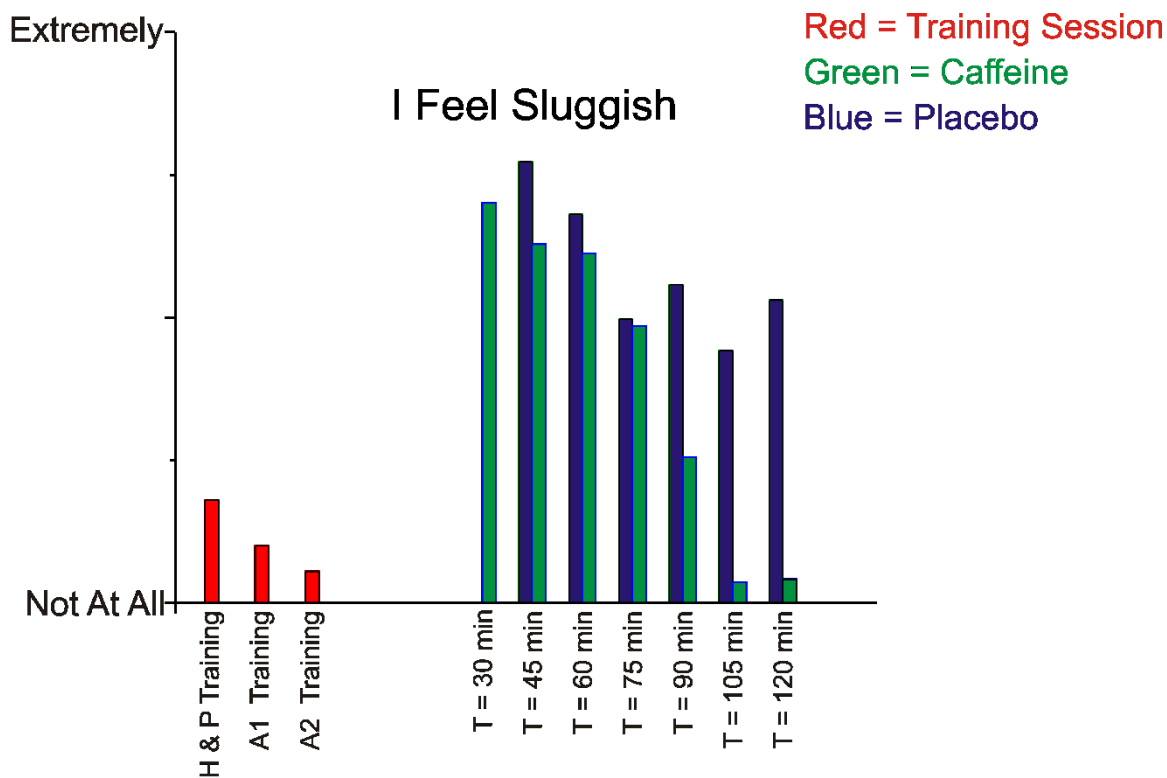


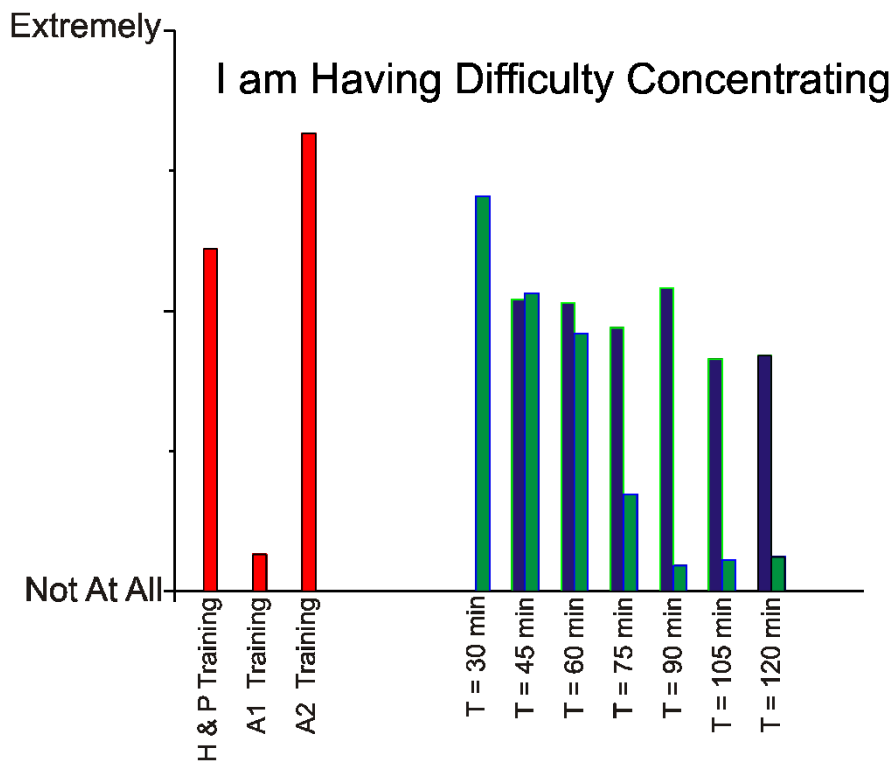
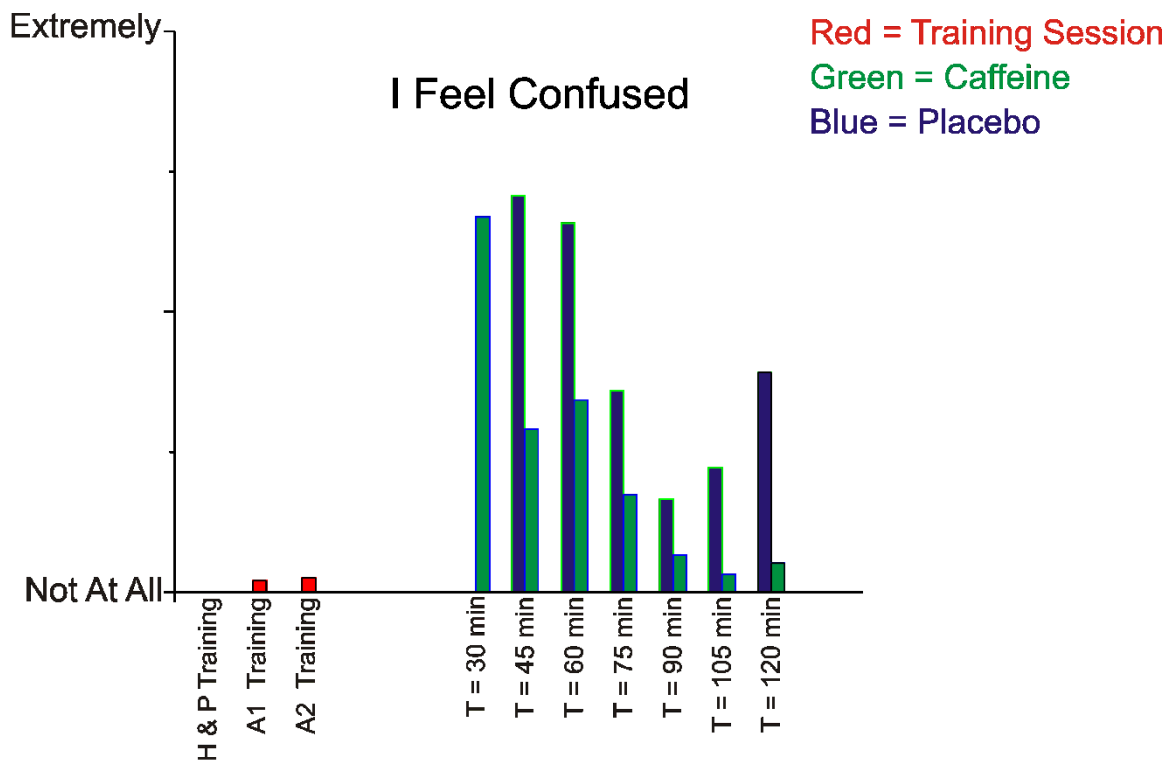
Memory Test – Anesthesia # 1 was with caffeine, while anesthesia #2 was placebo. This subject needed to be tested earlier in the anesthesia.

4 – **VAS Test** – In this test we ask the subject how they feel. This test takes advantage of the difference between a human and a rodent i.e. you can ask what is happening.

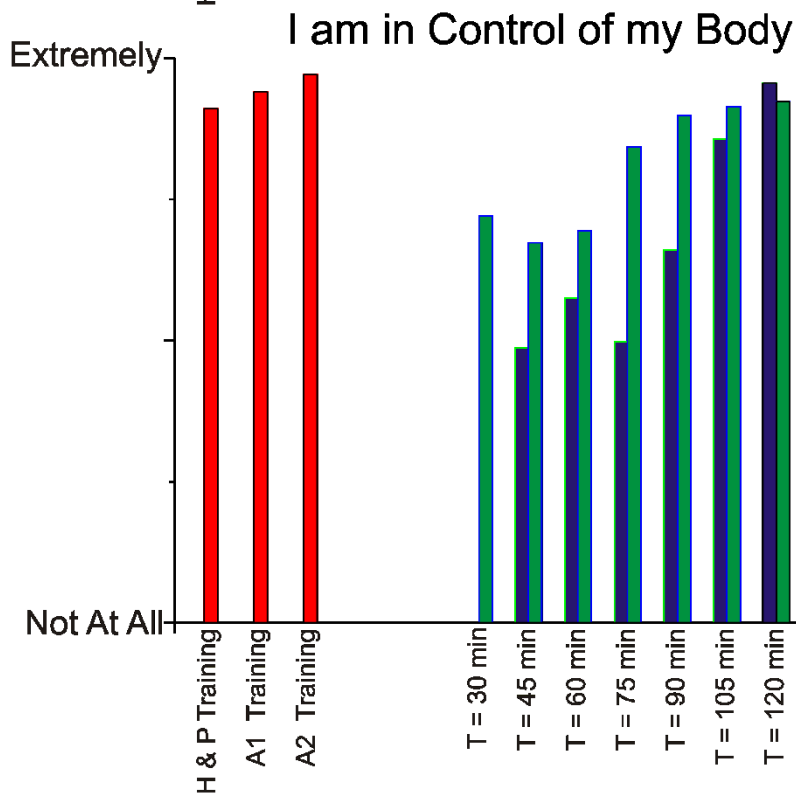
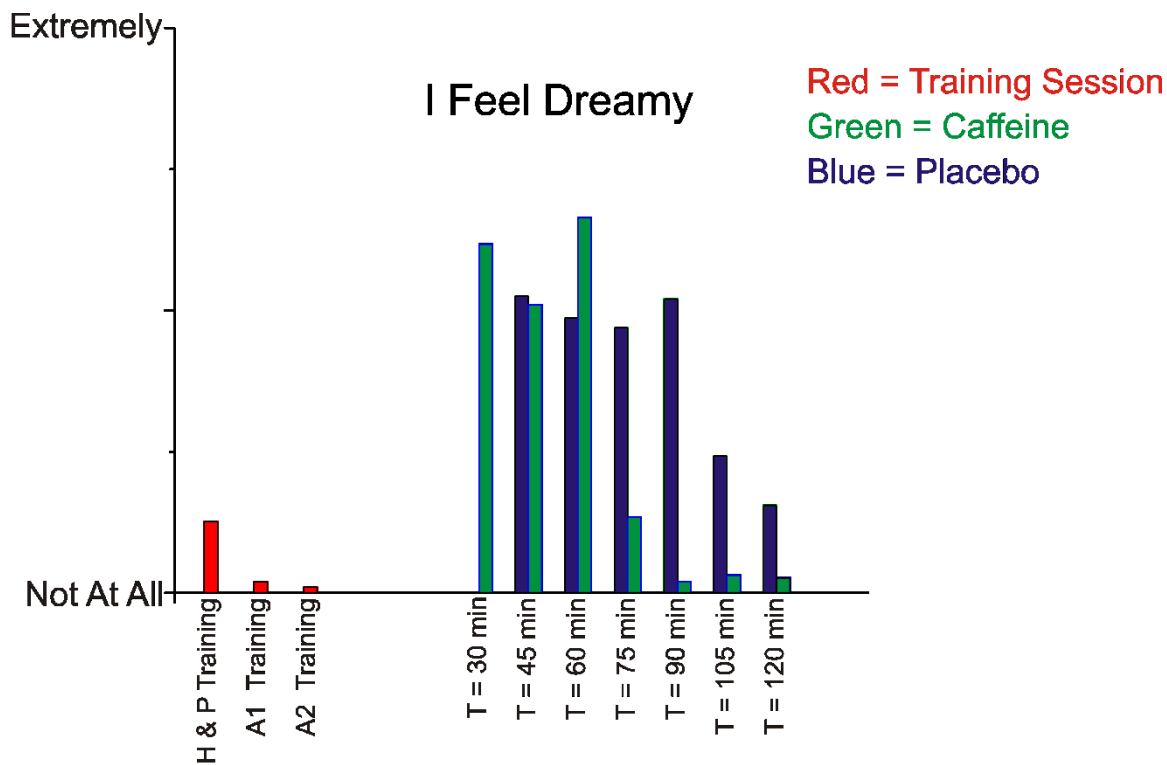


Subject #1

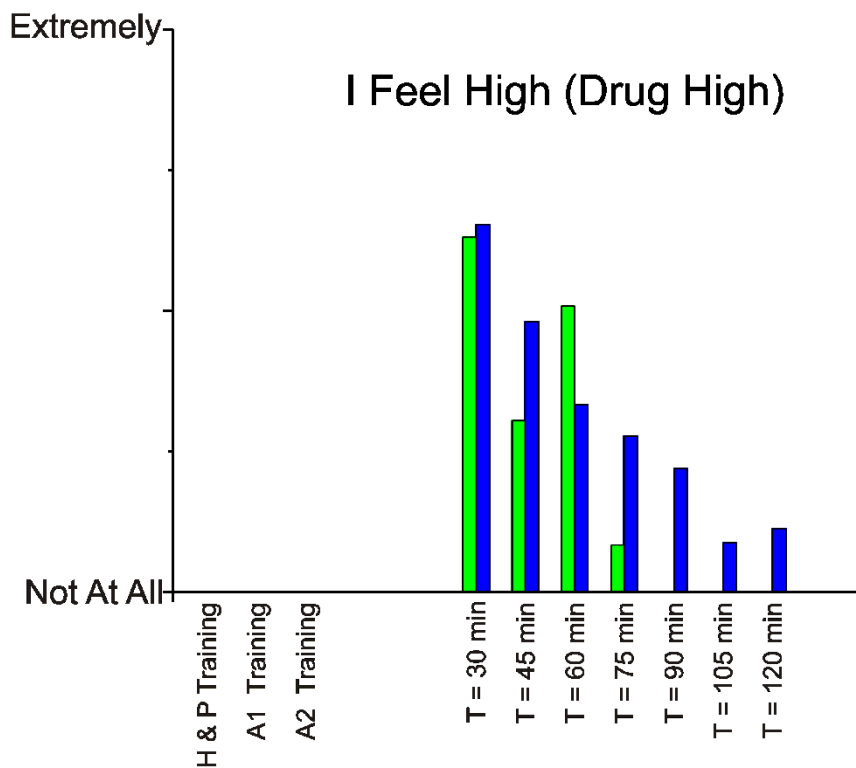
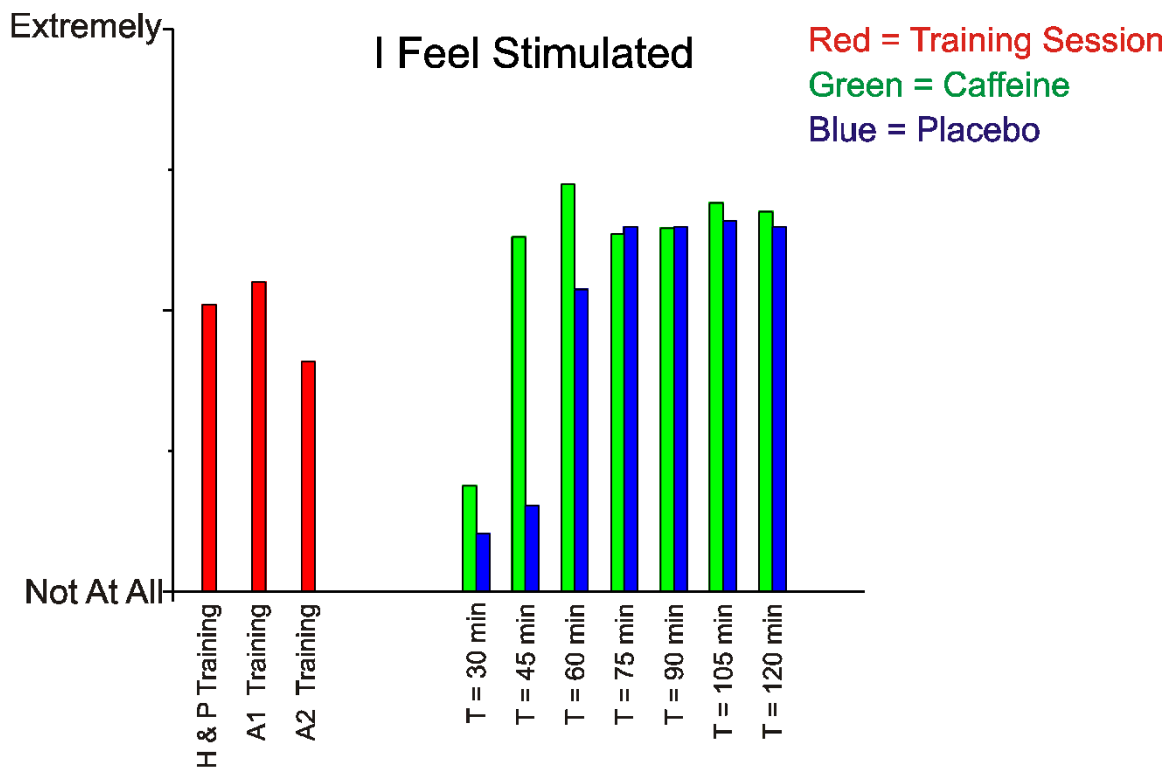




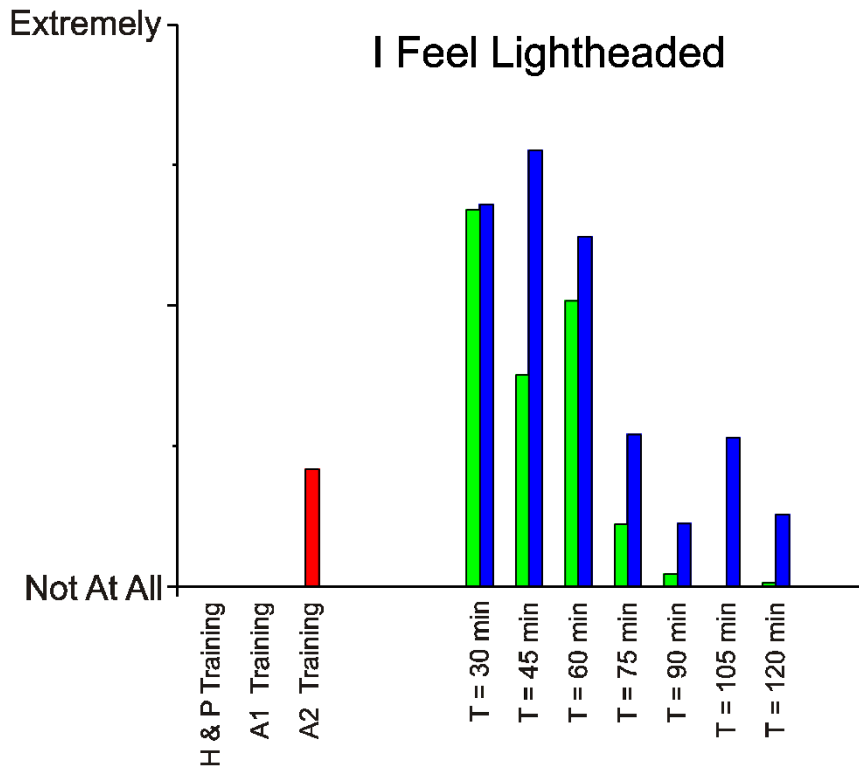
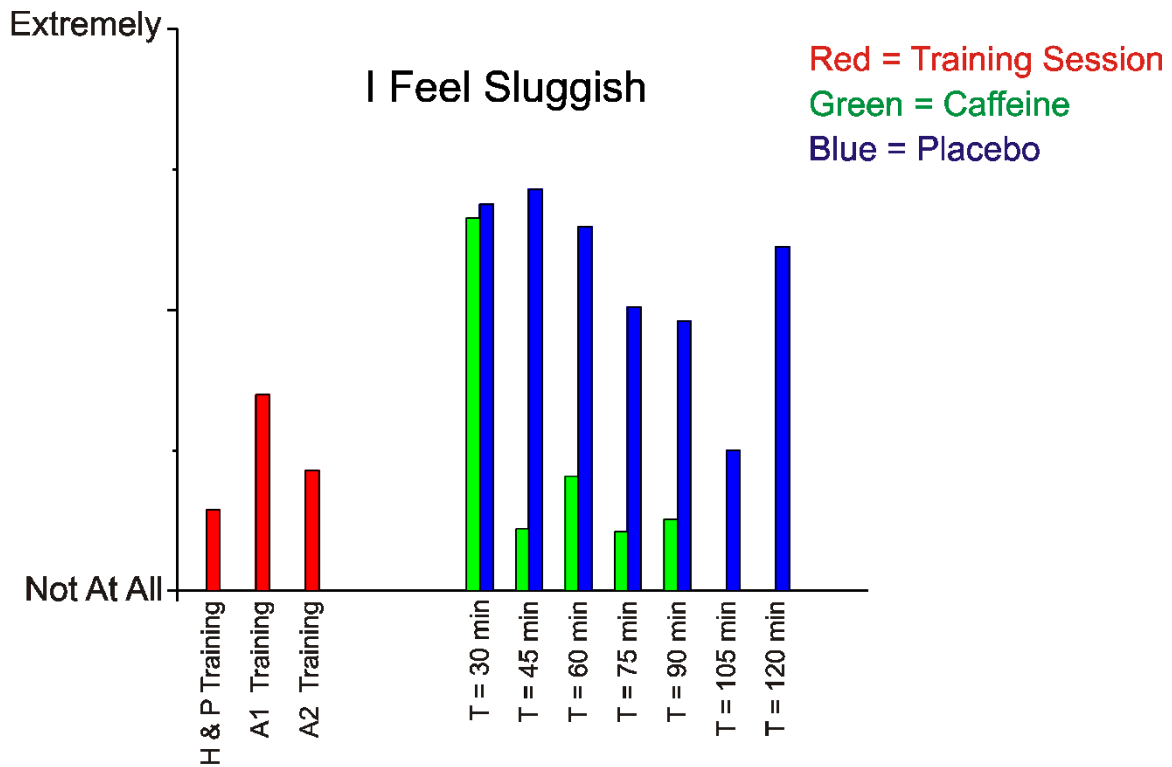
Subject #1



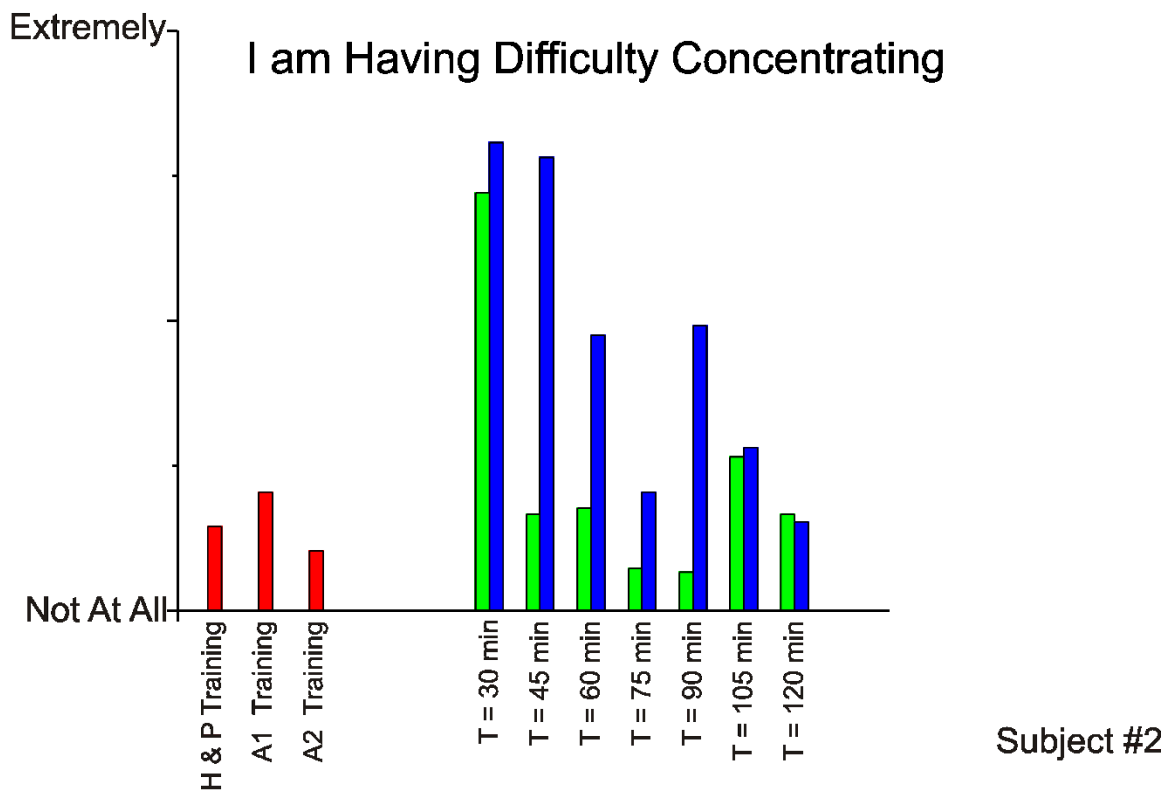
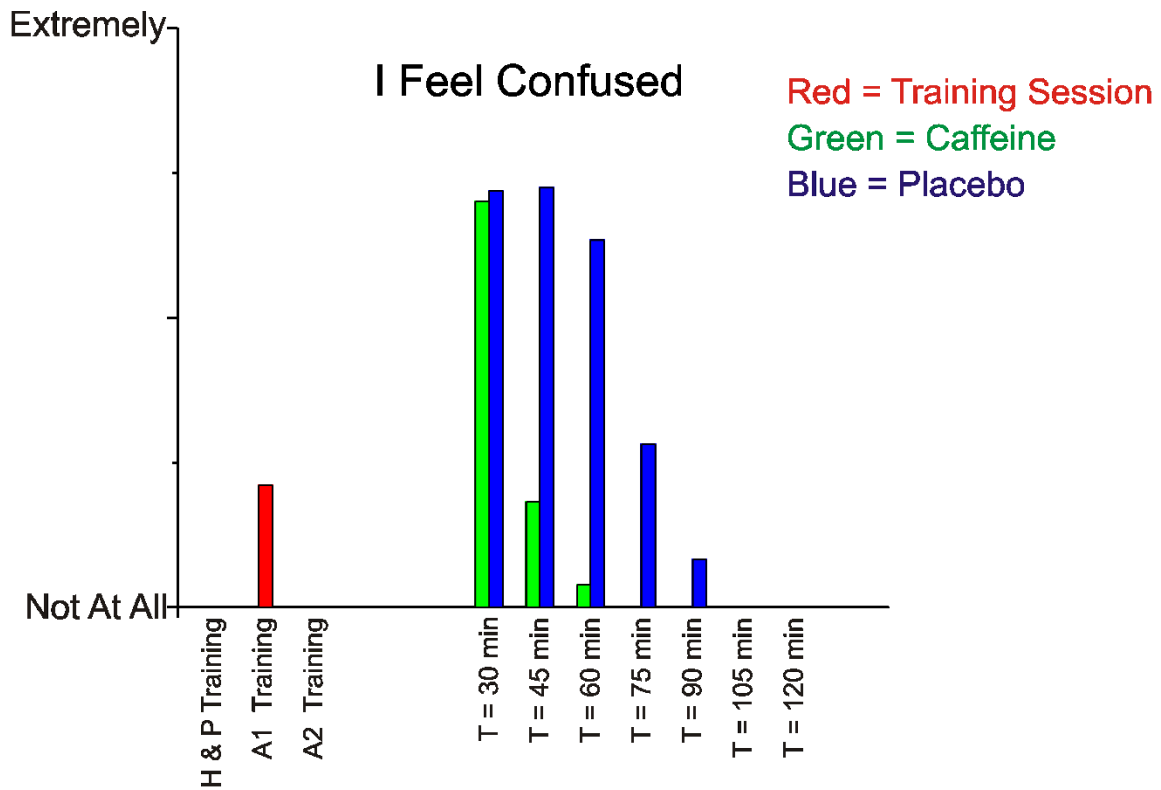
Subject #1

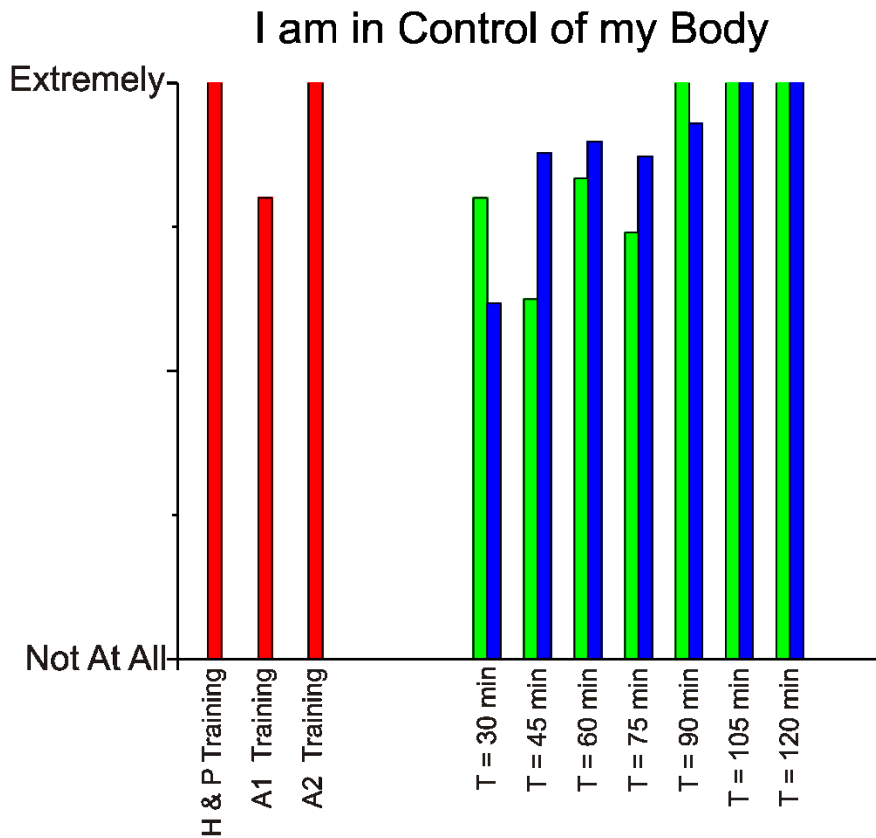
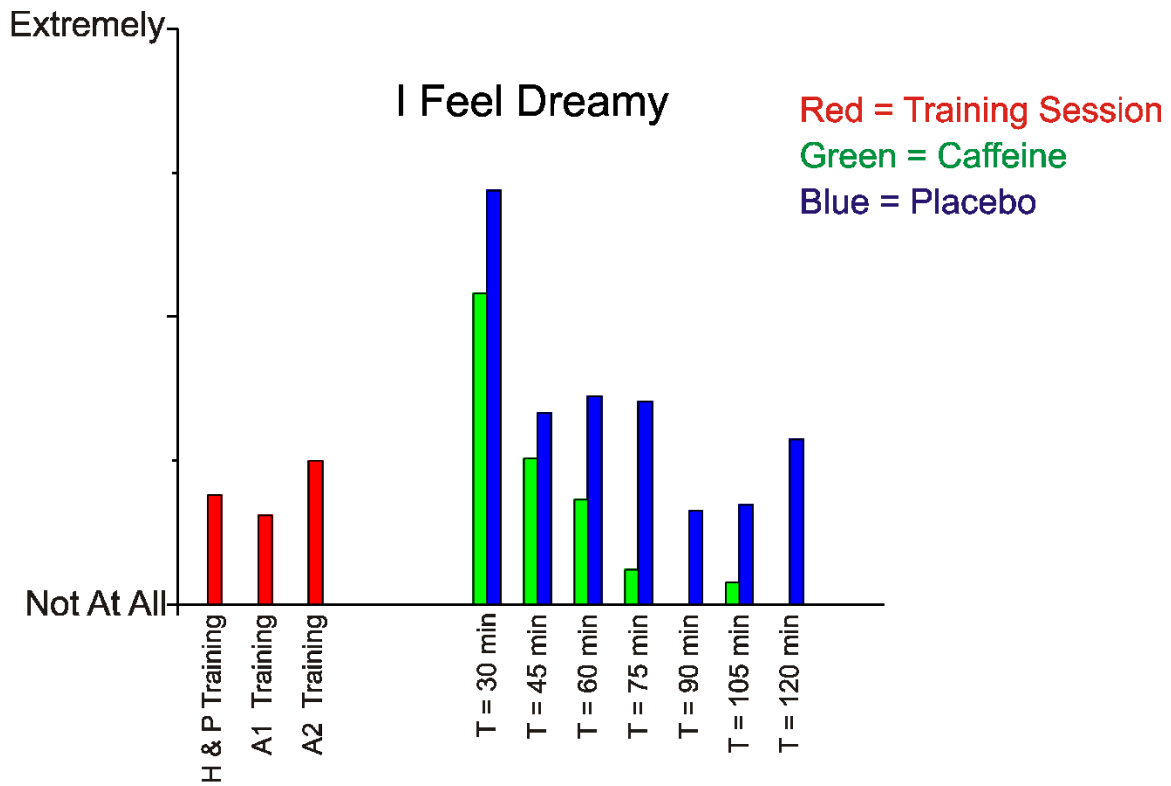


Subject #2



Subject #2





Subject #2

Summary of the Data from the Pilot Study:

This Pilot Study used an intermediate concentration of caffeine. In rats when we used an intermediate concentration of caffeine, the rats that emerged rapidly from anesthesia woke up more rapidly after caffeine, but the largest effect was observed with animals that emerged slowly from anesthesia. In this human trial, subject 1 woke with a placebo injection in 34 minutes and in 13 minutes after caffeine. This was a subject that emerged slowly from anesthesia. Subject 2 emerged from anesthesia in 14 minutes after receiving either caffeine or placebo injections. But due to a mistake with the anesthesia machine, we believe that he would have emerged 2-3 minutes more rapidly. This modest difference is what we saw in rats that emerged quickly from anesthesia, without any help from caffeine.

The isoflurane concentration where waking occurred was ~0.3% when caffeine was injected and ~0.2% when the subjects received a saline injection. This is already a large difference. The rat data suggests that as the caffeine concentration is increased, it works to antagonize the isoflurane more effectively. Thus increasing the caffeine from 10 mg/kg of the Pilot study to the 15 mg/kg requested in this study, we should see an even larger effect. That is the human subjects should wake from the isoflurane more rapidly with an even higher concentration of the anesthetic remaining in their bodies.

Although we do not have a time course for the memory test, anesthetics clearly impair memory. In fact, Subject 1 completed a test soon after emerging from anesthesia and then telling us that he had never completed the test. He became argumentative even after we showed him the completed test. Our best estimate at present is that complete restitution of memory took place 15-30 minutes more rapidly in the presence of caffeine. But this is anecdotal. We need better testing to demonstrate this effect.

Similarly when we asked the subjects how they felt, they clearly felt much better after caffeine. They came back to normal 30 – 60 minutes faster in every negative category (such as “I Feel Lightheaded” or “I feel Confused” or “I Feel Dreamy”).

1.1.5 Rationale for Conducting this Research

Our lab has studied anesthetics for over fifteen years. We have spent considerable time studying the inhibition of neurotransmitter release produced by anesthetics (Herring *et al.*, 2009; Herring *et al.*, 2011; Xie *et al.*, 2012; Xie *et al.*, 2013). We thought that if we could reverse this action that we would be able to reverse aspects of anesthesia. Several drugs were studied; these drugs normally facilitate neurotransmitter release. If inhibiting neurotransmitter release causes anesthesia then drugs that facilitate neurotransmitter release should aid in reversing anesthesia. Elevating intracellular cAMP, with drugs like caffeine, is well known to facilitate neurotransmitter release. We found that drugs that elevate intracellular cAMP levels caused rats to wake up very quickly from anesthesia.

A variety of drugs are used during general anesthesia that are reversed pharmacologically afterwards. For example opioid drugs, frequently used to suppress pain, can be reversed pharmacologically following surgery. Other drugs like anticoagulants and muscle relaxants are used in a similar manner. General anesthetics induce a coma-like state; unfortunately recovery from anesthesia induced loss of consciousness cannot be reversed (Solt *et al.*, 2011). Recovery is passive and is due to the

discontinuation of anesthetic. Problematically, recovery is somewhat random, dependent upon a variety of genetic, age, physical condition and gender related factors that are beyond the clinician's control (Solt *et al.*, 2011; Chemali *et al.*, 2012; Taylor *et al.*). Cognitive impairments persist for hours following anesthesia. Outpatients are released from hospital after anesthesia/ surgery after an ever diminishing recovery period in order to constrain hospital costs. This is a recipe for increasing anesthesia-induced accidents. It would be extremely beneficial to be able to time recovery from anesthesia in a reproducible manner and to have that recovery be complete. Anesthesia in rodents is thought to be very similar to that in humans (Thurman *et al.*, 2008). We have tested a variety of drugs in rats and we have identified four different drugs that appear to wake animals rapidly and reproducibly after terminating anesthesia. We have chosen one of the four drugs and would like to test it in humans. These studies have the potential to impact medicine in an important way and in a relatively short time frame.

1.1.6 Dose Rationale and Risks/Benefits

The drug that will be used in this study is caffeine which is commonly consumed by millions of people daily. It is the most popular drug used worldwide found in more than 60 plants. It is classified by the FDA as "Generally Recognized as Safe". This was a critical consideration for our study. To be able to find a drug that would be considered safe. An IV formulation of caffeine is in clinical use, for treating apnea of prematurity in neonates. The dosages of caffeine in this study are within the range used for the above clinical conditions. For example, the recommended loading dosage for premature infants being treated for apnea of prematurity is 20 mg/ kg, a 33% higher dosage that we propose in adult male volunteers. The dosage that we have chosen is not associated with serious side effects ("caffeine has few unwanted side effects and is safe even in very large doses". This quote is from (Rang *et al.*, 2001) the most widely used pharmacology textbook in medical schools. We hypothesize that injection of caffeine at the end of anesthesia at clinically relevant dosages will facilitate the emergence of a subject from general anesthesia. Additionally, caffeine may improve cognitive function recovery after general anesthesia.

1.2. OBJECTIVES

a) Hypothesis

Anesthesia is thought to work similarly in humans and animals (Thurman *et al.*, 2008). In our preliminary studies we found that drugs that elevate intracellular cAMP, like forskolin or caffeine, dramatically accelerated recovery from anesthesia in rats. Caffeine appeared to have a similar effect in two human test subjects. We hypothesize that drugs that elevate intracellular cAMP will dramatically accelerate recovery from anesthesia in humans.

Objectives

Primary Objective

To determine whether drugs that elevate cAMP levels accelerate recovery from anesthesia.

Secondary Objective(s)

To determine whether drugs that elevate cAMP help ameliorate the cognitive problems that persist for hours following anesthesia.

2. STUDY DESIGN

2.1 Type of Study

This is a Randomized, Double-Blinded, Placebo Controlled Study.

2.2 Duration of Study

Screening: 1 week

Intervention: 6 weeks total

Follow-up: 2 weeks

Part 1: We will enroll up to 8 test subjects in a full scale trial. The estimated duration of this trial will be approximately 16 weeks followed by a 2 week follow up period.

Week 1: Explanation of the trial and signature of informed consent form. Detailed medical exam and physical exam. Toxicology screen and acceptance into trial. Training with psychomotor tests.

Week 2: Volunteers will be exposed to isoflurane anesthesia as described below and then receive and injection of either a saline solution or caffeine citrate (both investigators and subjects will be blind to composition of injection). Psychomotor tests will be carried out. There will be a toxicology screen. Later that day and then the day after the session, there will be a telephone follow-up with the subject.

Week 3: There will be a telephone follow-up with both volunteers.

Week 4-6: Volunteers will be exposed to isoflurane anesthesia as described below and then receive and injection of either a saline solution or Caffeine (both investigators and subjects will be blind to composition of injection). Psychomotor tests will be carried out. There will be a toxicology screen. Later that day and then the day after the session, there will be a telephone follow-up with the subject.

Weeks 6: There will be telephone follow-ups with both volunteers.

Week 7: There will be a final series of telephone follow-up with both volunteers. If there are no issues identified, this will be the last contact with the volunteers.

We are suggesting that the trial take place over 16 weeks due to scheduling conflicts i.e. the possibility that subjects are not available. We hope to anesthetize 2 subjects per single lab day.

We are asking to be allowed to enroll up to 8 additional volunteers, but we will only require 6-8 to complete the study. If we need additional subjects due to dropout from the trial, we will ask the IRB for additional subjects. After 6 subjects are completed we will submit a continuing review after enrolling 6 additional subjects in order to inform the IRB of the study progress, how subjects tolerated the dosing changes, and if there were any subject safety issues.

If we are given IRB approval, the next set of test subjects will take 4-6 weeks to complete the trial. There will also be a two week follow-up period.

For the full trial, we are asking for extra time up to 26 weeks in the trial to allow for expected dropouts.

2.3 Schedule of Events

Each subject will attend 3 sessions to complete the study. In the first session, an anesthesiologist will explain the purpose of the trial and carefully go over the consent form. If the subject is willing to enroll the consent form will be signed. Then the anesthesiologist will take a detailed medical history and perform a physical examination of the subject. A baseline EKG and urine toxicology screen test will be obtained. If the subject meets the criteria for the study, the subject will be enrolled in the trial. At this point a pre-study training session will be provided to familiarize the subject with the psychomotor tests. In the following two sessions, each subject will receive general anesthesia in one session (with a saline injection as a placebo control) and receive general anesthesia plus an injection containing caffeine citrate (15 mg/ kg) in the other session in a randomized manner. For each subject, each session involving anesthesia will be at least 2 weeks apart. Detailed study procedure will be described in section 5 below.

Please Note: We will try to process two subjects on a single anesthesia day. One at 7:00 AM and another at 1:00 PM. In the Pilot study we tested one subject per day. The subjects arrived at UC at 7:00 AM and the entire anesthesia procedure including recovery was finished by 11:00 AM. That was wasteful, in terms of time. Normally anesthesiologists put multiple people to sleep per day. They don't finish their days at 11:00 AM. That is why we would like to have one subject in the morning and a second subject in the afternoon.

2.4 Sequence and Duration of all Study Periods

The total time of each study session, including the pre-anesthesia preparation, testing and recovery from anesthesia, will be ~4 hours.

2.5 Method for Assigning Subjects to Treatment Groups

The University of Chicago Investigational Drug Service (IDS) will prepare the injectable solutions for our trial. They will provide us with either saline or with caffeine citrate, 15 mg/ kg. They will not inform the research team of their preparations. There is no way to identify whether caffeine is present in a solution by looking at the solution (caffeine citrate is colorless and odorless). The entire experiment will be done blind by the research team and by the volunteer. The only requirement will be that at the end of the test the

subject will receive one round of anesthesia without caffeine and one round with caffeine. The assignment of which drug to use will be carried out with a random number sequence generated by random.org.

2.6 Blinding

This is a double-blinded study. Neither the experimenter nor the subject will know whether they are giving or receiving caffeine. The code will be broken only after the study is complete. At this point all the research team will be made aware of the results as will the IRB. During the study the blind may be broken only in emergencies when knowledge of the subject's treatment group is necessary for further subject management. When possible, the Investigator will discuss the emergency with the monitor (the IRB) prior to unblinding.

2.7 Primary Study Endpoint

The goal of the study is to determine whether caffeine speeds emergence from anesthesia. The primary endpoint will be a measurement made following termination of anesthesia in the human volunteers. This will be the time between terminating delivery of anesthetic and the subject opening his eyes. This measurement will constitute the "waking" time for each subject.

2.8 Secondary Study Endpoints

As a secondary endpoint for this study we will measure the time between terminating anesthetic and the subject being able to respond to a simple command ("squeeze my hand").

Normally patients receiving anesthesia exhibit significant cognitive problems for hours after anesthesia is terminated (references are provided below). Our goal is to determine whether caffeine helps ameliorate the cognitive issues. After waking, each subject will be asked to complete a series of psychomotor tests (described below), that will assess his reaction times, his memory and his motor control (ability to move his limbs). Details of the test are provided below.

2.9 Primary Safety Endpoints

Throughout each anesthesia visit, the subject's vital signs will be monitored and there will be two experienced anesthesiologists present throughout the anesthesia session. Both anesthesiologists will be present for the first hour of the recovery time following the anesthesia. Once the test subject is awake, oriented with regards to time and space, and showing stable vital signs, typically about an hour after terminating the isoflurane, one of the anesthesiologists may leave the room. There will be one anesthesiologist present at all times until the subject is discharged.

At the conclusion of each "anesthesia" visit, we will discharge the subject based on the criteria used in our post anesthesia care unit in the hospitals. In general, the subject should be awake and alert with the stable vital signs. He should be able to drink clear liquid, void and ambulate before discharge home. We will call a car service for him without cost to the subject. The subject will be expected to live within 30 miles of The University of Chicago to make sessions easily accessible. Upon discharge from the

hospital, the subject will be required to be accompanied by a responsible adult. Each session for any given subject will be at least 2 weeks apart. The total time of each session, including the pre-anesthesia preparation, testing and recovery from anesthesia, will be ~4 hours. We will call the subject later the same day and then again the next day to make sure the subject fully recovers without any adverse event. The subject will get another call a week after the procedure. In some cases we may use emails or text messages rather than phone calls.

After the 2nd and final anesthesia session the subject will receive phone calls for the next two weeks to ensure complete recovery and no adverse effects.

3. SUBJECT SELECTION AND WITHDRAWAL

3.1 Number of Subjects

For this study of up to 8 subjects will be tested. There will be a progress report to the IRB after 6 subjects.

3.2 Gender, Age, Racial and Ethnic Origin of Subjects

Healthy male, ages 25 – 40, all race and ethnic origin inclusive.

Our animal studies were carried out in adult male rats equivalent to the age range listed above. At present we would like to reproduce our animal studies in humans, introducing as few new variables as possible. The effects of anesthesia and the study drug on fetuses are not entirely known. The tests for early pregnancy are not 100% reliable. In males, pregnancy is not a possibility. Children can have adverse reactions to anesthetics, and they will not be recruited. Therefore, male volunteers are selected in this study. In order to further reduce the variables and minimize the risks for the subjects, exclusion criteria are used to select the subjects who are most suitable for this primary study.

3.5 Inclusion Criteria

1. Age 25-40
2. Male
3. Normal healthy subject without systematic diseases or conditions
4. Metabolic Equivalents of Functional Capacity ≥ 5
5. Low risk for Obstructive Sleep Apnea (OSA) based on the screening test (STOP-bang score established by American Society of Sleep Apnea): Yes to > 3 items- high risk of OSA
6. No History of Arrhythmia (Baseline EKG will be obtained during the history and physical session), seizure, liver and kidney diseases
7. BMI < 30 kg/m²
8. No prior difficulty with anesthesia
9. No personal or family history of malignant hyperthermia
10. No history of any mental illness
11. No history of drugs or alcohol abuse (urine drug screens required).
12. Subjects capable of giving consent.
13. Living less than 30 miles away from UC.
14. No history of seizure disorders.

15. No history of head trauma.

3.6 Exclusion Criteria

1. Age <25 or >40
2. Female
3. ASA physical status > 1 (normal healthy subject without systematic diseases or conditions)
4. Metabolic Equivalents of Functional Capacity (METs) < 5
5. High risks for Obstructive Sleep Apnea (OSA) based on the screening test (STOP-bang score established by American Society of Sleep Apnea): Yes to > 3 items- high risk of OSA
6. History Arrhythmia (Baseline EKG will be obtained during the history and physical session), seizure, liver and kidney diseases
7. BMI > 30 kg/m²
8. Prior difficulty with anesthesia
9. Personal or family history of malignant hyperthermia
10. History of any mental illness
11. History of drugs or alcohol abuse (urine drug screens required)
12. Subjects capable of giving consent
13. Living more than 30 miles away.
14. History of seizure disorders.
15. History of head trauma.

3.7 Vulnerable Subjects

No vulnerable subjects will be used.

3.8 Subject Identification & Recruitment

Brochures, flyers and posters will be distributed throughout Chicago (at malls, stores, community clubs, minority centers etc).

Women will not be recruited to this study. The effects of anesthesia and the study drug on fetuses are not entirely known. The tests for early pregnancy are not 100% reliable. In males, pregnancy is not a possibility. Therefore, male volunteers are selected in this study. In order to further reduce the variables and minimize the risks for the subjects, exclusion criteria are used to select the subjects who are most suitable for this primary study.

Children can have adverse reactions to anesthetics and they will not be recruited.

Minorities will be actively recruited.

3.9 Location

The University of Chicago CCD recovery room. This facility is outfitted with all the necessary emergency and equipment required to maximize safety. The isolation recovery room that we use is perfect for our purposes since it is soundproof.

3.10 Payment to Subjects

\$500 will be given to each subject for every day they are anesthetized. \$1000 will be provided to each subject for completing the entire trial. Since the subjects will be in the trial for 2 full days, the payments reflect an average salary for a working day of \$500/day. The subject will receive \$500 after each anesthesia session.

3.11 Informed Consent Process

One of the anesthesiologists will provide a verbal explanation of the study to the subject and answer all questions regarding this study. Then, a written description of the study will be provided to the subject. Afterwards the subject will be required to describe the study in their own words showing that they understand the purpose of the study, the risks involved and their own role in the study. If any risks are not well understood, the members of our team will explain them, in detail. The subject and the person who administered the informed consent must sign and date the document. A copy of the informed consent form will be given to the subject and the original will be kept in the subject's record.

3.12 Subject Withdrawal Following Study Completion

The final "anesthesia" visit will be no different than the previous one. After the subject wakes from anesthesia and completes the psychomotor tests, he will be released to home, in company with another adult, once he meets the safety criteria for release. This will be followed up by phone calls for the subsequent 2 weeks to ensure that the subject has not suffered any adverse effects from the treatment.

All subjects will be asked, upon completion of the study, to help us identify ways to make the study better. The idea for combining the informed consent and the history and physical sessions arose from complaints from test subjects.

3.13 Subject Withdrawal Before Study Completion (i.e., early termination)

All subjects are free to withdraw from participation in the trial at any time, for any reason, specified or unspecified, and without prejudice.

Not uncommon side effects of anesthesia are nausea and vomiting, mild to moderate sore throat, transient hypoxia (low oxygen level), transient blood pressure and heart rate changes (sinus arrhythmia). Most people do not experience any of these events. They are reversible and have no long-term consequence. But they can be unpleasant to the volunteer. Thus, the volunteers may decide not to complete the 2 rounds of anesthesia. Our expectation is that there will be significant drop out.

If there is a more serious event associated with the trial, then the IRB will be informed immediately and the subject will be monitored until the issue is resolved.

If we observe that the subject does not easily tolerate anesthesia, as indicated by changes in vital signs, or alternatively if the toxicology screen comes back positive for drugs or alcohol or alternatively if the subject does not bring a responsible adult with him to the session, they will be asked to leave the trial. Termination will have no adverse health consequences, but if the subject had previously received anesthesia there will be follow-up phone calls identical to those received by subjects who complete the trial.

3.14 Data Collection and Follow-up on Early Withdrawal Subjects

Most people suffer no adverse effects of any sort related to anesthesia. The adverse effects that are typically experienced like sore throat or vomiting are transient in nature and subjects will have no long-term consequence. These events will be monitored by the follow-up phone calls. More serious events will be monitored closely until the event is resolved, by clinicians associated with this project or alternatively by other clinicians at UC. No follow-ups on efficacy will be carried out, as the point of the research trial is to determine whether caffeine can cause a rapid emergence from anesthesia.

We ask the IRB for approval to recruit an additional 8 volunteers.

4. STUDY DRUG

A. Drug Study:

4.1A Formulation of Study Drug

Caffeine citrate (caffeine), is available commercially. It is manufactured by Bedford Labs.

Placebo control will be a sterile saline solution.

4.2A Treatment Regimen

The study drug (caffeine citrate) or the placebo (a saline solution) will be given intravenously 10 min before the end of 1 hour isoflurane anesthesia. Caffeine will be administered at a dose of 15 mg/ kg. There will be a single injection of either caffeine or a saline placebo control solution in the anesthetized test subjects. The drug will be injected slowly across a 10-minute interval, in order to prevent concentration spikes in the subject. A pump infuser will be used to introduce the drug. The subjects will be told not to eat or drink for 8 hours prior to anesthesia (see below for a more detailed description).

An example of the method of administration may be illustrative. The drug, caffeine citrate and comes in a single dosage of 20 mg caffeine per ml of solution. Let's say a volunteer weighs 65 kg. The dose we propose for this trial is 15 mg/ kg. Therefore the volunteer will get a 48.75 ml injection of caffeine citrate. This amount of solution (48.75 ml) can be injected as a bolus via an IV line in under a minute. This rapid infusion of drug is what we call a bolus injection. We plan on introducing the 48.75 ml of caffeine citrate more slowly, across a 10-minute time window, to prevent spikes in the circulating caffeine concentration. The caffeine will be introduced via a pump infuser. In this case the subject will get 975 mg of caffeine citrate that is equivalent to 487.5 mg of pure caffeine.

4.3A Preparation and Administration of Study Drug

All drugs will be stored and/ or prepared by the IDS group and provided to the research team on the day of each session.

The IDS office is located in the Wyler Pavilion, room C286, but is in the process of moving to CCD. The area has equipment necessary to store, prepare, and dispense all categories and dosage forms of drugs and biologics. IDS pharmacists and technicians along with other supporting pharmacy personnel provide services to faculty, investigators, pharmacy staff, nursing staff, and subjects enrolled in research protocols. The IDS office is open up to 10 hours per day on weekdays or a staff member is on call 7 days per week. Key staff members in the central pharmacy provide dispensing support twenty-four hours a day when IDS staff is not present

Required documents for every research study are placed in designated binders. They contain sections for: Randomization schedule, Drug Accountability Records, Drug Receipt Records, Subject scripts and consent signature pages, Drug Order Forms (if applicable) or information on how to get a re-supply of drug, and any other miscellaneous forms needed. For all studies, a protocol specific procedure sheet is prepared. Each subject's order is verified with the protocol assigned and with the subject's dispensing history prior to dispensing any study medications. Discrepancies are discussed with the study nurse or physician, as appropriate. The presence of the subject's signed informed consent is verified by the pharmacist. The protocol is reviewed for all pharmaceutical information prior to attempting to prepare a study medication. A pharmacy protocol specific procedure sheet is reviewed before drug preparation is set up by the pharmacist processing the order. The Central Pharmacy area maintains all investigational drug supplies handled by the inpatient pharmacy. The IDS provides training to essential staff to provide service to protocols that require continuous service 24 hours per day, 7 days per week, and 365 days per year.

IDS maintains drug accountability records in compliance with state and federal regulations and the specific requirements of each protocol. At study termination when study drug has been either returned to the sponsor or destroyed, all drug accountability records and study-related documents are transferred to the investigator.

IDS Information

Main Office: Room C286, Wyler Pavilion
Phone: 773-834-7466
Fax: 773-834-7461

4.4A Subject Compliance Monitoring

Compliance will be monitored with a toxicology screen. Data from the screen will be included in the study database containing recovery from anesthesia data. Any subjects that do not meet compliance guidelines (no drugs or alcohol) will be asked to leave the study. If they do not bring a responsible adult to the anesthesia sessions they will be asked to leave.

4.5A Concomitant Medications and Therapies

Our goal is to identify a relatively young cohort of healthy volunteers. Thus patients taking drugs for chronic conditions will not be chosen.

Propofol is the preferred agent for the induction phase because it does not cause airway irritation. Isoflurane will be used as the main anesthetic agent as it is an extremely safe anesthetic that has been used for decades. During this study, we do not plan to use other anesthetic agents, such as midazolam, fentanyl, and muscle relaxants to minimize the interactions of these drugs to our study drug. However, propofol and muscle relaxants are available at the bedside in case they are needed for the management of unexpected airway situations, such as laryngeal spasm. If an unexpected airway emergency occurs, the ASA difficult airway algorithm will be applied for airway management. The subject will be allowed to breathe spontaneously unless the tidal volume is less than 3 ml/kg. We will optimize the tidal volume at around 5 ml/kg to make sure each subject receives similar amount of isoflurane for an hour. Pressure support mode may be applied to optimize the tidal volume if necessary. The study drug (caffeine citrate) or the placebo control will be given intravenously 10 min before the end of 1-hour isoflurane anesthesia. The anesthesiologists will be blinded to the medication injected to the subject since the drug or normal saline is prepared by the pharmacists in the OR pharmacy. When isoflurane is turned off, the subject will eliminate isoflurane passively via breathing.

There will be at least two anesthesiologists present during the entire period that anesthesia is administered. Each subject will be given a dose of antiemetic medication, zofran (4 mg) 20 minutes before the end of the anesthesia to minimize the incidence of post anesthesia nausea and vomiting. During the period of anesthesia, we will keep the subject's BP within 20% of its pre-anesthesia value. If the BP falls more than 20% of the baseline, we will first give IV fluid to replete the fluid deficit due to the restriction of fluid intake prior to the study. If that is not sufficient to raise the BP, we will reduce the concentrations of isoflurane by 0.1% at a time until we can maintain the BP close to the normal range. Vasoactive medications will be given only if absolutely necessary but they will be available. Volunteer safety is our first priority.

[Intraoperative hypotension is not well defined (see (Bijker *et al.*, 2007)). In clinical practice, changes of 20 - 30% of the baseline mean MP or SBP are well accepted. Transient change of BP in this range is usually clinically insignificant. Healthy volunteers tolerate these changes better than sick surgical patients. In general, lowering of blood pressure by an anesthetic is also dose dependent. Hemodynamic changes are very fluid during anesthesia. It is important not to overcorrect the minor transient change of BP and heart rate. **Nonetheless, in this trial with healthy volunteer, we will use a change of 20% from the baseline (MBP or SBP) for more than 5 min as our trigger for intervention**].

4.6A Packaging and Labeling

The drug will arrive at UC packaged by the manufacturer, Bedford Labs Inc. It will be prepared for use by our IDS group as will be the placebo control. The drugs will be labeled with a numeric code that will be unknown to the research team and the research subjects. The code will be included in the database with the volunteer data. The study will be unblinded after completing the Pilot program.

4.7A Receipt of Drug Supplies

Caffeine is inexpensive and stable. The IDS group will be instructed to place an order sufficient for the entire Pilot Project and they will be required to store the drug. The placebo will be prepared as needed. It too should be stable for a period of time.

4.8A Product Storage and Stability

Caffeine is stable for months in the vials shipped by the manufacturer if stored at room temperature (15 – 30 degrees C). Caffeine is preservative free.

4.9A Dispensing of Study Drug

Caffeine or placebo will be injected via and intravenous line by one of the two attending anesthesiologists. Only a certified anesthesiologist will be allowed to dispense either the drug (caffeine citrate) or the IDS prepared placebo.

4.10A Return or Destruction of Study Drug

Any remaining caffeine will be discarded as will be any remaining saline.

5. STUDY PROCEDURES

Each subject will attend 4 sessions to complete the study.

5.1 Screening Session 1

In the first screening session, an anesthesiologist will explain the purpose of the trial and carefully go over the consent form. If the subject is willing to enroll the consent form will be signed. Next, the anesthesiologist will take a detailed medical history and perform a physical examination of the subject. A baseline EKG and urine toxicology screen text will be obtained. If the subject meets the criteria for the study, the subject will be enrolled in the trial. At this point a pre-study training session will be provided to familiarize the subject with the psychomotor tests.

5.2 Study sessions 2 – 3

In the following two sessions, each subject will receive general anesthesia in one session (with a saline injection as a placebo control) and receive general anesthesia and caffeine citrate (15 mg/ kg equal to 7.5 mg/ kg of pure caffeine) in the other session in a randomized manner. For each subject, each session involving anesthesia will be at least 2 weeks apart.

For each session, subjects will be asked not to eat and drink 8 hours prior to the study in order to minimize the potential risk for aspiration during anesthesia. They will be asked to refrain from alcohol or drug use for 24 hours prior to the sessions. A toxicology screen will be used for each session. Once the subject is checked in, a peripheral intravenous catheter (IV) will be inserted on one of the arms by the anesthesiologist (Xie, Fong or Apfelbaum). The IV is used to administer medications during the course of the study. After the IV insertion, American Society of Anesthesiologists (ASA) standard monitoring, including EKG, blood pressure (BP), respiratory rate, end tidal CO₂, pulse

oximetry, BIS monitor and temperature, will be used to assess the subject (see below for further details). The subject will then be asked to breathe 100% O₂ via a facemask for two minutes. After the lungs are filled with 100% O₂, the subject will be given propofol (2mg/kg), as an IV bolus, to induce anesthesia. Within 1-2 minutes, the subject will become unconscious. If the subject is still conscious, an extra dose of propofol (0.5 to 1 mg/kg) will be given until the subject is in a deeper stage of anesthesia. A Laryngeal Mask Airway (LMA), a rubber airway device, will be inserted via the mouth into the larynx. This device will help to optimize the patency of the airway while the subject is completely anesthetized. This allows the subject to breathe easier without the help of a ventilator. This is the induction phase of anesthesia. After induction, the subject will be given isoflurane (1.0 MAC, as adjusted by age and weight by the anesthesia machine) for 60 minutes. This period is called the maintenance phase of anesthesia. 60 minutes will ensure that each subject reaches anesthetic equilibrium. Oxygen flow will be kept at 2 L/min during the maintenance phase. Ten minutes before terminating the isoflurane, volunteers will be infused with a solution containing saline (control) or with caffeine (test). After the termination of isoflurane, the subject will be allowed to wake up. This phase is called the emergence phase of anesthesia. During the emergence phase, Oxygen flow will go up to 8 L/min to avoid rebreathing. We will test whether the caffeine shortens the emergence phase of anesthesia.

[EKG, respiratory rate, end tidal CO₂, pulse oximetry and temperature are monitored continuously, once the volunteer is connected to the monitoring machines. A bispectral index (BIS) monitor, will continuously measure depth of anesthesia. Prior to starting the procedure, blood pressure will be measured every 5 minutes; this measurement of blood pressure will provide the baseline for subsequent BP measurements. Blood pressure will be measured every 2 minutes during the first 10 min of the anesthesia, which includes the induction period. BP will be measured every 5 min throughout the course of anesthesia and the first 30 min in the PACU. After that, BP will be measured every 10 min until the subject is discharged. This data is stored automatically for each patient in our hospital's Epic database].

Propofol is the preferred agent for the induction phase because it does not cause airway irritation. Additionally, propofol is normally used for anesthesia induction in human patients. There are several advantages to use isoflurane as the main anesthetic agent. Most importantly, it is an extremely safe anesthetic that has been used for decades. All our preliminary animal data was generated with isoflurane. Because it takes slightly longer to "wake up" from isoflurane anesthesia, compared to other volatile anesthetics like sevoflurane, it will allow us to better differentiate the actions of the caffeine. During this study, we do not plan to use other anesthetic agents, such as midazolam, fentanyl, and muscle relaxants to minimize the interactions of these drugs to our study drug. However, propofol and muscle relaxants are available at the bedside in case they are needed for the management of unexpected airway situations, such as laryngeal spasm. If an unexpected airway emergency occurs, the ASA difficult airway algorithm will be applied for airway management. The subject will be allowed to breathe spontaneously unless the tidal volume is less than 3 ml/kg. We will optimize the tidal volume at around 5 ml/kg to make sure each subject receives similar amount of isoflurane for an hour. Pressure support mode may be applied to optimize the tidal volume if necessary. The

study drug (caffeine citrate) or the placebo (saline) will be given intravenously 10 min before the end of 1 hour isoflurane anesthesia. The anesthesiologists will be blinded to the medication injected to the subject since the pharmacists in the IDS GROUP prepare the drug or the placebo control. When isoflurane is turned off, the subject will eliminate isoflurane passively via breathing. We will measure the time between when the isoflurane is stopped to the awakening of the subject. We will record the time to recovery of the gag reflex, until the subject's eyes open, until the subject's mouth opens, and the time until the subject can respond to the command to grip the hand of the attending physician and the removal of the laryngeal mask. After the subject is awake and relatively alert, he will perform psychomotor tests to measure his cognitive function. The subject will be required to complete a simple set of cognitive tests to determine if they are still impaired by anesthetic. Both "control" and test subjects will be required to complete the same tests. In total there are 3 different tests. The tests will be repeated every 15 minutes in order to obtain a recovery time course.

There will be at least two anesthesiologists present during the entire period of anesthesia. Each subject will be given a dose of antiemetic medication, zofran (4 mg) 20 minutes before the end of the anesthesia to minimize the incidence of post anesthesia nausea and vomiting. During the period of anesthesia, we will keep the subject's BP within 20% of its pre-anesthesia value. If the BP falls more than 20% of the baseline, for more than 5 minutes, we will first give IV fluid to replete the fluid deficit due to the restriction of fluid intake prior to the study. Vasoactive medications will be given if necessary.

One hour after the isoflurane is stopped, if the subject is awake, oriented and showing stable vital signs, one of the anesthesiologists may leave the room to prepare for the afternoon session. There will always be at least one anesthesiologist with the test subject at all times. Most of the time there will be two anesthesiologists.

At the conclusion of the study, we will discharge the subject based on the criteria used in our post anesthesia care unit in the hospitals. In general, the subject should be awake and alert with the stable vital signs. He should be able to drink clear liquid, void and ambulate before discharge home. We will call a car service for him without cost to the subject. The subject will be expected to live within 30 miles of The University of Chicago to make sessions easily accessible. Upon discharge, the subject will be required to be accompanied by a responsible adult. Each session for any given subject will be at least 2 weeks apart. The total time of each session, including the pre-anesthesia preparation, testing and recovery from anesthesia, will be ~4 hours. We will call the subject on the same day and the next day after each session to make sure the subject fully recovers without any adverse event. The subject will get another call a week after the procedure.

Subjects will be tested in this manner a total of two times. In one of the tests they will be anesthetized with isoflurane and injected with a saline solution and in one test they will be anesthetized with isoflurane and then injected with caffeine citrate (15 mg/ kg).

After terminating anesthesia, the subject will perform psychomotor tests to measure his cognitive function. The subject will be required to complete a simple set of cognitive tests to determine if they are still impaired by anesthetic. Both "control" (saline injection) and test subjects (those that obtain caffeine) will be required to complete the same tests.

Memory will be tested with 20 words, after which the subject will be asked to recall the words, order not being important. Two hours later the subject will be asked for the same words. The Digit Symbol Substitution Test asks subjects to substitute symbols for numbers for 60 seconds. Each time there will be different symbol number codes. The subjects will also be asked to estimate a period of time, typically 30 seconds. Blood pressures, heart rate and O₂ saturation will be monitored. The entire suite of tests will be repeated two hours later. This latter test will allow us to assess whether the drugs allow recovery of cognitive ability.

***Please note:** We may recruit one or two additional anesthesiologists to our team. We will only recruit attending clinicians. They would take all the human clinical trial coursework and then we would have training sessions related to our protocol. The IRB should be aware that our protocol was designed to be as similar as possible to a standard surgical session with anesthesia, so that our research protocol will be instantly familiar to any experienced anesthesiologist.

Schema:

Procedure	Screening Session 1	Study Session 1	Study Session 2
Time	1 week prior to study	Start of Study: week 0	Week 2-4
Explain Trial to subject Obtain Consent	X		
Medical History	X		
Physical exam	X		
Vital signs	X	X	X
EKG	X	X	X
Urine Toxicology screen	X	X	X
Enroll Subject in Trial	X		
Psychomotor Training session	X		
Propofol Induction – Isoflurane maintenance		X	X
**Saline Control injection		X	
**caffeine (15 mg/ kg) injection			X
Psychomotor session 1 + 2		X	X
Adverse Event report		X	X

**Please note that the drugs will be applied in a double-blind manner and will be prepared by a hospital pharmacist.

6. STATISTICAL PLAN AND CONSIDERATIONS

6.1 Sample Size Determination

Members of the IRB should understand that the sample size described above is an estimate, since we could not find good data on human “waking” times from isoflurane anesthesia in the literature. Thus we used our own animal studies to estimate sample size. There are at least three reasons why our sample size might be in error. First, rats are not people. They may get rid of isoflurane differently than do humans due to their higher respiration rates. In addition, rats are genetically more similar to each other than are humans. That would tend to increase human variance relative to that of the rat. On the other hand, the equipment used to anesthetize rats is less sophisticated than that in humans. In humans, the expired anesthetic concentration is measured, thereby allowing for precise anesthetic concentration, in the lungs. This is not possible in rats and so the anesthetic dose applied is less precise. This will tend to lower human variance. This is a long-winded way to say that our sample size estimate may be wrong. In these studies rats were used 4 times (twice with a saline injection twice with an injection of saline containing caffeine), a strategy that reduced variance.

Additional caveats: The 15 mg caffeine citrate that we propose is equivalent to 7.5 mg/kg of pure caffeine. We have never tested 7.5 mg/kg caffeine in rats, nor have we ever studied isoflurane at a concentration of 1 MAC.

We use the following stringent criteria to determine sample size: Power will be set to 95% and $p \leq 0.01$.

Rats waking from isoflurane anesthesia with 5 mg/kg caffeine (isoflurane ~ 1.5 MAC):

(Control - saline injection) - Mean = 540 sec, SD = 159.04

(Test - 5 mg/kg caffeine in saline injection) - Mean = 269.67 sec, SD = 103.69

At this concentration of caffeine we would need $n=12$ measurements for control and $n=12$ measurements for caffeine to meet our stated stringent criteria. If each person were used for one control and one caffeine measurements we would need 12 people for the study.

We are using human volunteers twice, not four times like the rat population, we expect higher variance. Nonetheless, we expect 8 total volunteers will satisfy our study. This is because we are using a higher concentration of caffeine (7.5 mg/kg), which should produce a larger response than the 5 mg/kg shown above, and we will employ a lower concentration of isoflurane (1 MAC), which should be easier to antagonize.

6.2 Statistical Methods

We hope to use a simple paired t-test to analyze the data, using volunteers as their own controls.

Crossover study - Each volunteer receives anesthesia 2 times: once without drug (control) and once with caffeine (test). Our animal studies suggest that variance is dramatically minimized when test subjects can be used as their own controls, in this manner. If humans recapitulate the rodent studies then 8 volunteers should provide an adequately

sized sample to answer the research question. The study was originally divided into two parts. The first part, the Pilot study has been completed.

Part 1: In order to demonstrate feasibility and safety, we carried out a pilot study of 2 healthy human volunteers to assess whether caffeine aids these subjects in recovering from isoflurane anesthesia. Recovery times were measured in three ways: as the time between the time when anesthetic is terminated until the subject starts to gag, can open his eyes, and the subject is able to respond to simple commands (like squeeze the clinician's hand). Each of the three endpoints was monitored and plotted separately. In this pilot study caffeine citrate (caffeine) was used at 10 mg/kg. By comparing subjects that wake from anesthesia who are injected with a saline solution to subjects that are injected with caffeine we were able to show that caffeine is as efficient in humans as in rodents. Now that we have demonstrated safety and feasibility, if the IRB gives us permission, we will continue to Part 2 of our study. Time from the end of anesthesia to eye opening will be analyzed, as will be the time from the end of anesthesia to response to a simple command. Each parameter (gagging, eye opening and response to command) will be analyzed and plotted separately.

There are cognitive impairments that last for hours following anesthesia (Nathanson *et al.*, 1995; Larsen *et al.*, 2000; Chen *et al.*, 2001). Reaction times and memory are impaired following anesthesia (Nathanson *et al.*, 1995; Larsen *et al.*, 2000; Chen *et al.*, 2001). Outpatients are being released from surgery/ anesthesia on an ever shortening time scale to save money. It would be significant if clinicians could wake the patients safely, rapidly and completely with no cognitive issues. The psychomotor studies will allow us to assess whether caffeine helps ameliorate the cognitive problems associated with anesthesia. Subjects will be required to carry out a series of simple psychomotor tests thirty minutes after anesthesia and then again two hours later. By comparing results, memory and reaction times we will determine whether Caffeine helps ameliorate cognitive problems, relative to the saline treated controls. Data from each test will be analyzed and plotted separately.

Part 2: Up to 8 additional healthy human males will be recruited for this part of the study. If the effects of caffeine in humans mirror that of the rat, we will need ~8 more volunteers to complete the trial assuming that each volunteer attends all 4 anesthesia sessions. We anticipate some dropout. This is the part we are asking for permission to start.

6.3 Subject Population(s) for Analysis

The data from all subjects will be analyzed without knowledge of whether the data comes from a control or test condition. After breaking the code, data will be grouped accordingly. All data will be analyzed in two different ways. Using a paired t-test data from subjects that have completed the two anesthesia sessions of the study will be analyzed and plotted. Separately, using an unpaired t-test all data will be grouped together including that from subjects who did not finish the trial. In this way the data will be compared. Only data from subjects that failed the toxicology screen will be excluded.

7. RISKS AND BENEFITS

7.1 Risks and Likelihood of Occurrence

Anesthesia risks:

Overall, anesthesia is extremely safe. Our hospital administers anesthesia thousands time every year with minimal complications directly related to anesthesia. However, there are potential risks for general anesthesia. The likely risks in patients of the general population include, but are not limited to, post anesthesia nausea and vomiting (up to 30%), mild to moderate sore throat, transient hypoxia (low oxygen level), transient blood pressure and heart rate changes (sinus arrhythmia). Most people do not experience any of these events. They are reversible and have no long term consequence.

The more severe risks, but less likely, are awareness, under anesthesia, aspiration pneumonia (up to 0.03% - that is the reason that you are told not to eat and drink for 8 hours prior to the anesthesia so that your stomach can be as empty as possible), severe hypoxia due to aspiration or difficult airway, severe hyper or hypotension, sustained arrhythmia, dental damage, vocal cord damage and reactions to drugs we administrate. These events do not commonly occur in the healthy population. With the screening tests in this study, these events are very unlikely to occur. If they occur, they will be very unlikely to cause long term consequences with proper treatments.

The most severe risks are unexpected difficult airway, including difficult mask ventilation and difficult intubation, and malignant hyperthermia (MH), a genetic associated reaction to anesthesia (0.002-0.005%). These are considered rarely events in Anesthesia although severe consequences, such as organ damage or even death may occur. We will carefully screen the subjects based on our exclusion criteria to minimize these risks. Therefore, these risks will be further reduced in the population of our study because of careful screening carried out prior to the administration of anesthesia. In addition, our anesthesia location and anesthesiologists are well-equipped to handle these situations so that the damage will be as minimal as possible.

Assessing risks for this trial are somewhat complicated. We will be recruiting healthy volunteers for this trial, while most of the available data comes from the pool of people receiving anesthesia for surgery. Most of these surgical patients are quite sick and are therefore susceptible in ways that healthy people are not.

As an example: A common number used in anesthesia related deaths in ~1 per hundred thousand (see Fig. 1 in ref (Haller *et al.*, 2011)). Different recent studies have different numbers. Some studies have higher numbers of deaths (~2 per hundred thousand (Kawashima *et al.*, 2003)) while others have lower number of deaths (~4 per million, (Aitkenhead, 2005)). The problem with these numbers is that they include all anesthesia's including those in sick patients. Sick patients have most of the adverse reactions. I have only found a single study that examined ASA-1 patients by themselves (ASA-1 patients are healthy and are similar to those we hope to enroll). This study, which covered a span of 14 years found zero anesthesia related deaths in this 14 year interval in ASA-1 patients (Khan & Khan, 2011). This is why anesthesia studies in healthy human volunteers have been allowed in the past. There were ~22,000 ASA-1 patients tracked.

Definition of ASA categories:

ASA-1: No organic, physiologic, or psychiatric disturbance; excludes the very young and very old; healthy with good exercise tolerance

ASA-2: No functional limitations; has a well-controlled disease of one body system; controlled hypertension or diabetes without systemic effects, cigarette smoking without chronic obstructive pulmonary disease (COPD); mild obesity, pregnancy

ASA-3: Some functional limitation; has a controlled disease of more than one body system or one major system; no immediate danger of death; controlled congestive heart failure (CHF), stable angina, old heart attack, poorly controlled hypertension, morbid obesity, chronic renal failure; bronchospastic disease with intermittent symptoms

ASA-4: Has at least one severe disease that is poorly controlled or at end stage; possible risk of death; unstable angina, symptomatic COPD, symptomatic CHF, hepatorenal failure

ASA-5: Not expected to survive > 24 hours without surgery; imminent risk of death; multiorgan failure, sepsis syndrome with hemodynamic instability, hypothermia, poorly controlled coagulopathy

Risks of Caffeine:

Caffeine is very safe and commonly used in the household and also used clinically in certain conditions, such as post-dural puncture headache in post-partum women and sleep apnea in premature infants. The doses used in this study are lower than those used in infants for sleep apnea. The FDA categorizes caffeine as "Generally Recognized as Safe". However, caffeine can have side effects, such as palpitation, seizure, sleepless or nervousness. Seizures are exceptionally rare and occur at much higher concentrations than will be used in the current study. We don't expect caffeine's effects to be any different with or without anesthesia. Two clinical trials, one in adults and another in children, showed that it was safe to administer caffeine to anesthetized patients (Khalil *et al.*, 2008; Steinbrook *et al.*). Both these trials used caffeine and sodium benzoate.

In this protocol we describe a procedure where anesthetized healthy human volunteers would be given 15 mg/ kg caffeine citrate, 10 minutes before terminating anesthesia. The caffeine is to be administered slowly across a 10-minute time window so as to prevent a large spike in circulating caffeine levels. We propose using healthy, low body mass male volunteers exclusively in this trial. In these low body mass, low weight volunteers 15 mg/ kg caffeine and citrate will translate to 750-1200 mg caffeine citrate in total. Normalizing for the weight of the volunteers represents a more rigorous pharmacological approach than simply giving everyone the same amount of caffeine citrate. Note that 15 mg/ kg caffeine citrate is equivalent to ~7.5 mg/ kg of caffeine base. Thus 750-1200 mg caffeine citrate corresponds to 375-600 mg of pure caffeine.

The New Scientist website states that caffeine is the most commonly used psychoactive drug and in the United States more than 90% of adults use it daily. Caffeine has been used by humans at least since Roman times and has been found to be safe. Hundreds of millions of Americans drink caffeinated beverages every day. Worldwide the number is in the billions. In some cases the drinks contain large amounts of caffeine. For instance, a large cup (20 oz) of Starbucks' coffee contains over 415 mg of caffeine, an amount not so different than the low end of the range of caffeine that we propose to use in our study. Death Wish coffee is even more extreme in that it has 660 mg caffeine in a 12 oz cup. Most extreme is chameleon cold brew coffee, which has over 2000 mg in a 32 oz container. Caffeine is also used in medicine on a daily basis. Caffeine is used to treat neonatal apnea as well as certain types of headaches. It is a popular additive for drugs that treat pain, like Excedrin.

Citrate is used as a food additive in beverages, soda, ice cream, candy, fruit juice, wine, juice, jam, canned fruit and vegetables, frozen fruit, cheese spreads, dressings, preserves, cheese, mayonnaise etc. E numbers are codes for substances that can be safely used as food additives for use within the European Union and Switzerland. The E number for sodium citrate is E331. Caffeine and citrate are found in variety of energy drinks. For instance, Xtreme Shock Energy drink contains caffeine citrate, while drinks like RedBull, Monster and Full throttle contain caffeine and sodium citrate. Both caffeine and citrate are consumed by millions of people on a daily basis. Sodium citrate is generally considered inert.

Thousands and thousands of preterm infants have been given IV injections of caffeine citrate to treat apnea of prematurity (Reference 1 - (Parikka *et al.*, 2015)). Caffeine is a neonatal success story. It is one of the most commonly prescribed drugs in neonatal medicine. And it has been used successfully for about 40 years. See below for a more complete description.

In our trial we propose using caffeine in anesthetized patients. We found two studies where caffeine has been tested in anesthetized patients (Khalil *et al.*, 2008; Steinbrook *et al.*, 2013). In the Khalil *et al.* study, 20 mg/ kg caffeine was given to anesthetized children (Khalil *et al.*, 2008). This dosage is 33% higher than what we propose in our trial. The duration of the caffeine application is difficult to determine from the study. But it is important to note that no adverse effects were reported in the 36 children that received an injection of 20 mg/ kg caffeine with sodium benzoate.

The study that is most relevant to our proposed trial is that of Steinbrook *et al.* (Steinbrook *et al.*, 2013). In this study, anesthetized adult patients were given a bolus of caffeine (500 mg), 15 minutes before emergence from anesthesia. Both the dosage and the method of application were similar to what we propose. 65 patients received this treatment without adverse effect.

Caffeine and sodium benzoate has been used to treat post-dural puncture headaches for decades. In the Sechzer and Abel study (Sechzer & Abel, 1978), dozens of women were

treated for post-dural puncture headache with 500 mg caffeine and sodium benzoate, applied as a single 2 ml bolus. Both the dosage and the method of application were similar to what we propose in the IRB protocol. No adverse effects were reported, but the caffeine successfully treated most of the headaches.

Caffeine and sodium benzoate has been used in psychiatric studies. In one such study, caffeine and sodium benzoate was used in psychiatric patients at concentrations much higher than we plan to give to our volunteers. In this study 8 patients received IV caffeine sodium benzoate in the range of 500 – 2000 mg. The patients received the drug on multiple occasions at multiple concentrations (between 500 – 2000 mg). The drug was always applied as a single bolus. No adverse effects of the caffeine sodium benzoate were reported (Shapira *et al.*, 1987). In this study, the methodology, a bolus application and the concentration (up to 2000 mg), are more extreme than are proposed in our IRB protocol.

In a different psychiatric study, McCall (McCall, 1992) gave 10 patients caffeine sodium benzoate (500 mg) IV during amobarbital interviews to keep them from falling asleep. The caffeine was administered as a single bolus. This combination of caffeine concentration and bolus administration were similar to what we propose.

Thousands and thousands of preterm infants have been given IV injections of caffeine to treat apnea of prematurity (Parikka *et al.*, 2015). Caffeine is a neonatal success story. It is one of the most commonly prescribed drugs in neonatal medicine. And it has been used successfully for about 40 years. A brief excerpt from (Kreutzer & Bassler, 2014) - Kreutzer & Bassler, where they discuss caffeine in neonates: “Until 2006, it had only a few relatively small and short-term studies supporting its use. It is thanks to the efforts of Barbara Schmidt and the Caffeine for Apnea of Prematurity (CAP) Trial Group that we now have high-quality and reliable data not only on short-term but also long-term outcomes of caffeine use for apnea of prematurity. CAP was an international, multicenter, placebo-controlled randomized trial designed to determine whether survival without neurodevelopmental disability at a corrected age of 18 months is improved if apnea of prematurity is managed without methylxanthines in infants at a high risk of apneic attacks. CAP was kept simple and pragmatic in order to allow for maximum generalizability and applicability..... However, recent data suggest that the administration of prophylactic methylxanthine by neonatologists is now common practice”.

Caffeine citrate is functionally identical to caffeine and sodium benzoate. It has been used for decades as an injectable drug, with great success.

It is true that in none of the studies listed above have any subjects been given caffeine with a 10-minute injection, in a manner similar to what we propose. We would like to do this to prevent spikes in the circulating caffeine concentration. In several of the studies listed above caffeine was administered as a single bolus. Our preference, though, is a 10-minute infusion of caffeine applied via a pump. That is how we administered the caffeine in the Pilot study.

In infants and in some psychiatric patients higher concentrations of caffeine were tested than what we propose (see references 2 and 6 for examples). In all cases the drug was applied as an IV injection. No adverse responses were observed.

Finally, if there is a problem, the anesthesiologists are well trained to manage any unexpected events associated with anesthesia and the study drug. The area of the study will be well equipped to handle any unexpected events as well. For the most common side effects, such as nausea/vomiting, we will hydrate the subjects and administer antiemetic medications. There are rarely any long-term effects with post anesthesia nausea and vomiting. Mild or moderate sore throat usually goes away in a day or two. If any severe complications, such as aspiration, arrhythmia, persistent hypoxia, occur, the patient will be excused from the study. The anesthesiologists will manage the situation until the subject's conditions return to normal. Anesthesiologists are experts dealing with these situations in the operating rooms. The area of the study is adjacent to the operating rooms where airway management equipment, code cart (defibrillator, pacemaker and emergency drugs) and malignant hyperthermia cart are immediately available.

Risk of Data Loss:

There is also a risk that confidentiality will be lost. However, subject identity and data will be locked up in the PI's office. Only the investigators in this project have access to the data. The identity will not be included in the publication. The subject's identities will be destroyed once the analysis is completed for publication.

7.2 Potential Direct benefits to subject

Although the risks involved are more than minimal, the benefits to the general medical community are significant. Why is recovery from anesthesia important? Isn't recovery pretty fast normally? Recovery is rapid in most patients, but not in all. Small subsets of patients "wake" slowly. Even after "waking" from anesthesia patients can still exhibit cognitive impairment that can last for hours. This is especially true in the elderly. If drugs like caffeine were able to accelerate cognitive recovery in addition to accelerating "waking" times that would be extremely useful. Also, a large percentage of children (>20%) develop emergence agitation, a form of delirium, following anesthesia. Rather than re-anesthetize the child, the current practice, or use a variety of pre-medications, a safe and effective way to reverse the anesthesia, like caffeine, would be useful. If caffeine reverses anesthesia and calms the children, that would be extremely useful. Although we will not test any children, if the drug works in adults there will be a strong chance it will work in children.

Seniors are especially vulnerable to anesthesia. As more elderly undergo surgeries, it is apparent that recovery can take a long time (days). Memory especially appears to be labile. It would be very beneficial to have a drug that could reverse the effects of anesthesia in this population.

What is the goal of this trial? Our goal is to determine whether caffeine helps patients emerge from anesthesia quickly and reproducibly and to find out whether caffeine helps with the cognitive deficits that persist following anesthesia. Normally, recovery from anesthesia is somewhat random, dependent upon a variety of factors, like genetics or age

that are beyond the clinician's control. It would be extremely beneficial to be able to time recovery from anesthesia in a reproducible manner and to have that recovery be complete. Even though patients "wake" from anesthesia, there are still cognitive problems that persist for hours. Patient's reaction rates are slowed and memory can be impaired. Accidents may occur due to cognitive problems following anesthesia, which include problems with balance and with walking. And then there are outliers; patients that wake very slowly after anesthesia. None of this would be a problem, if there were a reliable drug that could speed recovery from anesthesia. Ideally you would design the drug to be almost completely innocuous and very inexpensive. If one had a really safe drug and one that was almost free, it might be possible to give it to everyone. Caffeine may be such a drug. It is the most commonly used psychoactive drug in the world, used by billions of people. If caffeine were to reverse cognitive deficits associated with anesthesia that would be valuable.

An additional benefit of caffeine is that it may reduce pain. Caffeine and sodium benzoate is used to reduce pain in post dural puncture headaches. Caffeine also magnifies the pain killing power of aspirin, ibuprophen and narcotics. That is why caffeine is an active ingredient in Excedrin Extra Strength or prescription drugs like Darvon Compound-65 or Triaminicin with Codeine. There may be several different mechanisms that enable caffeine to relieve pain of different kinds and differing intensities; intense pain may be directly sensitive to caffeine. It is possible that caffeine will be beneficial in reducing post-operative pain.

In a recent clinical trial in anesthetized children, caffeine was found to be safe (the trial was to determine whether caffeine could reduce the number of children who experience adverse post-extubation respiratory events – caffeine helped significantly).

Caffeine has been shown safe to use in anesthetized adults. In a clinical trial, to determine whether caffeine ameliorated post anesthesia nausea and vomiting, patients were given 500 mg caffeine during anesthesia. This combination, anesthesia and caffeine, was found to be safe. That trial used a dosage not too dissimilar to that we propose for our trial. Unfortunately, caffeine did not help with nausea and vomiting.

We hope that the IRB takes into account the potential benefit to medicine in a relatively short time frame.

The benefits to the subject are small, perhaps nonexistent. First, they will obtain a physical exam free of charge. Many people do not avail themselves of regular medical attention or don't have access. Identification of health-related issues may be important to the subject. Although the subjects will not be enrolled in the study, they will be informed of any issues identified by the clinicians. Second, is that many people have anesthesia during their lifetimes. Any beneficial changes to clinical standards will help them, but at an unidentified time in the future.

8. SAFETY AND ADVERSE EVENTS

8.1 Safety

Prior to induction of anesthesia and throughout the application of anesthetic, up until the anesthetic is discontinued and the subject opens their eyes, there will be two anesthesiologists with each subject continuously. After waking, there will be an

anesthesiologist present for the next 2 hours until the subject is released and the second anesthesiologist will be present for the first hour of recovery and then available but not necessarily in the room afterwards.

The exclusion criteria will eliminate any subject with high risks for this study. The study will be carried out adjacent to the adult operating rooms that are fully equipped with monitors, resuscitation equipment, oxygen, suction, airway and cardiac code carts etc.

Note that having two accredited anesthesiologists present throughout the anesthesia is a standard higher than that used in a typical operating room. This is done to minimize risk. If we can use each subject as their own control, that will lead to the fewest total number of anesthetics due to reduced population variance.

8.2 Adverse Events

Definitions

Adverse Event (AE):

Any problematic or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g. abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's involvement in the research, whether or not considered related to participation in the research.

Serious Adverse Event (SAE):

- Any adverse event that:
- Results in death
- Is life threatening, or places the subject at immediate risk of death from the event as it occurred
- Requires or prolongs hospitalization
- Causes persistent or significant disability or incapacity
- Results in congenital anomalies or birth defects
- Causes cancer
- Is an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

Unanticipated Problem:

Any incident, experience, or outcome that meets **all** of the following criteria:

- **Unexpected**, in terms of nature, severity, or frequency, given (a) the research procedures that are described in the protocol-related documents, such as the IRB approved research protocol and informed consent document; and (b) the characteristics of the study population;
- **Related or possibly related** to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research);

- Suggests that the research places subjects or others at a **greater risk** of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.3 Responsibilities

Investigators

Investigators conducting clinical research are responsible for:

- Assurance that their protocols are conducted in compliance with these guidelines.
- Submission of protocol to IRB and FDA. The protocol must include a DSM Plan (data & safety monitoring, section 8.6) that is commensurate with the study risk and reflects this guideline.
 - DSM plan describes plans for monitoring and reporting adverse events, serious adverse events, and unanticipated problems commensurate with nature and complexity of study.
 - Recipients of Serious Adverse Event and Unanticipated Problem reports must include IRBs, DSMB (data & safety monitoring board) and the FDA.
- Adherence to the DSM with respect to timely submission of adverse events, serious adverse events, and unanticipated problems.

Sponsors

Sponsors are responsible for:

- Following FDA Adverse Event Reporting guidelines and regulations for IND (investigational new drug) & IDE (investigational device exemption) -regulated trials through the Medwatch Program.
- Assistance to extramural investigators in understanding and applying adverse event and serious adverse event guidelines and for ensuring compliance with OHRP guidance.
- Oversight of these guidelines, which includes periodic review and revision as relevant rules and regulations change.
- Assurance that the Data and Safety Monitoring Plan (DSM Plan) addresses reporting of adverse events, serious adverse events, and unanticipated problems (if a DSMP is required).
- Verification that all corrective action plans have been adequately implemented.
- Assurance that the study has an independent data and safety monitoring body commensurate with study risk (Data and Safety Monitoring Board or Safety Officer).
- Ongoing oversight of the safety reporting process to ensure that potential safety issues are addressed.

Sponsor - Investigators

- Implement and adhere to all items cited for both investigators and sponsors.

8.4 Documenting and Reporting Adverse Events

8.4.1 Adverse Event Reporting

Data will be reviewed after each session and all Adverse Events will be summarized and reported to the IRB at the time of IRB Continuing Review.

8.4.2 Serious Adverse Event Reporting

All SAEs will be reported according to FDA guidelines for receipt of Adverse Event reporting:

- Unexpected fatal or life threatening experiences associated with the use of the drug (21 CFR 312[.32[c] [ii] [2]) - must be reported to the FDA by telephone or by facsimile transmission as soon as possible but no later than seven calendar days after the sponsor's initial receipt of the information if the intervention is under an Investigative New Drug (IND) application.
If an adverse drug experience was not initially determined to be reportable but the results of an investigation show that the experience is reportable under the above paragraph, the experience should be submitted to the FDA in a written safety report as soon as possible, but in no event later than 15 calendar days after the determination is made.
- Other adverse experiences associated with the use of the drug that are both serious and unexpected must be reported to the FDA within 15 calendar days after the sponsor's initial receipt of the information.
- If the drug is marketed and not under an IND (21 CFR 314.80[(c) (1) (i)]), a serious and unexpected adverse drug experience must be reported to the FDA within 15 calendar days of initial receipt of the information by the applicant.

8.4.3 Unanticipated Problems (UP) Reporting

All Unanticipated Problems that meet the stated criteria (section 8.2) will be reported within 10 days using the Unanticipated Problem Report Form to the IRB. For **Internal Fatal/Life-Threatening Unanticipated Problems**, the PI should notify the IRB Chair by phone **immediately** and consider voluntarily halting subject enrollment.

Table1. Time Line for Reporting Events

	Fatal/Life Threatening – Changing Risk-Benefit	Serious, Unexpected, & Related – Changing Risk-Benefit	All Other Events & Minor Protocol Deviations
IRB	Immediately by phone. Follow-up written notice within 10 days	10 working days	Continuing Review
FDA	7 days by IND/IDE holder	15 days by IND/IDE holder	Annual Report

8.5 Classifying Adverse Events

The Adverse Events will be classified according to severity, expectedness and potential relatedness to the study drug.

1. Severity

Classifications often include the following:

- **Mild:** Awareness of signs or symptoms, but easily tolerated and are of minor irritant type causing no loss of time from normal activities. Symptoms do not require therapy or a medical evaluation; signs and symptoms are transient.
- **Moderate:** Events introduce a low level of inconvenience or concern to the subject and may interfere with daily activities, but are usually improved by simple therapeutic measures; moderate experiences may cause some interference with functioning.
- **Severe:** Events interrupt the subject's normal daily activities and generally require hospitalization.

2. Expectedness

AEs must be assessed as to whether they were expected to occur or unexpected, meaning not anticipated based on current knowledge found in the protocol, informed consent form, investigator brochure, product insert, or label. Categories are:

- **Unexpected** - nature or severity of the event is not consistent with information about the condition under study or intervention in the protocol, consent form, product brochure, or investigator brochure.
- **Expected** - event is known to be associated with the intervention or condition under study.

3. Relationship to the Study Drug

The potential event relationship to the study intervention and/or participation must be assessed by the site investigator. A comprehensive scale in common use to categorize an event is:

- **Definitely Related:** The adverse event is clearly related to the investigational agent/procedure – i.e. an event that follows a reasonable temporal sequence from administration of the study intervention, follows a known or expected response pattern to the suspected intervention, that is confirmed by improvement on stopping and reappearance of the event on repeated exposure and that could not be reasonably explained by the known characteristics of the subject's clinical state.
- **Possibly Related:** An adverse event that follows a reasonable temporal sequence from administration of the study intervention and follows a known or expected response pattern to the suspected intervention, but that could readily have been produced by a number of other factors.
- **Not Related:** The adverse event is clearly not related to the investigational agent/procedure - i.e. another cause of the event is most plausible; and/or a clinically plausible temporal sequence is inconsistent with the onset of the event and the study intervention and/or a causal relationship is considered biologically implausible.

8.6 Unblinding procedures

The safety of the subject always comes first. If a serious event occurs, although very unlikely to result from caffeine, we will unblind study for the subject in order to ensure the subject's safety. This procedure is covered in the consent form. The unblinding will be part of managing an SAE, and will be reported with the SAE. We will use the same timeline requirements for investigator reporting of SAEs.

8.7 Data Safety Monitoring Plan

A final analysis will be done at the end of the study. However, data will be reviewed after each session with a volunteer (excluding the screening sessions). Particularly, any safety issues, such as unexpected vitals signs change or adverse events, will be recognized immediately. Therefore, corrections can be made quickly.

Any serious/unexpected adverse events will be immediately reported to the IRB. An investigation will be carried out immediately. If the adverse events are observed, then the caffeine dosage will be reduced. If similar adverse events keep being caused by the study drug, we will stop the study unless we can find a solution to avoid these events.

We will review each session every day so that we can deal with any adverse events in a timely manner. We will have follow up phone calls to the subject to make sure that no adverse effects occur away from UC.

The team that evaluates subject safety will include three anesthesiologists (Apfelbaum, Wong and Xie) all of whom are faculty at UC and all are experienced anesthesiologists. Data quality will be monitored by the three anesthesiologists and by Fox, the PI.

Our protocol is designed to be conservative in order to prevent any unexpected or adverse effects. The concentrations anesthetics and caffeine used in this study are safe based on the data in the literature. Even so, unexpected events can occur. For example, seizure or arrhythmia will be reported immediately to the IRB. Similarly a fall in BP that triggers a response (see above), but which does not respond quickly to treatment will be reported. If any of these events, seizure or arrhythmia or prolonged depression of BP, occurs in more than 1 subject the study will be stopped.

9. DATA HANDLING AND RECORD KEEPING

9.1 Data Management Responsibilities

Fox will carry out data handling and record-keeping. All data will be stored in a password-protected computer in a locked room in the Fox office. A backup of the data will be kept on a password-protected thumbdrive. Another backup is kept on a second password-protected computer in the Fox office. (Note that password-protected means that all data is kept in an encrypted form in a military grade 256 bit AES encrypted container). All paperwork will be kept in a locked file cabinet in the Fox lab. There will be no grouping of data until the study is complete and unblinding occurs. The IDS group will

prepare the injectable solution for use in each volunteer in a randomized manner. Members of our study group will not know what is being injected. At the end of each section of the trial the IDS group will provide to us the information required to unblind the trial. At this time data will be grouped into a “control” and “caffeine” group. The data will be analyzed with a t-test and then graphed, using Origin software. This data will then be sent off for publication.

Clinical data related to the anesthesia procedure will be stored in our hospital’s hipaa-compliant Epic database.

9.2 Data Capturing Methods

The data from the trial will be stored in a computer file. The file will be updated following each volunteer session. Analysis will not take place until all the the trial is complete.

9.3 Source Documents

All trial data (findings, pharmacy information, vital signs, lab notes, clinical notes etc) will be entered by Fox into a computer. This data will be kept on a password protected computer in a locked room in the Fox office. The data will be encrypted. Only members of the research team (Fox, Xie, Apfelbaum and Fong) will have access to the data. The data from the trial will be kept indefinitely. After the study is complete all identifiers to the subjects will be eliminated, as they are not required. Any documents i.e. signed, consent forms, original lab notes, clinical notes, will be kept in a locked file cabinet in the same locked room in the Fox lab. Only Fox will have a key.

Please note that all the clinical data generated during the procedure itself (BP, heart rate, respiratory rate etc.) will be stored in The University of Chicago’s hipaa compliant, Epic database.

9.4 Records Retention

It is the investigator’s responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

Institutional policy requires that all study records be kept for a minimum of seven years.

9.5 Confidentiality

All study data will be kept on a password protected computer in a locked room in the Fox lab. The data will be encrypted. Only members of the research team (Fox, Xie, Apfelbaum and Fong) will have access to the data. The data from the trial will be kept indefinitely. After the study is complete all identifiers to the subjects will be eliminated,

as they are not required. Any documents i.e. signed consent forms, will be kept in a locked file cabinet in the same locked room in the Fox office.

10. STUDY MONITORING, AUDITING AND INSPECTING

10.1 Study Monitoring Plan

There are two aspects that will be tightly monitored. Subject safety will be monitored by Dr. Xie while data quality will be monitored by Drs. Fox and Xie. Dr. Xie is an experienced clinician well versed in patient care as well as in data collection/ analysis. Dr. Fox has been running a lab at UC for over 25 years, collecting different types of scientific data.

After each session with a subject our research team will meet to go over any subject safety issues. Any serious issues will be immediately reported to the IRB. At the same session the team will examine the data collected to ensure that our protocols were carefully followed. This will be done in a blinded fashion.

Our plan is to publish our results as soon as humanly possible. The referees provided by the journal as well as the editor will provide an objective outside review of the data.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the OCR, IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

11. FINANCIAL CONSIDERATIONS

The single largest cost in the study is the time of the three clinicians. For this study, lab days, kindly donated by their department (Anesthesia and Critical Care) will be used for some subject sessions. Other will be paid for by the NIH grant that supports this study. Equipment for the study will be loaned as well (Anesthesia and Critical Care). The other costs (payments to subjects, car service, supplies, drugs, IDS costs, light refreshments) are to be paid by a NIH grant.

12. ETHICAL CONSIDERATIONS

12.1 Institutional Review Board (IRB) approval

The investigator-sponsor will obtain, from the University of Chicago Institutional Review Board (IRB), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e., directed at potential research subjects) for study recruitment.

The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the investigator-sponsor will promptly notify the University of Chicago IRB of the deviation.

The University of Chicago IRB operates in compliance with FDA regulations at [21 CFR Parts 50](#) and [21 CFR 56](#), and in conformance with applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (GCP).

In the event that the University of Chicago IRB requires, as a condition of approval, substantial changes to a clinical protocol submitted under an FDA-accepted IND application, or in the event of an investigator-sponsor's decision to modify the previously accepted clinical protocol:

- for a Pilot clinical study: The investigator-sponsor will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change to the Phase 1 clinical protocol that significantly affects the safety of the subjects. For changes that do not affect critical safety assessments, the revisions to the clinical protocol will be addressed in the Annual Report to the IND.

12.2 Ethical and Scientific Conduct of the Clinical Study

The clinical study will be conducted in accordance with the current IRB-approved clinical protocol; ICH Guidelines on GCP; and relevant policies, requirements, and regulations of the University of Chicago IRB, University of Chicago and UCMC, State of Illinois, and applicable federal agencies.

12.3 Subject Informed Consent

The sponsor-investigator will make certain that an appropriate informed consent process is in place to ensure that potential research subjects, or their authorized representatives, are fully informed about the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research subjects. The investigator-sponsor, or a sub-investigator(s) designated by the investigator-sponsor, will obtain the written, signed informed consent of each subject, or the subject's authorized representative, prior to performing any study-specific procedures on the subject. The date and time that the subject, or the subject's authorized representative, signs the informed consent form and a narrative of the issues discussed during the informed consent process will be documented in the subject's case history. The investigator-sponsor will retain the original copy of the signed informed consent form, and a copy will be provided to the subject, or to the subject's authorized representative.

The investigator-sponsor will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled subjects are adequately addressed and that the subjects are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial

changes to the clinical study or the risk-to-benefit ratio of study participation, the investigator-sponsor will obtain the informed consent of enrolled subjects for continued participation in the clinical study

13. CONFLICT OF INTEREST

The investigators responsible for this study does not have any Conflict of Interests with regard to this Clinical Study.

14. PUBLICATION PLAN

Data from the trial will be published as soon as the trial is complete regardless of whether the results confirm our working hypothesis. All subject identifiers will be removed as they are not required for publication. In addition to the primary endpoints (eye, opening and response to command) and secondary endpoints (psychomotor results), we will also publish vital sign information in the presence and absence of caffeine.

15. REFERENCES

To aid the IRB review I have included brief descriptions of a few references. References 1 and 2 were studies where caffeine was given to anesthetized patients.

3-9 were all carried out at The University of Chicago in healthy human volunteers and are similar to the studies we propose. Similar studies from other institutions are included below from elsewhere in the USA or other countries (references 10-18). Note that our literature search yielded thousands of similar studies. At the end are included a small number of crossover studies where healthy human volunteers were given drugs that carried more than minimal risk (19-23). The final reference (number 24) is from a review that covers 3323 healthy volunteers used in a variety of phase 1 trials to test new experimental drugs. The review covers a variety of single-dose studies as well as crossover studies where volunteers were exposed to drugs multiple times. Afterward are a compilation of all the references used to prepare this application, in alphabetical order.

1) Khalil, S. N., Maposa, D., Ghelber, O., Rabb, M. F., Matuszczak, M., Ganesan, B. A., Tabrizi, H. K., & Chuang, A. Z. (2008), Caffeine in children with obstructive sleep apnea. *Middle East J Anesthesiol* 19(4), 885-99.

In this clinical trial in anesthetized children, caffeine was found to be safe. The trial was carried out in order to determine whether caffeine reduced the number of children who experience adverse post-extubation respiratory events – caffeine helped significantly.

2) Steinbrook, R. A., Garfield, F., Batista, S. H., & Urman, R. D. (2013), Caffeine for the prevention of postoperative nausea and vomiting. *J Anaesthesiol Clin Pharmacol* 29(4), 526-9.

This clinical trial was carried out to determine whether caffeine ameliorated post anesthesia nausea and vomiting. Patients were given 500 mg caffeine

during anesthesia. This combination, anesthesia and caffeine, was found to be safe. Note that 500 mg in that trial is what we propose for our trial. Unfortunately, the caffeine did not help with the nausea or vomiting.

- 3) Galinkin JL, Janiszewski D, Young CJ, Klapfta JM, Klock PA, Coalson DW, Apfelbaum JL & Zacny JP. (1997). Subjective, psychomotor, cognitive, and analgesic effects of subanesthetic concentrations of sevoflurane and nitrous oxide. *Anesthesiology* 87, 1082-1088.

In this study, a cohort of 12 healthy human volunteers was exposed multiple rounds of sevoflurane or nitrous oxide anesthesia. This was done to test efficacy of sevoflurane. Sevoflurane is a volatile anesthetic similar to isoflurane. This study was carried out at The University of Chicago.

- 4) Young CJ, Coalson D, Klock PA, Klapfta JM, Goldsher G, Apfelbaum JL, Zacny JP. Analgesic and psychomotor effects of thiopental at subanesthetic concentrations in human volunteers. *Acta Anaesthesiol Scand.* 1997 Aug;41(7):903-10.

In this study, a cohort of 12 healthy human volunteers was exposed multiple rounds of thiopental to test effects on pain. This study was carried out at The University of Chicago.

- 5) Zacny JP, Janiszewski D, Sadeghi P, Black ML. Reinforcing, subjective, and psychomotor effects of sevoflurane and nitrous oxide in moderate-drinking healthy volunteers. *Addiction.* 1999 Dec;94(12):1817-28.

A cohort of 14 healthy volunteers were exposed to sevoflurane and nitrous oxide. This study was carried out at The University of Chicago.

- 6) Zacny JP, Paice JA, Coalson DW. Separate and combined psychopharmacological effects of alprazolam and oxycodone in healthy volunteers. *Drug Alcohol Depend.* 2012 Aug 1;124(3):274-82. doi: 10.1016/j.drugalcdep.2012.01.023. Epub 2012 Feb 25.

A cohort of 20 healthy volunteers were given restricted drugs. This study was carried out at The University of Chicago.

7) Zacny JP, Paice JA, Coalson DW. Characterizing the subjective and psychomotor effects of carisoprodol in healthy volunteers. *Pharmacol Biochem Behav.* 2011 Nov;100(1):138-43. doi: 10.1016/j.pbb.2011.08.011. Epub 2011 Aug 23.

A cohort of 15 healthy volunteers were given carisoprodol. This study was carried out at The University of Chicago.

8) Weafer J, de Wit H. Inattention, impulsive action, and subjective response to d-amphetamine. *Drug Alcohol Depend.* 2013 Jun 18. pii: S0376-8716(13)00196-8. doi: 10.1016/j.drugalcdep.2013.05.021. [Epub ahead of print]

A cohort of 165 healthy adults were exposed to d-amphetamine. This study was carried out at The University of Chicago.

9) Baggott MJ, Childs E, Hart AB, de Bruin E, Palmer AA, Wilkinson JE, de Wit H. Psychopharmacology of theobromine in healthy volunteers. *Psychopharmacology (Berl).* 2013 Jul;228(1):109-18. doi: 10.1007/s00213-013-3021-0. Epub 2013 Feb 19.

A cohort of 80 healthy volunteers were exposed to a variety of theobromine concentrations. In addition, they were exposed to caffeine. Theobromine is a drug that is similar to caffeine. This study was carried out at The University of Chicago.

10) Holaday, Duncan A., Smith, Frederick R., *Anesthesiology.* 1981 Feb;54(2):100-6. Clinical Characteristics and Biotransformation of Sevoflurane in Healthy Human Volunteers.

The anesthetic sevoflurane was tested in healthy human volunteers. Volunteers were anesthetized for an hour. This study was carried out at the University of Miami.

11) Saxena N, Muthukumaraswamy SD, Diukova A, Singh K, Hall J, Wise R. *PLoS One.* 2013;8(3):e57685. doi: 10.1371/journal.pone.0057685. Epub 2013 Mar 6. Enhanced stimulus-induced gamma activity in humans during propofol-induced sedation.

In this study, the general anesthetic propofol was administered to 15 healthy human volunteers while their brain waves were monitored. This study was carried out at Cardiff University, Great Britain.

12) Russo SG, Cremer S, Eich C, Jipp M, Cohnen J, Strack M, Quintel M, Mohr A. *Br J Anaesth.* 2012 Dec;109(6):996-1004. doi: 10.1093/bja/aes314. Epub 2012 Sep 25.

Magnetic resonance imaging study of the in vivo position of the extraglottic airway devices i-gel™ and LMA-Supreme™ in anaesthetized human volunteers.

Magnetic resonance study in a cohort of 12 healthy human volunteers that were anesthetized as part of the study. This study was carried out at Gottingen University, Germany.

13) Långsjö JW, Alkire MT, Kaskinoro K, Hayama H, Maksimow A, Kaisti KK, Aalto S, Aantaa R, Jääskeläinen SK, Revonsuo A, Scheinin H. J Neurosci. 2012 Apr 4;32(14):4935-43. doi: 10.1523/JNEUROSCI.4962-11.2012. Returning from oblivion: imaging the neural core of consciousness.

A cohort of 20 healthy volunteers were anesthetized with a variety of anesthetics including propofol while their brain activity was monitored. This study was carried out at University of Turku, Finland.

14) Neukirchen M, Hipp J, Schaefer MS, Brandenburger T, Bauer I, Winterhalter M, Kienbaum P, Werdehausen R. Br J Anaesth. 2012 Dec;109(6):887-96. doi: 10.1093/bja/aes303. Epub 2012 Sep 3. Cardiovascular stability and unchanged muscle sympathetic activity during xenon anaesthesia: role of norepinephrine uptake inhibition.

A cohort of 8 healthy volunteers were anesthetized with Xenon while physiological measurements were obtained. This study was carried out at Heinriche-Heine University, Germany.

15) Schlünzen L, Juul N, Hansen KV, Cold GE. Acta Anaesthesiol Scand. 2012 Feb;56(2):248-55. doi: 10.1111/j.1399-6576.2011.02561.x. Epub 2011 Oct 19. Regional cerebral blood flow and glucose metabolism during propofol anaesthesia in healthy subjects studied with positron emission tomography.

A cohort of 8 healthy volunteers were anesthetized with propofol while brain activity measurements were obtained. This study was carried out at Aarhus University, Denmark.

16) Hines CS, Fujita M, Zoghbi SS, Kim JS, Quezado Z, Herscovitch P, Miao N, Ferraris Araneta MD, Morse C, Pike VW, Labovsky J, Innis RB.
J Nucl Med. 2013 Jan;54(1):64-9. doi: 10.2967/jnumed.112.106872. Epub 2012 Nov 12.

Propofol decreases in vivo binding of 11C-PBR28 to translocator protein (18 kDa) in the human brain.

A cohort of 10 healthy human volunteers were anesthetized with propofol and then had PET scans. This study was carried out at The National Institutes of Health, USA.

17) Alkire MT, Gruver R, Miller J, McReynolds JR, Hahn EL, Cahill L. Proc Natl Acad Sci U S A. 2008 Feb 5;105(5):1722-7. doi: 10.1073/pnas.0711651105. Epub 2008 Jan 28. Neuroimaging analysis of an anesthetic gas that blocks human emotional memory.

A cohort of 39 volunteers were exposed to the general anesthetic sevoflurane. Memory tests were given. This study was carried out at The University of California at Irvine.

18) Shiratsuchi H, Kouatli Y, Yu GX, Marsh HM, Basson MD. Am J Physiol Cell Physiol. 2009 Jun;296(6):C1400-10. doi: 10.1152/ajpcell.00345.2008. Epub 2009 Apr 8. Propofol inhibits pressure-stimulated macrophage phagocytosis via the GABA_A receptor and dysregulation of p130cas phosphorylation.

In this study volunteers were anesthetized with propofol; blood was drawn 30 minutes after anesthesia was started and measurements were made. This study was carried out at Wayne State University, Detroit.

19) Hull JH, Findlay JW, Rogers JF, Welch RM, Butz RF, Bustrack JA. Drug Intell Clin Pharm. 1982 Nov;16(11):849-54. An evaluation of the effects of smoking on codeine pharmacokinetics and bioavailability in normal human volunteers.

In this study 12 healthy volunteers were given codeine two times at one week intervals.

20) Donny EC, Bigelow GE, Walsh SL. Drug Alcohol Depend. 2003 Apr 1;69(3):289-301. Choosing to take cocaine in the human laboratory: effects of cocaine dose, inter-choice interval, and magnitude of alternative reinforcement.

In this study human volunteers (in this case cocaine-dependent volunteers) were given multiple dosages of cocaine.

21) Liu X, Chen D, Zhang R. Drug Dev Ind Pharm. 2003 Aug;29(7):813-9. Evaluation of monolithic osmotic tablet system for nifedipine delivery in vitro and in vivo.

In this study 11 healthy human volunteers were given nifedipine, a calcium channel blocker, multiple times in a new delivery system.

22) Hawkey CJ, Jones JJ, Atherton CT, Skelly MM, Bebb JR, Fagerholm U, Jonzon B, Karlsson P, Bjarnason IT. Gut. 2003 Nov;52(11):1537-42. Gastrointestinal safety of AZD3582, a cyclooxygenase inhibiting nitric oxide donator: proof of concept study in humans.

In this study 31 human volunteers were exposed to a variety of drugs at different concentrations including a new experimental drug. This is not an uncommon way to test new drugs.

23) la Porte C, Verweij-van Wissen C, van Ewijk N, Aarnoutse R, Koopmans P, Reiss P, Stek M Jr, Hekster Y, Burger D. J Clin Pharmacol. 2005 Feb;45(2):211-8. Pharmacokinetic interaction study of indinavir/ritonavir and the enteric-coated capsule formulation of didanosine in healthy volunteers.

In this study a variety of drugs used to treat HIV were given to 8 healthy volunteers. Each volunteer received multiple drugs.

24) Buoen C, Bjerrum OJ & Thomsen MS. (2005). How first-time-in-human studies are being performed: a survey of phase I dose-escalation trials in healthy volunteers published between 1995 and 2004. J Clin Pharmacol 45, 1123-1136.

This review covers a subset of studies carried out in the 1995-2004 time frame. It examines data from 3323 healthy human volunteers in multiple studies where the subjects were given escalating doses of drugs. Some studies used single-dose methodologies while other studies used crossover studies where subjects were given multiple doses of drugs.

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16. APPENDIXES

A. INFORMED CONSENT FORM

B. SCHEDULE OF EVENTS (SCHEMA)

C. DATA SAFETY AND MONITORING PLAN

Our research group (Fox and three anesthesiologists) will monitor patient vital signs and any adverse results immediately following each session. Any problems will immediately be reported to the IRB. Our research team will also track data quality.