PHARMACOKINETIC-PHARMACODYNAMIC MODELING OF ARMODAFINIL: EFFECTS ON ELECTROENCEPHALOGRAM AND NEUROCOGNITION OF SLEEP DEPRIVED ADULTS

By

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To my Grandparents
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Subjective measures are extensively used to assess drug effects on the central nervous system. Electroencephalogram (EEG) offers the possibility of an objective and continuous measure which also does not require active cooperation of the subjects. The aim of this study was to evaluate EEG as a measure of wakefulness-promoting effect.

We performed a double-blind, placebo-controlled, cross-over study consisting of three 48-hour sessions (placebo, 150 mg and 250 mg armodafinil oral tablets). In each session, the subjects (n=6) underwent 36-hour sleep deprivation period during which blood sampling, EEG recording and neurobehavioral assessments were simultaneously performed. The effects of sleep deprivation were significantly mitigated by armodafinil when compared to placebo. Shortly, (1) armodafinil increased the performance of the subjects during the behavioral tasks (i.e., psychomotor vigilance task, PVT, and go/no-go association task, GNAT, as measured by the mean reciprocal reaction time and error rates); (2) armodafinil increased the event-related brain activity in the central region of the brain during the execution of the both behavioral tasks, PVT and GNAT; (3) armodafinil mitigated the increase in the EEG delta power over the frontal, temporal and
occipital region of the brain. Using a pharmacokinetic-pharmacodynamic modeling approach, the armodafinil effect on the behavioral measures and event-related brain activity was best described by an excitatory (Emax) drug effect model. On the other hand, armodafinil effect on the EEG delta power was best described by an inhibitory (Imax) drug effect model. In all the aforementioned models, drug effect was linked to the hypothetical drug concentrations in the site of action (i.e., effect compartment approach). A statistically significant correlation between the population predictions for the drug effect on the mean 1/RT of the PVT (i.e., a well-established measure of alertness) and EEG-based measures (brain activity and delta power) suggests EEG as a potential biomarker of armodafinil effect building foundation for further research on this topic.
Study Rationale

The CTSI funding opportunity has led to the consolidation of a multi-disciplinary research team involving investigators from the College of Pharmacy, College of Engineering and College of Medicine. As a result of this collaboration, the present research addresses a genuine need in the pharmaceutical area through integration of clinical pharmacology and biomedical engineering-related concepts and approaches.

A therapeutic effect is obtained when the minimum effective concentration of a drug reaches the site of action and binds to its target. While the drug-target interaction constitutes the pharmacodynamics (PD), the amount of drug available in the biophase will be determined by its pharmacokinetic (PK) properties. Although drug concentrations are easily measured and sometimes used to guide treatment, they are not always directly correlated with drug effect. The concentration-effect relationship may vary between subjects, and even within subjects under the influence of pathophysiological variables. Defining how concentration and effect are correlated and which parameters affect this relationship is the objective of the PK-PD modeling (Figure 1-1). It allows an accurate assessment of the clinical significance of PK and/or PD changes due to drug-drug interactions, disease states, age, gender, and other genetic and environmental factors. Moreover, a reproducible, precise and accurate pharmacodynamic measure has the ability to enhance drug development, by optimization of study design, it can reduce cost and time of development.

While assessing the pharmacokinetic component is relatively straightforward, measuring pharmacological effect may be cumbersome. The latter is particularly true for
central nervous system (CNS)-acting drugs.\textsuperscript{3} Most methods for measuring central drug effects rely on subjective assessments.\textsuperscript{5-7} Self-rated scales require individuals to rate their perceived intensity of some outcome measure, such as sleep quality after treatment with a sedative drug.\textsuperscript{8} This limitation presents an opportunity to investigate more objective pharmacodynamics measures to describe drug response. 

Electroencephalogram (EEG) brings the possibility of an objective, nearly-continuous and non-invasive measure of central effects.\textsuperscript{3,9-11} There are a few drug classes for which EEG has been systematically investigated with the prime examples being benzodiazepines,\textsuperscript{12} anesthetics and opioids.\textsuperscript{9} The goal of the present research was to evaluate EEG-based approaches as measures of stimulant effect. Armodafinil (Nuvigil\textsuperscript{®}), a novel non-amphetamine stimulant medication, was selected due to its low potential for abuse.\textsuperscript{13} In addition, its closely-related drug modafinil has demonstrated effects detectable with EEG.\textsuperscript{11,14} The main hypothesis is that armodafinil-related changes on EEG are correlated with a well-established neurocognitive measure of alertness (i.e., psychomotor vigilance task) and drug concentrations. Such a wide range of measures may provide insight of the clinical meaning of changes on EEG. This hypothesis can be stratified and investigated as below.

**Hypothesis and Specific Aims**

**Hypothesis 1.** Armodafinil effect on the neurocognitive performance of sleep deprived healthy adults is correlated with its concentrations.

**Specific aim 1.** To describe the relationship between neurocognitive performance and armodafinil concentrations. We (a) developed and validate an analytical method to measure armodafinil concentrations in human plasma; (b) determined the pharmacokinetics after single oral dose administration of armodafinil
150 mg and 250 mg in sleep deprived healthy adults; (c) determined and compared the individual performance of the subjects by two neurocognitive tests (psychomotor vigilance task, PVT, and go/no-go association task, GNAT) after armodafinil and placebo administration; (d) established a non-linear mixed effects PK-PD model to describe the relationship between armodafinil effect on neurocognition and its concentration-time profile.

**Hypothesis 2.** Armodafinil effect on the EEG power spectrum and event-related brain activity of sleep deprived healthy adults are correlated with its concentrations.

**Specific aim 2.** To describe the relationships between ERPs and EEG frequency-specific power and armodafinil concentrations. We will (a) analyze and compare the ERP produced during the execution of two neurocognitive tests (PVT and GNAT) after armodafinil and placebo administration; (b) establish a non-linear mixed effects PK-PD model to describe the relationship between armodafinil effect on event-related brain activity and its concentration over time; (c) perform a power spectral analysis of eyes-closed and eyes-open EEG recording after armodafinil and placebo administration; (d) establish a non-linear mixed effects PK-PD model to describe the relationship between armodafinil effect on specific frequency ranges and its concentration-time profile.

**Hypothesis 3.** Armodafinil-related EEG changes are correlated with its effect on alertness of sleep deprived healthy adults.

**Specific aim 3.** To describe the relationship between drug-related EEG changes and behavioral alertness. We will (a) describe the relationship between armodafinil
effect on EEG power spectrum and alertness (i.e., PVT); (b) describe the relationship between armodafinil effect on event-related brain activity and alertness (i.e., PVT).

Background

Excessive Sleepiness

Current scenario

Excessive daytime sleepiness (EDS) is a common problem among patients visiting sleep clinics. It is defined as sleepiness occurring in situations when an individual would typically be expected to be alert. It has been estimated by epidemiologic studies that EDS affects up to 13% of the population. The four major causes of EDS are: (1) CNS pathologic disorders such as narcolepsy and idiopathic hypersomnia; (2) qualitative or quantitative sleep deficiencies such as sleep apnea and sleep deprivation; (3) circadian rhythm misalignments such as jet lag or shift work; and (4) medication side effects. Chronic excessive sleepiness is related to substantial morbidity such as reduced quality of life, impaired work or academic performance and psychosocial distress. It has also been associated to an increased risk of accidents with a consequent impact on public health. The National Highway Traffic Safety Administration reported that sleepiness is the main leading factor in about 100,000 of the annual police-reported crashes in the United States. Approximately 4% of all fatal motor vehicle accidents (i.e., 1,500 deaths) occurring every year has been associated to drowsy driving. Moreover, it has been suggested that sleep deprivation played an important role in some of the major disasters of the last decades. Those include the nuclear accident at the Three Mile Island (1979), the nuclear meltdown at Chernobyl (1986), the explosion of the space shuttle Challenger (1986) and the grounding of the Exxon Valdez oil tanker (1989).
Management strategies and armodafinil

Management strategies for EDS depend on the etiology of the disorder. In respect to the pharmacotherapy, EDS patients are normally treated with amphetamine-like stimulants. The most frequently utilized ones are dexamphetamine, methamphetamine, methylphenidate, mazindol and pemoline.\textsuperscript{21} Even though approved to alleviate EDS, these agents are associated with potential for abuse, adverse cardiovascular and central effects.\textsuperscript{22}

Modafinil (Provigil\textsuperscript{®}), a more recent non-amphetamine wakefulness-promoting agent, has demonstrated less potential for abuse.\textsuperscript{23} It is a racemic mixture of R-modafinil and S-modafinil approved to promote wakefulness in individuals with excessive sleepiness related to obstructive sleep apnea/hypopnea syndrome (OSAHS), narcolepsy and shift work sleep disorder (SWSD).

Armodafinil (Nuvigil\textsuperscript{®}), approved by the U.S. Food and Drug Administration agency (FDA) in 2007, is the R-enantiomer of modafinil. While approved for the same indications, armodafinil has an elimination half-life time that is three to four times longer than that of the S-enantiomer.\textsuperscript{24} Armodafinil, hence, can sustain higher concentrations late in the day when compared to the racemate.\textsuperscript{25} The consequence of this has been demonstrated in studies with OSAHS\textsuperscript{26} and narcoleptic\textsuperscript{27} patients where armodafinil sustained wakefulness throughout the day. Armodafinil exact mechanism of action remains unknown.\textsuperscript{28}

Although armodafinil is a relatively new drug, its analogous compound, modafinil, has received extensive attention in the literature. Studies on modafinil are considered to shed light on armodafinil pharmacology given that both enantiomers demonstrated
similar pharmacological profile in pre-clinical and in vitro studies. Modafinil potential mechanisms of action have been extensively presented in a systematic review: its effect has been related with enhanced adrenergic, glutamatergic, histaminergic and hypocretin activity and reduced \( \gamma \)-aminobutyric acid (GABA) activity in certain areas of the brain. There is evidence supporting that modafinil has a distinct mechanism of action when compared to the conventional amphetamine-like stimulants. Interestingly, modafinil produces neuronal activation that is more restricted to wakefulness areas (e.g., hypothalamus) as opposed to amphetamine-like compounds which have a more widespread neuronal activation. Unlike conventional stimulants, modafinil has been shown to exclusively reduce the release of GABA and only weakly enhance the release of dopamine in the nucleus accumbens of rats. Moreover, it has been suggested that modafinil modulates the hypocretin system by causing activation of hypocretin-secreting neurons. Hypocretin or orexin is a hypothalamic peptide that plays a role in the regulation of sleep and wakefulness. This peptide seems to be able to stimulate glutaminergic and histaminergic systems leading to arousal. Therefore, modafinil effect on monoamine systems seems to be less important than modulation of GABA, glutamate, hypocretin and histamine. Indeed, compared to amphetamines-like compounds, modafinil does not produce feelings associated to drug abuse and does not affect the sleep style.

Several potential neurological and psychiatric indications have been investigated for modafinil and are likely to be extended for armodafinil. A systematic review points out that compelling evidence exists for the use of modafinil in attention-deficit and
hyperactive disorder, post-anesthetic sedation, cocaine withdrawal and as an adjunct in major depressive disorder.\textsuperscript{29}

**Armodafinil pharmacokinetics**

Armodafinil pharmacokinetics has been previously described in a pooled analysis of three randomized clinical trials.\textsuperscript{34} Armodafinil exhibited linear pharmacokinetics in the dose range of 50 to 400 mg. The rate but not the extent of absorption was affected by food intake. Maximum plasma concentrations were reached at approximately 2.3 and 6.0 hours post-dose in fasted and fed state (after consumption of a fatty meal), respectively. After the maximum plasma concentration, armodafinil concentrations declined in a monoexponential manner having a half-life time of approximately 15 hours. Armodafinil lipophilic nature precluded the intravenous administration of the drug and, hence, the determination of its absolute oral bioavailability.\textsuperscript{13} After the multiple-dose administration, steady-state seemed to be reached by the 7\textsuperscript{th} day post-dosing and the systemic exposure was 1.8 times when compared to the single-dose.\textsuperscript{34}

Armodafinil mass balance data is not available. Modafinil is metabolized by the liver with less than 10% of the parent compound being excreted in the urine.\textsuperscript{28} Armodafinil suffers hydrolytic deamidation, S-oxidation and aromatic ring hydroxylation with subsequent glucuronide conjugation of the hydroxylated products.\textsuperscript{28} Amide hydrolysis is the most noticeable metabolic pathway followed by sulfone formation through cytochrome P450 (CYP) 3A4/5.\textsuperscript{28} In a dedicated drug-drug interaction study in healthy subjects, armodafinil did not induce CYP1A2 but was a moderate CYP3A4 inducer and a moderate CYP2C19 inhibitor.\textsuperscript{35} Although armodafinil was well tolerated when co-administered with midazolam and omeprazole,\textsuperscript{35} one cannot exclude the need
to perform dose adjustment of other co-administered CYP3A4 and CYP2C19 substrates.

**Pharmacodynamic Measures of Central Drug Effects**

CNS-acting drugs are an important part of the therapeutic arsenal constituting more than 14% of the dispensed prescriptions in the U.S. market. While therapeutic drug monitoring is a common practice for many drugs in this class, assessment solely of concentration levels may be of limited importance. Drug concentration and effect may be correlated in a complex and/or indirect manner. The concentration-effect relationship may vary between subjects, and within subjects due to the influence of pathophysiological or external factors. As a consequence, it becomes important to describe the PK-PD relationship to account for the pharmacodynamic component of the variability in drug response. A pharmacodynamic measure can be classified as biomarker, surrogate endpoint or clinical outcome. Although clinical outcomes are considered the primary assessment of effectiveness and safety, biomarkers normally occur earlier and can be determined in a more robust manner. Moreover, a reproducible, precise and accurate pharmacodynamic measure has the ability to enhance PK-PD modeling and consequently expedite drug development.

**Psychometric tests.** Measuring pharmacological effect is still cumbersome for many CNS-acting drugs. Central drug effects are commonly measured using psychometric tests. Although they might focus on assessment of sensory, cognitive or motor functions, it is the integration of sensory and motor systems through the cognitive processing what is measured. Psychometric tests can be more or less subjective and robust. They normally require active cooperation of the individuals. In addition, they will likely produce correlated measures when learning effects occur with repeated testing.
**Electroencephalogram-based measures**

Unlike psychometric tests, EEG is considered to meet almost all criteria for an ideal pharmacodynamic measure. Learning effects in EEG-based measures have not been reported; they yield an objective, (nearly-)continuous, sensitive and non-invasive assessment of central effects without requiring subjects’ active collaboration.\(^3,9,39\).

**Source of electroencephalogram activity.** The electroencephalogram converts small electrical signals arising from neuronal synaptic activity into waveforms.\(^40\) Neuronal communication occur through synapses: (a) an action potential leads the presynaptic terminal to release neurotransmitter; (b) the neurotransmitter binds to the membrane post-synaptic terminal leading to a change in the membrane potential; (c) a sequence of change in the membrane potential are summed in the body of the target neuron resulting in a post-synaptic potential; (d) as a consequence, the ion channels open resulting in an action potential which, in turn, will lead to the same sequence of events previously described.\(^40\) The post-synaptic potentials from these cells are summed together in the extracellular fluid around them and conducted in the following sequence: (a) the volume of the brain; (b) cerebrospinal fluid; (c) blood; (d) bone; (e) muscle; (f) skin comprising the head; (g) scalp electrodes; (h) EEG amplifier.

**Electroencephalogram frequencies.** The clinical utility of a typical scalp EEG resides in four major frequency ranges: (a) beta (over 12 or 13/sec or Hz), usually anterior; (b) alpha (8 – 12 or 13 Hz), usually posterior; (c) theta (4 – 8 Hz), usually widespread; (d) delta (under 4 Hz), associate to drowsiness/sleep.\(^40,41\) Another less specific nomenclature is calling waves under 8 Hz as slow waves and waves over 13 Hz as fast waves.\(^41\)
The alpha rhythm is not only characterized by its frequency, but also by its distribution and reactivity; as a normal consequence, alpha rhythm and alpha frequency are not synonymous.\textsuperscript{41} This rhythm occurs during wakefulness in the posterior part of the brain showing high voltage in the occipital area; although the amplitude can vary, it should be mostly below 50 $\mu$V.\textsuperscript{42} Alpha rhythm is best seen with eyes closed and in situations of physical relaxation and fairly mental inactivity; indeed, it is characteristically blocked by attention, in particular, visual and mental efforts.\textsuperscript{42} It has been also pointed out that alpha rhythm disappears with drowsiness.\textsuperscript{42}

Conversely, beta activity is more prominent in the frontal area when the subject is allowed to fall sleep; in addition, drugs such as barbiturates and benzodiazepines has been shown to increase beta activity.\textsuperscript{42} The theta and delta rhythm play an important role in the childhood, but are also present in the adult life, and are evident in states of drowsiness and sleep.\textsuperscript{42}

**Quantitative electroencephalogram analysis.** Quantitative EEG can defined as “the transformation of a particular EEG feature into a numerical value”.\textsuperscript{41} The quantification of EEG signal can be done in the time domain (i.e., aperiodic analysis) or in the frequency domain (i.e., fast Fourier analysis).\textsuperscript{9,41,43}

In the aperiodic analysis the quantitation is performed directly from the analysis of the signal represented in the time domain; a wave is defined as an oscillation in voltage between two local minima.\textsuperscript{9} Therefore, the frequency and the amplitude of an EEG signal are calculated wave by wave generating two main variables: (a) the total number of waves per second and (b) total voltage per second for a determined frequency range.\textsuperscript{43}
Fast Fourier transform assumes that the raw EEG waveforms are a summation of waves; as a consequence, this approach decomposes the raw EEG signal into multiple sine waves with different phases, frequencies and amplitudes.\textsuperscript{41} In this sense, the squaring of the Fourier coefficients (i.e., numerical coefficients representing these sine waves) within a specific frequency range generates the power spectrum in a certain time period. The power spectrum contain considerably less data than the raw EEG making the storage of data more efficient.\textsuperscript{41} A drawback is that the original EEG signal cannot be reconstructed from the power spectrum since it does not comprise phase information.\textsuperscript{41} 

**Event-related brain activity.** Event-related brain activity or event-related potential (ERP) is another type of EEG-based measure corresponding to “an increase in the brain activity due to a specific event which can be an internal or external stimulus”.\textsuperscript{44} As defined by Vaughan in 1969, “ERP is proposed to designate the general class of potentials that display stable time relationships to a definable reference event”.\textsuperscript{44} ERPs offer the possibility of a continuous assessment of brain processing between a stimulus and a response, allowing one to determine which stage of the information processing is altered when applying an experimental paradigm (e.g., sensory, cognitive, motor process).\textsuperscript{44} In other words, ERPs are not composed of an overlap of individual cognitive processes.\textsuperscript{44} Conversely, the output of a behavioral measure comes from innumeros individual cognitive processes, and the variation in reaction time and accuracy are difficult to associate with a specific variation of a cognitive process.\textsuperscript{44} Another advantage is that ERPs can be measured in the absence of a behavioral response allowing one to
continuously monitor brain processing and compare between conditions of attended with unattended stimulus.\textsuperscript{44}

ERPs are obtained by recording EEG during the time where the subjects are being exposed to multiple repetitions of a certain stimulus (e.g., visual, somatosensory or auditory stimulus).\textsuperscript{45} In order to filter out background noise, one has to record the exact time in which the stimulus is presented, align the trials based on stimulus appearance and average the trials altogether (i.e., stimulus-locked average).\textsuperscript{44} The assumption is that the ERP waveforms (related to the stimulus appearance) are identical in each trial repetition but the noise occurs randomly and, hence, cancels out during the average.\textsuperscript{44} The result of trials average is a sequence of positive and negative voltage deflections called ERP peaks, waves or components representing the flow of information through the brain (Figure 1-3).\textsuperscript{44} The early peak (P1) is a mandatory sensory response provoked by visual stimulus and highly influenced by physical attributes of the stimulus (i.e., exogenous component); conversely, the late peak (P3) depends completely on the task performed by the subject not being directly influenced by the physical attributes of the stimulus (i.e., endogenous component).\textsuperscript{44} The P300 latency is related to the time required to categorize a stimulus; P300 amplitude, in turn, is related to attention and perception and gets larger as the target probability gets smaller.\textsuperscript{44,46}

Finally, as early components, such as P1, are related to sensory processing they are more noticeable in the occipital region of the brain; later components, such as P3, reflect complex cognitive process (e.g., categorization of stimulus) being more noticeable in the parietal/central/frontal regions of the brain.\textsuperscript{44}
**Pharmaco-electroencephalogram.** Pharmaco-EEG has been defined as “the study of drug effects on the electroencephalograms of animals, healthy volunteers or patients with respect to the possibility of applying the information obtained to drug effects on human brain function and disease”.\(^{47}\) EEG has been systematically studied in a few drug classes. Changes in EEG parameter not only were able to differentiate benzodiazepines with a wide range of intrinsic efficacy and affinity by the GABA-benzodiazepine receptor complex,\(^ {48}\) but also reflected the depth of anesthesia after administration of intravenous anesthetics.\(^ {9}\) Likewise, remifentanil development was expedited based on a well-established EEG parameter\(^ {49,50}\) which showed to be correlated with its analgesic efficacy.\(^ {51}\) The greatest challenging is that the correlation between EEG parameters and drug effect is not clear yet in many situations.\(^ {52}\) It has been claimed that a better understanding of the clinical meaning of EEG parameters would be obtained if an alternative measure of drug effect were performed in combination with EEG recording.\(^ {3}\) Although it has been noticed more than two decades ago, studies investigating correlations between changes in EEG parameters and well-established measures of specific drug effects are still limited.

**Significance and Innovation**

In light of the previous observations, this research consists of a thorough evaluation of the armodafinil effect in sleep deprived healthy adults. In order to minimize the likelihood of empirical correlations, we conducted EEG recording with simultaneous assessment of armodafinil concentrations as well as alternate measures of armodafinil effect. Among the utilized pharmacodynamic measures, PVT is a well-established measure of armodafinil effect on alertness. Investigating the presence of correlation between a specific EEG parameter and alertness will provide insight of the clinical
meaning of changes on EEG. Indeed, a PK-PD model integrating both pharmacodynamic measures would allow us to suggest EEG as a potential biomarker of armodafinil effect. Finally, we believe that a PK-PD modeling approach incorporating an objective CNS effect measure such as EEG can provide a means to optimize the dose regimen under various clinical scenarios (e.g., disease states and concomitant medications). It will be of great value to guide appropriate dose selection in clinical trials pursuing new indications for armodafinil and expedite development of novel compounds with an analogous mechanism of action. This research project demonstrates innovation given that no published studies have applied such a wide range of CNS-based measures in the context of a PK-PD analysis of armodafinil or its analogous compound (i.e., modafinil).
Figure 1-1. Schematic representation of the pharmacokinetic (PK)-pharmacodynamic (PD) modeling approach. Adapted from Derendorf H, Meibohm B. Modeling of pharmacokinetic/pharmacodynamic (PK/PD) relationships: Concepts and perspectives. Pharmaceutical Research 1999;16:176-85 (Page 177, Figure 1).
Figure 1-2. Schematic representation of different event-related potential components. Negative voltage is plotted upward. Adapted from Luck, S. J. What is an ERP. 2012. (Accessed 10/17, 2012, at http://erpinfo.org/what-is-an-erp, Figure 2).
CHAPTER 2
RESEARCH DESIGN AND METHODS

Clinical Study

A double-blind, placebo-controlled, crossover clinical study was performed at the UF Clinical Research Center, Shands Hospital, University of Florida. The study protocol was in accordance with the Declaration of Helsinki and was approved by the local Institutional Review Board. The study consisted of three 48-hour visits separated by washout periods of at least 7 days. In each visit, the subjects underwent a 24-hour sleep deprivation period. After that a single dose of the study drug was administered (placebo, 150 mg, or 250 mg armodafinil oral tablets). The subjects then underwent additional 12 hours of sleep deprivation during which pharmacodynamics and pharmacokinetics assessments were simultaneously carried out.

Six subjects were enrolled following a set of inclusion and exclusion criteria. They were healthy adults, between 18 and 35 years of age, with a normal body weight (BMI between 18.5 and 29.9), and without clinical sleep abnormalities. At the screening, the study physician conducted an Epworth Sleepiness Scale evaluation and discussed with the subject about his or her sleep history. Eligible subjects agreed not to consume alcohol three days prior to and during any study session. In addition, they did not use any other medication one week prior to and during any study session. They were recommended to avoid caffeine for one week prior to a study session, but required to avoid caffeine for 24 hours prior to and during a study session. Females had a negative pregnancy test at screening and admission and were abstinent, sterile, postmenopausal, or practicing an effective method of birth control except steroidal contraceptives. Smokers or subjects who used a nicotine-containing product within 12
months of screening were not eligible. Likewise, subjects with evidence of use of drugs of abuse (including but not limited to barbiturates, opiates, cocaine, cannabinoids, amphetamines, and benzodiazepines) were excluded.

On the day of any study session, a set of pre-specified procedures were performed. On admission, subjects were interviewed about duration of sleep as well as inclusion and exclusion criteria. Urine drug screen (for all subjects) and pregnancy test (for female subjects) were also performed. Subjects had a training session on each neurobehavioral assay before the “resting” baseline session was performed. The latter consisted of simultaneous EEG recording and execution of the neurobehavioral assays. The subjects then underwent 24 hours of sleep deprivation after which a “fatigue” baseline session was performed. The procedures were identical to the resting baseline session. Subsequently, the subjects received the study drug and 10 experimental sessions were performed along the period of 12 hours. The experimental sessions were identical to the baseline session but blood sampling was also performed. Nature and order of the procedures are provided in Figure 2-1. Blood collection was performed through an intravenous catheter placed in the arm vein of the subjects. Blood sampling was conducted prior to drug dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours after dosing. Blood samples were approximately 4 mL in volume and were collected into EDTA-containing collection tubes and centrifuged at 1,300 g for 10 min at 4 °C. Plasma was stored at -80 °C until analysis. Subjects received a standardized and caffeine-free diet during each study visit. In the morning of the drug dosing, subjects were encouraged to have breakfast prior to the administration of the study drug because of the full schedule of data collection.
Protein binding determination was conducted at 2 hours and 12 hours after drug dosing. Once the blood samples were obtained from the subjects, those were processed as previously described. An aliquot of plasma was taken to determine the armodafinil total concentration. The remaining volume was placed in a 37°C water bath for 30 minutes. After this time, 1 mL of the sample was added to a filtrate device (Centrifee; Millipore Corporation, Billerica, MA) which was centrifuged at 1,800 g for 25 minutes at 37°C. The filtrate device underwent previous acclimation in the centrifuge at 37°C. Upon completion of centrifugation, the filter was removed from the device and the ultrafiltrate (<11% of the total plasma volume) was submitted to visual inspection for membrane damage. The ultrafiltrate yielded armodafinil unbound concentration. Simultaneously, samples of armodafinil in aqueous solution (50–4200 ng mL⁻¹) were run to assess armodafinil binding to the membrane of the filtration device (i.e., non-specific binding). The difference of armodafinil total and unbound concentrations corrected by non-specific binding was assumed to be the armodafinil plasma protein binding.

Bioanalytical Analysis

Solvents and Chemicals

Prednisolone acetate and analytical grade ammonium acetate were purchased from Sigma-Aldrich (St. Louis, MO). High performance liquid chromatography grade methanol was purchased from Fisher Scientific (Fair Lawn, NJ). Analytical grade acetic acid was obtained from Fisher Scientific (Fair Lawn, NJ). Double distilled water was obtained in house (Department of Pharmaceutics, University of Florida).

Preparation of Standard Solutions and Quality Control Samples

Two individual weighing of armodafinil was performed to prepare standard stock solutions of 480 μg mL⁻¹ in methanol. One stock solution was used to generate the
working solutions for the calibration curve standards and the other, the quality control (QC) samples. The calibration curve samples were prepared by a sequence of dilution steps: (a) 100 μL of the 480 μg mL⁻¹ stock solution was diluted with 100 μL of methanol-double distilled water (1:1) to yield a 240 μg mL⁻¹ solution of armodafinil; (b) 20 μL of the 240 μg mL⁻¹ solution was diluted with 980 μL of human plasma to yield a final concentration of 4.8 μg mL⁻¹; (c) 250 μL of the 4.8 μg mL⁻¹ armodafinil solution was diluted with 250 μL of human plasma to yield a final concentration of 2.4 μg mL⁻¹. This last step was repeated 7 times in order to obtain armodafinil final concentrations of 1.2, 0.6, 0.3, 0.15, 0.075, 0.0375, 0.01875 μg mL⁻¹. QCs were obtained by three dilution steps: (a) 400 μL of the 4.8 μg mL⁻¹ armodafinil solution was diluted with 80 μL of human plasma to yield a final concentration of 4 μg mL⁻¹; (b) 96 μL of the 4.0 μg mL⁻¹ armodafinil solution was diluted with 384 μL of human plasma to yield a final concentration of 0.8 μg mL⁻¹; (c) 30 μL of the 0.8 μg mL⁻¹ armodafinil solution was diluted with 450 μL of human plasma to yield a final concentration of 0.05 μg mL⁻¹. A standard stock solution of prednisolone (IS) was obtained by dissolving 25 mg of the standard in methanol-acetonitrile (1:1) to a final volume of 25 mL to yield a final concentration of 0.2 μg mL⁻¹.

**Instrumentation**

Analyses were performed on a high performance liquid chromatography system (Perkin Elmer; Waltham, MA). Detection was performed on a triple quadrupole mass spectrometer (API4000; Applied Biosystems, Carlsbad, CA) equipped with an electrospray ionization interface.
**Sample Preparation**

Prior to chromatographic analysis, plasma samples (100 μL each) were deproteinized with 400 μL methanol:acetonitrile (1:1) containing 0.2 μg mL⁻¹ of IS, vortexed for 25 s and centrifuged at 10,000 rpm for 10 minutes. The respective supernatant was separated and 5 μL were injected into the system. IS final concentration into the samples was 0.16 μg mL⁻¹. Calibration curve, QC, and experimental plasma samples were processed in the same manner.

**Chromatographic and Mass Spectrometer Conditions**

The mobile phase used for the chromatographic separation consisted of methanol-water (90:10, v/v) containing 10 mM ammonium acetate buffer. The apparent pH was adjusted to 4 with an aqueous solution of acetic acid. The mobile phase was filtered and isocratically delivered at a flow rate of 1.0 mL min⁻¹ (split ratio 3:4). The analysis was performed utilizing a reversed-phase analytical column (Supelco C18, 5 μm, 25 cm x 4.6 mm; Sigma-Aldrich, St. Louis, MO).

Detection was performed on a triple quadrupole mass spectrometer (API4000; Applied Biosystems, Carlsbad, CA) equipped with an electrospray ionization interface operating in a positive mode (ESI⁺). The spectrometer was programmed in the multiple reaction monitoring mode to allow for the transition of the precursor ion to its respective fragment. The decay of the mass-to-charge ratio (m/z) 274.2 precursor ion to the m/z 167.2 product ion for armodafinil and the decay of the m/z 403.3 precursor ion to the m/z 307.1 product ion for the IS were monitored.
Validation Procedure

Selectivity, linearity, intra- and inter-day precision, accuracy and lower limit of quantification were evaluated through standard calibration curves and quality control sets. Each concentration level of the standard calibration curve and QC samples were analyzed in triplicate in three different days. Different stock solutions were used for each curve and QC samples set. Prednisolone acetate was utilized as an IS to correct for potential loss of the analyte during sample preparation and analysis; in additional it helped correct potential variability in the detection stage.

The linearity of the calibration curves based on the peak area ratio (armodafinil/IS) as a function of nominal concentration was investigated in the concentration range of 18.75–4800 ng mL\(^{-1}\) (weighted linear regression, concentration\(^{-1}\)). Intra- and inter-day accuracy and precision of the bioanalytical method were determined by analyzing nine replicates (three/per day during three days) of individually prepared QC samples for each concentration level (0.05, 0.8 and 4.0 \(\mu\)g mL\(^{-1}\)).

Precision (%) was determined by calculating the relative standard deviation (RSD) of the experimental concentrations; accuracy (%) was calculated as the percentage of the experimental and the nominal sample concentration ratio. The utilized criteria for acceptability of the validation procedure included accuracy from 85 to 115% and precision within 15%. In particular, the lower limit of quantification (LLOQ) should not exceed 20% of precision and not extrapolate the range of 80-120% for accuracy. The extraction efficiency was determined by comparing extracted calibration curve and QC samples with unextracted standards (i.e., recovery of 100%).
Stability Studies

Studies were carried out to evaluate armodafinil stability under the (potential) experimental conditions. First, bench top stability was determined in order to mimic plasma samples handling at room temperature for 4 hours. Second, stability of the processed plasma samples was determined at room temperature for 6 h in the autosampler. Third, armodafinil stability in human plasma was also determined after three freeze and thaw cycles. Stability studies were performed using three replicates of the low and high concentration level of the QC samples.

Behavioral-Based Measures

The subjects executed two neurobehavioral assays: a psychomotor vigilance task (PVT) and a go/no-go association task (GNAT). Both tasks were based on published literature.\textsuperscript{55-57}

In the 10-minute PVT, the subjects were requested to focus on a cross symbol located at the center of a computer screen and respond to the appearance of a visual stimulus. The visual stimulus consisted of a bull’s eye picture displayed at random time intervals (Figure 2-2). The subjects were instructed to respond as quickly as possible by pressing a response button on an external keyboard logic (EXKEY; BeriSoft Cooperation, Frankfurt, Germany). Responses with reaction time (RT) less than 100 milliseconds post-stimulus were not regarded as valid (i.e., false starts or errors of commission). The primary PVT output variables were: (a) the number of false starts (errors of commission) plus number of performance lapses (errors of omission: no response within 500 milliseconds post-stimulus), and (b) the mean reciprocal reaction time (1/RT).\textsuperscript{57} In order to calculate the mean 1/RT, individual RTs were divided by 1,000 before the reciprocal transformation was applied.
In the 10-minute GNAT, the subjects were requested to respond to one form of visual stimuli and hold a response to another form. The visual stimulus consisted of four squares arranged as a (a) right-slanted line, (b) left-slanted line, (c) right slanted diamond, or (d) left-slanted diamond (Figure 2-3). One of the four stimuli was displayed on the screen after a random time delay. The subjects initiated each trial by pressing and holding a button on the keyboard. They were instructed to release the button (go condition) for line, and keep holding it (no-go condition) for diamond disregarding orientation of the stimulus. Go and no-go trials occurred in a random fashion with equal probability in every session. The output variables were: (a) the mean 1/RT for the go trials; (b) the number of failures to respond for the go trials (errors of omission); (c) the number of failures to inhibit a response for the no-go trials (errors of commission); (d) the total number of errors (errors of omission plus errors of commission). The mean 1/RT was determined as aforementioned for the PVT task. All output variables were recorded by a dedicated computer (ERTS; BeriSoft Cooperation, Frankfurt, Germany) and then extracted for analysis (MATLAB; MathWorks Inc, Natick, MA).

**Electroencephalogram-Based Measures**

Scalp-EEG recordings were acquired using a battery-operated 128-channel amplifier with a 1024 Hz sampling rate and a 24-bit converter (Biosemi; Amsterdam, The Netherlands). 128 scalp electrodes were placed using a spandex electrode cap. Four electro-oculogram (EOG) electrodes – below and above the left eye (vertical EOG) and in the left and right side of the eyes (horizontal EOG) – were attached to control for eye artifacts.
EEG recording was quantitatively analyzed by two different approaches: (1) the event-related potential (ERP) analysis was applied to the EEG recording performed during the execution of the behavioral assays (i.e., PVT and GNAT); (2) the EEG power spectral analysis was applied to the 2-minute EEG recording alone (Figure 2-1).

In the ERP analysis, the data were pre-processed and stimulus-locked average was performed (BESA 5.3; BESA GmbH, Gräfelfing, Germany). A low cutoff filter of 0.1 Hz (6 db/oct, forward) and a high cutoff filter of 40 Hz (24 db/oct, zero phase) were applied to the data. An adaptive artifact correction was utilized in order to correct for vertical EOG. The maximum amplitude value of accepted trials was set to 120 μV. The data were epoched from -500 to 1000 ms with zero millisecond corresponding to the stimulus onset. Baseline was defined in the time window of -200 to 0 ms. The original recording was re-referenced and interpolated to the 10-10 average virtual montage with 27 channels (Figure 2-4). The output variables were amplitude, mean amplitude and area of the peaks.

In the EEG power spectral analysis, fast Fourier transform was applied and power estimates were obtained (BESA 5.3; BESA GmbH, Gräfelfing, Germany). The data were epoched in 4-second intervals (0.25 Hz of frequency resolution). The maximum amplitude value of accepted epochs was 120 μV. Likewise, the original recording was re-referenced and interpolated to the 10-10 average virtual montage with 27 channels (Figure 2-4). Power estimates were averaged within the following frequency ranges: δ (1-4 Hz), θ (4-8Hz), α (8-12 Hz) and β (12-25 Hz) (Figure 2-5).
Pharmacokinetic-Pharmacodynamic Modeling

The PK-PD modeling was conducted using a non-linear mixed effects model approach (NONMEM 7.2; ICON Development Solutions, Ellicot City, MD). Based on preliminary studies, we started by utilizing a one-compartment model with first-order elimination and first-order absorption to described the armodafinil pharmacokinetics. Inclusion of a lag-time was tested given the reported effect of meal in the rate of absorption. Using the outcome of the non-linear mixed effects pharmacokinetic model, we performed a sequential fit of the PK-PD data. Specifically, we fixed the population pharmacokinetic parameter estimates and respective variabilities, including the individual pharmacokinetic data, and fit the pharmacodynamic data. The observed effect was defined by two components: time-varying baseline and drug effect. Two options were tested: additive and proportional drug effect relative to baseline. The time-varying baseline function was determined by fitting of the placebo group data. Different functions (e.g., constant, linear, exponential, Weibull, inverse bateman function, polynomial, cosine) were tested in order to best describe the data over time. Likewise, several functions to describe the drug effect were tested (e.g., linear, Emax, sigmoidal Emax, Richards’ model). Diagnostics for time-delay of the drug effect with respect to the concentration were performed. These included data based (e.g., hysteresis plot) and PK-PD model based (e.g., basic goodness-of-fit and conditional weighted residuals, CWRES, vs. derivative of concentration, CDER) approaches.

Overall model selection was guided by objective function values (NONMEM 7.2; ICON Development Solutions, Ellicot City, MD). In addition, visual predictive check and
goodness-of-fit plots were generated using the Perl-coded PsN-Toolkit\textsuperscript{59} and the R-based package Xpose.\textsuperscript{59}

**Statistical Analysis**

Output variables among the three treatment groups were compared using a generalized linear mixed model (SAS 9.3; SAS Institute Inc., Cary, NC). Data that were not normally distributed underwent transformation (i.e., log or reciprocal transformation). The fixed effects of treatment, time, fatigue baseline, prior treatment (carry-over) and treatment*time interaction were investigated. Subject and visit were set as random effects. Different covariance structures were investigated to account for the repeated measures performed on each subject. Tukey’s test was used for post-hoc multiple comparisons.
Figure 2-1. Schematic representation of the study design

**Session tasks:**

i. Blood sampling  
ii. 2 minutes eyes open electroencephalogram recording  
iii. 10 minutes psychomotor vigilance task and simultaneous electroencephalogram recording  
iv. 10 minutes go/no-go task and simultaneous electroencephalogram recording  
v. 2 minutes eyes closed electroencephalogram recording

Nature and order of the tasks were identical for every experimental session.
Figure 2-2. Schematic representation of the psychomotor vigilance task. The figure shows a regular computer screen with the visual stimulus corresponding to a bull’s eye picture.
Figure 2-3. Schematic representation of the go/no-go task. The figure shows a regular computer screen with the visual stimulus consisting of four squares arranged as a right-slanted line, a left-slanted line, a right slanted diamond, or a left-slanted diamond. Adapted from reference Zhang Y, Chen Y, Bressler SL, Ding M. Response preparation and inhibition: the role of the cortical sensorimotor beta rhythm. Neuroscience 2008;156:238-46 (Page 239, Figure 1).
Figure 2-4. Schematic representation of the 10-10 average virtual montage with 27 channels. The original recording was re-referenced and interpolated using BESA 5.3 (BESA GmbH, Gräfelfing, Germany).
Figure 2-5. Schematic representation of the electroencephalogram power spectral analysis. Preprocessing and analysis were performed in BESA 5.3 (BESA GmbH, Gräfelfing, Germany).
CHAPTER 3
BIOANALYTICAL ANALYSIS

Rationale
Developing and validating an analytical method to measure armodafinil concentrations in human plasma.

Results

Selectivity and Recovery
The selectivity of the analytical method was demonstrated by comparing representative chromatograms: blank plasma, blank plasma spiked with armodafinil (18.75 ng mL\(^{-1}\)) and processed with IS solution (160 ng mL\(^{-1}\)); blank plasma spiked with armodafinil (4800 ng mL\(^{-1}\)) and processed with IS solution (160 ng mL\(^{-1}\)) (Figure 3-1). The extraction recovery of armodafinil was higher than 90%.

Linearity and Lower Limit of Quantification
Calibration curves of peak area ratio (armodafinil/IS) as a function of nominal concentration were linear (weighted linear regression, concentration\(^{-1}\)) in the investigated concentration range (18.75–4800 ng mL\(^{-1}\)). The lower limit of quantification (LLOQ) was 18.75 ng mL\(^{-1}\) corresponding to the lowest concentration that could be determined with precision of 20% and accuracy from 80 to 120% under the experimental conditions (Tables 3-1 and 3-2).

Precision and Accuracy
The intra- and inter-day relative standard deviation (R.S.D.) values for armodafinil determination in human plasma are shown in Table 3-1. The intra- and inter-assay precision in the quantification of QC samples were less than or equal to 13.3 and
6.6%, respectively. The accuracy in their determination was from 84.9 to 110% (Table 3-2).

**Stability**

The accuracy and precision in the quantification of QC samples (low and high level of concentration) for bench top (four hours) and autosampler (six hours) are shown in Table 3-3. After the three freeze-thaw cycles the precision in the quantification of QC samples were less than or equal to 8.6%; the accuracy in their determination was from 94.6 to 101% (Table 3-4). A long-term stability study on both R- and S-modafinil in human plasma showed that both enantiomers were stable for at least 2 months at -20°C. Moreover, an even longer stability study showed that modafinil was stable for approximately 6 months at -20°C in human plasma.
### Table 3-1. Intra- and inter-day variation of armodafinil determination in human plasma

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<th>Nominal concentration (ng mL(^{-1}))</th>
<th>Day</th>
<th>Experimental concentrations(^a)</th>
<th>Mean (ng mL(^{-1}))</th>
<th>SD</th>
<th>RSD (%)</th>
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</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>3769</td>
<td>61</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td>3953</td>
<td>206</td>
<td>5.2</td>
</tr>
<tr>
<td><strong>Inter-day variation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.75 (LLOQ)</td>
<td></td>
<td></td>
<td>18.69</td>
<td>0.33</td>
<td>1.8</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
<td>50</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>800</td>
<td></td>
<td></td>
<td>804</td>
<td>53</td>
<td>6.6</td>
</tr>
<tr>
<td>4000</td>
<td></td>
<td></td>
<td>3918</td>
<td>134</td>
<td>3.4</td>
</tr>
</tbody>
</table>

\(^a\)n=3 observations. SD, standard deviation; RSD, relative standard deviation; LLOQ, lower limit of quantification.

### Table 3-2. Intra- and inter-day variation of armodafinil determination in human plasma

<table>
<thead>
<tr>
<th>Concentration (ng mL(^{-1}))</th>
<th>Range (ng mL(^{-1}))</th>
<th>Accuracy (%)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.75 (LLOQ)</td>
<td>15.06 – 21.56</td>
<td>80.3 – 115</td>
</tr>
<tr>
<td>50</td>
<td>42 – 55</td>
<td>84.9 – 110</td>
</tr>
<tr>
<td>800</td>
<td>733 – 880</td>
<td>91.6 – 110</td>
</tr>
<tr>
<td>4000</td>
<td>3700 – 4160</td>
<td>92.5 – 104</td>
</tr>
</tbody>
</table>

\(^a\)n=9 observations. LLOQ, lower limit of quantification.

### Table 3-3. Bench top and autosampler stability of armodafinil in human plasma

<table>
<thead>
<tr>
<th>Nominal concentration (ng mL(^{-1}))(^a)</th>
<th>Bench top stability</th>
<th>Autosampler stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean measured concentrations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>46</td>
<td>52</td>
</tr>
<tr>
<td>4000</td>
<td>4093</td>
<td>3812</td>
</tr>
<tr>
<td>Precision (RSD)</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>92.4</td>
<td>2.4</td>
</tr>
</tbody>
</table>

\(^a\)n=3 observations per concentration level in each condition.
Table 3-4. Freeze-thaw stability of armodafinil in human plasma

<table>
<thead>
<tr>
<th>Nominal concentration (ng mL(^{-1}))(^a)</th>
<th>Freeze-thaw Cycle 1</th>
<th>Freeze-thaw Cycle 2</th>
<th>Freeze-thaw Cycle 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>4000</td>
<td>50</td>
</tr>
<tr>
<td>Mean measured concentrations</td>
<td>50</td>
<td>3787</td>
<td>47</td>
</tr>
<tr>
<td>Precision (RSD)</td>
<td>1.9</td>
<td>3.5</td>
<td>8.6</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>101</td>
<td>94.7</td>
<td>94.6</td>
</tr>
</tbody>
</table>

\(^a\)n=3 observations per concentration level in each cycle.
Figure 3-1. Representative chromatograms of calibration curve in human plasma. Multiple reaction mode with positive electrospray ionization interface is shown using m/z ratios of 274.2 > 167.2 and 403.3 > 307.1 for armodafinil and internal standard (prednisolone), respectively. Blank plasma A) MRM 274.2 > 167.2 B) MRM 403.3 > 307.1. Blank plasma spiked with armodafinil (18.75 ng mL\(^{-1}\)) and processed with IS solution (160 ng mL\(^{-1}\)) C) MRM 274.2 > 167.2 D) MRM 403.3 > 307.1. Blank plasma spiked with armodafinil (4800 ng mL\(^{-1}\)) and processed with IS solution (160 ng mL\(^{-1}\)) E) MRM 274.2 > 167.2 F) MRM 403.3 > 307.1.
CHAPTER 4
BEHAVIORAL-BASED MEASURES

Rationale

In an attempt to assess the armodafinil effect on the subjects, the present study comprised two different approaches: behavioral- and EEG-based measures. This chapter addresses the first approach by involving two neurocognitive tests (PVT and GNAT). PVT has been selected for three main reasons: i) it meets the criteria to an accurate assessment of neurobehavioral degradation due to sleep loss;\textsuperscript{55} ii) it has been recently pointed out as the most widely used measure of behavioral alertness;\textsuperscript{57} and iii) it has been used as an efficacy endpoint in clinical trials of armodafinil.\textsuperscript{62} In this sense, a PK-PD model involving a reliable measure of armodafinil effect on alertness will give insight about the drug effect and help to apply the knowledge to the development of compounds with an analogous mechanism of action. GNAT, in turn, brings foundation to the ERP analysis, since it has been previously applied to investigate the effect of response inhibition and production on ERP studies.\textsuperscript{63-65} In additional, it is a well-known measure of impulsive behavior being applied to study the effect of stimulant drugs such as methylphenidate in children with ADHD;\textsuperscript{66,67} it becomes interesting due to the fact that modafinil, the analogous compound of armodafinil, has showed to alleviate the symptoms of ADHD.\textsuperscript{68}

Results

Psychomotor Vigilance Task

Armodafinil increased the PVT performance as measured by number of lapses plus false starts and mean reciprocal response time (1/RT) when compared to placebo (armodafinil 150 mg vs. placebo: lapses plus false starts, $P = .0081$, mean 1/RT, $P <$
Armodafinil 250 mg vs. placebo: lapses plus false starts, $P = .0218$, mean $1/RT$, $P = .0254$). A significant treatment versus time interaction was observed in both PVT metrics (lapses plus false starts: $P < .0001$; mean $1/RT$: $P = .0012$), indicating that the change in alertness over time varies with treatment. Fatigue baseline adjustment was performed in order to account for the significantly different baseline values across the different treatments (lapses plus false alarms: $P < .0001$; mean $1/RT$: $P < .0001$). Armodafinil produced a significant decrease of the number of lapses plus false starts from 1.6 to 11.6 hours post-dose (Figure 4-1) and a significant increase of the mean $1/RT$ from 2.6 to 9.6 hours post-dose (Figure 4-2). The two doses of armodafinil were not significantly different from each other in respect to both PVT metrics (lapses plus false starts, $P = .9644$, mean $1/RT$, $P = .5095$).

**Go/No-Go Association Task**

Armodafinil increased the GNAT performance as measured by mean $1/RT$ of go trials, number of errors of omission (go trials) and errors of commission (no-go trials) (armodafinil 150 mg vs. placebo: mean $1/RT$, $P < .0001$, errors of omission, $P = .0038$, errors of commission, $P < .0001$; armodafinil 250 mg vs. placebo: mean $1/RT$, $P < .0001$, errors of omission, $P = .0048$, errors of commission, $P < .0001$). A significant treatment versus time interaction was observed in all three GNAT metrics (mean $1/RT$: $P = .0257$; errors of omission: $P < .0001$; errors of commission: $P < .0001$). Fatigue baseline adjustment was performed for the three metrics given their significant difference across the treatments ($1/RT$: $P = .0001$; errors of omission: $P < .0021$; errors of commission: $P < .0001$). Armodafinil improved the mean $1/RT$ of go trials from 1.8 to 9.8 hours post-dose (Figure 4-3). In respect to accuracy, armodafinil significantly reduced errors of omission from 1.8 to 11.8 hours post-dose (Figure 4-4); errors of
commission were also significantly reduced from 3.8 to 9.8 hours post-dose (Figure 4-5). The two doses of armodafinil were not significantly different from each other in respect to the three GNAT metrics (mean 1/RT, $P = .9362$, errors of omission, $P = .9979$, errors of commission, $P < .5417$). Finally, a statistically significant correlation was observed between the mean 1/RT during GNAT and PVT (Figure 4-6).
Figure 4-1. Psychomotor vigilance task number of lapses plus false starts. A) Mean observed data. B) Least square mean fatigue baseline adjusted data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significant different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 4-2. Psychomotor vigilance task mean reciprocal reaction time. A) Mean observed data. B) Least square mean fatigue baseline adjusted data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significant different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 4-3. Go/no-go association task mean reciprocal reaction time. A) Mean observed data. B) Least square mean fatigue baseline adjusted data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significant different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 4-4. Go/no-go association task number of errors of omission. A) Mean observed data. B) Least square mean fatigue baseline adjusted data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significant different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 4-5. Go/no-go association task number of errors of omission. A) Mean observed data. B) Least square mean fatigue baseline adjusted data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significant different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 4-6. Pearson’s correlation analysis between the psychomotor vigilance task, PVT, and go/no-go association task, GNAT. Observed mean reciprocal reaction time after administration of placebo, 150 mg and 250 mg armodafinil.
CHAPTER 5
ELECTROENCEPHALOGRAM-BASED MEASURES

Rationale

We aim to propose EEG-based approaches as a potential measure of armodafinil effect given their numerous advantages over behavioral measures. Differences in reaction time and error rates obtained from behavioral measures are not easily attributed to a particular cognitive process. ERP, a measure of brain activity due to a specific event, adds to our research in the sense that it yields a continuous monitoring of neural sub-processes (e.g., sensory, cognitive and motor processes) with a high time resolution.\(^4\) Another advantage of ERP is that it allows to measure brain activity even in the absence of behavioral response. EEG spectral power, in turn, offers the possibility of assessing alertness during a resting waking state. Indeed, it presents the advantage of requiring no active subjects’ cooperation among all the other aforementioned advantages of an EEG-based measure.

Results

Event-Related Potentials

ERP analyses were performed during execution of the PVT and GNAT.

During the PVT an armodafinil effect was evident in the Cz channel where the amplitude of the positive ERP peak at around 380 ms was increased (Figure 5-1; armodafinil 150 mg vs. placebo: \(P = .0526\); armodafinil 250 mg vs. placebo: \(P = .0001\)). No significant treatment versus time interaction (\(P = .2827\)) and fatigue baseline difference (\(P = .7199\)) were observed. The average amplitude over time for the three treatment groups is presented in the Figure 5-2. The two doses of armodafinil were not significantly different from each other in respect to the ERP peak at around 380 ms (\(P = \))
Statistically significant correlations were observed between the average ERP amplitude and the both PVT metrics (Figures 5-3 and 5-4).

Likewise, during the GNAT an armodafinil effect was pronounced in the Cz channel where the amplitude of the positive ERP peak at around 380 ms was increased (Figure 5-5; armodafinil 150 mg vs. placebo: go condition, \( P < .0001 \), no-go condition, \( P < .0001 \); armodafinil 250 mg vs. placebo: go condition, \( P < .0001 \), no-go condition, \( P < .0001 \)). No significant treatment versus time interaction was observed for the go (\( P = .8377 \)) and no-go conditions (\( P = .7242 \)). Fatigue baseline adjustment was performed in order to account for the significantly different baseline values across the different treatments during the no-go condition (\( P < .0001 \)); for consistency, it was kept in the model even when no significant difference was observed during the go condition (\( P = .2625 \)). The average amplitude over time for the three treatment groups during the go and no-go conditions is presented in the Figures 5-6 and 5-7, respectively. The two doses of armodafinil were not significantly different from each other in respect to the ERP peak at around 380 ms for both conditions (go condition: \( P = .9943 \); no-go condition: \( P = .8583 \)). Statistically significant correlations were observed between the average ERP amplitude for both conditions and the GNAT metrics (Figures 5-8, 5-9, 5-10) as well as the PVT mean reciprocal reaction time (Figures 5-11).

**Spectral Analysis**

Armodafinil decreased the EEG power in the delta frequency range (1-4 Hz, delta power) over the frontal, temporal and occipital region of the brain (Figure 5-12) when compared to placebo (armodafinil 150 mg vs. placebo: F3, \( P = .0018 \), F4, \( P = .0008 \), T7, \( P < .0001 \), T8, \( P < .0001 \), O1, \( P = .0081 \), Oz: \( P = .0018 \), O2: \( P < .0001 \); armodafinil 250 mg vs. placebo: F3, \( P = .0348 \), F4, \( P = .0032 \), T7, \( P = .0040 \), T8, \( P = .0173 \), O1, \( P
<.0001, Oz: \( P < .0001 \), O2: \( P < .0001 \)). No significant treatment versus time interaction was observed in any of the aforementioned regions (F3: \( P = .9343 \); F4: \( P = .7949 \); T7: \( P = 0.1750 \); T8: \( P = .1426 \); O1: \( P = .7100 \); Oz: \( P = .7016 \); O2: \( P = .6342 \)). Fatigue baseline adjustment was performed in order to account for the significantly different baseline values across the different treatments (T7: \( P = .0216 \); O1: \( P < .0001 \); Oz: \( P < .0002 \); O2: \( P = .0026 \)); for consistency, it was kept in the model even when no significant difference was observed (F3: \( P = .6507 \); F4: \( P = .2744 \); T8: \( P = .6074 \)). The average delta power over time for the three treatment groups in the F3, F4, T7, T8, O1, Oz and O2 electrodes are presented in the Figures 5-13 through 5-19, respectively. The two doses of armodafinil were not significantly different from each other in respect to delta power at any tested region (F3: \( P = .5233 \); F4: \( P = .9200 \); T7: \( P = .5120 \); T8: \( P = .2841 \); O1: \( P = .2173 \); Oz: \( P = .3264 \), O2: \( P < .8441 \)). A statistically significant correlation was observed between the delta power and the mean PVT 1/RT (Figure 5-20).
Figure 5-1. Grand average event-related brain activity during the execution of the psychomotor vigilance task. A) The vertical bar (time=0) corresponds to the appearance of the visual stimulus (time window: -200 to 500 ms; positive potentials are plotted downward). B) Topographic map at approximately 6 hours after the treatment administration (380 ms after appearance of visual stimulus).
Figure 5-2. Average event-related brain activity, ERP, during the execution of the psychomotor vigilance task, PVT (Cz channel, positive peak at around 380 ms). A) Mean observed data. B) Least square mean fatigue baseline adjusted-data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significantly different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 5-3. Pearson’s correlation analysis between the event-related brain activity, ERP, during the execution of the psychomotor vigilance task, PVT, and the PVT mean reciprocal reaction time. Observed data after administration of placebo, 150 mg and 250 mg armodafinil.
Figure 5-4. Pearson’s correlation analysis between the event-related brain activity, ERP, during the execution of the psychomotor vigilance task, PVT, and the PVT number of lapses plus false starts. Observed data after administration of placebo, 150 mg and 250 mg armodafinil.
Figure 5-5. Grand average event-related brain activity during the execution of the go/no-go association task (no-go condition). A) The vertical bar (time=0) corresponds to the appearance of the visual stimulus (time window: -200 to 700 ms; positive potentials are plotted downward). B) Topographic map at approximately 6 hours after the treatment administration (380 ms after appearance of visual stimulus).
Figure 5-6. Average event-related brain activity, ERP, during the execution of the go/no-go association task, GNAT (go condition, Cz channel, positive peak at around 380 ms). A) Mean observed data. B) Least square mean fatigue baseline adjusted-data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significantly different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 5-7. Average event-related brain activity, ERP, during the execution of the go/no-go association task, GNAT (no-go condition, Cz channel, positive peak at around 380 ms). A) Mean observed data. B) Least square mean fatigue baseline adjusted-data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significantly different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 5-8. Pearson’s correlation analysis between the event-related brain activity, ERP, during the go/no-go association task, GNAT, and the GNAT mean reciprocal reaction time. Observed data for ERP during the go A) and no-go B) condition after administration of placebo, 150 mg and 250 mg armodafinil.
Figure 5-9. Pearson's correlation analysis between the event-related brain activity, ERP, during the go/no-go association task, GNAT, and the GNAT number of errors of omission. Observed data for ERP during the no-go condition after administration of placebo, 150 mg and 250 mg armodafinil.
Figure 5-10. Pearson's correlation analysis between the event-related brain activity, ERP, during the go/no-go association task, GNAT, and the GNAT number of errors of commission. Observed data for ERP during the go condition after administration of placebo, 150 mg and 250 mg armodafinil.
Figure 5-11. Pearson’s correlation analysis between the event-related brain activity, ERP, during the go/no-go association task, GNAT, and the psychomotor vigilance task, PVT, mean reciprocal reaction time. Observed data for ERP during the go A) and no-go B) condition after administration of placebo, 150 mg and 250 mg armodafinil.
Figure 5-12. Frontal (F), temporal (T) and occipital (O) regions of the brain shown in the 10-10 average virtual montage. Electrodes represented by filled blue circles evidenced a significant decrease in the EEG power in the delta frequency range in the armodafinil groups when compared to placebo.
Figure 5-13. Electroencephalogram delta band spectral power in the electrode F3 of the frontal area. A) Mean (± S.E.M.) observed data. B) Least square mean fatigue baseline adjusted-data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significantly different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 5-14. Electroencephalogram delta band spectral power in the electrode F4 of the frontal area. A) Mean (± S.E.M.) observed data. B) Least square mean fatigue baseline adjusted-data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significantly different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 5-15. Electroencephalogram delta band spectral power in the electrode T7 of the temporal area. A) Mean (± S.E.M.) observed data. B) Least square mean fatigue baseline adjusted-data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significantly different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 5-16. Electroencephalogram delta band spectral power in the electrode T8 of the temporal area. A) Mean (± S.E.M.) observed data. B) Least square mean fatigue baseline adjusted data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significantly different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 5-17. Electroencephalogram delta band spectral power in the electrode O1 of the occipital area. A) Mean (± S.E.M.) observed data. B) Least square mean fatigue baseline adjusted-data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significantly different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 5-18. Electroencephalogram delta band spectral power in the electrode Oz of the occipital area. A) Mean (± S.E.M.) observed data. B) Least square mean fatigue baseline adjusted-data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significantly different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 5-19. Electroencephalogram delta band spectral power in the electrode O2 of the occipital area. A) Mean (± S.E.M.) observed data. B) Least square mean fatigue baseline adjusted-data. Simple effect comparisons of treatment*time by time. Specific time points represented by different letters are statistically significantly different (Adj. Tukey p<0.05). rBL, resting baseline; fBL, fatigue baseline.
Figure 5-20. Pearson’s correlation analysis between the delta power the psychomotor vigilance task, PVT, mean reciprocal reaction time. Observed data after administration of placebo, 150 mg and 250 mg armodafinil.
CHAPTER 6
PHARMACOKINETIC-PHARMACODYNAMIC MODELING

Rationale

We believe in the value of establishing a model to correlate armodafinil concentrations with neurocognitive and EEG-based measures of drug effect. Furthermore, we went further by investigating the clinical meaning of EEG parameters. In other words, we investigated the correlation between a well-established measured of the armodafinil effect on alertness, which is PVT, and EEG parameters bringing insight of the clinical meaning of changes on EEG.

Results

Pharmacokinetics

Armodafinil pharmacokinetics was determined in order to establish the pharmacokinetic-pharmacodynamic (PK-PD) relationship of the drug. The plasma concentration profile of armodafinil was best described by a one-compartment model with first-order elimination and absorption with lag-time as follows:

$$C = \frac{F \cdot D \cdot \text{k}_a}{(\text{k}_a - \text{k}_e) \cdot V_d} \cdot (e^{-\text{k}_e(t - t_{lag})} - e^{-\text{k}_a(t - t_{lag})})$$

(6-1)

where $C$ is the total plasma concentration over time $t$; $F$ is the oral bioavailability of the drug; $k_a$ is the first-order absorption rate constant; $k_e$ is the first-order elimination rate constant; $V_d$ is the apparent volume of distribution; $t_{lag}$ is the delay between the dosing time and the appearance of concentration in the sampling compartment.

The final parameter estimates and variability are presented in Table 6-1. The visual predictive check plots (Figure 6-1) together with the goodness-of-fit plots (Figure
6-2) for armodafinil plasma concentrations demonstrated the conformity between the model predictions and the observed data.

**Pharmacokinetic-Pharmacodynamic Modeling**

In this study, behavioral and EEG-based assessments were investigated as measures of drug effect. For the purpose of the PK-PD modeling we focused on a specific metric for each PD measure: 1) PVT, mean 1/RT; 2) GNAT, mean 1/RT; 3) ERP during PVT, amplitude of the positive peak at around 380 ms in the Cz electrode; 4) ERP during GNAT, amplitude of the positive peak at around 380 ms in the Cz electrode; 5) Spectral analysis, delta power of the occipital region of the brain (O2 electrode).

A delay in effect (counterclockwise hysteresis) was observed for all PD measures suggesting disequilibrium between plasma and effect-site concentration after oral administration of armodafinil (Figure 6-3). An effect compartment approach was used to account for this delay: in the one-compartment pharmacokinetic model, the effect compartment is linked to the plasma concentrations (central compartment) by the rate constant \( k_{1e} \); \( k_{e0} \), in turn, represents the rate constant for the drug elimination from the effect compartment (Figure 6-4).\(^{69}\) The rate of change of the drug amount in the effect compartment can then be described as follow:\(^{69}\)

\[
\frac{dX_e}{dt} = X \cdot k_{1e} - X_e \cdot k_{e0}
\]  

(6-2)

where \( X_e \) represents the hypothetical drug amount in the effect compartment, \( X \) represents the drug amount in the central compartment.

In the steady-state, we have that \( \frac{dX_e}{dt} = 0 \) or:
\[ X \cdot k_{le} - X_e \cdot k_{e0} = 0 \]  \hspace{1cm} (6-3)

Since \( X = C \cdot V_d \) and \( A_e = C_e \cdot V_e \):

\[ C \cdot V_d \cdot k_{le} - C_e \cdot V_e \cdot k_{e0} = 0 \]  \hspace{1cm} (6-4)

where \( C \) is the drug concentration in the central compartmental, \( V_d \) is the volume of the central compartment, \( C_e \) is the drug concentration in the effect compartment and \( V_e \) is the volume of the effect compartment. As \( C = C_e \) in the steady-state, we can define:

\[ V_e = \frac{V_d \cdot k_{le}}{k_{e0}} \]  \hspace{1cm} (6-5)

Now, dividing Equation 6-2 by \( V_e \), we have:

\[ \frac{dC_e}{dt} = k_{e0} \cdot (C - C_e) \]  \hspace{1cm} (6-6)

Behavioral-based measures

The observed effect was defined by two components: time-varying baseline and drug effect. The time-varying baseline function was determined by fitting the data corresponding to the placebo group.

Placebo model or baseline. The following models described the placebo effect on the mean \( 1/RT \) for the PVT and GNAT, respectively:

\[ S(t)_{PVT} = S_0 \cdot \left[ 1 + \alpha \cdot \cos \left( \frac{2\pi}{24} (t + \phi) \right) \right] \]  \hspace{1cm} (6-7)

\[ S(t)_{GNAT} = S_0 \cdot \left[ 1 - \alpha \cdot \cos \left( \frac{2\pi}{24} (t - \phi) \right) \right] \]  \hspace{1cm} (6-8)

where \( S(t) \) is the placebo effect over time, \( S_0 \) is the mean baseline, \( \alpha \) is the amplitude, \( \phi \) is the peak-related parameter and \( t \) is time.
**Drug effect model.** For the groups receiving armodafinil treatment, the mean 1/RT increased as a function of the drug concentration in the effect compartment (i.e., hypothetically the site of action). Therefore, an excitatory model was used to describe the drug effect on the mean 1/RT for both behavioral measures, PVT and GNAT:

\[
E(t)_{PVT} = S(t)_{PVT} + \frac{E_{max} \cdot C_e(t)}{EC_{e50} + C_e(t)}
\]

\[
E(t)_{GNAT} = S(t)_{GNAT} + \frac{E_{max} \cdot C_e(t)}{EC_{e50} + C_e(t)}
\]

where \(E_{max}\) is the maximum excitatory drug effect and \(EC_{e50}\) is the apparent drug concentration at the effect site producing 50% of the \(E_{max}\). The final parameter estimates and variability for the placebo and drug effect model corresponding to both behavioral measures are presented in Table 6-2 and 6-3. The visual predictive check plots together with the goodness-of-fit plots for armodafinil effect on both behavioral measures demonstrated the conformity between the model predictions and the observed data (Figures 6-5 through 6-8).

**Electroencephalogram-based measures**

Likewise, the observed effect was defined by two components: time-varying baseline and drug effect. The time-varying baseline function was determined by fitting the data corresponding to the placebo group.

**Placebo model or baseline.** The following models were used to describe the placebo effect on the amplitude of the positive peak at around 380 ms in the Cz electrode (i.e., event-related brain activity) during the execution of both behavioral measures, PVT and GNAT:
\[ S(t)_{ERP-PVT} = S_0 \]  
\[ S(t)_{ERP-GNAT} = S_0 \cdot \left[ 1 - \alpha \cdot \cos \left( \frac{2\pi}{24} (t - \phi) \right) \right] \]

To describe the placebo effect on the delta power of the occipital region (O2 electrode), the following equation was used:

\[ S(t)_{DELTA POWER} = S_0 \cdot \left[ 1 + \alpha \cdot \cos \left( \frac{2\pi}{24} (t - \phi) \right) \right] \]

**Drug effect model.** For the groups receiving armodafinil treatment, the event-related brain activity increased as a function of the drug concentration in the effect compartment (i.e., hypothetically the site of action). Therefore, an excitatory model was used to describe the drug effect on the brain activity during the execution of both behavioral measures, PVT and GNAT:

\[ E(t)_{ERP-PVT} = S(t)_{ERP-PVT} + \frac{E_{max} \cdot C_e(t)}{E_{c50} + C_e(t)} \]  
\[ E(t)_{ERP-GNAT} = S(t)_{ERP-GNAT} + \frac{E_{max} \cdot C_e(t)}{E_{c50} + C_e(t)} \]

The final parameter estimates and variability for the placebo and drug effect model corresponding to the brain activity during both behavioral measures are presented in Table 6-4 and 6-5. The visual predictive check plots together with the goodness-of-fit plots for armodafinil effect on brain activity demonstrated the conformity between the model predictions and the observed data (Figures 6-9 through 6-12).
To describe the drug effect on the delta power of the occipital region (O2 electrode), an inhibitory model was used given that armodafinil mitigated the increase in delta power due to the sleep loss:

\[
E(t)_{\text{DELTA POWER}} = S(t)_{\text{DELTA POWER}} - \frac{I_{\text{max}} \cdot C_e(t)}{IC_{50} + C_e(t)} \quad (6-16)
\]

where \(I_{\text{max}}\) is the maximum inhibitory drug effect and \(IC_{50}\) is the drug concentration producing 50% of the \(I_{\text{max}}\). The final parameter estimates and variability for the placebo and drug effect model corresponding to the delta power is presented in Table 6-6. The visual predictive check plots together with the goodness-of-fit plots for armodafinil effect on delta power demonstrated the conformity between the model predictions and the observed data (Figure 6-13 and 6-14).

**Correlation between behavioral alertness and electroencephalogram-based measures**

The correlation between behavioral alertness and EEG-based measures was investigated in order to bring insight into the clinical significance of the drug-related changes on EEG. PVT represents the most widely used measure of behavioral alertness with the mean 1/RT being one of the primary metrics. A statistically significant correlation between the population predictions for the drug effect on the mean 1/RT of the PVT and EEG-based measures (brain activity and delta power) is shown in Figure 6-15.
Table 6-1. Pharmacokinetic parameters of armodafinil.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (RSE%)</th>
<th>BSV% (RSE%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/F (L·h(^{-1}))</td>
<td>2.2 (7.7)</td>
<td>9.4 (87)</td>
</tr>
<tr>
<td>V/F (L)</td>
<td>41 (9.8)</td>
<td>21 (27)</td>
</tr>
<tr>
<td>(k_a) (h(^{-1}))</td>
<td>1.3 (18)</td>
<td>27 (52)</td>
</tr>
<tr>
<td>(t_{lag}) (h)</td>
<td>1.1 (18)</td>
<td>54 (25)</td>
</tr>
</tbody>
</table>

A proportional between-subject variability (BSV) was used for all parameters. RSE%, estimate of standard error divided by parameter estimate; CL/F, total clearance divided by oral bioavailability; V/F, apparent volume of distribution divided by oral bioavailability; \(k_a\), absorption rate constant; \(t_{lag}\), time delay between drug dosing and its appearance in the sampling compartment.

Table 6-2. Pharmacodynamic parameters of the armodafinil effect on the psychomotor vigilance task mean reciprocal reaction time.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (RSE%)</th>
<th>BSV% (RSE%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S_0) (ms(^{-1}))</td>
<td>3.6 (6.4)</td>
<td>14 (32)</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>0.066 (27)</td>
<td>NE</td>
</tr>
<tr>
<td>(\phi)</td>
<td>6.9 (16)</td>
<td>17 (65)</td>
</tr>
<tr>
<td>(k_e0) (h(^{-1}))</td>
<td>0.78 (41)</td>
<td>NE</td>
</tr>
<tr>
<td>(E_{max}) (ms(^{-1}))</td>
<td>1.3 (8.2)</td>
<td>NE</td>
</tr>
<tr>
<td>(EC_{e50}) (µg·mL(^{-1}))</td>
<td>4.1 (33)</td>
<td>138 (58)</td>
</tr>
</tbody>
</table>

A proportional between-subject variability (BSV) was used in the case where it was estimated. RSE%, estimate of standard error divided by parameter estimate; \(S_0\), mean baseline; \(\alpha\), amplitude; \(\phi\), peak; \(k_e0\), elimination rate constant from the effect compartment; \(E_{max}\), maximum excitatory drug effect; \(EC_{e50}\), apparent drug concentration at the effect site producing 50% of \(E_{max}\); NE, not estimated.

Table 6-3. Pharmacodynamic parameters of the armodafinil effect on the go/no-go association task mean reciprocal reaction time.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (RSE%)</th>
<th>BSV% (RSE%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S_0) (ms(^{-1}))</td>
<td>2.2 (6.4)</td>
<td>14 (34)</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>0.044 (65)</td>
<td>NE</td>
</tr>
<tr>
<td>(\phi)</td>
<td>4.7 (9.4)</td>
<td>NE</td>
</tr>
<tr>
<td>(k_e0) (h(^{-1}))</td>
<td>0.69 (38)</td>
<td>NE</td>
</tr>
<tr>
<td>(E_{max}) (ms(^{-1}))</td>
<td>1.2 (21)</td>
<td>NE</td>
</tr>
<tr>
<td>(EC_{e50}) (µg·mL(^{-1}))</td>
<td>7.6 (21)</td>
<td>123 (75)</td>
</tr>
</tbody>
</table>

A proportional between-subject variability (BSV) was used in the case where it was estimated. RSE%, estimate of standard error divided by parameter estimate; \(S_0\), mean baseline; \(\alpha\), amplitude; \(\phi\), peak; \(k_e0\), elimination rate constant from the effect compartment; \(E_{max}\), maximum excitatory drug effect; \(EC_{e50}\), apparent drug concentration at the effect site producing 50% of \(E_{max}\); NE, not estimated.
Table 6-4. Pharmacodynamic parameters of the armodafinil effect on the event-related brain activity during the psychomotor vigilance task. A proportional between-subject error was used unless stated otherwise.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (RSE%)</th>
<th>BSV% (RSE%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_0$ ($\mu$V)</td>
<td>1.2 (36)</td>
<td>213 (22)*</td>
</tr>
<tr>
<td>$k_{e0}$ (h$^{-1}$)</td>
<td>0.88 (28)</td>
<td>1.9 (34)</td>
</tr>
<tr>
<td>$E_{max}$ ($\mu$V)</td>
<td>3.5 (9.6)</td>
<td>NE</td>
</tr>
<tr>
<td>$EC_{e50}$ ($\mu$g.mL$^{-1}$)</td>
<td>5.3 (18)</td>
<td>143 (51)</td>
</tr>
</tbody>
</table>

*Additive between-subject variability (BSV). RSE%, estimate of standard error divided by parameter estimate; $S_0$, mean baseline; $k_{e0}$, elimination rate constant from the effect compartment; $E_{max}$, maximum excitatory drug effect; $EC_{e50}$, apparent drug concentration at the effect site producing 50% of $E_{max}$; NE, not estimated.

Table 6-5. Pharmacodynamic parameters of the armodafinil effect on the event-related brain activity during the go/no-go association task (no-go condition). A proportional between-subject error was used unless stated otherwise.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (RSE%)</th>
<th>BSV% (RSE%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_0$ ($\mu$V)</td>
<td>5.7 (34)</td>
<td>494 (30)*</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.14 (25)</td>
<td>NE</td>
</tr>
<tr>
<td>$\phi$</td>
<td>2.6 (24)</td>
<td>NE</td>
</tr>
<tr>
<td>$k_{e0}$ (h$^{-1}$)</td>
<td>1.1 (45)</td>
<td>88 (84)</td>
</tr>
<tr>
<td>$E_{max}$ ($\mu$V)</td>
<td>8.0 (15)</td>
<td>NE</td>
</tr>
<tr>
<td>$EC_{e50}$ ($\mu$g.mL$^{-1}$)</td>
<td>5.7 (19)</td>
<td>121 (70)</td>
</tr>
</tbody>
</table>

*Additive between-subject variability (BSV). RSE%, estimate of standard error divided by parameter estimate; $S_0$, mean baseline; $\alpha$, amplitude; $\phi$, peak; $k_{e0}$, elimination rate constant from the effect compartment; $E_{max}$, maximum excitatory drug effect; $EC_{e50}$, apparent drug concentration at the effect site producing 50% of $E_{max}$; NE, not estimated.

Table 6-6. Pharmacodynamic parameters of the armodafinil effect on the delta power of the occipital region of the brain.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (RSE%)</th>
<th>BSV% (RSE%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_0$ ($\mu$V$^2$)</td>
<td>14 (21)</td>
<td>44 (24)</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.23 (33)</td>
<td>NE</td>
</tr>
<tr>
<td>$\phi$</td>
<td>7.0 (5.5)</td>
<td>NE</td>
</tr>
<tr>
<td>$k_{e0}$ (h$^{-1}$)</td>
<td>0.50 (38)</td>
<td>NE</td>
</tr>
<tr>
<td>$I_{max}$ ($\mu$V$^2$)</td>
<td>10 (24)</td>
<td>NE</td>
</tr>
<tr>
<td>$IC_{e50}$ ($\mu$g.mL$^{-1}$)</td>
<td>2.2 (12)</td>
<td>NE</td>
</tr>
</tbody>
</table>

A proportional between-subject variability (BSV) was used in the case where it was estimated. RSE%, estimate of standard error divided by parameter estimate; $S_0$, mean baseline; $\alpha$, amplitude; $\phi$, peak; $k_{e0}$, elimination rate constant from the effect compartment; $I_{max}$, maximum inhibitory drug effect; $IC_{e50}$, apparent drug concentration at the effect site producing 50% of $I_{max}$; NE, not estimated.
Figure 6-1. Visual predictive check of the final armodafinil pharmacokinetic model. Observed data (blue circles) are shown with their median (red circles). The median (solid black line) and the 5-95th percentile prediction interval (black broken lines) from the model are also presented.
Figure 6-2. Basic goodness-of-fit plots of the final armodafinil pharmacokinetic model.
Figure 6-3. Anticlockwise hysteresis loop between armodafinil pharmacokinetics and pharmacodynamics. The plot shows that the peak of armodafinil effect on the psychomotor vigilance task, PVT, reaction time is delayed with respect to its peak plasma concentration (subject 6 after administration of armodafinil 250 mg).
Figure 6-4. Schematic representation of the effect compartment approach. Adapted from Sheiner LB, Stanski DR, Vozeh S, Miller RD, Ham J. Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to d-tubocurarine. Clin Pharmacol Ther 1979;25:358-71 (Page 360, Figure 1).
Figure 6-5. Visual predictive check of the final pharmacokinetic/pharmacodynamic model of the armodafinil effect on the psychomotor vigilance task mean reciprocal reaction time. Observed data (blue circles) are shown with their median (red circles). The median (solid black line) and the 5-95th percentile prediction interval (black broken lines) from the model are also presented.
Figure 6-6. Basic goodness-of-fit plots of the final pharmacokinetic/pharmacodynamic model of the armodafinil effect on the psychomotor vigilance task mean reciprocal reaction time. A) Population predictions. B) Individual predictions. C) Individual weighted residuals. D) Conditional weighted residuals.
Figure 6-7. Visual predictive check of the final pharmacokinetic/pharmacodynamic model of the armodafinil effect on the go/no-go association task mean reciprocal reaction time. Observed data (blue circles) are shown with their median (red circles). The median (solid black line) and the 5-95th percentile prediction interval (black broken lines) from the model are also presented.
Figure 6-8. Basic goodness-of-fit plots of the final pharmacokinetic/pharmacodynamic model of the armodafinil effect on the go/no-go association task mean reciprocal reaction time. A) Population predictions. B) Individual predictions. C) Individual weighted residuals. D) Conditional weighted residuals.
Figure 6-9. Visual predictive check of the final pharmacokinetic/pharmacodynamic model of the armodafinil effect on the event-related brain activity, ERP, during the psychomotor vigilance task, PVT. Observed data (blue circles) are shown with their median (red circles). The median (solid black line) and the 5-95th percentile prediction interval (black broken lines) from the model are also presented.
Figure 6-10. Basic goodness-of-fit plots of the final pharmacokinetic/pharmacodynamic model of the armodafinil effect on the event-related brain activity during the psychomotor vigilance task. A) Population predictions. B) Individual predictions. C) Individual weighted residuals. D) Conditional weighted residuals.
Figure 6-11. Visual predictive check of the final pharmacokinetic/pharmacodynamic model of the armodafinil effect on the event-related brain activity, ERP, during the go/no-go association task, GNAT (no-go condition). Observed data (blue circles) are shown with their median (red circles). The median (solid black line) and the 5-95th percentile prediction interval (black broken lines) from the model are also presented.
Figure 6-12. Basic goodness-of-fit plots of the final pharmacokinetic/pharmacodynamic model of the armodafinil effect on the event-related brain activity during the go/no-go association task (no-go condition). A) Population predictions. B) Individual predictions. C) Individual weighted residuals. D) Conditional weighted residuals.
Figure 6-13. Visual predictive check of the final pharmacokinetic/pharmacodynamic model of the armodafinil effect on the delta power of the occipital region of the brain. Observed data (blue circles) are shown with their median (red circles). The median (solid black line) and the 5-95th percentile prediction interval (black broken lines) from the model are also presented.
Figure 6-14. Basic goodness-of-fit plots of the final pharmacokinetic/pharmacodynamic model of the armodafinil effect on the delta power of the occipital region of the brain. A) Population predictions. B) Individual predictions. C) Individual weighted residuals. D) Conditional weighted residuals.
Figure 6-15. Correlation between behavioral alertness and electroencephalogram-based measures. A) Correlation between event-related brain activity, ERP, during the psychomotor vigilance task, PVT, and PVT mean reciprocal reaction time (alertness). B) Correlation between EEG delta power and PVT mean reciprocal reaction time (alertness). Blue circles are population predictions after armodafinil administration.
CHAPTER 7
DISCUSSION AND CONCLUSIONS

To our knowledge, the present study is the first one correlating armodafinil (or its analogous compound modafinil) effect on quantitative EEG with a well-established measure of alertness and drug concentrations through a PK-PD modeling approach. We developed and compared PK-PD models describing the armodafinil effect on EEG- and behavioral-based measures in sleep deprived healthy adults. We observed that armodafinil increased the subject’s performance in the two behavioral tasks (i.e., PVT and GNAT), increased the event-related brain activity and decreased the EEG power in the delta frequency range when compared to placebo; overall, the drug groups started to differentiate from the placebo group after two hours post-dose with the difference becoming less evident toward the last experimental time point (i.e., approximately 12 hours post-dose). All pharmacodynamics measures were correlated with the apparent armodafinil concentrations at the site of action (i.e., effect compartment approach). PVT, in particular, is a widely-used measure of alertness; therefore, establishing the correlation between a specific EEG parameter and alertness provides insight of the clinical meaning of changes on EEG in the context of a wakefulness-promoting drug.

Spectral Analysis

EEG has been systematically investigated on a few drug classes such as benzodiazepines, anesthetics and opioids. PK-PD models using the EEG beta-frequency band amplitudes as a measure of effect have been successfully established for diazepam, flunitrazepam, midazolam, clobazam, oxazepam, and bretazenil. Alfentanil, fentanyl, sufentanil and remifentanil have their analgesic effects reflected on the EEG spectral edge. In particular, a PK-PD model utilizing EEG spectral edge as
an effect measure identified the importance of taking into account age and lean body mass when determining the dose regimen of remifentanil.\textsuperscript{72}

Systematic PK-PD studies of the effect of CNS stimulant drugs on quantitative EEG along with a clinically relevant correlation still lacks in the literature. In a previous study, quantitative EEG was evaluated as a potential pharmacodynamic measure of the CNS stimulant effect of dextroamphetamine in non-sleep deprived healthy male subjects.\textsuperscript{73} Although the EEG alpha power was compared with alternative measures of CNS stimulation (e.g., continuous performance, self-rated mood scales and neuroendocrine tests), a well-established measure of alertness and assessment of drug concentrations were not part of the study. In addition, armodafinil’s pharmacodynamics profile is not identical to that of the amphetamine-like stimulants\textsuperscript{29-31,62} and any generalization among these different therapeutic classes would be premature. It becomes important to highlight that modafinil effect on the EEG power spectrum has been reported in the literature\textsuperscript{11,14,74,75} but a systematic correlation of the drug-related EEG changes with simultaneous plasma concentrations and alertness was not conducted.

In the present study, we observed that armodafinil mitigated the slowing of the brain activity due to sleep deprivation. In other words, it decreased the EEG power in the delta frequency range (1-4 Hz, i.e., EEG delta power) over the frontal, temporal and occipital region of the brain when compared to placebo. This effect was evident during the eyes-open in opposed to the eyes-closed EEG recording sessions. Drug-related effects on either theta, alpha or beta frequency ranges were not observed in both eyes-open and -closed conditions. Our findings are virtually consistent with a helicopter
simulation study where modafinil reduced EEG activity in the delta and theta but had no effect in the alpha and beta frequency ranges of sleep deprived healthy pilots. The absence of armodafinil effect on the theta activity in the present study may be related to the different study design. In our study a single dose of armodafinil 150 mg or 250 mg was administered after 24 hours of sleep deprivation; on the other hand, three repeated doses of modafinil 200 mg after 16, 20 and 24 hours of sleep deprivation were administered to the sleep deprived healthy pilots. Another study in healthy subjects showed that modafinil 300 mg not only decreased the EEG power in the delta and theta frequencies but also increased the EEG power in the alpha frequency range. In that study, the subjects underwent 60 hours of sleep deprivation and received three doses of modafinil: the first dose aimed to prevent decrease in vigilance (at 11:30 pm previously sleep deprivation); the second dose aimed to restore vigilance (5:30 am, after approximately 45 hours of sleep deprivation), and (3) investigate the effect of drug on recovery sleep (3:30 pm, ten hours after administration of the second dose). Importantly, the authors noted that between the second and third dose administrations, drug and placebo did not differ much in respect to the EEG power in the alpha frequency range. This period between the third and second dose would more closely represent our study design since the second drug dose was administered after a period of sleep deprivation in order to restore vigilance. Indeed, the authors suggested that when the alpha activity is largely reduced (i.e., after a period of sleep deprivation), modafinil may not be effective enough to increase the EEG alpha power. In addition, the authors performed exclusively eyes-closed EEG recording and stated that only the waking portions of the EEG recordings were considered. In our study, we made efforts
to include epochs that well represented the respective recording sessions to prevent
any potential selection bias; naturally, it was not always possible to avoid transition
periods between wakefulness and sleep during eyes-closed sessions.

**Psychomotor Vigilance Task**

PVT, a measure of vigilant attention, meets the criteria to an accurate
assessment of neurobehavioral degradation due to sleep loss.\textsuperscript{55} It has been recently
pointed out as the most widely used measure of behavioral alertness.\textsuperscript{57} Indeed,
degradation in attention, particularly vigilant attention, is one of the most reliable effects
of sleep deprivation.\textsuperscript{76,77} PVT fulfills the requirements for an accurate measurement of
the neurocognitive effect of sleep loss in that: (a) it reflects an aspect of waking
cognitive function (e.g., ability to use attention); (b) it is easily and relatively quickly
performed; (c) it has minor learning effects; (d) it is sensitive; (e) its output variables are
of easy interpretation; (f) it has been suggest to reflect “real world” tasks such as alcohol
and drowsy-related impaired driving.\textsuperscript{55} In the present study, the number of lapses plus
false starts and mean 1/RT were utilized as primary PVT metrics. In a previous study,
these both metrics showed the highest sensitivity in measuring sleep deprivation when
considering both acute and chronic sleep deprivation. The mean 1/RT, in particular,
scored very high effect sizes in measuring sleep loss after acute total sleep deprivation
(i.e., a 33-hour period of total sleep deprivation) and chronic sleep deprivation (i.e., four
hours of sleep per night during five consecutive nights).\textsuperscript{55} The number of lapses
combined with the number of false starts, in turn, presented a comparable high effect
size in the condition of acute total sleep deprivation but its effect size was slightly lower
in the condition of chronic sleep deprivation.\textsuperscript{55}
The PVT has advantages over the maintenance of wakefulness test (MWT) and multiple sleep latency test (MSLT), which were used as primary efficacy endpoint in clinical trials for modafinil and armodafinil. In the MWT, OSAHS and narcoleptic patients were requested to stay awake in a dark room, and four 20-minute sessions were performed every 2 hours. The metric for each visit was the mean time for the patient to fall sleep over the four sessions. In the MSLT, SWSD patients basically underwent the same procedure, except that now the patients were requested not to resist falling sleep.\textsuperscript{62} Both MWT and MSLT are normally performed in a laboratory setting and, hence, provide artificial conditions that may stimulate sleep onset in children\textsuperscript{78} and inhibit sleep onset in adults. Moreover, the PVT allows for a repeated use and, consequently, concomitant assessment of alertness in a rich pharmacokinetic study. The result is a better description of the time course of effect over time, an important feature of an exploratory study (i.e., biomarker investigation) such as ours.

Armodafinil increased the PVT performance as measured by our primary metrics when compared to placebo. Both armodafinil doses produced a significant cumulative increase in the level of alertness during the 12-hour experimental period; subjects not only responded faster to the appearance of the stimulus but also improved their response accuracy (i.e., fewer errors of commission and omission). A significant treatment versus time interaction was observed for both PVT metrics reflecting the change in alertness over time with treatment. We performed a fatigue baseline adjustment to account for the fact that the subjects did not respond to the sleep deprivation period in an identical manner previous each treatment visit. This adjustment along with a placebo-controlled crossover study helped us to eliminate potential
confounder factors. PVT has been used as a secondary pharmacodynamic endpoint in a large phase one clinical trial of armodafinil in healthy subjects undergoing acute sleep deprivation. In that trial, the PVT was applied every 2 hours starting at approximately 1.5 hours post-dose. The investigators started to evidence a significant armodafinil effect (as measured by the RT and lapses of performance) at the second time point what is consistent with our results. Likewise, modafinil improved the reaction time of OSAHS\textsuperscript{79} and SWSD\textsuperscript{80} patients as well as sleep deprived healthy subjects.\textsuperscript{81} Moreover, we observed that two doses of armodafinil were not significantly different from each other in respect to both PVT metrics. Consistently, the FDA concluded that no consistent increase in armodafinil effect was observed between the different doses tested in OSAHS, narcolepsy and SWSD patient populations.\textsuperscript{62} The Agency further stated that the high dose of 250 mg once a day was accepted since there was no safety concern.

**Go/No-Go Association Task**

The GNAT is a well-known measure of impulsive behavior.\textsuperscript{56,63-67} The capacity to inhibit an irrelevant response is a fundamental component of cognitive tasks.\textsuperscript{82} Deficit in response inhibition have been associated with several conditions such as sleep deprivation and attention deficit and hyperactivity disorder (ADHD).\textsuperscript{82-85} Indeed, a study comparing adult patients with narcolepsy, idiopathic hypersomnia and ADHD demonstrated a high percentage of overlapping symptoms which may lead to misdiagnosing;\textsuperscript{86} in particular, the inattention score, as measured by the ADHD Rating Scale, was significantly correlated with excessive daytime sleepiness, as measured by the Epworth Sleepiness Scale. A systematic review suggested that children with ADHD had higher daytime sleepiness and more “disturbed sleep” compared with controls.\textsuperscript{78} In
another study including 2,463 children 6-15 years old, sleep-related problems (e.g., dyssomnia, parasomnia, daytime inadvertent napping) were found to be correlated with ADHD-like symptoms (e.g., inattention, hyperactivity and impulsivity). Despite of the overlapping symptoms of sleep disorders and ADHD, further studies are required to establish their pathophysiological relationship.

Armodafinil increased the GNAT performance as measured by mean 1/RT of go trials, number of errors of omission (go trials) and errors of commission (no-go trials). Subjects not only responded faster to the appearance of the go-stimuli but also improved their response accuracy (i.e., fewer errors of commission and omission). A significant treatment versus time interaction was observed for all GNAT metrics reflecting the important change in performance with treatment. A fatigue baseline adjustment was also performed to account for the fact that the subjects did not respond to the sleep deprivation period in an identical manner previous each treatment visit. Likewise evidenced in the PVT, we observed that two doses of armodafinil were not significantly different from each other in respect to GNAT metrics. Response inhibition tasks have been applied to study the effect of stimulant drugs in children with ADHD. Methylphenidate, a classical stimulant drug, was able to improve the GNAT performance by reducing the number of failures to inhibit a response in the no-go trials (errors of commission). In another response inhibition task, ADHD patients demonstrated a greater ability to inhibit response when compared to placebo. Indeed, several studies proposed modafinil as an effective therapy in the treatment of ADHD. Children and adults with ADHD demonstrated reduced inattention, hyperactivity and impulsivity when treated with modafinil.
GNAT also brings foundation to the ERP analysis, since it has been previously applied to investigate the effect of response inhibition and production on ERP studies.\(^{63-65}\) This topic will be further discussed in the next section.

**Event-Related Brain Activity**

ERP is an increase in the brain activity due to a specific event which can be an internal or external stimulus.\(^{44}\) In the present study, the event corresponds to the external visual stimuli presented during the execution of the neurocognitive assays (PVT and GNAT). Analyses of the ERP during execution of the PVT and GNAT have been performed. Armodafinil effect has been more evident in the Cz channel where the amplitude of the positive ERP peak at around 380 ms was increased when compared to the placebo group. Overall, no significant treatment versus time interaction and fatigue baseline difference were observed; the exception was seen in the no-go condition of the GNAT where significantly different fatigue baseline values of ERP amplitude across the different treatments were observed. The overall non-significance of fatigue baseline values of ERP amplitude suggests that the effect of sleep deprivation on this measure has a lower within-subject in respect to behavioral measures where fatigue baseline was always significantly different. Importantly, we conducted selective averaging in that only correct response trials were averaged using a stimulus-locked average approach; hence, error-related interference was prevented.

The GNAT has been applied to study the effect of response inhibition and production on ERP with most of the studies using visual stimuli.\(^{63-65}\) By using visual letter and symbol stimuli, it has been found a P3 with maximum at Pz in go trials, and similar amplitude at Cz and Pz in no-go trials.\(^{93}\) A response inhibition task was used to investigate the effect of methylphenidate on inhibitory control in children with attention
deficit/hyperactivity disorder (ADHD). The no-go P3 mean amplitude was increased by methylphenidate when compared to placebo. This results is consistent with our findings and with the fact that modafinil showed to improve the symptoms of ADHD in children and adults. In an ERP study using a visual odd-ball paradigm in patients with narcolepsy, the latency of the positive peak around 300 ms (P3) was able to differentiate between responders and non-responders to modafinil therapy.

**Pharmacokinetics**

The observed monophasic decline of the armodafinil concentrations is consistent with a pooled pharmacokinetic analysis from three randomized studies on armodafinil. The estimates of armodafinil clearance and volume of distribution are similar to the mean values reported in the literature (2.32 L/h and 42.4 L, respectively). The delayed appearance of armodafinil in the systemic circulation (i.e., lag time) can be attributed to the fact that the subjects received the study drug after breakfast; indeed, it has been reported that food affects the rate of absorption of armodafinil. Considering armodafinil neutral character (negative logarithm of the acid dissociation constant, pKₐ, 19.25), this decrease in the rate of armodafinil absorption with food could be attributed to the altered gastric emptying rate instead of a pH-dependent dissolution. It is coherent with the fact that the dissolution profiles of armodafinil tablets were basically superimposed in media of different pH (i.e., 2.0, 6.4 and 7.4); in those, a complete release of the active content was evidenced in approximately 15 minutes.

**Pharmacokinetic-Pharmacodynamic Modeling**

In the present study, behavioral and EEG-based measures of armodafinil effect were correlated with the apparent drug concentrations at the site of action by an effect compartment approach. The rate constant for the drug elimination from the hypothetical
effect compartment, $k_{e0}$, characterizes the temporal component of the equilibration between drug concentration and effect. Based on the established PK-PD models, the population estimates for $k_{e0}$ ranged from 0.5 to 1.1 h$^{-1}$ implying an average half-time of equilibration between armodafinil plasma concentration and effect of around 1 hour. The limitation of the effect compartment approach, though, resides in its inability to identify which of the possible factors would be the rate-limiting step in the equilibration process between drug concentration and effect; namely, tissue perfusion, drug diffusion from capillaries to the tissue, tissue:blood partition coefficient for the drug, or post-receptor events; in this case, we are referring to the tissue where the site of action belongs to.

At least two mechanisms could be proposed to justify the disequilibrium between armodafinil plasma concentration and effect. First, armodafinil was demonstrated to be a substrate of the P-glycoprotein through a permeability in vitro assay (Madin-Darby canine kidney cells transfected with the human MDR1 gene, MDR-MDCK). The P-glycoprotein, a well-known efflux transporter, plays an important role in limiting the cellular uptake of drugs not only from the intestinal lumen into the epithelial cells, but also from the systemic circulation into the brain. Interestingly, the MDR-MDCK permeability assay has been proposed as a blood-brain barrier permeability model. Second, despite of the fact that armodafinil mechanism of action is not well-understood, modafinil studies have suggested that modafinil modulates the hypocretin system by causing activation of hypocretin-secreting neurons, which, in turn, stimulate glutaminergic and histaminergic systems leading to arousal. Therefore, the hypothesis that such a sequence of events would constitute the rate-limiting step for armodafinil onset of effect cannot be ruled out.
To our knowledge, there is only one PK-PD study of armodafinil/modafinil in the literature. That study was conducted by armodafinil sponsor (Cephalon®) and consisted of a pooled analysis of two randomized, double-blind, placebo-controlled, multiple dose trials in 463 SWSD patients treated for 12 weeks with modafinil or armodafinil. The PD measure, however, was a 20-minute MSLT which corresponded to the primary clinical endpoint in the SWSD clinical trials. Under the assumption that modafinil and armodafinil are equipotent, the authors estimated an EC$_{50}$ of 4.6 $\mu$g mL$^{-1}$ for both drugs. Interestingly, in the present study, we estimated a similar EC$_{50}$ of 4.1 $\mu$g mL$^{-1}$ using PVT as our “gold standard” measure of alertness in sleep deprived healthy subjects receiving a single dose of armodafinil. This observation could help substantiate (a) the utility of our model of total acute sleep deprivation in mimicking a chronic condition; (b) the sensitivity of the PVT to sleep loss; (c) our single dose study as a good predictor of a multiple dose condition.

In this study, we developed PK-PD models for five different pharmacodynamic measures. Based on their expected clinical relevance, we can categorize them in (a) alertness- and (b) impulsive behavior-related models. The first category includes the models for PVT (i.e., a behavioral measure), event-related brain activity during PVT, and EEG spectral analyses (i.e., the EEG-based measures). The second category includes the models for GNAT (i.e., a behavioral measure) and event-related brain activity during GNAT (i.e., the EEG-based measures). There is no clear trend in the EC$_{50}$ values when comparing behavioral and EEG-based measures. Focusing on the alertness-related models, the EC$_{50}$ estimates were relatively close between the model for PVT and brain activity (EC$_{50}$ 4.1 vs. 5.3 $\mu$g mL$^{-1}$, respectively) in opposed to the one
for spectral analysis ($EC_{e50} \ 2.2 \mu g \ mL^{-1}$). Focusing on the impulsive behavior-related models, the $EC_{e50}$ estimates was higher in the model for GNAT than brain activity (7.6 vs. 5.7 $\mu g \ mL^{-1}$, respectively). In this scenario where we have used an empirical model to describe armodafinil effect, we would like to raise a hypothesis to explain these differences in the $EC_{e50}$ estimates. The concept of a translation mechanism between drug-receptor interaction (i.e., occupancy) and drug response, the so called stimulus-response relationship, has been originally proposed to explain why two agonists with similar a receptor occupancy profile could lead to significantly different responses.\textsuperscript{100,101} This concept was later applied to characterize the stimulus-response relationship (or transducer function) for GABA\textsubscript{A} receptor agonists.\textsuperscript{70,102} In this context, we could not rule out the presence of different transducer functions translating the stimulus – produced by the drug-receptor interaction – to the different measure of response.

**Study Limitations**

The present study has some limitations that need to be considered. First, we conducted the study in a relatively small sample size. We made efforts to increase the the power by designing a placebo-controlled cross-over study. Second, our subjects underwent a total of 36 hours sleep deprivation in the context of a single dose study. In respect to the EEG spectral analysis, it could explain the absence of a significant armodafinil effect in the theta frequency range, a phenomenon observed in modafinil studies where the subjects underwent longer sleep deprivation periods and multiple armodafinil administrations. Third, it was not always possible to avoid transition periods between wakefulness and sleep during the eyes-closed sessions; however, we sought to include epochs that well-represented each recording session. Finally, the effect
compartment approach consists of an empirical model and does not allow us to identify which of the possible factors is the rate-limiting step in the equilibration process between drug concentration and effect. Nevertheless, we believe that the use of a mechanistic model to describe the armodafinil effect is still premature given that its mechanism of action is not well-understood.

**Study Strengths**

Besides the innovative and significant aspects of this study, we can highlight some other following strengths. First, we conducted a placebo-controlled cross-over study which increased the power and allowed us to separate the placebo effect or time-varying baseline. Second, a rich data collection was performed with simultaneous assessment of pharmacokinetics and pharmacodynamics yielding a more accurate description of armodafinil effect over time. Third, EEG metrics were correlated not only with drug concentrations but also with behavioral measures. The latter, including a well-established measure of alertness, suggested the clinical relevance of EEG as a measure of alertness. Finally, the similarity between the EC$_{50}$ estimates for alertness (PVT and MSLT models, respectively) obtained in our study and in a pooled analysis of two phase-3 clinical trials studies on modafinil/armodafinil could advocate for the clinical utility of our model: (a) our model of total acute sleep deprivation seemed to mimic a chronic condition reasonably well; (b) the single dose study seemed to be a reasonable predictor of a multiple dose condition.

**Conclusions**

Armodafinil mitigated the neurobehavioral degradation due to sleep loss. The correlation between armodafinil-related changes on EEG and alertness suggests EEG as a potential biomarker of armodafinil effect. Ultimately, it may guide dosage selection
in phase 3 clinical trials pursuing new indications for armodafinil and expedite the development of novel compounds with an analogous mechanism of action.
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BIOGRAPHICAL SKETCH

Daniela Joice Conrado was born in Vacaria (Rio Grande do Sul, Brazil). She received her high school diploma in 1997 focusing on analytical chemistry. In 1999, she got into the College of Pharmacy Federal University of Rio Grande do Sul (Porto Alegre, Rio Grande do Sul, Brazil) receiving her Bachelor in Science degree in 2003. Her final undergraduate project was a scientific review on the issues involving pharmacokinetic of antimicrobial agents in burn patients. In 2004, she joined a Master of Science program in the same aforementioned university conducting a research work on “Preclinical Pharmacokinetic Evaluation of LASSBio-579: An N-phenylpiperazine Antipsychotic Prototype”. In 2006, she received a Master of Science degree as well as a diploma of specialist in industrial pharmacy by the same university. Then, she worked as a lecturer in the Federal University of Rio Grande do Sul (2006-2007) and Integrated Regional University of Alto Uruguai and Missões (Erechim, Rio Grande do Sul, Brazil; 2007-2008); the main courses were pharmacokinetics, pharmacodynamics and pharmaceutical practice and cosmetology. In 2009, Daniela joined Professor Hartmut Derendorf’s research group at the Department of Pharmaceutics, College of Pharmacy University of Florida (Gainesville, Florida). She focused on quantitative electroencephalography as a means to assess a wakefulness-promoting effect.