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RESIDUAL NEXT-DAY EFFECTS OF ALPRAZOLAM ON PSYCHOMOTOR PERFORMANCE AND SIMULATED DRIVING IN HEALTHY NORMAL VOLUNTEERS

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DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Medicine
at the University of Kentucky

By
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Lexington, Kentucky
2019

ABSTRACT OF DISSERTATION

RESIDUAL NEXT-DAY EFFECTS OF ALPRAZOLAM ON PSYCHOMOTOR PERFORMANCE AND SIMULATED DRIVING IN HEALTHY NORMAL VOLUNTEERS

The prevalence of drugged driving has increased in the United States, and some prescription medications (e.g., zolpidem) cause impairment after the predicted duration of therapeutic action has elapsed. The aim of this study is to determine if bedtime administration of alprazolam similarly impacts driving performance the following day.

Volunteers were 14 healthy adults (6 males) who completed a double-blind, double-dummy within-subjects design study examining the effects of alprazolam (0.5, 1, & 2mg), zolpidem (10mg), and placebo administered at bedtime on driving performance the following day. The positive control condition was alprazolam (1mg) administered on the test morning. Driving simulator measures, cognitive and psychomotor tasks, and questionnaires querying drug effects were collected the afternoon before drug administration and for 5.5 hours the next day and analyzed using symmetry and mixed-model approaches. The positive control was robustly impairing. Driving impairment equivalent to that seen with alcohol at the legal limit was observed up to 12.5hr after bedtime alprazolam 2mg and for 8.5hr after bedtime zolpidem 10mg. Volunteers were not fully aware of their own level of impairment. These results suggest that alprazolam used before bed may pose an as yet unrecognized public safety risk in the form of next-day drugged-driving.

KEYWORDS: Alprazolam, Driving Impairment, Benzodiazepines, Zolpidem, Sex Differences

Marion A. Coe

February 1, 2019

Date

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CHAPTER I. INTRODUCTION

PART I. Driving Under the Influence of Alcohol and/or Drugs (DUIAD)

Traffic accidents are a leading cause of unintentional death in the United States, second only to unintentional poisonings (Murphy et al. 2017). Approximately 40,000 persons died as a result of a motor vehicle accident in 2017 (NSC 2018). Unfortunately, traffic fatality rates have been increasing since 2014, even after accounting for population growth and increased vehicle miles driven annually (see Table 1.1). Many factors contribute to traffic accidents and resultant deaths (e.g., speeding, bad weather, use of a seatbelt, etc.), but one consistently significant predictor of traffic fatality risk is driver impairment; more specifically, impairment due to use of alcohol and/or drugs. While the number of deaths associated with drunk driving has decreased over the past 10 years (Berning et al. 2015), the frequency of drugged driving (i.e., driving under the influence of a potentially impairing medication or drug) has increased. These trends are consistent across self-report surveys (i.e., National Survey on Drug Use and Health), voluntary roadside drug tests (i.e., National Roadside Survey), and drug test results from drivers involved in fatal accidents recorded in the National Highway Traffic Safety Administration's (NHTSA) federal registry (Fatality Analysis Reporting System). The increase in drugged driving frequency and associated deaths represents a nascent target for public health and safety efforts to prevent further morbidity and mortality. Consequently, both the White House Office of Drug Control Policy and the National Institute on Drug Abuse have identified drugged driving as a research priority (NIDA 2014, ONDCP 2014).

Prevalence

According to the National Survey on Drug Use and Health (NSDUH), an annual nationwide survey of persons aged 12 and older, in 2014, 27.7 million people aged 16 or older (11.1 percent) drove under the influence of alcohol in the past year. The percentage of people aged 16 or older who drove under the influence of alcohol in 2014 was lower than the percentages in 2002 through 2012 (ranging from 11.8 to 15.3 percent). Approximately 10.5 million people over the age of 16

drove under the influence of illicit drugs in the past year in 2005 (SAMHSA 2006) and 11.8 million people drove under the influence of illicit drugs in 2016 (SAMHSA 2017). The 2016 number includes 10.9 million persons who report use of marijuana (even in states where it had been legalized for recreational use [i.e., Colorado, Washington, Oregon, Alaska] before 2016; the year of the most recent survey wave). The survey did not query behavior related to driving under the influence of potentially impairing prescription drugs; nevertheless, the results are demonstrative of an increased pervasiveness of drug-induced impaired driving.

The National Roadside Survey, conducted in 1973, 1986, 1996, 2007, and most recently in 2014, collects information from ~11,000 drivers who voluntarily stop at 300 research sites around the U.S. Road signs alert drivers to a voluntary paid survey ahead. The drivers answer questions about their driving habits, health, and can choose to provide biological samples (e.g., breath, saliva, urine) for alcohol and/or drug testing. Results are completely anonymous. Testing of urine and/or saliva for illegal drugs, prescription medicines, and over-the-counter drugs was conducted in the most recent two survey waves (2007 and 2014) only. Since the first NRS was conducted in 1973, the proportion of drivers with measurable alcohol levels has declined by nearly 80%, and in the most recent survey wave, there were 8.3% of drivers with some measurable alcohol level (Kahn 2015). In contrast, approximately 20% of drivers tested positive for at least one potentially impairing drug (illicit or prescription) in 2014 (Berning et al. 2015), up from 16.3% in 2007 (Lacey et al. 2009). Because the survey is voluntary, these numbers may be an underrepresentation of the percentage of drivers who would test positive for alcohol and/or drugs in a truly random sample, as impaired drivers (or those who believe they would test positive) may choose to not participate out of fear of legal repercussions. These survey results (indicative of falling drunk driving and rising drugged driving frequency) are substantiated by a rising prevalence of positive drug test results obtained from drivers involved in fatal accidents.

DUIAD as a Causal Factor in Traffic Fatalities

Fatality Analysis Reporting System Statistics: Since 1975, the National Highway Traffic Safety Administration (NHTSA) has maintained the Fatality Analysis Reporting System (FARS), a federal registry with data on traffic crashes resulting in a death (either of the vehicle occupant or a non-occupant such as pedestrian) within 30 days of the crash. The database allows for imputation of over 100 coded variables describing the driver (e.g., age, race, previous DUIs, license state, drug test results), vehicle (e.g., make, model, year, previous damage, impact points), pre-crash conditions (e.g., weather, time of day, road type, location), occupant (e.g., seating position, seatbelt use, injury severity), and non-occupants (e.g., age, sex, alcohol and drug involvement). Once collated, NHTSA provides these data to the public.

Data in Tables 1.2 through 1.6 were derived from the FARS interface (NHTSA), and detailed query terms used for each outcome are documented in Appendix 1. Tables 1.2 and 1.4 describe alcohol and drug test statistics for all fatally injured drivers who tested positive for alcohol or drugs at autopsy. Statistics from this group are those typically described in briefings and government documents regarding drunk and drugged driving. Also relevant are all the drivers involved in a fatal accident—whether the death was the driver themselves or another person (pedestrian, passenger, etc.). These numbers reflect all traffic deaths possibly attributable to impaired driving, and the alcohol and drug testing statistics for this group are displayed in Tables 1.3 and 1.5. The trends are similar for both groups (drivers involved in fatal accidents and fatally injured drivers) across the 10 most recent years for which data are available (2007 – 2016). The total number of driver deaths was lowest in 2011 and has increased since that time—8,100 more driver deaths occurred in 2016 than 2011.

The number of drivers involved in fatal accidents who were tested for alcohol remained steady at ~49% from 2007 to 2015 but dropped precipitously to ~40% in 2016. Fatally injured drivers

were tested for alcohol ~75% of the time up until 2016 when the percentage dropped to ~61. The absolute number of drivers (either involved or fatally injured) testing positive for alcohol are generally decreasing, and this is not likely a function of the decrease in the number of drivers tested for alcohol as the percentage testing positive is also generally decreasing. The percentage of drivers involved in fatal accidents and those fatally injured who are tested for drugs remained steady from 2007 to 2015, but (as with alcohol testing) dropped precipitously in 2016, so that only about a third of drivers involved in a fatal accident are tested for drugs. Unlike the alcohol results, there has been a general increase in the number of drivers testing positive for drugs. In fact, in 2011, a higher percentage of drivers tested positive for drugs than alcohol and this has been the case for every year since. Fatally injured drivers are more likely to be tested for alcohol and drugs than drivers involved in a fatal accident; though the percentage testing positive for drugs and alcohol in both groups are similar.

Challenges in data collection and interpretation: Estimating the number of deaths attributed to drugged driving is methodologically challenging for several reasons. First, not all drivers involved in fatal accidents are tested for drugs (and those who are drug tested do not always have results available in the federal registry). The percentage of fatally injured drivers who are tested for drugs has remained relatively stable for the past decade, except for a marked drop in 2016 (see Table 1.4). Second, states use different methods of drug testing both in surviving drivers and during the autopsy of fatally injured drivers; there is not a consistent policy governing when or how drug testing must proceed. As such, there is considerable variation in who is tested, which drugs are tested for, the type of test/equipment that is used, the sensitivity of the tests that are used, and which biological specimen (blood, urine, or saliva) is tested. Furthermore, if alcohol is present (as is the case for ~ 23 – 30% of fatally injured drivers per year), the driver is less likely to be also tested for drugs because state laws often fail to distinguish between alcohol and drug impairment. An alcohol test is easy and cheap compared to a drug test; particularly if the drug

assay used is sophisticated enough to determine drug concentrations (e.g., GCMS). Finally, a causal relationship between any detected drugs and the fatal crash cannot be definitely established for several reasons.

The presence of a drug in a person's blood is not a guarantee of impairment. Some drugs, like marijuana and diazepam, can be detected in the blood for weeks after their use (long after the impairing effects would have dissipated). This is particularly relevant because in 2015, over one-third of the fatally injured drivers who were tested for drugs during autopsy tested positive for marijuana. For impairing drugs with shorter windows of detection or with half-lives that more closely mirror duration of action, there is perhaps a greater possibility of impairment at the time of a positive test; however, factors such as drug tolerance and individual differences in drug response still make assumptions of impairment based on positive drug test results tenuous. There are multiple types of tolerance which can develop in response to repeated exposure to alcohol or drugs, and all can affect the level of impairment caused by ingestion of the substance. As an example, functional tolerance manifests in chronic heavy drinkers when they show few overt signs of intoxication (e.g., slurred speech, increased body sway, etc.) even at blood alcohol concentrations that would be incapacitating or even fatal in those who drink less frequently (Lipscomb and Nathan 1980, Bennett et al. 1993). Environment-dependent tolerance occurs when a substance is administered in the same environment or context (e.g., at a bar). Rats repeatedly administered alcohol in one room and placebo in a different room exhibited tolerance to the sedative and body temperature-lowering effects of alcohol only in the alcohol-specific environment (Mansfield and Cunningham 1980). A similar procedure used in humans revealed similar results: alcohol-induced heart rate elevations were blunted in the alcohol-paired room compared to a novel room (Dafters and Anderson 1982). Similar tolerance was observed for social drinkers performing simple psychomotor tasks in a bar-like environment compared to an office. Performance was better in the bar than the office (McCusker and Brown 1990). Learned

tolerance refers to when a person displays improved task performance while intoxicated after practicing that task repeatedly while under the influence of that impairing substance. Importantly, the learning is state dependent, so practicing the task while sober does not help as much as practicing while under the influence (Sdao-Jarvie and Vogel-Sprott 1992). Environment-dependent and learned tolerance have important implications for driving while under the influence of drugs and/or alcohol. While practicing a task (e.g., driving) under the influence of an impairing substance can lead to task improvement, it is crucially important to note that the tolerance developed is context specific and does not apply to novel situations such as a new route, a deer running in front of the car, or bad weather (Siegel and Sdao-Jarvie 1986).

Despite the limitations inherent to survey and toxicology data, what can be said with some degree of certainty is that: 1) the percentage of persons self-disclosing drugged driving and/or drivers testing positive for drugs and 2) the percentage of drivers involved in and those killed in traffic accidents who test positive for at least one drug (illicit or prescription) are both increasing.

The Role of Prescription Drug Use in DUIAD and Traffic Fatalities

Drugged driving can occur after use of illicit drugs such as methamphetamine, cocaine, and heroin but also after use of prescription drugs such as opioids or benzodiazepines. Almost 20% of drivers queried about prescription drug use as part of the NRS (Berning et al. 2015) disclosed use within the past 2 days, with the most common drug classes reported as: sedatives including sleep aids and benzodiazepines (8.0%), followed by antidepressants (7.7%), narcotics including opiates (7.5%), and stimulants (3.9%). 78.2% reported that the drug was prescribed by a doctor for their use. Of course, these prescription drugs may cause impairment even when taken as prescribed by a doctor. While medication labels caution patients not to drive or operate machinery until they know how the drug will affect them, it is well established that people are

poor judges of their own level of impairment (Brumback et al. 2007, Echizenya et al. 2007). For drugs that may cause impairment the day after ingestion, there may be even greater risk that the person will be unaware of their own degree of impairment. The most common prescription medications detected during autopsy of fatally injured drivers for the past 10 years are listed in Table 1.4. Notably, alprazolam is in the top three most commonly detected drugs in fatally injured drivers for 8 of the past 10 years. In some cases, patients may be unaware of their own degree of impairment after use of a prescription medication, and the impairment can be significant. In the case of zolpidem, a sleeping medication, the impairment caused by its use was of significant scope and severity that the FDA required a labeling change to reduce starting doses for women (FDA 2013a) in 2013.

Zolpidem: Zolpidem is an imidazopyridine non-benzodiazepine hypnotic indicated for short-term treatment of insomnia; specifically, for insomnia characterized by difficulty falling asleep. It was approved by the FDA in 1992, and a controlled-release formulations was later approved for insomnia characterized by difficulty staying asleep (Sanofi-Aventis 2005). At the time of approval, the adult starting dose for immediate-release (IR) zolpidem was 10mg. Zolpidem is structurally similar to the benzodiazepines (see Figure1.1) and binds to the benzodiazepine site on $GABA_A$ receptors, but zolpidem exerts mostly hypnotic effects (as opposed to benzodiazepines which have hypnotic, anxiolytic, amnesiac, and anticonvulsant effects). It has specificity for the $\alpha 1$ subunit-containing $GABA_A$ receptors, low affinity for $\alpha 2$ and $\alpha 3$ subunit-containing receptors, and negligible affinity for the $\alpha 5$ subunit-containing receptors (Crestani et al. 2000). This subunit specificity is thought to be the reason that zolpidem has poor anxiolytic and anticonvulsant properties compared to the benzodiazepines which have equal affinity for all receptor subtypes (Hanson et al. 2008). Zolpidem is administered immediately before bed, and causes significant sedation as well as cognitive, psychomotor, and memory impairment within 15min of administration. The immediate-release (IR) zolpidem formulation has a reported half-life of 2-

3hr (Sanofi-Aventis 2014), and the duration of action was originally reported to be 3hr; meaning that persons using zolpidem before bed and getting a full night of sleep should no longer exhibit impairment upon waking. Post-marketing surveillance revealed that this was not the case. From 2007 to 2013, the FDA received approximately 700 reports, submitted to the Adverse Event Reporting System, of driving impairment and traffic accidents linked to previous day use of zolpidem (FDA 2013b).

These reports were substantiated by driving simulation studies and pharmacokinetic analyses conducted the morning after zolpidem administration. Leufkens and colleagues performed psychomotor performance evaluations using an on-the-road driving task and several classical cognitive and psychomotor tasks to assess impairment the morning after administration of multiple hypnotic agents, including zolpidem. They report that 10 – 11hr after 10mg zolpidem administration, driving performance was significantly impaired. The next-day impairment was not limited to driving: zolpidem administration also caused significant impairment in the critical tracking test, the divided attention task, the Digit Symbol Substitution Test, as well as significant increases in body sway compared to placebo (Leufkens et al. 2009). Although the studies that the FDA used to link zolpidem blood concentrations to impairment levels are not publically available, press releases state that 15% of women and 3% of men had blood concentrations above 50 ng/mL (the concentration determined by the FDA to correlate with significant impairment) 8hr after administration of 10mg IR zolpidem products (FDA 2013c). The percentage of persons exceeding the 50ng/mL threshold 8hr after ingestion of CR zolpidem products was 25% and 33% for men and women, respectively (FDA 2013c) but the vast majority (i.e., 90%) of zolpidem prescriptions dispensed are for the IR formulations. With ~40 million zolpidem prescriptions dispensed in 2011 , a conservative estimate of the possible number of impaired drivers on the road that year exceeds 400,000 (assuming all prescriptions were for 12 months and an equal gender split in prescribing), presenting a serious threat to public safety. Importantly, body weight

did not completely account for the sex differences in pharmacokinetics (FDA 2013c), which are thought to be due in part to reduced clearance processes in women compared to men. In a study comparing IR zolpidem pharmacokinetic parameters between men and women in Asian populations (Guo et al. 2014), maximum plasma concentration (male: 169.11 μg/L vs. female: 217.83 μg/L), half-life (male: 0.94 hr vs. female: 0.79 hr), volume of distribution (male: 66.59 L vs. female: 47.50 L), clearance (male: 27.51 L/hr vs. female: 13.58 L/hr), and area-under-the-curve (male: 507.86 μg*hr/L vs. female: 784.20 μg*hr/L) parameters were all significantly different between sexes after controlling for weight. The direction of these pharmacokinetic differences all support the increased duration of impairment experienced by women than men.

From 1992 to 2013, the recommended dose of IR zolpidem for all adults was 10mg to be taken before bedtime. Based on driving and pharmacokinetic data, the FDA required labeling changes to all IR zolpidem-containing products to reduce the starting dose for women to 5mg in 2013. No changes were made to the CR zolpidem product doses. In addition to the new IR dosing recommendations, zolpidem-containing drug labels now have stronger warnings about the risks of next-day impairment. Specifically, driving or engaging in any task requiring mental alertness is cautioned against if: zolpidem is taken with less than a full night's sleep remaining; if a higher than recommended dose is taken; if co-administered with other CNS depressants; or if co-administered with drugs that could increase blood levels of zolpidem. These include CYP3A4 inhibitors such as ketoconazole, amiodarone, and fluconazole. Patients should be cautioned that they might be impaired even if they feel awake. There is a need for accurate and thorough labeling to inform patients and prescribers of potential risks, side effects, and duration of possible impairment for each medication. Medications with characteristics similar to zolpidem may also cause previously unknown cognitive or psychomotor impairment the morning after ingestion, and may present dangers to public health and safety.

PART II. Legislation and Measurement of Impaired Driving

It is illegal in the United States to drive under the influence of impairing amounts of alcohol or other drugs, but defining "impairing amounts" of drugs (including prescription and over-the-counter medications) presents a unique challenge compared to such a definition for alcohol. Nevertheless, it is important to understand how and why the current drunk driving laws were instituted so that law enforcement, legislators, and other stakeholders can learn from, and improve on, the current system.

Historical Context and Evidence Supporting Current Drunk Driving Legislation

Breath alcohol can be quickly, easily, and inexpensively measured at the scene of an accident or traffic stop, allowing law enforcement to ascertain whether a driver has a BAC at or above the established illegal level of .08%. Importantly, it illegal *in and of itself* to operate a motor vehicle with an alcohol concentration measured at or above the established illegal level, regardless of whether or not the driver exhibits visible signs of intoxication. This .08% BAC *per se* limit was not accepted by all states until 2004. Before that time, laws varied between states; and as recently as 2001, the states were evenly split between establishing the legal limit as .08% or .10% BAC. Ultimate adoption of the .08% limit by all states was largely due to financial incentives from the federal government; the Department of Transportation's 2001 Appropriations Act (HR4475) provided that states must pass a .08% *per se* law by 2004 or begin losing federal highway construction funds (2% penalty, with the penalty increasing by 2% each year, until reaching 8%; representing a significant amount of funding – as an illustrative example, the last state to adopt this policy, Delaware, would have forfeited ~3.3 million dollars in 2004).

The support for establishment of a .08% BAC *per se* legal limit originated from laboratory and epidemiological evidence. A review of 112 laboratory studies determined that at .08 BAC,

virtually all (94%) persons exhibit substantial impairment in driving-related skills such as braking, steering, lane changing, judgment and divided attention (Moskowitz and Fiorenintо 2000). An analysis of traffic fatalities in five states (CA, ME, OR, UT, VT) before and for at least two years after implementation of state-mandated .08% BAC per se laws reported that there were significant decreases in alcohol related crashes (ranging from a 4 percent decrease in the level of driver involvement in alcohol related fatal crashes at .10% BAC to a 40% decrease in the level of driver involvement in fatal crashes estimated to be alcohol involved) after adoption of the .08% BAC limit (Johnson and Fell 1994). Likewise, a review of seven retrospective case-control studies comparing BAC between drivers involved in crashes and those not involved in crashes concluded that there was some increase in relative crash risk between 0.00% and 0.05% (the estimates of relative risk for drivers with a positive BAC less than .05% are less than or equal to 1.5 times the risk of drivers with a 0.00% BAC), followed by a more rapid increase in relative risk around 0.08% to 0.10% (between 2 and 20 times the risk of drivers with a 0.00% BAC) (NHTSA 1991). Based on epidemiological and experimental evidence, a blood alcohol concentration above 0.08% is considered to be significantly impairing, and it is therefore illegal to drive with concentrations exceeding 0.08%. For drugs and medications, the evidence linking blood concentrations to crash risk are less robust.

Implementing Drugged Driving Legislation

Defining a blood concentration at which there is a reasonable expectation of impairment for every drug and medication is both impracticable and of questionable utility. There have been three general strategies employed for drugged driving legislation: zero-tolerance policies, defining legal limits for blood concentration of certain drugs, and no legal limit (but law enforcement may test a driver suspected of drug-induced impairment and charge them with a DUI if the result is positive). The zero-tolerance policy enacted by some countries (e.g., Italy) and 16 states

mandates that driving with any detectable trace of a designated impairing substance (including opioids and benzodiazepines) is against the law; meaning that a positive drug test for an illegal drug such as marijuana or a prescription drug such as oxycodone would have similar legal repercussions for the driver, such as fines, license revocation, and/or jail time (Ferreira 2007). In places that have imposed legal limits for specific drugs based on concentrations estimated to be impairing, there is not a consensus threshold. Even for marijuana—the most commonly used impairing substance after alcohol—the laws vary widely between states (GHSA 2017). Montana and Washington have per se limits for THC concentrations above 5ng/mL, and Colorado law considers there to be a reasonable inference of impairment at THC concentrations above 5ng/mL. The limits are different for Nevada, Ohio (2ng/mL) and Pennsylvania (1ng/mL). Moreover, laws vary on legal limits of metabolites. The third strategy, employed by 28 states, relies on a driver's judgement to not drive if impaired. Individual tolerance (see above section for details) can vary significantly from one person to the next (e.g., after use of a therapeutic dose of oxycodone [30mg], a person who chronically uses opioids at or above that dose would almost certainly be less impaired than a person who has used opioids only a few times). If a law enforcement officer suspects a driver of impairment, a breath alcohol test and/or field sobriety test is typically administered, and law enforcement officials may also require a drug test in cases of suspected drug-related impaired driving. If the test is positive for a drug, regardless of the concentration, the driver could be charged with driving under the influence.

Ideally, whatever legislative strategy is employed would be evidence-based. Accurate data are needed to determine which drugs cause impairment and the expected profile of that impairment. These data would support both legislative decisions and appropriate warnings on medication labels. However, determining which drugs cause impairment and fully characterizing the time-course and severity of that impairment is methodologically challenging. The first step in

determining which drugs impair driving would be to define what constitutes meaningful driving impairment.

Methods for Determining Meaningful Driving Impairment and Elevated Crash Risk after Use of a Potentially Impairing Substance

An expert panel of behavioral scientists, epidemiologists, pharmacologists, toxicologists, and traffic safety professionals was convened by NHTSA in 2008 and 2009 in order to determine if it was feasible to develop two lists: one list of medications or classes of medications that may impair driving and a second list of medications that do not impair driving (Kay and Logan 2011). These lists could be used to inform both prescribers and patients about the likelihood of impaired driving due to use of the constituent medications. The expert panel concluded that the absence of a standardized protocol for assessing the impairment potential of drugs was a serious barrier to categorization based on impairment risk. For the few drugs that had extensive behavioral testing completed, assessing driving impairment risk based on extant literature remained difficult due to the inconsistency in testing methodologies used. Factors that vary widely from one research protocol to another (even for evaluation of the same drug) included elements such as: dose range, interval between dosing and assessment, interaction between drug and condition being treated (i.e., study population made up of patients or non-patients), chronicity of use, and inclusion of appropriate positive and negative controls. Based on review of existing behavioral assessment protocols and scientific literature, the panel concluded that a tiered, parallel process involving pharmacological, toxicological, epidemiological reviews and a standardized behavioral assessment (based on the Essential Driving Ability Domains model) would provide the most comprehensive evaluation of driving impairment and risk. Notably, the FDA also recommends this tiered multi-modal approach to assessing driving impairment risk (FDA 2015).

The Essential Driving Ability Domains (EDAD) model suggests that the following five behavioral domains are relevant to driving ability: 1) alertness/arousal, 2) attention and processing speed, 3) reaction time/psychomotor functions, 4) sensory-perceptual functions, and 5) executive functions. Alertness/arousal refers to the person's degree of consciousness. Drowsiness (or lack of alertness/arousal) is associated with increased standard deviation of lane position and speed—two driving outcomes positively associated with crash risk (Verster and Mets 2009, Penning et al. 2010). The panel concluded that any drug causing impairment in performance in any of these five domains *at a magnitude known to be associated with increased crash risk* is presumed to have a negative impact on driving safety. As technology has evolved, so have the methods available for assessing driving performance.

Simple laboratory measures: Older measures of cognitive and psychomotor impairment include tasks such as the Digit Symbol Substitution Task, Choice Reaction Time, Critical Flicker Fusion Task, Balance Test, etc. These simple tasks are easy to administer, quick, and relatively inexpensive, but they can only capture one facet of behavior required by an action as complex as driving. Moreover, it is not clear how well each assessment (or even a battery of these assessments) maps on to driving ability. In one study comparing several psychometric tasks to on-the-road driving, it was reported that results from each of the psychometric tasks (tracking, divided attention, reaction time, Critical Flicker Fusion Test, memory, and tapping) individually had low to modest correlations ($r = 0.2$ to 0.4) to SDLP (Ramaekers 2003). Because each of these psychometric tests assesses separate skills and abilities (e.g., alertness, attention, reaction time, etc.) that are thought to be important to driving ability (ICADTS), the results from a combination of these tasks may provide greater correlation to SDLP. Verster and Roth analyzed results from a battery of seven psychometric tasks compared to SDLP and still found that the tasks together had a predictive validity of only 33.4% for on-the-road driving (Verster and Roth 2012a).

On-the-road driving task: On-the-road driving assessment is the gold standard methodology of driving impairment research as it almost exactly mimics the real world. The test is performed in normal traffic on a 100km stretch of a public highway. Because traffic is present, participants have to react to other cars, and unexpected circumstances and events (e.g., traffic jams, emergency stops, etc.). Participants are instructed to drive with a steady lateral position within the right lane and to maintain a constant speed of 95 km/hr. They are allowed to pass slower-moving vehicles, but otherwise are to maintain the indicated course and speed. A trained instructor with override pedals and steering is in the passenger seat in the event that the participant is too impaired to safely operate the vehicle. The car is outfitted with specialized machinery to track the lateral movement (i.e., "weaving") of the car, and the primary outcome measure is standard deviation of lane position (SDLP). These tests have superior external validity but are costly, time-consuming, and require specialized equipment and highly trained staff to administer. While no traffic accidents have occurred in the 30-year history of these experiments (Verster and Roth 2011), safety concerns may preclude the examination of extremely impairing doses of alcohol or drugs. As seen in Table 1.7, the highest dose of alcohol tested in these on-the-road driving studies is ~.10% BAC.

Driving simulator task: The third method that is commonly used to assess driving performance is with a computerized driving simulator, which obviates any safety concerns inherent to allowing potentially impaired persons driving a vehicle in traffic. This allows for testing of higher doses of alcohol and more impairing drugs than on-the-road driving tasks. There is a range of driving simulator systems available, ranging from simple, single-screen systems on one end of the spectrum to high fidelity and immersive systems on the other. The most basic systems consist of a single computer screen mounted directly in front of the volunteer, a gas and brake pedal and steering wheel. The most complex simulators (i.e., National Advanced Driving Simulator-1)

consist of an actual car mounted on a movable base to mimic turning, pitch, and vibration from different road surfaces. The car is housed within a dome, and 360-degree scenery is projected on the inside of the dome. While this complex system eliminates the safety concerns inherent to on-the-road driving assessment of drug-impaired participants, it is still quite costly and requires highly technically proficient staff for daily operations and maintenance. There are many other systems with intermediate complexity. The more immersive and realistic the system is, the more generalizable the results should be to real-world driving.

Use of a driving simulator allows for standardized and reproducible testing of the operational and tactical aspects of driving. Every detail of the driving experience is customizable: investigators can select road surface, visibility, traffic conditions, vehicle type, scenery, time of day, etc. Unlike on-the-road tests in which real-world weather and traffic conditions will vary, the driving simulator ensures that these (and thousands of other) variables are identical every time the driving task is completed. Of course, this customizability and range of options means that no two research groups have identical driving tasks. Simulators are capable of capturing thousands of data points per second; thus, the outcomes and possibilities for data analysis are almost endless. Some commonly used tasks and outcomes are described below.

Car following: In this task, participants are instructed to follow a lead car at a safe distance. The lead car can be programmed to randomly change speed (e.g., (Brookhuis et al. 1994)) or maintain a constant speed (e.g., Kenntner-Mabiabla et al. 2015). Outcome measures for this task include gap (distance between the front bumper of the volunteer's car and the rear bumper of the lead car), minimum time-to-collision, and coherence (i.e., the extent to which the patterns of speed changes of the lead- and following car are the same).

Gap acceptance: This task is used to measure risk-taking behavior (e.g., Fillmore et al. 2005; Fillmore et al. 2008). Though there are variations in task specifics; generally, the participant must make a turn into traffic that has the right-of-way. The time between each of the oncoming vehicles range from 1sec to 12sec, so the driver must consider the waiting time versus the risk of causing an accident. Shorter gap time is indicative of riskier behavior.

Incursion: This task assesses reaction time and alertness. A vehicle, pedestrian, or other object moves into the participant's lane, requiring them to either brake or steer to avoid a collision with the object. Outcomes include minimum time-to-collision and reaction time.

Speed tracking: It has been demonstrated that participants will vary speed in order to compensate for sedating effects or because perception of risk is decreased (e.g., (Brookhuis et al. 2004)). In an attempt to increase arousal, sedated participants will temporarily increase their speed. Over the course of a driving scenario, this results in a more variable standard deviation of speed compared to non-sedated drivers (Brookhuis 1998).

Lane tracking/Standard deviation of lane position (SDLP): For both on-the-road driving and computerized driving simulator tests, the primary outcome measure reported is almost exclusively SDLP (i.e., a measure of weaving). Larger SDLP is associated with increased crash risk (O'Hanlon 1984, Verster and Mets 2009, Penning et al. 2010). Table 1.5 displays the SDLP results from 16 cross-over design studies in which healthy volunteers received placebo and alcohol in different sessions. Results are displayed from studies using on-the-road driving and driving simulator tasks. Breath alcohol concentrations of 0.05% and 0.08% are considered meaningful benchmarks because 0.05% is the legal limit in most of the countries in the European Union and 0.08% BAC is the legal limit in the United States. Epidemiological data on the correlation between BAC and accident risk revealed that crash risk increases at a blood alcohol

concentration of 0.04% and exponentially rises above 0.10% (Borkenstein 1978). Drugs that cause an increase in SDLP equivalent to what is observed with a breath alcohol concentration of .05% or .08% could be considered significantly impairing.

Importantly, absolute SDLP values are not reliable predictors of impairment seen after drug or alcohol administration because individual differences in SDLP are large. Instead, calculating SDLP changes from placebo to the active condition is the preferred method of determining drug- or alcohol-induced impairment. This is the case because group-level SDLP changes are stable and independent of the magnitude of baseline or placebo SDLP values. For these reasons; when conducting a drugged driving study with either the on-the-road or driving simulator task, it is imperative that the study utilizes a within-subject, repeated measures design. Landmark work conducted by Louwerens and colleagues calibrated ascending BACs (group BAC means: 0.024%, 0.06%, 0.085%, and 0.122%) with changes in SDLP in 24 healthy volunteers participating in an on-the-road driving test. The group mean increases in SDLP from placebo to BACs .05% and .08% were 2.4 cm and 4.1 cm, respectively (Louwerens et al. 1987). These numbers have since been used as benchmarks of impairment in over 50 on-the-road driving studies, and the results have been corroborated in other on-the-road driving studies with placebo and similar alcohol administration conditions (see Table 1.7). Owens and Ramaekers found a very high correlation ($r = 0.99$) between SDLP increases at ascending BACs and epidemiological studies relating BAC and accident data (Owens and Ramaekers 2009). Although direct data relating change in SDLP to accidents are lacking, these correlative data support the utility of SDLP measurements in its relationship to crash risk.

Because on-the-road driving tests and driving simulator tasks are both used so frequently in drugged-driving research, it is important to compare SDLP outcomes between these two methods to ensure that the impairment benchmarks used for on-the-road driving tests are also well-suited

for simulator studies. While a review of the literature reveals no studies that compare SDLP outcomes derived from on-the-road driving studies and driving simulator tasks in a within-subject design, Table 1.7 lists the group-level SDLP changes across 16 studies (both on-the-road and simulator). From these studies, it is clear that, at least for the SDLP changes from placebo to BACs of .05% and .08%, on-the-road and simulator tasks are strikingly comparable. The average SDLP changes for on-the-road vs. simulator tasks differ little at BAC .05% (2.0cm and 2.1cm) and at BAC 0.08% (4.1cm and 4.1cm).

PART III. Multi-Modal Evidence of Alprazolam-induced Driving Impairment

General Background and Prescription Rates

Alprazolam is a triazolo benzodiazepine indicated for the treatment of panic and anxiety disorders. It was first approved in the U.S. in 1981 for panic disorder and an extended-release (ER) formulation was subsequently approved in 2003. Alprazolam has consistently been one of the most frequently prescribed psychiatric drugs in the U.S. Data from IMS Health show that it was the most frequently prescribed psychiatric drug in 2005, 2009, 2011 & 2013—most recently in 2013, with a record ~48.5 million prescriptions written. Although more recent data for the number of alprazolam prescriptions written are not publically available, alprazolam was still the second most-prescribed psychiatric medication in 2016 (Grohol 2017).

While only five benzodiazepines are FDA-approved for insomnia (i.e., flurazepam, temazepam, triazolam, estazolam, and quazepam), these older benzodiazepines are no longer commonly prescribed; instead, doctors now more commonly prescribe benzodiazepines such as diazepam, alprazolam, lorazepam, and clonazepam for sleep disturbances, particularly in patients with mood or anxiety disorders (Lader 2011). Although these newer benzodiazepines are not indicated for sleep disturbances, off-label prescribing (i.e., prescribing currently available and marketed

medications for an indication that has not received FDA approval) is legal and common. An estimated 1 in 5 prescriptions are written for off-label use (AHRQ 2015). Benzodiazepines are prescribed to women almost twice as often as to men (Olfson et al. 2015) and in 2013, an estimated 5.6% of the adult U.S. population filled a benzodiazepine prescription in the past year (Bachhuber et al. 2016).

Pharmacology/Toxicology

Alprazolam is a 1,4-triazolobenzodiazepine analog that differs in structure from classical benzodiazepines, such as diazepam, by the presence of a triazole ring (see Figure 1.1). The addition of the triazole ring to the diazepine scaffold promotes activity at different receptor subtypes than benzodiazepines with a carbonyl or other functional groups. Like other benzodiazepines, alprazolam acts as a positive allosteric modulator of the gamma-amino butyric acid$_A$ ($GABA_A$) receptor, a ligand-gated chloride-selective ion channel found on neurons. GABA is the primary inhibitory neurotransmitter in the central nervous system, and it binds to approximately 30% of all brain synapses—resulting in widespread and non-specific inhibitory effects. Each $GABA_A$ receptor complex is made up of five glycoprotein subunits (two α subunits, two β subunits, and one γ subunit) and contains three binding sites (two GABA binding sites [(Macdonald and Olsen 1994)] and one benzodiazepine binding site). Benzodiazepines enhance the activity of GABA in two ways: by increasing the probability that GABA will bind to its receptor and by amplifying the magnitude of effect after GABA is bound. When benzodiazepines bind to their binding site in the pocket between the α and γ subunits, it induces a conformational change that increases the affinity of the GABA binding site for GABA. Once GABA is bound to its receptor, it causes hyperpolarization of the neuronal cell via chloride influx and subsequent inhibition of action potentials. Additionally, electrophysiological studies have demonstrated that benzodiazepines increase the frequency of chloride channel opening in

response to GABA binding, providing another mechanism of hyperpolarization and inhibition of cellular activity (Gallager et al. 1984, Smith and Gallager 1987).

Multiple types of benzodiazepine receptors exist; they are classified by the isoform of the $GABA_A$ receptor α subunit on which the benzodiazepine binding pocket is found and by their clinical effect. The differences in receptor subtypes and the way ligands interact with different residues in the benzodiazepine binding site are important considerations for drug development, as researchers aim to exploit these differences to develop selective drugs with improved specificity and reduced off-target effects (McKernan et al. 2000, Atack 2005, Skolnick 2012). Because of the numerous and varied interactions between ligands and receptor, drugs that interact with the benzodiazepine binding site can be anxiolytics, hypnotics, or myorelaxants (Sternbach 1979, Villar et al. 1991, McKernan et al. 2000), though overlapping effects among these drugs is common. The two benzodiazepine receptor types about which the most is known are types 1 and 2. The benzodiazepine-1 receptor contains the α1 subunit, is present in 60% of all $GABA_A$ receptors, and is highly concentrated in the cortex, thalamus, and cerebellum. Binding of this receptor subtype is thought to be responsible for the sedative, anticonvulsant, and amnesic effects of benzodiazepines. The benzodiazepine-2 receptor contains the α2 subunit, is present in an unknown percentage of all $GABA_A$ receptors, and is highly concentrated in the limbic system, on motor neurons, and the dorsal horn of the spinal cord. It is thought to mediate the anxiolytic and myorelaxant properties of benzodiazepines.

Approximately 90% of alprazolam is absorbed from the digestive tract after oral administration, and rate of absorption is dose-independent (Greenblatt et al. 1993), meaning that peak concentrations are not achieved sooner after larger doses. There is no evidence for metabolic tolerance to benzodiazepines after repeated exposure (in contrast to what is observed after chronic alcohol administration). In patients with panic disorder either chronically using alprazolam or not

being treated with benzodiazepines, plasma concentrations after acute diazepam administration did not differ between groups (Cowley et al. 1995). Metabolism of alprazolam occurs by hepatic microsomal oxidation and is mediated by cytochrome P4503A4. While 29 metabolites of alprazolam have been identified, none are considered to significantly contribute to its clinical effects. The two primary metabolites of alprazolam, 4-hydroxy alprazolam and α-hydroxy alprazolam, are present at low concentrations (i.e., concentrations of less than 0.04 those of parent drug) and show significantly reduced benzodiazepine receptor affinity (between 0.20 and 0.66) compared to alprazolam itself (Pfizer 2011). Alprazolam is highly protein bound. It is widely distributed in the body, easily crosses the blood-brain barrier, and preferentially accumulates in lipid-rich areas, such as the central nervous system and adipose tissue. The elimination half-life is estimated to be 6 to 27hr, and approximately 80% of alprazolam is excreted unchanged by the kidney. The estimated half-life is similar for both the immediate-release (IR) and extended-release (ER) formulations (~11hr and 10.7—15.5hr, respectively) (Pfizer 2011, Pharmacia and Upjohn 2011). The pharmacological actions described above are responsible for the clinical and behavioral effects of alprazolam.

Behavioral Effects of Alprazolam

Numerous laboratory studies report that acute benzodiazepine exposure causes cognitive and psychomotor impairment (see, for example, (Evans et al. 1994, Verster et al. 2002b, Stewart 2005)). Single-dose behavioral studies in healthy volunteers have administered alprazolam doses of 0.25 to 2.0mg, and generally report that acute alprazolam administration results in dose-related decrements in task performance (Verster and Volkerts 2004). In non-patient populations, acute impairment due to alprazolam administration is evident across a wide range of tasks commonly used in psychopharmacology research, including the Digit Symbol Substitution Task [DSST: e.g., (Ellinwood et al. 1985, Fleishaker et al. 1995)], Card Sorting Task [e.g., (Ellinwood et al. 1987)], Critical Flicker Fusion Test [CFFT: e.g., (Subhan et al. 1986)], vigilance [e.g., (Kozena et al.

1995)], reaction time [e.g., (Crombez et al. 1991)], and mental recall [e.g., (Barbee et al. 1991)] tests. Alprazolam-induced impairment in healthy volunteers has also been demonstrated during an on-the-road driving task performed 1hr after 1mg alprazolam administration compared to placebo (Verster et al. 2002b). When administered chronically (i.e., one week or longer) to healthy volunteers, alprazolam causes little significant psychomotor and/or cognitive impairment (Aranko et al. 1985, Allen et al. 1991, Hart et al. 1991, Bourin et al. 1998) as measured by the same tasks that showed significant impairment after single dose studies (e.g., DSST, CFFT). Likewise, patients with panic disorder using alprazolam chronically displayed no increase in sedation or motor impairment after the acute alprazolam administration (Cowley et al. 1995). In these repeated dosing studies, the tasks are completed at baseline and then repeated under placebo or chronic alprazolam administration conditions; thus, a practice effect may explain improvements in participant performance. The other likely explanation is that participants and patients became tolerant of alprazolam effects.

It is well-known that tolerance develops to some of the therapeutic effects of benzodiazepines, thereby limiting their use for some indications. Benzodiazepine use in epilepsy is limited in part due to the development of tolerance to their anticonvulsant effects (Browne and Penry 1973, Isojarvi and Tokola 1998). Anxiolytic tolerance develops slowly in patient populations. After 8 weeks of alprazolam treatment, patients with panic disorder did not display anxiolytic tolerance (i.e., their ratings of alprazolam efficacy remained high), nor did they require dose increases (a hallmark of tolerance development) (Schweizer et al. 1993). Similar results have been found for long-term alprazolam anxiolytic efficacy for patients with generalized anxiety disorder (Cohn and Wilcox 1984). GABAergic activity underlies the cognitive and psychomotor impairment seen after alprazolam administration, and this impairment may have a role in traffic accidents.

Epidemiology of Driving Impairment and Crash Risk from Benzodiazepines Including Alprazolam

Epidemiological studies consistently report that benzodiazepine use correlates with driving impairment. One observational study looking at benzodiazepine concentrations in the bodily fluids of drivers arrested for reckless driving reported higher blood concentrations in more impaired drivers (Bramness et al. 2002). A meta-analysis of published epidemiological studies determined that drivers with benzodiazepine prescriptions were at 60 – 80% greater risk of traffic accidents and were 40% more likely to be the party responsible for the accident (Dassanayake et al. 2011). Risk of traffic accidents may be highest for those recently prescribed benzodiazepines. Using the Saskatchewan Health database information (which includes prescription information and hospital admissions for over 98% of the population), 148,000 adults with an anxiolytic benzodiazepine prescription (and 98,000 age and sex-matched controls) were identified and monitored for traffic-accident-related hospitalization for two months after the first prescription fill date (Neutel 1995). Risk of hospitalization for a traffic-accident-related condition was highest within one week of benzodiazepine prescription (OR = 13.5) and remained elevated when the timeframe examined extended to two weeks (OR = 5.6) and four weeks (OR = 2.5) of benzodiazepine prescription. Oster and colleagues likewise used insurance claim data to assess traffic accident and injury rates in 7,271 persons prescribed an anxiolytic benzodiazepine compared to 65,439 controls and found that a significantly higher percentage of those prescribed benzodiazepines needed medical attention and hospitalization than controls (Oster et al. 1987). Perhaps the most compelling data would be those describing risk of traffic accidents before and after benzodiazepine prescription initiation in the same persons; unfortunately, only one study of this type could be found. Oster and colleagues (Oster et al. 1990) examined traffic-accident-related injury three months before and for six months after initial anxiolytic benzodiazepine prescription in 4,554 persons. Post-prescription initiation risk of traffic-accident-related injury was increased compared to the three months before prescription initiation (OR = 1.15). Notably,

when compared to a group of 13,662 controls, the future benzodiazepine users were more likely to require medical attention for a traffic-accident-related injury (OR = 1.5), suggesting that anxiety may by itself increase the risk of traffic accidents. Using prescription databases to follow drivers in the United Kingdom, Barbone and colleagues reported that drivers using benzodiazepines on the day of the accident were at higher risk of being involved in a traffic accident (odds ratio [OR] 1.63) compared to drivers without reported benzodiazepine use. The odds of crash risk were higher for benzodiazepines than for selective serotonin-reuptake inhibitors (OR = 0.85), tricyclic antidepressants (OR = 0.93) or 47 other psychoactive drugs (OR = 0.88) and crash risk was higher for anxiolytic benzodiazepines with long half-lives (OR = 2.22) than intermediate half-lives (OR = 1.59) (Barbone et al. 1998).

Time course of alprazolam effects: From the previous sections, it is clear that benzodiazepines in general, and alprazolam specifically, cause acute cognitive and psychomotor impairment in both preclinical and clinical populations as measured by a multitude of assessment methodologies. Extant literature does not; however, adequately describe the complete time course of alprazolam impairment. As is common in psychopharmacology research, the outcome measures reported were from the expected time of peak effects, but because alprazolam possesses a long half-life (~11hr), it could be expected that deleterious effects on cognition, reaction time, processing speed, and psychomotor performance could still be present long after peak effects are exhibited. This hypothesis has indirect support in the literature from a few studies but has not been directly investigated.

The sedating effects of alprazolam display a counter-clockwise hysteresis (i.e., Emax [maximum drug effect] occurs after Cmax), which may have clinically significant implications. Smith and Kroboth (Smith and Kroboth 1987) determined that the peak sedating effect of a single alprazolam dose occurred *four hours after* Cmax was reached. This lag between Cmax and Emax

was still present after 4 days of twice-daily alprazolam dosing and may be due to delayed drug entry into the central nervous system. Alprazolam Cmax is reached ~2hr after oral administration, suggesting that sedative effects may be greatest around 6hr after administration.

A small trial evaluated reaction time and critical flicker fusion, a measure of visual persistence, 1 and 11hr after 2mg nighttime alprazolam or placebo administration. At both measurement times, choice reaction time was impaired after the alprazolam condition compared to placebo. The critical flicker fusion scores were also slower following alprazolam at both 1 and 11hr, but this effect did not reach statistical significance (Norman et al. 1990). A placebo-controlled trial assessing on-the-road driving ability 10-11hr and 16-17hr after nighttime administration of lormetazepam and flurazepam found that 2mg lormetazepam impaired driving performance 10-11hr after administration (Brookhuis et al. 1990). Importantly, lormetazepam possesses a half-life comparable to alprazolam (i.e., 10-12hr) and is also metabolized via CYP3A4.

Evidence from epidemiological surveys, laboratory studies and the pharmacology of alprazolam together suggest that alprazolam may indeed produce residual psychomotor effects the morning after nighttime use. Those who use benzodiazepines at night for sleep or anxiety likely believe that the impairing effects would dissipate by the next morning; but, as was the case for zolpidem, this may not be true. There are many important factors which may affect the profile of alprazolam impairment, but one important and often overlooked factor is sex.

PART IV. Sex Differences in Drug Response

Sex differences in drug response can be extensive, but too often these differences are not examined during drug development and approval processes. Since the 1938 establishment of the FDA until the end of 2017, 1,607 new molecular entities (i.e., new medications, not generics)

have survived the rigorous approval process to be designated as safe and effective for the treatment of a human disease (Kinch et al. 2014, Kinch 2015, FDA 2016, Griesenauer and Kinch 2017, Kinch and Griesenauer 2018); however, over 1,000 of those medications were approved without ensuring that they were actually safe and effective for a demographic that comprises 50% of the U.S. population—namely, women. This is due, in large part, to the fact that before 1993, "women of child-bearing potential" were prohibited in participating in many clinical trials. Even after this rule was eliminated, men continued to comprise the majority of clinical trial participant populations. Women participate in clinical trials about a third as often as men (Pinnow 2009), which is particularly problematic because women use prescription medications more frequently than men (Manteuffel et al. 2012). The consequences of this disproportionate participation in the medication testing process have at times been dire. Once medications are FDA-approved and on the market, sex differences in drug response can sometimes become readily apparent, and the FDA can appropriately require changes to indication or dosage or even remove dangerous drugs from the market.

Causes of Sex Differences in Drug Response

Many drugs removed from the market posed a greater danger to women than men (e.g., fenfluramine and terfenadine). One common mechanism of this increased danger is in the form of drug-induced torsade de pointes, a potentially deadly form of ventricular tachycardia arising as a complication from long QT syndrome(Al-Khatib et al. 2003). The underlying cause of the increased risk for drug-induced torsade de pointes and symptomatic long QT syndrome in women may be due to higher intrinsic heart rate, longer corrected QT interval and/or a shorter sinus nodal recovery time than men (Wolbrette et al. 2002). Many drugs which prolong QT interval are still marketed (e.g., erythromycin, fluoxetine, quetiapine), but knowledge of the potentially fatal sex differences allows prescribers to make more informed risk/benefit calculations when dosing and prescribing these drugs to men and women (Roden 2004). For medications that elicit less easily

measured (but not necessarily less hazardous) sex-specific consequences, the process of changing prescribing habits takes longer and often requires careful research to characterize those effects (e.g., as was the case for the 2013 sex-specific re-labeling of zolpidem, which had been on the market for over 20 years before it was discovered that zolpidem clearance was significantly slower in women than men, resulting in over-medication and a higher than expected incidence of adverse drug reactions in women using zolpidem-containing medications).

In addition to physical differences (height, weight) between men and women, disparities in pharmacokinetic processes (i.e., absorption, distribution, metabolism, excretion) between men and women may contribute to sex differences in drug response. Using a scintillation camera and computer to measure the rate of gastric emptying in 15 men and women after ingestion of a standardized radioactive-labeled meal, Datz and colleagues determined that women empty both solids and liquids from their stomachs significantly more slowly than men. This difference in gastric emptying may contribute to sex-specific differences in drug absorption (Datz et al. 1987). Plasma binding protein levels can affect free vs. bound fraction of drug. The volume of distribution for non-polar, lipid-soluble drugs (e.g., theophylline, chloroquine) is dependent on body fat percentage, which is typically higher in women than men (Anderson 2008). Because adipose tissue is poorly vascularized and drug distribution is determined by perfusion, distribution for drugs that accumulate in adipose tissue is slow. This drug sequestration in fat can result in extension of drug effects or elimination half-life (e.g., seen with repeated THC exposure [Ashton 2001]). Several important plasma binding proteins (i.e., alpha-1 acid glycoprotein [AAG] and alpha globulins) are affected by estrogen (estrogen decreases AAG and increases globulin levels) (Soldin and Mattison 2009). Blood flow has been shown to differ between men and women for certain organs/tissues (adipose M=5%, F=8.5%; heart M=4%, F=5%; kidney M = 19%, F=17%; liver M=25%, F=27%; and muscle M = 17%, F =12%), affecting the rate of drug distribution, metabolism, and elimination (Soldin and Mattison 2009). The activity of liver

cytochrome P450s may differ between men and women, and women can have slower drug clearance due to slower glomerular filtration rate (Greenblatt et al. 2000).

Relative amounts of sex hormones may also influence drug effects—not only between men and women but also between women in different stages of the menstrual cycle (Spoletini et al. 2012). Use of oral contraceptives may even further complicate the characterization of sex-related differences in drug response (Ellinwood et al. 1984, Stoehr et al. 1984, Kroboth et al. 1985). Variations in any one of these pharmacokinetic factors can alter drug concentrations in the body, resulting in deviation from the expected range of effects. This may manifest in several ways, such as women experiencing more adverse drug effects (Franconi et al. 2007); requiring different doses than men [e.g., in some cases of anesthesia (Pleym et al. 2003) and antipsychotics (Seeman 2004)]; or unintentional drugged driving resulting in more accidents the morning after using a sedative medication (FDA 2013a).

Sex Differences in Drugged Driving Research

Little research has been conducted to describe potential sex differences in driving performance after administration of drugs. Drugged driving studies have been typically conducted in females only [e.g., (O'Hanlon and Volkerts 1986)], in males only [e.g., (Volkerts et al. 1992)], or included both sexes but were not powered to detect sex differences [e.g., (Brookhuis et al. 1990, Hartman et al. 2015)]. Indeed, a 2012 review (Verster and Roth 2012b) of studies assessing driving performance after bedtime administration of sleeping medications found that only one of 14 studies conducted a sex difference analysis (and importantly, found that women drove significantly worse than men the morning after middle-of-the-night zolpidem administration). Because of the dearth of studies analyzing sex differences in driving performance, the evidence for greater driving impairment in females than males after drug administration is sporadic. In a pooled analysis of nine within-subject, blinded, placebo-controlled, on-the-road driving studies in

which a total of 148 (75 male) volunteers were administered placebo and alcohol before completing a driving task, Jongen and colleagues demonstrated that significant impairment (i.e., SDLP increase of 2.4 cm or greater) was displayed by 42.7% of males and 52.1% of females at a BAC of ~.05% compared to placebo (Jongen et al. 2017). Other studies have reported no significant sex differences in driving simulator outcomes after zopiclone and ramelteon (Mets et al. 2011) and marijuana (Anderson et al. 2010), although SDLP was not assessed in the latter study.

Sex Differences in Alprazolam Response

Because alprazolam was approved before women were generally included in clinical trials, unknown sex differences in pharmacokinetics and/or pharmacodynamics may exist. Indeed, alprazolam pharmacokinetic studies typically included only males (Ellinwood et al. 1985, Smith and Kroboth 1987, Wright et al. 1997); however, scattered evidence exists to support the presence of sex differences in response to alprazolam. Alprazolam is a lipophilic drug (i.e., its volume of distribution depends on body fat percentage—which is typically higher in women). Its mechanism of action is similar to that of zolpidem, a drug known to display sex-specific pharmacokinetics. One study (Kirkwood et al. 1991) reported that women in the luteal phase reached higher peak alprazolam area-under-the-curve concentrations than women in the follicular phase; these concentrations also were higher compared to peak and area-under-the-curve concentrations in men. The results were not statistically significant, but sample sizes were small (n = 3 - 4 per group) and only tested a single dose (1mg) of alprazolam. Importantly, a study demonstrating that the peak sedating effect of a single alprazolam dose occurred 4hr after reaching peak alprazolam plasma concentration enrolled only males (Smith et al. 1984); therefore, it is unknown how sex-specific metabolic differences could affect the delay between peak plasma concentrations and peak effects. Sex differences in pharmacokinetics have been reported for benzodiazepines as a class, if not for alprazolam specifically. The elimination half-

life of oxazepam has been demonstrated to be significantly longer in females than males and increases with age in females but not males (Greenblatt et al. 1980). Women using oral contraceptives have also been reported to be more sensitive to the psychomotor effects of single doses of benzodiazepines than women not using oral contraceptives (Kroboth et al. 1985). The potential pharmacokinetic and/or pharmacodynamic differences in alprazolam response between men and women are not fully characterized.

PART V. Summary

Results from national surveys (both household and roadside) and alcohol/drug tests from drivers involved in fatal traffic accidents concur that while the prevalence of drunk driving has decreased, there has been an increase in persons driving under the influence of potentially impairing drugs (i.e., "drugged driving"). Drugged driving is associated with significant mortality, both of the driver and of others on the road. The most commonly detected drugs in drivers involved in a fatal accident include prescription medications (e.g., opioids and benzodiazepines), which may or may not have been used as directed by a doctor. Even when used as directed, prescription medications can cause significant impairment that may be unbeknownst to the patient using them (or even to the doctors prescribing them). More than 20 years after its initial approval by the FDA, the sleeping medication, zolpidem, was discovered to produce significant impairment (resulting in elevated risk of involvement in traffic accidents) the morning after its use. Despite having survived a rigorous pre-approval process, the time course of zolpidem effects was not completely characterized. There may be other drugs with similar mechanisms of action for which the duration of effects is not fully elucidated.

While the laws governing alcohol use and driving are standardized across the U.S. and are relatively simple to enforce, similar legislation for drug use is hampered by unique challenges

related to the definition and measurement of drugged driving. Defining a blood concentration threshold for impairment (akin to a 0.08% BAC) for every possible drug is impractical; thus, there are laws which 1) define thresholds (of questionable scientific merit) for some drugs, 2) implement a zero-tolerance policy for all drugs, and 3) do not define any blood concentration thresholds but rely on police judgement of driver impairment. Regardless of the legislative strategy ultimately used, it is important to accurately and completely inform patients of potential driving impairment (including the expected time course) caused by drugs prescribed to them so that they may be safe and in compliance with the law. As exemplified in the 2013 case of zolpidem, it is clear that these data are not available for all prescription drugs currently on the market. These data are also needed for drugs not yet approved. Determining which drugs cause impairment and fully characterizing the time course and severity of that impairment is methodologically challenging.

Measurement of drug effect on driving ability has historically been accomplished in one of three ways: with simple laboratory tasks, by using on-the-road driving tasks, or with a driving simulator. The first method indirectly assesses some of the same skills ostensibly used for driving (e.g., reaction time, spatial awareness), but the external validity is low. On-the-road driving tasks have obvious external validity, but their use is quite limited due to factors such as cost and safety. The use of driving simulators is an alternative with superior validity to simple laboratory measures and obviate any safety concerns inherent to an impaired person operating a vehicle in normal traffic. An increase in vehicle standard deviation of lane position (i.e., lateral movement/weaving) caused by a drug is associated with increased crash risk, and this is the primary outcome measure reported in both on-the-road and simulated driving studies.

Alprazolam is a benzodiazepine often prescribed off-label for sleep. Effects are mediated by increased GABAergic activity and include sedation and psychomotor impairment; and with a

half-life of 11hr, it is possible that these effects would persist long after falling asleep. Like zolpidem, this may result in increased crash risk the morning after its use. That hypothesis is supported by epidemiological studies indicating that prescription of benzodiazepines as a class (and alprazolam specifically) is associated with a higher risk of traffic accidents. The time course of alprazolam-induced impairment has not been fully characterized.

There are many factors which may influence individual drug response. Sex differences in drug pharmacokinetics and pharmacodynamics have been underappreciated and understudied, in part due to grossly disproportionate clinical trial participation by men and women. Women are prescribed drugs more often and have more adverse drug reactions than men, but for drugs approved before 1993 (e.g., alprazolam), women were prohibited from participating in the clinical trials determining their safety and efficacy. Many drugs exhibit sex-specific effects. The increased crash risk associated with zolpidem use was almost exclusively seen in women due to their reduced clearance of the drug compared to men. Alprazolam is a lipophilic drug that may display a similar pattern of sex-specific driving impairment. Few studies assessing drug-induced driving impairment have included adequate numbers of both men and women to perform a sex difference analysis (and no such study has been conducted for alprazolam)—representing a significant gap in the literature.

Pharmacodynamic evaluation of the time course of alprazolam effects, specifically on driving ability, the day after bedtime administration is needed, as is an assessment of possible sex-differences in those effects.

Table 1.1 United States traffic fatality statistics, 2007-2016

	Total Deaths [a]	Deaths per 100,000 population [b]	Deaths per 100 million vehicle miles driven [a]
2007	41,259	13.70	1.36
2008	37,423	12.32	1.26
2009	33,883	11.05	1.15
2010	32,999	10.67	1.11
2011	32,479	10.42	1.1
2012	33,782	10.76	1.14
2013	32,893	10.40	1.05
2014	32,744	10.28	1.08
2015	35,485	11.06	1.15
2016	37,461	11.59	1.18

[a] Deaths and vehicle miles driven data from NHTSA (NHTSA 2017)
[b] Population statistics from US Census Data (USCB 2018)

Table 1.2 Alcohol test statistics for fatally injured drivers, 2007-2016

	Total	Tested for Alcohol [a]		Positive Test Result		
	(#)	(#)	(%)	(#)	(% of total)	(% of those tested)
2007	26,570	19,434	73.14	8,127	30.59	41.82
2008	24,254	18,415	75.93	7,762	32.00	42.15
2009	21,835	16,761	76.76	7,044	32.26	42.03
2010	21,072	16,405	77.85	6,657	31.59	40.58
2011	20,815	15,846	76.13	6,362	30.56	40.15
2012	21,490	16,097	74.90	6,524	30.36	40.53
2013	20,943	15,661	74.78	6,264	29.91	40.00
2014	20,788	15,352	73.85	6,048	29.09	39.40
2015	22,348	16,469	73.69	6,275	28.08	38.10
2016	23,560	14,453	61.35	5,473	23.23	37.87

[a] Numbers reflect only those drivers tested who have a known result recorded in the FARS database. Approximately 2 – 4% of drivers listed as receiving an alcohol test have unknown results.

Table 1.3 Alcohol test statistics for drivers involved in fatal accidents, 2007-2016

	Total	Tested for Alcohol [a]		Positive Test Result		
	(#)	(#)	(%)	(#)	(% of total)	(% of those tested)
2007	56,019	27,048	48.28	10,535	18.81	38.95
2008	50,416	26,046	51.66	10,000	19.83	38.39
2009	45,291	23,906	52.78	9,114	20.12	38.12
2010	44,599	24,291	54.47	8,779	19.68	36.14
2011	43,840	23,303	53.15	8,291	18.91	35.58
2012	45,664	23,631	51.75	8,496	18.61	35.95
2013	44,803	22,597	50.44	8,127	18.14	35.96
2014	44,671	22,053	49.37	7,827	17.52	35.49
2015	49,162	23,855	48.52	8,125	16.53	34.06
2016	51,914	20,945	40.35	6,997	13.48	33.41

[a] Numbers reflect only those drivers tested who have a known result recorded in the FARS database. Approximately 2 – 4% of drivers listed as receiving an alcohol test have unknown results.

Table 1.4 Drug test statistics for fatally injured drivers, 2007-2016

	Total	Tested for Drugs [a]		Positive Drug Test Result		
	(#)	(#)	(%)	(#)	(% of total)	(% of those tested)
2007	26,570	14,921	56.16	4,198	15.80	28.13
2008	24,254	14,394	59.35	4,258	17.56	29.58
2009	21,835	12,371	56.66	4,323	19.80	34.94
2010	21,072	13,033	61.85	4,551	21.60	34.92
2011	20,815	12,608	60.57	4,696	22.56	37.25
2012	21,490	13,049	60.72	5,074	23.61	38.88
2013	20,943	12,650	60.40	5,042	24.07	39.86
2014	20,788	12,665	60.92	5,171	24.87	40.83
2015	22,348	13,777	61.65	5,977	26.75	43.38
2016	23,560	12,296	52.19	5,365	22.77	43.63

[a] Numbers reflect only those drivers tested who have a known result recorded in the FARS database. Approximately 3 – 5% of drivers listed as receiving a drug test have unknown results.

Table 1.5 Drug test statistics for drivers involved in fatal accidents, 2007-2016

	Total	Tested for Drugs [a]		Positive Drug Test Result		
	(#)	(#)	(%)	(#)	(% of total)	(% of those tested)
2007	56,019	18,645	33.28	5,262	9.39	28.22
2008	50,416	18,335	36.37	5,422	10.75	29.57
2009	45,291	16,176	35.72	5,500	12.14	34.00
2010	44,599	17,445	39.12	5,946	13.33	34.08
2011	43,840	16,970	38.71	6,096	13.91	35.92
2012	45,664	17,459	38.23	6,572	14.39	37.64
2013	44,803	16,761	37.41	6,540	14.60	39.02
2014	44,671	16,806	37.62	6,640	14.86	39.51
2015	49,162	18,530	37.69	7,738	15.74	41.76
2016	51,914	16,215	31.23	6,982	13.44	43.06

[a] Numbers reflect only those drivers tested who have a known result recorded in the FARS database. Approximately 3 – 5% of drivers listed as receiving a drug test have unknown results.

Table 1.6 Most commonly detected prescription drugs in fatally injured drivers, 2007-2016

	Drug (number of drivers testing positive)				
	1st	2nd	3rd	4th	5th
2007	AMP (253)	BZO (163)	HC (147)	METH (118)	MTD (105)
2008	AMP (226)	HC (193)	BZO (187)	ALP (138)	METH (109)
2009	AMP (215)	HC (213)	BZO (207)	ALP (131)	OXY (121)
2010	HC (241)	AMP (231)	ALP (198)	OXY (153)	DIA (124)
2011	HC (284)	AMP (248)	ALP (229)	OXY (152)	BZO (147)
2012	AMP (318)	HC (280)	ALP (188)	BZO (186)	OXY (152)
2013	AMP (390)	HC (269)	ALP (200)	BZO (178)	OXY (145)
2014	AMP (371)	HC (215)	ALP (212)	METH (182)	BZO (173)
2015	AMP (488)	HC (254)	ALP (237)	BZO (227)	METH (222)
2016	AMP (449)	ALP (239)	METH (207)	HC (180)	OXY (151)

ALP = alprazolam, AMP = amphetamine, BZO = benzodiazepine (unspecified type), DIA = diazepam, HC = hydrocodone, METH = methamphetamine, MTD = methadone, OXY = oxycodone

Methamphetamine is prescribed under the trade-name Desoxyn, but may also cross-react with amphetamine (prescribed under the trade name Adderall) depending on the sensitivity of the drug assay utilized for testing.

Table 1.7 Standard deviation of lane position (SDLP) after placebo and alcohol in 16 studies

	SDLP (cm) at BrAC at beginning of driving task								Δ SDLP	
	.00	.02	.03	.04	.05	.06	.08	.10	.05 – 0.00	.08 – 0.00
Study & task										
On-the-road driving task (100km, highway)										
Kuypers et al. (2006)	20.6			23.5						
Louwerens et al. (1987) [a]									2.4	4.1
Ramaekers et al. (2000)	22.3			24.4						
Schumacher (2011)	18.3				20.7				2.4	
Schumacher et al. (2014)	18.0				20.7				2.7	
Van der Sluizen et al. (2016)	17.0			19.4						
Vermeeren et al. (2002a)	20.0			22.3						
Vermeeren et al. (2002b)	17.7			19.4						
Verster and Roth (2012) [a]									1.3	
Average									**2.0**	**4.1**
Driving simulator task										
Berthelon and Gineyt (2014)										
Highway route (15min)	26.4		25.9		28.4		31.9		2.0	5.5
Following route (10min)	18.4		19.2		19.7		23.3		1.3	4.9
Hartman et al. (2016)	31.4	32.3			33.6		34.8	35.7	2.2	3.4
Lenne et al. (2003)	31.3					40.5				
Lenne et al. (2010)	36	41			41				5	
Kenntner-Mabiala et al. (2015)										
Rural nighttime (24km)	17				19		20		2	3
Car following (2km)	20				23		25		3	5
Winding road (5km)	21				21		22		0	1
Mets et al. (2011)	28.0				29.7		33.8	36.3	1.7	5.8
Veldstra et al. (2012)	15.8		17.7		17.9		19.9		2.1	4.1
Average									**2.1**	**4.1**

[a] Indicates that raw SDLP data not reported; thus, only change from placebo scores are listed.

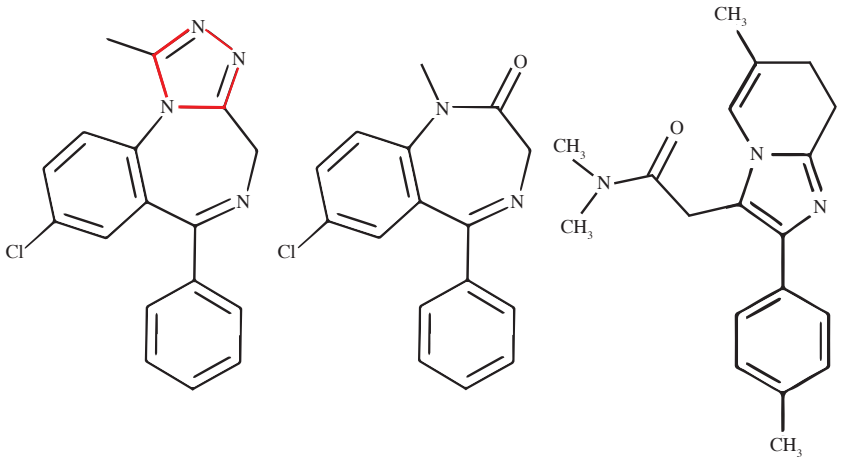

Figure 1.1 Chemical structure of alprazolam, diazepam, and zolpidem. Alprazolam (left) and diazepam (middle) are both comprised of the benzodiazepine core: a benzene ring (six-membered ring comprised of carbon atoms with one hydrogen atom attached to each) fused to a diazepine ring (a seven-membered heterocyclic compound with two nitrogen atoms) as well as an additional aryl ring attached at the 5' position of the diazepine ring. Alprazolam has an additional triazole ring (highlighted in red). Zolpidem (right) contains structures similar to the benzodiazepines: a imidazopyridine (six-membered ring with five carbon and one nitrogen atom fused to a five-membered ring with two nitrogen atoms) and an aryl ring.

CHAPTER II. HYPOTHESIS

Bedtime administration of alprazolam will dose-dependently cause psychomotor impairment that is detectable after a night's sleep.

a) Participants will be unaware of the extent of their own impairment.

b) Impairment will be of greater magnitude and longer duration in females compared to males.

CHAPTER III. MATERIALS & METHODS

Volunteers

Study volunteers were 14 (6 male) non-nicotine-product-using adults (age 18 to 50) who possessed a valid driver's license. Volunteers were carefully screened to exclude those with a seizure disorder, acute narrow-angle glaucoma (as it is contraindicated for alprazolam), lifetime history of benzodiazepine physical dependence, current physical dependence on any substance except caffeine, or clinically significant ongoing medical problems (e.g., diabetes, hypertension, etc.). All volunteers were determined to be in good physical and psychiatric health by medical history and physical examination, an ECG and laboratory tests, and exam by a psychiatrist. A complete list of the inclusion/exclusion criteria for the study can be found in Appendix 2. Screening assessments included the NEO Personality Inventory (Costa and McCrae 2008), Symptom Checklist-90 (Derogatis et al. 1976), Beck Depression Inventory (Beck et al. 1961), Columbia Suicide Severity Scale (Posner et al. 2011), and the Wide Range Achievement Reading Test (Jastak 1984). Volunteers provided urine and breath samples to be tested for illicit drugs (American Screening, LLC; Shreveport, LA), alcohol, carbon monoxide, and pregnancy during each screening visit. The 11-panel drug test card detected the following substances: benzodiazepines, barbiturates, cocaine, tetrahydrocannabinol (THC), methamphetamine, morphine-derivative opiates (e.g. heroin), methadone, oxycodone, phencyclidine (PCP), amphetamines, and buprenorphine. Volunteers were recruited through local print and online advertisements and by word of mouth, and they were paid for their participation. The University of Kentucky Institutional Review Board approved this study, and volunteers gave their written informed consent prior to the conduct of study procedures. This study was conducted in accordance with the Helsinki guidelines for ethical human research.

Study Design and Setting

This study employed a randomized, placebo-controlled, mixed-factorial design (with sex as the between-subject factor). It was conducted at the University of Kentucky Center on Drug and Alcohol Research and the Center for Clinical and Translational Sciences inpatient unit located in the University of Kentucky Hospital. After screening procedures were finished, volunteers completed a practice session to familiarize them with the session tasks. No drugs were administered during this session. Volunteers then completed a total of six overnight experimental sessions scheduled at least two days apart to ensure adequate wash-out of drugs between sessions. Breath and urine samples were collected twice during each session—once upon arrival in the afternoon and again in the morning to ensure that no alcohol or drugs (other than those administered as part of the study) were consumed while the volunteer was on the research unit for the night. Women were tested for pregnancy at the same interval. No study procedures (i.e., drug administration) were conducted if a volunteer tested positive for illicit drugs, alcohol, or pregnancy at the beginning of a session.

Study Drugs

Each session included two drug administrations: one at bedtime (~11:30 PM) and one the following morning (~8:30 AM), though only one dose per session was active—the other dose was placebo. Six dose conditions were evaluated during the study (bedtime dose/morning dose): alprazolam 0.5mg/placebo, alprazolam 1.0mg/placebo, alprazolam 2.0mg/placebo, zolpidem 10mg/placebo, placebo/placebo, and placebo/alprazolam 1.0mg. All doses were placed into size 00 capsules and loose-filled with lactose powder. Placebo doses consisted of identically appearing capsules loose-filled with lactose powder. All study medication was prepared in the University of Kentucky Investigational Drug Service Pharmacy by a licensed pharmacist. Doses administered on the inpatient unit were stored at the pharmacy until dosing. Drugs administered in the morning at the Center on Drug and Alcohol Research Building were stored in a locked

drawer in a limited-access drug storage room. All drugs, capsules, and filler were obtained from commercial sources through the University of Kentucky hospital formulary.

Dose Selection Rationale

Previous laboratory studies have safely administered alprazolam doses of 2mg to non-patient populations (Smith and Kroboth 1987, Norman et al. 1990). Alprazolam doses used clinically range from .75mg to 4mg daily, given in divided doses. While controlled data for alprazolam doses used off-label for sleep are lacking, internet drug abuse-oriented forums suggest doses of 0.125 to 2mg are used (Bluelight 2012). Commonly prescribed doses of 0.5, 1 & 2mg alprazolam were ultimately selected to 1) ensure volunteer safety and 2) maximize public health relevance. The daytime positive control (alprazolam 1mg) has been shown to cause acute impairment in psychomotor tasks (including on-the-road driving). Zolpidem 10mg was also included because it is the dose at which the FDA considers significant impairment to occur in women the morning after bedtime use; thus, it serves as a regulatory benchmark and clinical control condition. Although controlled-release (CR) zolpidem formulations result in the highest blood concentrations and subsequent impairment, the immediate-release formulation is of greater public health relevance as zolpidem CR is prescribed at approximately 1/10th the rate of zolpidem IR. The inclusion of zolpidem allows for exploration of relative impairment caused by an FDA-approved sleeping medication to that of a medication often used off-label for sleep.

Experimental Sessions

Volunteers were initially trained on all study tasks during a practice session. For the six experimental sessions, volunteers arrived at the laboratory in the afternoon and were evaluated for continued qualification (i.e., inclusion criteria all met and no exclusion criteria met). This evaluation included a history and physical exam update conducted by a study physician. Baseline (pre-drug administration) assessments were completed, and volunteers were escorted to

the Center for Clinical and Translational Science inpatient unit. Volunteers were allowed to eat until three hours prior to drug administration, after which food was restricted. Water was allowed at all times. Nursing staff supervised the administration of the single capsule at 11:30 PM, and volunteers were instructed to sleep immediately following drug administration. A research assistant woke the volunteers 7.5hr after drug administration and escorted them to the driving simulator laboratory, where the remainder of the session took place. A light breakfast (including a single cup of coffee or caffeinated tea if desired) was consumed and the morning baseline assessments were conducted. The second drug administration occurred nine hours after the first (at approximately 8:30 AM). Volunteers completed driving simulations, responded to computer questionnaires, and performed other tasks under the supervision of a trained research assistant. Data were collected for 5hr after the morning drug administration. Table 2.1 details the timing of all pharmacodynamic measures.

Outcome Measures

Driving Simulator Task

The Driving Simulator: A National Advanced Driving Simulator MiniSim™ computerized driving simulator was used to conduct driving tasks. Operation of the simulator required the use of a steering wheel, accelerator and brake pedals. Computer-generated graphics were displayed on three 20in LCD monitors, one fixed 15in directly in front of the driver, and two at 150-degree angles providing peripheral coverage. An additional monitor displayed the instruments panel (speedometer, gear indicator, etc.). Audio output included engine noise, wind, tire squeal, Doppler Effects of passing vehicles, and impact sounds in cases of collision. Also in the event of a collision, a tactile transducer created low-frequency vibrations to shake the MiniSim™ apparatus and driver. The simulation lasted approximately 10min. The driving scenario began as a rural two-lane highway in which the volunteer accelerated up to the speed limit. The highway continues for approximately two miles before on-screen and spoken

instructions indicate that the volunteer should take the next exit. After the off-ramp, the simulation becomes a rural road followed by suburbs and finally a city. Several incursion events are placed throughout the simulation: a car braking suddenly on the highway, a pedestrian walking directly in front of the volunteer car, a car pulling into the road in front of the volunteer car, and a poorly parked car blocking part of the volunteer's lane which they must navigate around. Outcome measures include standard deviation of lane position (SDLP; a marker of swerving and the primary outcome measure), standard deviation of speed, and tactical measures (e.g., deceleration in response to an obstacle).

The primary outcome measure, SDLP, was calculated in three steps:

1. Data were edited to include only valid data points and data points of interest. Two measures of SDLP were initially calculated: one from the entire drive and one from a short straight section of highway. On-the-road driving tests are conducted in a highway setting; thus, this second measure may be more easily compared to SDLP outcomes in those studies. Because the 'whole drive' SDLP includes many more data points than the 'highway' SDLP, it would be expected that the whole drive SDLP would be smaller. Any occasion in which the volunteer makes a full lane change was removed from the data. See Figure 2.1 for driving data editing details.

2. The lateral position [X] for each valid data point was determined and the mean (μ) calculated. A single mean lateral position (MLP) for the simulation section of interest was generated from the following formula:

$$MLP\ [X] = \mu$$

3. The standard deviation of the MLP across all valid samples [X] was calculated using the following formula:

$$SDLP = \sqrt{MLP\ [(X\text{-}\mu)^2]}$$

In order to promote conscientious driving, volunteers were paid a performance-based bonus of $5 for each drive if they followed the rules of the road and instructions from study staff.

Subjective Assessments Related to the Driving Simulator Task

Driving Visual Analog Scales (VAS): Immediately after each driving simulation, volunteers rated the following items: 'I drove the previous simulation well, I drove within the posted speed limits, and I feel safe to drive on the road' on a 100-point line with the terms 'Disagree Completely' on the far left (at 0) and 'Agree Completely' on the far right (at 100).

Simulator Sickness Questionnaire (SSQ) (Kennedy et al. 1993): The SSQ is a validated tool for quantifying adverse reactions to simulated driving environments. It uses a four-point scale (from none [scored 0] to severe [scored 3]) to rate the following 27 symptoms which are commonly experienced by users of virtual reality systems: general discomfort, fatigue, headache, eyestrain, difficulty focusing, increased salivation, sweating, nausea, difficulty concentrating, blurred vision, fullness of head, dizzy, vertigo, stomach awareness, and burping. Scores for three subscales (nausea, oculomotor problems, disorientation) were calculated.

Other Psychomotor Assessments

The Circular Lights Task: Also known as the Wayne Saccadic Fixator™ task, this task consists of a square panel with 33 red light switches which the volunteer presses to extinguish.

Lights appear randomly on the panel, and volunteers were instructed to use their pointer finger to extinguish as many lights as possible in 1min. Performance on this task is related to reaction time, hand-eye coordination, and peripheral awareness.

The Digit Symbol Substitution Task (DSST) (McLeod et al. 1982): The DDST is a timed task requiring volunteers to replicate a pattern presented on a computer screen using a numeric keypad, and performance is related to attention, visual scanning and memory. Volunteers were instructed to complete as many correct patterns as possible in 90sec. Additionally, volunteers were asked to rate 'How well do you think you will perform on the DSST compared to normal?' prior to starting and 'How well do you think you did perform the DSST compared to normal?' after finishing. Ratings were completed on a 100mm VAS anchored at -50 (much worse), 0 (normal), and 50 (much better).

The SafeDrive Application (Miceli et al. 2015): This phone and tablet application assesses auditory and visual reflexes and is currently being used by the Italian police force to assess impairment in drivers who test negative for breath alcohol. It consists of four short tests (simple visual, complex visual, simple auditory, complex auditory) and provides results in deciles compared to the general population. Outcomes analyzed were test completion time and whether each test was passed or failed.

Assessments of Subjective Drug Effect

Drug Visual Analog Scales (VAS): Volunteers rated the following items: 'Do You Feel…Any Drug Effect, Good Effects, Bad Effects, High?' and 'Does the Drug Make You Feel…Nauseated, Confused, Dizzy, Tired, Irritable?' on a 100-point line anchored with the terms 'none at all' at the far left (at 0) and 'extremely' at the far right (at 100).

The Adjective Checklist: Volunteers rated (on a scale from 0 [not at all] to 4 [extremely]) the following signs/symptoms of alprazolam and zolpidem: dizzy, upset stomach, irritable, relaxed, drunken, slurred speech, difficulty concentrating, heavy/sluggish, dry mouth, lightheadedness, sleepy, weak, nervous, uncoordinated, and confused.

The Observer Adjective Checklist: Trained research assistants rated (on a scale from 0 [not at all] to 4 [extremely]) the visually observable signs and symptoms from the Adjective Checklist (i.e., nervous, drunken, irritable, slurred speech, relaxed, sleepy, clumsy, sluggish, confused, and dazed/spaced-out).

Sleep Measures

Sleep VAS: Approximately 1hr after waking each session, volunteer perception of sleep quality was assessed with the following items: 'I slept well last night', 'I fell asleep easily last night', 'I feel clear-headed this morning', 'I woke up often last night', I am satisfied with my sleep last night.' Responses were recorded on a 100-point line anchored with the terms 'not at all' at the far left (at 0) and 'extremely' at the far right (at 100). These questions have been used previously to detect drug effects on subjective sleep quality (Haney et al. 2004).

Fitbit: A wrist monitor was worn from a volunteer's arrival at the inpatient unit until waking the next morning to track sleep duration and quality.

Physiological Measures

In-session measures: Heart rate, blood pressure, and respiration rate were collected every 30 minutes during the experimental session. Heart rate and blood pressure were collected using a

Dinamap Non-Invasive Patient Monitor (GE Medical Systems, Tampa, FL, USA) and respiration rate was assessed visually over 60 seconds by a trained research assistant or nurse.

Body fat percentage: A dual-energy x-ray absorptiometry (DXA) scan was performed on one visit to determine total body fat percentage.

Statistical Analyses

Standard deviation of lane position (SDLP) was the primary outcome measure. Each drive yielded two SDLP outcomes—one calculated using mean lateral position from the entire drive and one calculated using mean lateral position during a short (~1-mile) section of uneventful highway within the drive. In order to determine which of these two outcomes was more appropriate to use as the primary outcome measure, the mean, range, and variance in both were calculated for each volunteer's pre-drug baseline drives in all six sessions. On-the-road driving studies have demonstrated that SDLP is a stable measure within subject; thus, the SDLP outcome measure with the least within-subject variability across six sessions was selected as the primary outcome measure.

Two analytic approaches were utilized for hypothesis testing—symmetry analysis (Laska et al. 2012) and mixed models analysis. The primary outcome measure was analyzed using both methods. All other outcomes were analyzed using a mixed-models approach only. Analyses were conducted using SAS 9.3, and statistical significance was set at $p < .05$.

Symmetry analyses

Because analyses of mean (group-level) changes may be insensitive to clinically meaningful impairment (due to large inter-person variability in pharmacodynamic effects—see introduction),

the FDA recommends symmetry analysis to evaluate drug effects on the ability to operate a motor vehicle (FDA 2015). Symmetry in SDLP implies that the probability of improvement after an active dose (relative to the negative control—i.e., placebo) is the same as the probability of impairment. An increase in SDLP of 2.4cm relative to placebo reflects the impairment seen at a BAC of 0.05% (Leufkens et al. 2009, Verster et al. 2014); thus, 2.4cm was used as the initial threshold for impairment. For each individual volunteer, the difference in SDLP (active – placebo) was calculated at each post-drug administration timepoint. The distribution of the difference in SDLP at each active dose and timepoint was then analyzed with a McNemar test to detect asymmetry. If the distribution of SDLP differences after active dosing show a random pattern (i.e., the number of volunteers with changes in SDLP above +2.4cm and below -2.4cm are not different), then the active dose has no impairing effect. If significantly more volunteers exhibit changes in SDLP above +2.4cm than below -2.4cm after an active dose compared to placebo, that dose increases the risk of impaired driving performance. To further characterize the magnitude of driving impairment risk, additional McNemar tests were used to determine asymmetry in distributions around thresholds of 4.1cm and 5.3cm (indicative of impairment equivalent to that at 0.08% and 0.10% BAC, respectively).

Mixed model analyses

All measures were first analyzed as raw time course data using a three-factor (Sex x Dose x Time) model with sex as the between-subject factor. Emax scores (either peak or trough as appropriate) were then calculated for each individual subject and dose condition and analyzed in a two-factor (Sex x Dose) mixed model. Both time course and Emax mixed-models analyses incorporated the following variables as covariates: age, body fat percentage, and subjective sleep quality reported (on a 100-point visual analog scale) approximately 1hr after waking each session. *Tukey's post-hoc* tests were performed to examine time course of drug effects, active dose effects compared to the placebo and zolpidem doses, and differences between males and females.

Table 2.1 Schedule of data collection during experimental sessions

Time (hours, relative to bedtime drug)		0	7.75	8	8.5	8.75	9	10	10.5	11	11.5	12	12.5	13	13.5	14
Time (hours, relative to daytime drug)		-8.5	-.75	-.5	0	.25	.5	1	1.5	2	2.5	3	3.5	4	4.5	5
Drug Visual Analog Scales	■		■	■		■	■	■	■	■	■	■	■	■	■	■
Driving Simulation	■			■			■		■		■		■		■	
Driving Visual Analog Scales	■			■			■		■		■		■		■	
Simulator Sickness Questionnaire	■			■			■		■		■		■		■	
Safe Drive Application Adjective	■			■			■		■		■		■		■	
Checklist	■		■			■	■	■	■	■	■	■	■	■	■	■
Observer Adjectives Checklist	■		■			■	■	■	■	■	■	■	■	■	■	■
Vitals	■		■			■	■	■	■	■	■	■	■	■	■	■
Circular Lights Task	■			■		■	■	■	■	■	■	■	■	■	■	■
Digit Symbol Substitution Task	■			■		■	■	■	■	■	■	■	■	■	■	■

Bedtime Drug Administration

Daytime Drug Administration

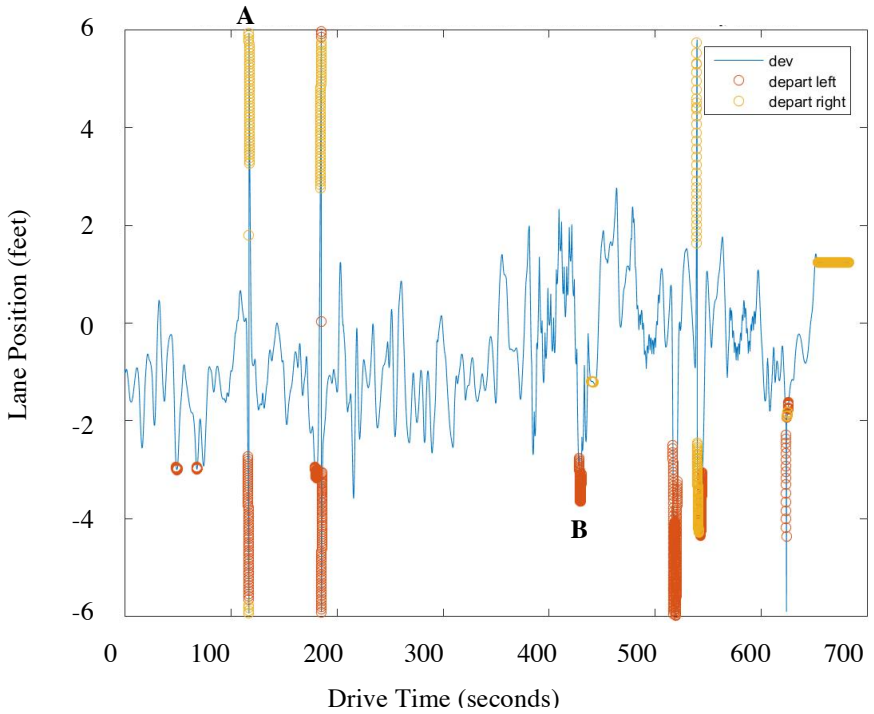

Figure 2.1 Editing raw lane position data from a driving simulation. The blue line shows the lateral position of the car throughout the drive. If the car is perfectly centered in the lane, the line is at 0. Deviation toward the left is represented by a negative number, and deviation toward the right of the lane is represented by a positive number. The yellow and red circles indicate when the car is departed from the lane (i.e., when more than 50% of the car crosses over the lane lines. **A**) A lane change maneuver: these data are removed from calculation of SDLP. **B**) A lane departure: these data are included in calculation of SDLP.

CHAPTER IV. RESULTS

Recruitment Statistics and Baseline Subject Characteristics

Figure 3.1 displays the flow of volunteer recruitment and participation. Demographic characteristics and results of screening assessments are listed in Table 3.1. The only characteristic or screening assessment results that were significantly different between male and female volunteers were height and body fat percentage. Of the eight female volunteers, three used hormonal contraception (two intra-uterine devices and one oral contraceptive) and two had a tubal ligation.

Part I. Validating the Primary Outcome Measure

Figure 3.2 displays the SDLP from the baseline drive of all six study sessions for each volunteer, and within-subject descriptive statistics (mean, range, variance) are listed in Table 3.2. Two separate measures of SDLP were calculated for each drive: the first is from the entire simulation and the second is from a stretch of straight highway (more akin to the conditions used in the on-the-road driving studies). As can be seen in both Figure 3.2 and Table 3.2, baseline SDLP variability was greater for the highway section of the drive than the whole drive; for this reason, the more stable whole drive SDLP measurement was used for analyses below.

SDLP was also generally greater in females than males. A visual inspection of the data suggested that the subjects with the largest SDLP variability in their baseline drives were the oldest in the sample. Pearson correlations (age x baseline drive SDLP variance) indicated that there was a positive (though non-statistically significant) relationship between volunteer age and baseline drive SDLP variance (see Figure 3.3).

Part II. Hypothesis Testing and Study Results

Driving Simulator Task

Failure to complete the driving task

One female volunteer was unable to complete the driving task 1hr after the daytime alprazolam 1mg dose was administered. Approximately four minutes after the task began, the volunteer nodded off and subsequently drove the car off the side of a highway off-ramp situated on a steep hill. At that point, the simulation was automatically terminated by the computer because the car moved outside the programmed area. Her data were included in the statistical analyses. She was able to complete the subjective and other psychomotor task battery.

Standard deviation of lane position (SDLP)

Symmetry analysis: SDLP changes from placebo for each active dose for the first four drives are displayed in Figure 3.4. Impairment equivalent to that seen with a .10% BAC was detected 8.5hr after the bedtime zolpidem 10mg condition (McNemar Statistic = 8, $p = .005$); this effect was strongest in females and dissipated by 9.5hr after drug administration. The bedtime alprazolam 2mg dose was equally impairing at the first morning timepoint in both sexes (McNemar Statistic = 12, $p = .001$). Significant impairment equivalent to 0.05% BAC was still detected at the group (n = 14) level at 12.5 and 13.5hr after bedtime alprazolam 2mg administration (McNemar Statistic = 4.45 – 5.00, $p < .05$). The daytime alprazolam 1mg dose was robustly impairing in both males and females starting 1.5hr after drug administration (see Figure 3.4). Significant impairment equivalent to 0.10% and 0.05% BAC was still detected at the group (n = 14) level at 3.5 and 4.5hr after daytime alprazolam 1mg administration, respectively (McNemar Statistic = 4.45 – 8, $p < .05$).

Because body fat percentage was predicted to be a significant contributing factor to time course of alprazolam impairment effects, Pearson and Spearman Correlations were conducted to

explore potential associations between body fat percentage and SDLP change from placebo at doses and timepoints for which significant impairment was found using symmetry analyses. Correlations were all non-significant ($p > .05$), and no clear association was observed.

Time course analysis: As seen in the top row of Figure 3.5, dose effects on SDLP were evident for the daytime alprazolam 1mg dose and bedtime alprazolam 2mg doses in both males and females. For the active bedtime doses, the largest effects were observed at the first morning timepoint (~8:30 AM), but after the daytime alprazolam 1mg dose, the largest effects were observed approximately 1hr after dosing (~9:30 AM). Visual inspection of the data indicates that SDLP remained elevated compared to placebo for longer in females (timepoint 1.5hr - 3.5hr) than males (timepoint 1.5hr), though this effect was non-significant (*Tukey's post hoc test*, $p > .05$). Likewise, an area-under-the-curve analysis by sex was non-significant, $p > .05$.

Emax analysis: The largest SDLP was observed after the daytime alprazolam 1mg dose and the bedtime alprazolam 2mg dose (See Table 3.3 for means and SEMs). Although peak SDLP did not vary as a function of sex, the difference was largest after the bedtime zolpidem 10mg dose (see top left of Figure 3.6).

Secondary weaving variables

Time course analysis: Figure 3.5 (middle row) displays the time course for percent of the drive departed from lane. For active bedtime doses, more time departed from the lane was seen at the earliest timepoints. For the daytime alprazolam 1mg condition, there was an increase 1.5hr after drug administration. This increase persisted in females, but not males, for 3hr. The total number of lane departures made during a drive followed a similar pattern of results, with a significant effect of Dose [$F(5,60) = 3.25$, $p = .012$] and a Dose x Time interaction [$F(30,360) = 2.02$, $p = .002$].

Emax analysis: When analyzed as a peak score, there was a significant main effect of Dose for both number of lane departures [$F(5,58) = 8.44$, $p < .0001$] and percent of time departed from the lane [$F(5,58) = 6.65$, $p < .0001$]. Means (SEMs) are listed in Table 3.3. Although peak percent time departed from lane did not vary as a function of sex, the difference was largest after the bedtime zolpidem 10mg dose (see top right of Figure 3.6), with females spending more time departed from their lane than males.

Speed-related variables

Time course analysis: Standard deviation of speed (SDS) was insensitive to dose effects (see the bottom of Figure 3.5); but when collapsing across dose conditions, males (M ± SEM: 15.67 ± 0.03mph) displayed significantly greater variability in speed than females (M ± SEM: 14.85 ± 0.05mph). There were no significant main effects or interactions for percent time driving 5mph or more over the speed limit, $p > .05$.

Emax analysis: When analyzed as a peak score, there were no significant effects or interactions for SDS or percent time driving 5mph or more over the speed limit, $p > .05$. As seen in the lower right of Figure 3.6, the trough mean speed (i.e., the mean speed of the drive which the volunteer completed the slowest) was typically lower in females than males.

Collisions

Collisions were rare. During the course of 168 post-drug administration drives, only 20 collisions occurred. Three collisions occurred during baseline drives; but as these are not attributable to drug effect, they were excluded from further description. Females crashed more frequently than males (15 vs. 5) and were most likely to crash after the daytime alprazolam 1mg

dose (7 collisions). Four of those seven collisions occurred 4.5hr after dosing—during the last drive of the session. For males, one collision occurred after each dose condition except the bedtime alprazolam 2mg dose.

Incursion events

Time course analysis: Three-factor (Sex x Dose x Time) revealed no significant effects or interactions for distance between the incursion object and the volunteer car at the time of braking, minimum time-to-collision with the incursion object, or reaction time to press the brake in response to the incursion object, $p > .05$.

Emax analysis: There were no significant main effects or interactions for peak reaction time to press the brake in response to the incursion object, trough distance between the incursion object and the volunteer car at the time of braking, or trough minimum time-to-collision with the incursion object, $p > .05$.

Subjective Assessments Related to the Driving Simulator Task

Driving Visual Analog Scales (VAS)

Time course analysis: For males and females, driving difficulty was rated high after the daytime alprazolam 1mg condition; and for males at the first morning drive after the bedtime alprazolam 1mg (23.33± 15.91) and 2mg (43.33 ± 13.87) conditions. After administration of the daytime alprazolam 1mg dose, males reported driving difficulty dropped to 4.33 ± 4.33 (i.e., not at all difficult) by the final drive 4.5hr after drug administration, but females reported driving difficulty at 24.38 ± 11.77 (out of 100) at that same dose and timepoint (see Figure 3.7). Volunteers rated their ability to drive safely in a real car lowest after the daytime alprazolam 1mg

condition. In males, there was also a decrease in perceived safety after the bedtime alprazolam 1mg and 2mg conditions (see Figure 3.7). Obeying speed limits was rated lowest after the daytime alprazolam 1mg condition for both males and females (see Figure 3.7). An AUC analysis by sex was non-significant.

Emax analysis: There was a main effect of Dose for peak score for the Driving VAS item "Driving was difficult," and for trough scores for the items "I performed the previous simulation well," and "I could drive safely in a real car right now." Means (SEMs) collapsed across sex are listed in Table 3.3. Although Driving VAS Emax scores did not vary as a function of sex, $p > .05$, females reported that driving was more difficult and that they felt less safe to drive in a real car than males after the bedtime zolpidem 10mg dose (see Figure 3.8).

Simulator Sickness Questionnaire (SSQ)

Time course analysis: Three-factor (Sex x Dose x Time) mixed-model analyses revealed significant effects of Sex, Dose, and Time for the three subscale scores (see Figure 3.9). In general, females reported more symptoms at each dose condition, and symptoms remained elevated longer than for males. The highest SSQ scores were reported after the daytime alprazolam 1mg condition.

Emax analysis: Peak subscale scores are displayed as a function of dose and sex in Figure 3.10. Peak scores were higher for females than males after all active doses, with the largest difference observed after the bedtime zolpidem 10mg dose. These differences were non-significant in the two-factor model, and means (SEMs) collapsed across sex for scores with a significant effect of Dose (oculomotor, disorientation) are listed in Table 3.3. Scores were dose-related.

Other Psychomotor Assessments

The Circular Lights Task

Time course analysis: Three-factor (Sex x Dose x Time) mixed-model data are displayed in the top of Figure 3.11. In general, the fewest lights were extinguished after administration of the daytime alprazolam 1mg dose and the bedtime alprazolam 2mg dose. While sex was not a significant factor, females typically extinguished fewer lights than males (M ± SE collapsed across dose: [males 85.42 ± 0.46, females 80.50 ± 0.41]), particularly after the daytime alprazolam 1mg dose.

Emax analysis: Trough means (SEMs) for each dose collapsed across sex are listed in Table 3.3, and displayed by dose and sex in the bottom of Figure 3.11. For both males and females, the fewest lights were extinguished after the daytime alprazolam 1mg dose.

The Digit Symbol Substitution Task (DSST)

Time course analysis: Three-factor (Sex x Dose x Time) mixed-model analysis revealed significant effects of Dose [$F(5,60) = 3.0, p = 0.018$] and Time [$F(12,135) = 4.19, p < .0001$)] for number of trials attempted. Likewise, there were significant effects of Dose [$F(5,60) = 3.92, p = 0.004$] and Time [$F(12,135) = 3.71, p < .0001$] for number of correct trials entered. Typically, the fewest trials were attempted and correctly entered after the daytime alprazolam 1mg and the bedtime alprazolam 2mg doses. There was an effect of Sex [$F(1,11)$] for predictions of how well the volunteer thought they would perform prior to task administration ($F = 52.15, p < .0001$), the percentage of correct trials entered ($F = 14.60, p = .003$), and estimates of how well the volunteer thought they did perform immediately after task completion ($F = 32.04, p < .0001$), with males

predicting better performance, entering a greater percentage of correct trials, and retrospectively rating better performance than females.

Emax analysis: Trough means (SEMs) values for DSST measures that varied significantly by dose are listed in Table 3.3. The only item for which there was a main effect of Sex [$F(1,11) = 4.96, p = .048$] was for the question 'How well do you think you will perform?', and females proactively estimated their performance would be worse than males (M ± SE collapsed across dose with "normal performance" scored at 0 and negative numbers indicating poorer relative expected performance: [males -2 ± 1.96, females -11.19 ± 1.82]).

The SafeDrive Application

Time course analysis: Three-factor (Sex x Dose x Time) mixed-model analysis revealed no significant effects or interactions for completion time of either the simple visual or simple auditory tests, $p > .05$. For the complex visual test, there were effects of Sex [$F(1,11) = 10.67, p = .008$] and Time [$F(6,72) = 2.28, p = .045$] and a Dose x Time interaction [$F(60,673) = 1.94, p = .003$]. Irrespective of dose, females completed the test quicker than males (M ± SE: [males: 524.64 ± 3.10, females: 515.23 ± 4.03]). For both males and females, completion time was quickest at the pre-drug baseline and at the first morning timepoint but became slower for the next 4hr. There was an improvement in completion time at the last timepoint. A similar pattern of results was observed for the complex auditory test, with significant effects of Sex [$F(1,11) = 6.73, p = .025$] and Time [$F(6,72) = 4.50, p = .001$]. Irrespective of dose, females completed the test quicker than males (M ± SE: [males: 652.28 ± 4.34, females: 644.83 ± 5.72]). There were no differences in the failure rate for any the four tests as a function of sex, dose, or time.

Emax analysis: There was a main effect of Dose for peak (i.e., slowest) completion time for the simple [$F(5,58) = 4.47$, $p = .002$] and complex [$F(5,58) = 3.24$, $p = .012$] visual tests. Completion times were slowest in the daytime alprazolam 1mg and bedtime alprazolam 2mg conditions (see Table 3.3).

Assessments of Subjective Drug Effect

Drug Visual Analog Scales (VAS)

Time course analysis: Figure 3.12 displays the time course effects for the representative VAS questions, 'Do you feel any drug effects?' and 'Do you feel any bad drug effects?'. Dose effects were observed with the daytime alprazolam 1mg dose and bedtime alprazolam 2mg doses. For the active bedtime doses, the largest effects were observed at the first morning timepoint (~8:30 AM), but after the daytime alprazolam 1mg dose, the largest effects were observed approximately 1hr after dosing (~9:30 AM). Similar dose-related and time-action profiles were observed for other VAS measures (e.g., 'Do you feel high?' and 'Does the drug have any sedating effects?'). Sex was a significant factor for 'Does the drug have any stimulating/arousing effects?' [$F(1,11) = 15.74$, $p = .036$] only, with females reporting significantly more stimulating/arousing effects than males, though the means were below 1 (out of a possible 100) for both groups (M ± SE: males 0.37 ± .09, females 0.91 ± .23). The time-action profile of subjective drug VAS ratings differed between males and females. After the daytime alprazolam 1mg dose, females reported drug effects longer than males (though this was nonsignificant: *Tukey's post hoc test*, $p > .05$). In contrast, after the bedtime alprazolam 2mg dose, males reported drug effects longer than females (though this was nonsignificant: *Tukey's post hoc test*, $p > .05$). No significant dose effects were detected for the items 'Does the drug have any good effects?' and 'Does the drug have any stimulating/arousing effects?'. An AUC analysis by sex was non-significant.

Emax analysis: Items with a significant Dose effect in the two-factor (Sex x Dose) model are listed in Table 3.4 and are displayed as a function of dose and sex in Figure 3.13. Peak effects were generally dose-related and highest in the daytime alprazolam 1mg condition.

The Adjective Checklist

Time course analysis: Figure 3.14 displays the time course effects for the representative Adjective Checklist items, difficulty concentrating and sleepy. The largest scores were observed after the daytime alprazolam 1mg and bedtime alprazolam 2mg doses. For items with a significant effect of Sex (relaxed, difficulty concentrating, dry mouth, heavy/sluggish, and sleepy), females reported greater effects than males (M ± SE: [relaxed: males 0.75 ± 0.04, females 1.69 ± 0.05], [difficulty concentrating: males 0.12 ± 0.02, females 0.37 ± 0.03], [dry mouth: males 0.09 ± 0.01, females 0.01 ± 0.00], [heavy/sluggish: males 0.38 ± 0.03, females 0.38 ± 0.03], and [sleepy: males 0.86 ± 0.05, females 0.88 ± 0.04]). An AUC analysis by sex was non-significant.

Emax analysis: Means (SEMs) for items with a significant Dose effect in the two-factor (Sex x Dose) model are listed in Table 3.4. The only Adjective Checklist peak score that differed between males and females was lightheadedness [$F(1,11) = 4.88$, $p < .05$], with females (0.19 ± 0.06) reporting more lightheadedness than males (0.06 ± 0.04). Although other peak Adjective Checklist scores did not vary as a function of Sex, there were differences between males and females for specific doses (see Figure 3.15). Females reported upset stomach and difficulty concentrating after the bedtime zolpidem 10mg dose, while males did not.

The Observer Adjective Checklist

Time course analysis: Three-factor (Sex x Dose x Time) mixed-model results are listed in Table 3.4. For the Observer Adjective Checklist items with a significant effect of time (relaxed, sleepy, clumsy, sluggish, and dazed/spaced-out), scores were generally higher in the morning and tapered off towards the end of the session (see Figure 3.14 for representative examples). Observer Adjective Checklist items with a significant effect of dose (relaxed, sleepy, and sluggish) were typically highest for the positive control condition. Females were rated as clumsier than males (M ± SE: males 0.38 ± 0.01, females 0.99 ± 0.01). As can be seen in Figure 3.14, sluggish scores are higher for the daytime alprazolam 1mg condition for both males and females, but the peak occurred earlier for males (1hr post drug administration) than females (1.5hr post drug administration). The effect also appears to be of longer duration in females compared to males. This pattern of results was similar for the item, dazed/spaced-out. An AUC analysis by sex was non-significant.

Emax analysis: Means (SEMs) for items with a significant effect of Dose in the two-factor (Sex x Dose) model are listed in Table 3.4. Peak Observer Adjective Checklist scores did not vary as a function of Sex for any item, $p > .05$.

Sleep Measures

Sleep Visual Analog Scales (VAS)

In general, volunteers reported that they fell asleep more easily and woke up less often after any of the positive bedtime dose administrations. Volunteers reported feeling most clear-headed after the bedtime alprazolam 0.5mg dose (81.63 ± 5.55) and least clear-headed after the bedtime alprazolam 2mg dose (43.86 ± 8.57). While Sleep VAS outcomes were not significantly different

between males and females, as can be seen in Figure 3.16, males reported more positive sleep indices for Sleep VAS items.

Fitbit

Two-factor (Sex x Dose) mixed-model analysis revealed no significant effects or interactions for total sleep time, see the bottom of Figure 3.16. Due to equipment malfunction and user error, approximately 30% of fitbit data were missing.

Physiological measures

Blood pressure

Time course analysis: Three-factor (Sex x Dose x Time) mixed-model results are displayed in the top of Figure 3.17. For all dose conditions, blood pressure measurements were lower for females than males at all timepoints. Blood pressure decreased slightly 15 min after morning drug administration and then generally leveled off near the baseline measurements. Blood pressure was unaffected by dose.

Emax analysis: There was an effect of Sex [$F(1,11) = 21.65 – 22.12, p < .05$] for peak and trough blood pressure. Males reached higher peak systolic (M ± SE: [males: 132.94 ± 1.78, females: 114.96 ± 2.35]) and diastolic (M ± SE: [males: 81.50 ± 0.99, females: 74.83 ± 1.58]) blood pressure than females. Females reached lower trough systolic blood pressure than males (M ± SE: [males: 112.42 ± 1.60, females: 96.02 ± 1.92).

Heart rate

Time course analysis: Three-factor (Sex x Dose x Time) mixed-model results are displayed in the bottom of Figure 3.17. Heart rate decreased from baseline until approximately 2hr after drug administration and then increased to near baseline levels by the end of session. Heart rate was unaffected by dose and did not vary significantly by sex (though mean heart rate measurements were lower in females than males for all timepoints except the first taken approximately 1hr after waking).

Emax analysis: There was a main effect of Dose [$F(5,58) = 2.96$, $p = .019$] for peak heart rate. The highest heart rate was observed during the daytime alprazolam session (M ± SE: 81.79 ± 2.96) while the mean peak heart rate from the other five sessions was 76.3 ± 3.29 bpm, but post hoc dose comparison tests were non-significant, $p > .05$.

Respiration rate

Time course analysis: Three-factor (Sex x Dose x Time) mixed-model analysis revealed significant effects of Sex on respiration rate measured over 60 seconds [$F(1,11) = 9.57$, $p = .01$], with females breathing slightly slower than males (M ± SE: [males: 16.17 ± 0.19, females: 15.06 ± 0.06]). Neither dose nor time significantly affected respiration rate, $p > .05$.

Emax analysis: There were no main effects or interactions for respiration rate when analyzed as a peak or trough value, $p > .05$.

Table 3.1 Subject demographics and baseline characteristics

	Mean (SEM)	
	Males (n = 6)	**Females (n = 8)**
Age	26.83 (2.02)	33.50 (3.43)
Height (in)	**70.58 (1.36)**	**66.86 (0.95)**
Weight (lb)	176.00 (8.47)	150.75 (10.21)
BMI	24.92 (1.45)	23.68 (1.43)
Body Fat Percentage	**23.75 (2.68)**	**34.43 (3.49)**
Years of Education	15.67 (0.99)	16.25 (0.96)
WRAT	50.33 (1.98)	50.88 (0.52)
SCL-90	9.00 (5.29)	9.86 (3.20)
Beck	2.00 (0.58)	2.00 (0.80)
PSQI	6.00 (1.35)	4.25 (0.84)
MAST	2.83 (0.98)	2.38 (0.89)
30-Day Alcohol Use	5.50 (2.01)	8.50 (2.39)

Bolded values indicate a significant difference between males and females with t-test statistic, $p < .05$.

Table 3.2 Standard deviation of lane position (SDLP) descriptive statistics from baseline drives of all six sessions

	Whole Drive			Highway Section		
	Mean	Range	Variance (s^2)	Mean	Range	Variance (s^2)
Males (n = 6)						
1	28.45	26.19 – 30.37	2.54	19.07	15.94 – 25.03	11.73
2	39.38	34.52 – 44.81	17.57	21.99	17.61 – 28.59	19.17
3	30.73	28.70 – 33.14	3.57	18.46	11.84 – 28.71	33.48
4	37.73	33.31 – 43.15	12.76	27.22	18.67 – 32.73	24.69
5	36.92	35.87 – 38.11	0.84	22.26	11.05 – 40.17	93.10
6	30.26	27.65 – 34.06	6.44	19.03	13.28 – 28.01	26.91
Average	33.91		7.29	21.34		34.85
Females (n = 8)						
7	43.11	37.84 - 49.61	20.28	22.43	14.13 – 30.04	31.28
8	30.42	27.4 - 32.36	2.83	25.26	22.33 – 27.72	4.04
9	41.17	34.7 - 49.68	26.33	34.68	24.14 – 50.75	125.89
10	38.11	35.5 - 41.10	4.57	21.67	12.55 – 33.48	64.38
11	32.56	27.6 – 39.96	9.50	25.14	17.92 – 31.87	34.74
12	42.56	39.4 - 47.00	6.73	33.36	26.61 – 37.99	23.29
13	30.29	27.8 - 35.04	7.45	23.93	11.63 – 36.55	80.10
14	31.15	25.6 - 35.33	13.02	11.76	9.59 – 14.45	3.35
Average	36.17		11.34	24.78		45.88
Total (n = 14)						
Average	**35.20**		**9.60**	**23.30**		**41.15**

Table 3.3 Emax (peak and trough) values for psychomotor and related subjective assessments

	Dose (mg) Combination (Bedtime/Daytime)						
	F(5,58)	PLC/PLC	Z(10)/PLC	PLC/A(1.0)	A(0.5)/PLC	A(1.0)/PLC	A(2.0)/PLC
Driving Simulator							
Standard deviation of lane position	**8.90**	1.32 (0.08)	1.48 (0.12)	**1.71 (0.08)**	1.33 (0.06)	1.35 (0.06)	**1.56 (0.09)**
Number of lane departures	**6.65**	18.07 (1.26)	20.50 (1.93)	**28.00 (2.18)**	18.14 (1.15)	19.36 (1.39)	22.07 (1.82)
Time departed from lane (%)	**8.44**	5.35 (0.63)	7.31 (1.62)	**10.21 (1.14)**	5.27 (0.50)	6.01 (0.76)	7.82 (1.09)
Driving Visual Analog Scales							
Driving was difficult	**3.76**	14.29 (5.96)	20.29 (8.21)	**44.07 (10.20)**	14.86 (7.04)	26.64 (9.30)	31.79 (8.58)
I performed well (T)	**2.55**	62.86 (7.08)	67.86 (7.92)	49.43 (8.25)	73.71 (6.72)	63.86 (8.25)	64.57 (6.82)
I could drive safely (T)	**4.21**	79.36 (8.25)	76.64 (8.80)	**48.07 (9.69)**	79.71 (7.95)	64.29 (8.84)	65.07 (10.54)
Simulator Sickness Questionnaire							
Oculomotor	**7.51**	1.57 (0.43)	1.86 (0.58)	**4.57 (0.89)**	1.36 (0.50)	2.71 (0.67)	3.07 (0.82)
Disorientation	**3.07**	0.64 (0.25)	0.86 (0.35)	**2.21 (0.59)**	1.21 (0.55)	1.1 (0.40)	0.57 (0.25)
SafeDrive Application (time, ms)							
Simple visual test	**4.47**	362 (20.53)	364 (20.26)	405 (19.50)	371 (22.63)	390 (26.22)	**407 (30.51)**
Complex visual test	**3.24**	566 (28.42)	558 (18.76)	599 (19.12)	557 (13.09)	561 (12.71)	615 (26.60)
Circular Lights Task							
Lights extinguished	**7.48**	77.71 (3.21)	78.21 (2.16)	**67.36 (2.65)**	79.07 (2.05)	75.43 (1.85)	69.64 (2.52)
Digit Symbol Substitution Task							
How well do you think you will perform? (T)	**4.69**	9.29 (3.21)	8.14 (2.54)	**6.00 (2.20)**	7.50 (2.22)	10.64 (3.37)	6.21 (2.37)
Trials attempted (T)	**8.29**	41.29 (1.22)	41.86 (1.08)	**39.86 (1.16)**	41.29 (1.17)	40.71 (1.05)	**39.50 (1.24)**
Number of correct trials (T)	**8.66**	40.43 (1.17)	40.64 (1.01)	**39.21** (1.19)	40.50 (1.20)	40.29 (1.09)	**38.79 (1.25)**
Percent of correct trials (T)	**2.85**	92.32 (1.02)	90.99 (1.40)	86.67 (2.89)	92.54 (1.16)	90.94 (1.55)	88.06 (2.47)

PLC = placebo, Z = zolpidem, A = alprazolam. Data are means (SEMs) collapsed across sex. Two factor analysis (Sex x Dose) detected significant main effects of Dose for all items listed, $p < .05$. Items marked with (T) indicate trough scores are shown; all other items are peak. Bolded values indicate a significant *Tukey post hoc test* from the placebo/placebo dose combination, and boxed items indicate a significant *Tukey post hoc* test from the zolpidem/placebo condition, $p < .05$.

Table 3.4 Emax (peak and trough) values of subjective and observer-rated measures

	Dose (mg) Combination (Bedtime/Daytime)											
	F(5,58)	PLC/PLC		Z(10)/PLC		PLC/A(1.0)		A(0.5)/PLC		A(1.0)/PLC		A(2.0)/PLC	
Drug Visual Analog Scales													
Any Drug Effect	**8.80**	13.86	(5.32)	20.07	(6.11)	**49.14**	**(8.59)**	11.29	(4.77)	25.71	(5.77)	**41.00**	**(6.72)**
High	**2.98**	8.29	(4.44)	8.64	(4.54)	28.71	(7.96)	4.29	(2.33)	9.71	(5.29)	11.79	(4.43)
Bad Effects	**7.12**	2.71	(1.48)	12.07	(3.65)	**34.71**	**(7.68)**	5.64	(3.06)	13.64	(5.94)	**20.07**	**(6.41)**
Sedating Effects	**6.80**	13.00	(5.40)	16.43	(4.92)	**51.57**	**(8.58)**	13.14	(5.36)	29.29	(8.14)	38.07	(8.83)
Adjective Checklist													
Dizzy	**2.52**	0.07	(0.07)	0.07	(0.07)	0.36	(0.17)	0.07	(0.07)	0.07	(0.07)	0.07	(0.07)
Upset Stomach	**3.27**	0.00	(0.00)	**0.36**	**(0.17)**	0.07	(0.07)	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)
Drunken	**2.76**	0.07	(0.07)	0.14	(0.10)	**0.07**	**(0.07)**	0.14	(0.14)	0.36	(0.17)	0.50	(0.20)
Slurred Speech	**3.90**	0.00	(0.00)	0.00	(0.00)	**0.50**	**(0.20)**	0.07	(0.07)	0.21	(0.11)	0.21	(0.11)
Difficulty Concentrating	**7.31**	0.29	(0.13)	0.57	(0.23)	**1.43**	**(0.31)**	0.50	(0.20)	0.79	(0.28)	**1.14**	**(0.25)**
Heavy/Sluggish	**8.51**	0.43	(0.17)	0.86	(0.18)	**1.71**	**(0.30)**	0.64	(0.17)	1.07	(0.30)	**1.93**	**(0.30)**
Sleepy	**6.74**	1.29	(0.29)	1.36	(0.27)	**2.57**	**(0.29)**	1.64	(0.27)	**2.29**	**(0.34)**	**2.50**	**(0.27)**
Weak	**5.34**	0.07	(0.07)	0.29	(0.13)	**0.79**	**(0.28)**	0.14	(0.10)	0.29	(0.16)	0.86	(0.29)
Uncoordinated	**4.11**	0.29	(0.13)	0.43	(0.14)	**1.00**	**(0.31)**	0.36	(0.17)	0.50	(0.17)	0.71	(0.22)
Observer Adjective Checklist													
Sleepy	**2.63**	1.64	(0.17)	1.36	(0.23)	2.21	(0.26)	1.57	(0.20)	1.50	(0.17)	2.00	(0.26)
Clumsy	**2.86**	0.36	(0.17)	0.07	(0.07)	0.71	(0.19)	0.29	(0.16)	0.14	(0.10)	0.57	(0.17)
Sluggish	**3.72**	0.86	(0.18)	0.86	(0.23)	**1.93**	**(0.35)**	1.07	(0.22)	1.21	(0.19)	1.14	(0.23)

PLC = placebo, Z = zolpidem, A = alprazolam. Data are means (SEMs) collapsed across sex. Two factor analysis (Sex x Dose) detected significant main effects of Dose for all items listed, $p < .05$. Items marked with (T) indicate trough scores are shown; all other items are peak. Bolded values indicate a significant *Tukey post hoc test* from the placebo/placebo dose combination, and boxed items indicate a significant *Tukey post hoc* test from the zolpidem/placebo condition, $p < .05$.

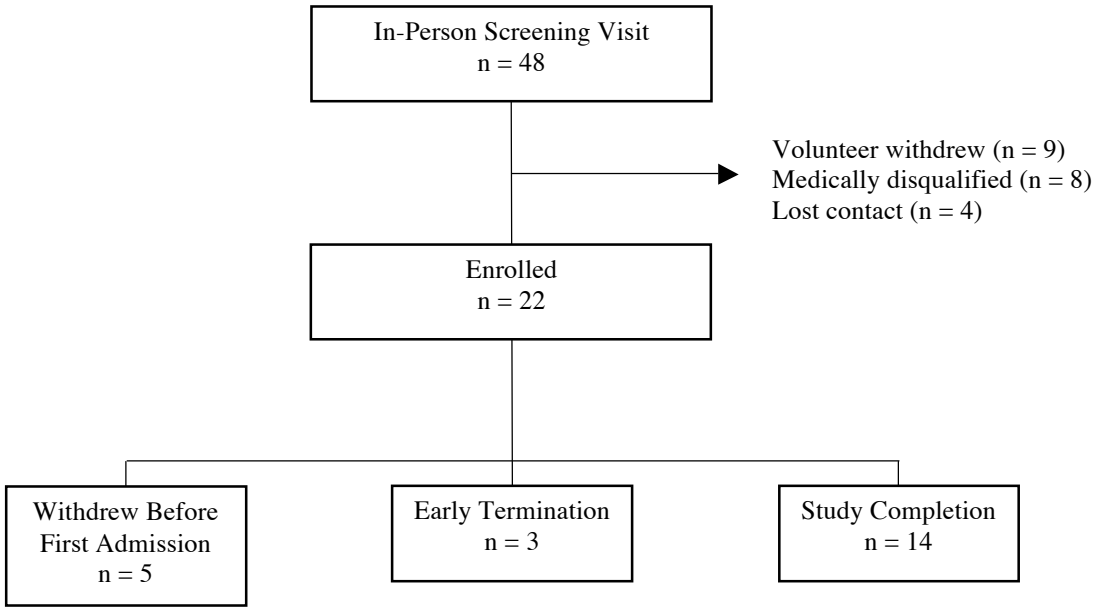

Figure 3.1 Participant flow

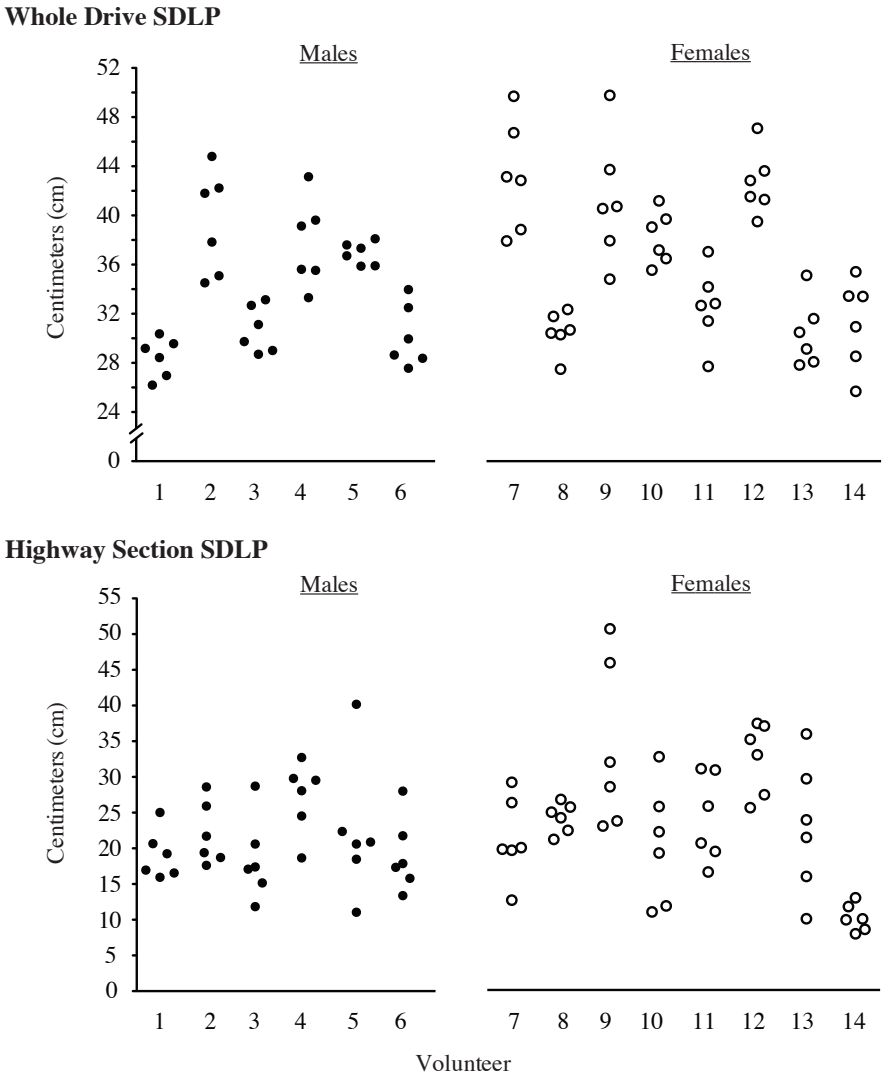

Figure 3.2 Baseline drive standard deviation of lane position (SDLP) variability across all six study sessions. Each point represents the SDLP (from the whole drive [top row] or the highway section of the drive [bottom row]) of a volunteer's first drive (pre-drug administration baseline) of each of the six study sessions. Points clustered closer together in a column (e.g., Volunteer 14) indicate less variability in SDLP from one session to the next than for points more spread out (e.g., Volunteer 13).

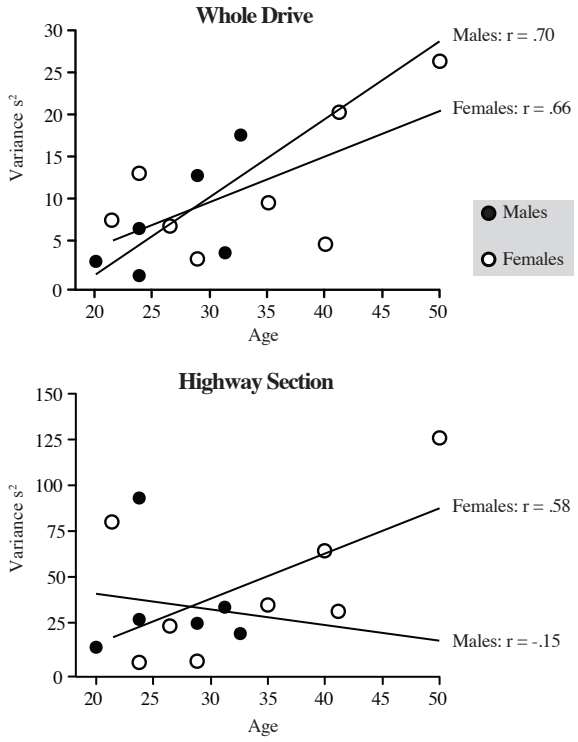

Figure 3.3 Variance in baseline drive standard deviation of lane position (SDLP) correlation with age. n = 14 (6 male). Data are individual volunteer variance in SDLP (from the whole drive [top row] or the highway section of the drive [bottom row]) calculated from the first drive (pre-drug administration baseline) of each of the six study sessions displayed as a function of age and sex. Pearson correlations were non-significant, $p > .05$, both when analyzing sexes separately and together.

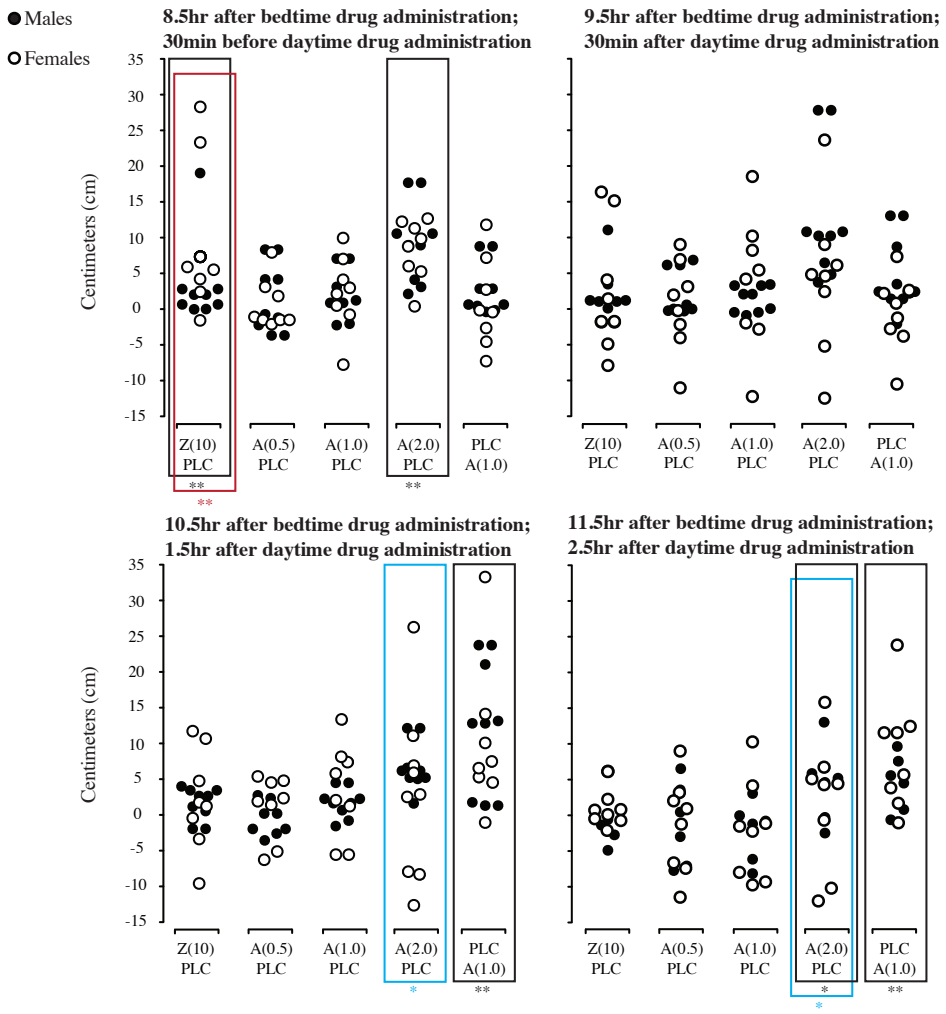

Figure 3.4 Standard deviation of lane position change from placebo for each volunteer across all active dose conditions, from 8.5hr to 11.5hr after bedtime drug administration. Each symbol represents a single volunteer's drive at the corresponding dose and timepoint. Significant impairment (equivalent to ≥ .05% BAC) based on symmetry analysis is indicated by boxes [black boxes indicate the dose was impairing for the group as a whole (n = 14); red boxes indicate impairment for females (n = 8) analyzed alone; blue boxes indicate impairment for males (n = 6) analyzed alone], $p < .05$. Sex-specific boxes are only pictured when whole group outcomes were not consistent with sex-collapsed outcomes. * Indicates impairment equivalent to ≥ .08% BAC. ** Indicates impairment equivalent to ≥ .10% BAC.

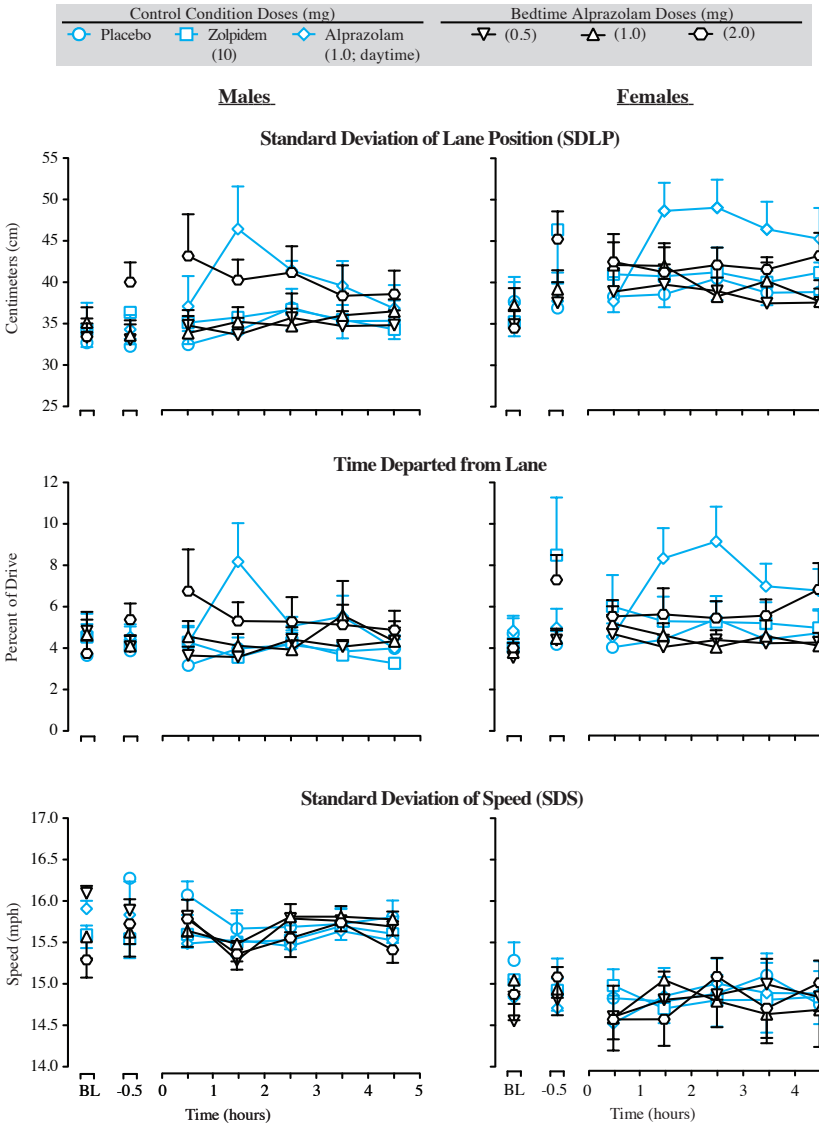

Figure 3.5 Driving simulator outcomes measured across the whole drive, from pre-drug baseline (BL) through the end of the 5hr session. n = 14 (6 males). Data are means (SEM) and are displayed as a function of dose (mg), time, and sex (males in left column, females in right column). Time course analyses indicated main effects of Dose [$F(5,60) = 3.72 - 4.13$, $p < .05$] and Time [$F(6,72) = 2.26 - 8.82$, $p < .05$] and a Dose by Time interaction [$F(30,360) = 2.47 - 3.08$, $p < .005$] for standard deviation of lane position (SDLP) and percent of drive departed from lane. There was a main effect of Sex for standard deviation of speed [$F(1,11) = 24.71$, $p < .0001$], with females varying speed significantly less than males (*Tukey's post hoc test*, $p < .05$).

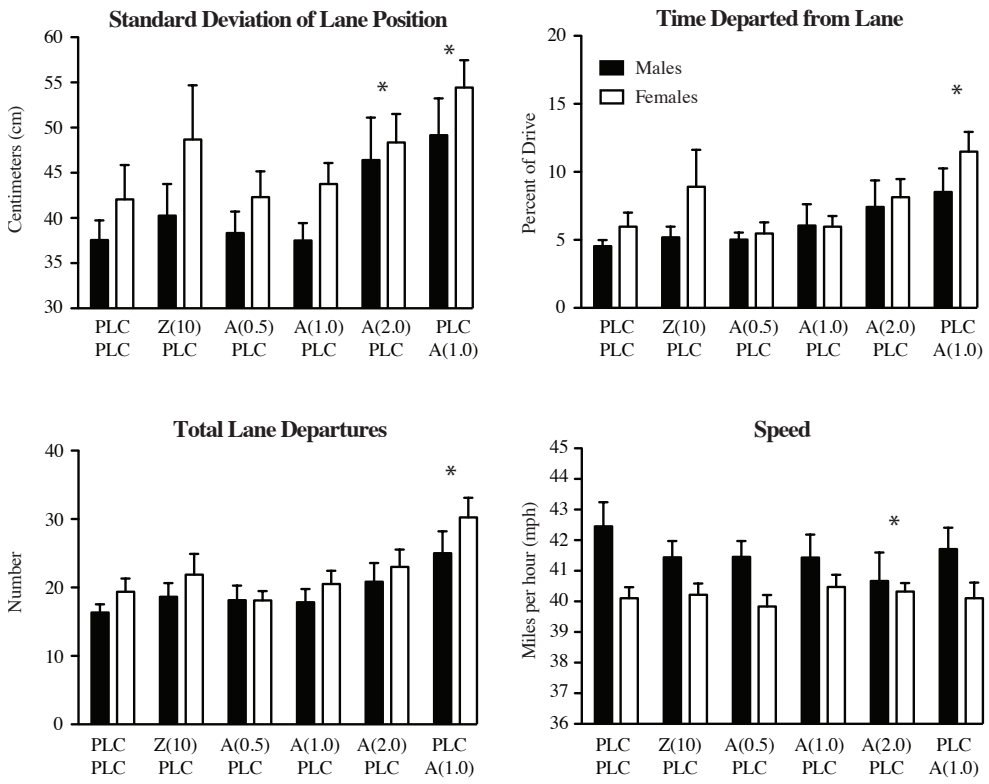

Figure 3.6 Mean peak (standard deviation of lane position [SDLP], time departed from lane, total lane departures) and trough (speed) effects for driving simulator outcomes. n = 14 (6 males). Data are means (SEM) and are displayed as a function of dose (mg) and sex. Dose combinations below each graph are shown with the bedtime dose listed above the daytime dose (PLC = placebo, A = alprazolam, Z = zolpidem). Two factor analyses (Dose x Sex) detected a significant main effect of Dose [$F(5,58) = 6.65 – 8.44$, $p < .0001$] for peak SDLP, time departed from lane, total lane departures and a Sex by Dose interaction for trough speed [$F(5,58) = 3.36$, $p = .01$]. * Indicates a significant peak effect of dose compared to placebo (*Tukey's post hoc test, $p < .05$*).

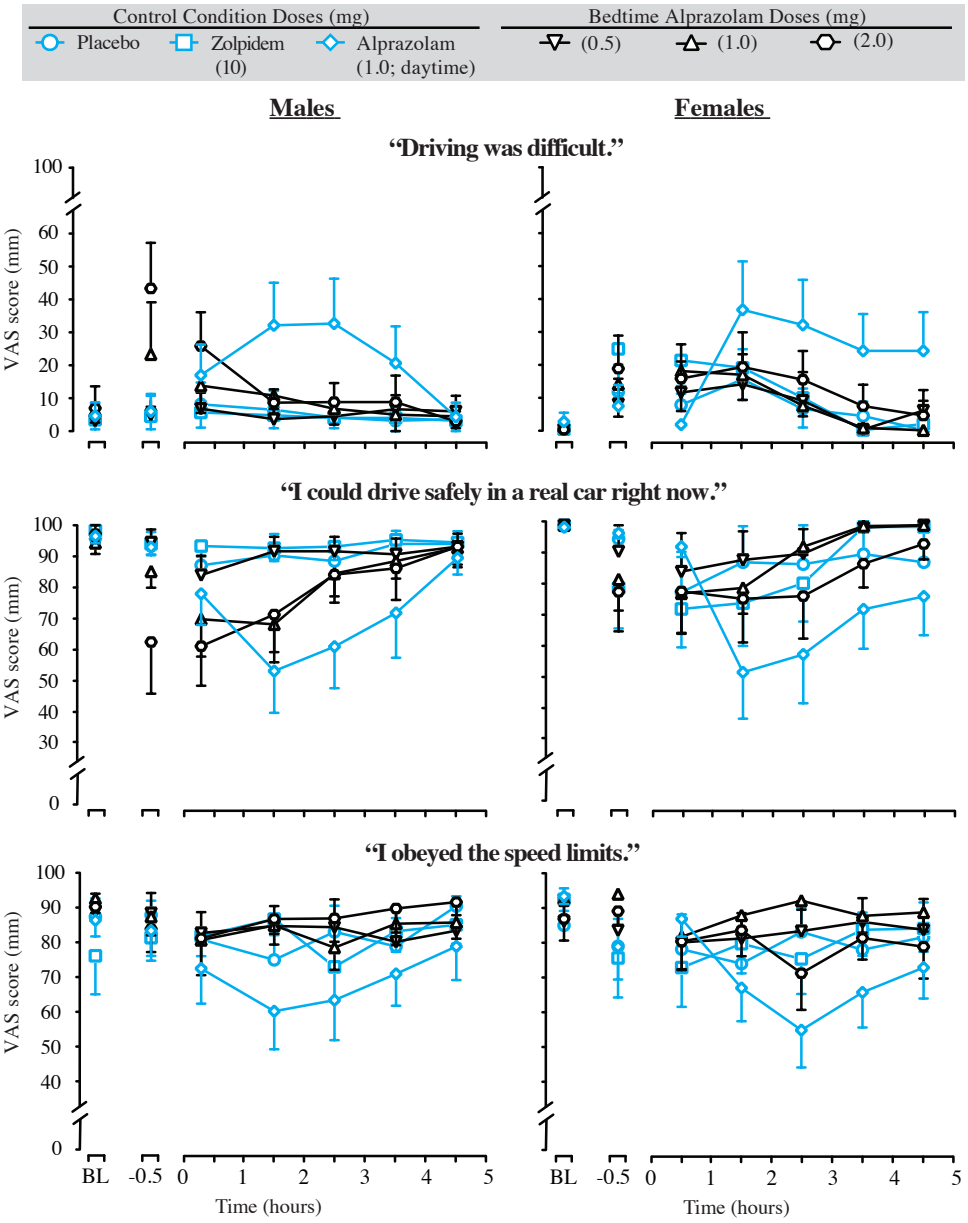

Figure 3.7 Time course of mean driving visual analog scale (VAS) outcomes, from pre-drug baseline (BL) through the end of the 5hr session. n = 14 (6 males). Three-factor (Sex x Dose x Time) analyses revealed a main effect of Time [$F(6,72) = 3.48 – 711.19, p < .005$] for all three items. For the items "Driving was difficult" and "I could drive safely in a real car right now," there was also a main effect of Dose [$F(5,60) = 2.76 - 3.41, p < .05$] and a Dose by Time interaction [$F(3,360) = 1.83 – 2.46, p < .01$).

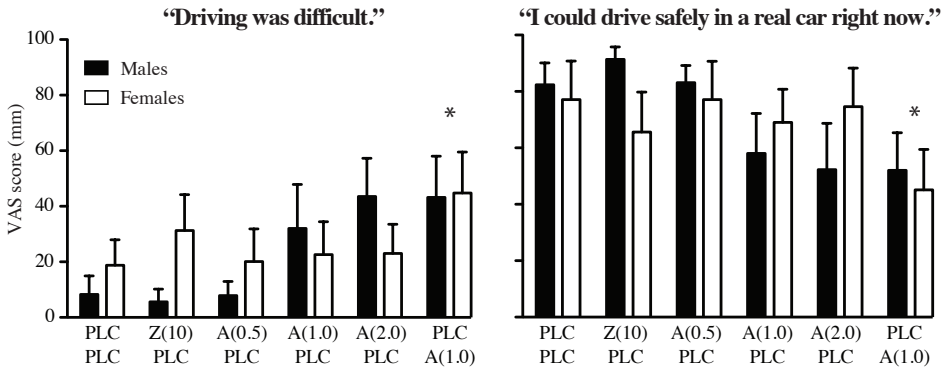

Figure 3.8 Mean Emax of driving simulator visual analog scale (VAS) outcomes. n = 14 (6 males). Data are means (SEM) and are displayed as a function of dose (mg) and sex. Dose combinations below each graph are shown with the bedtime dose listed above the daytime dose (PLC = placebo, A = alprazolam, Z = zolpidem). Two factor analyses (Dose x Sex) detected a significant main effect of Dose [$F(5,58)$] for peak response to "driving was difficult," [$F = 3.76, p = .005$] and trough response to "I could drive safely in a real car right now" [$F = 4.21, p = .003$].

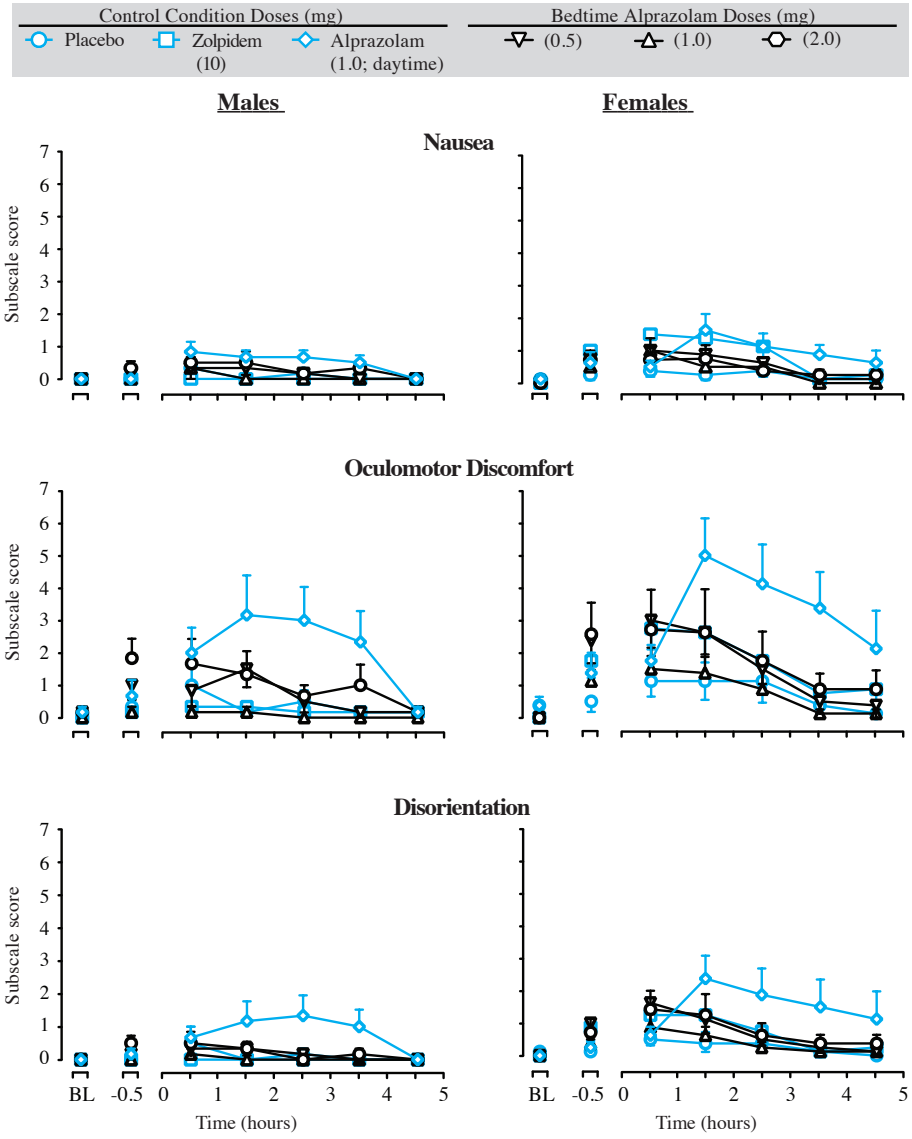

Figure 3.9 Mean time course of simulator sickness questionnaire subscale scores, from pre-drug baseline (BL) through the end of the 5hr session. n = 14 (6 males). Three-factor (Sex x Dose x Time) analyses revealed main effects of Sex [$F(1,11) = 13.67$ - 19.70, $p < .005$], Dose [$F(5,60) = 2.50 – 7.22$, $p < .05$], and Time [$F(6,72) = 9.17 – 16.22$, $p < .0001$], and a Sex x Time [$F(6,72) = 3.37 – 4.19$, $p < .01$], interaction for all three subscales.

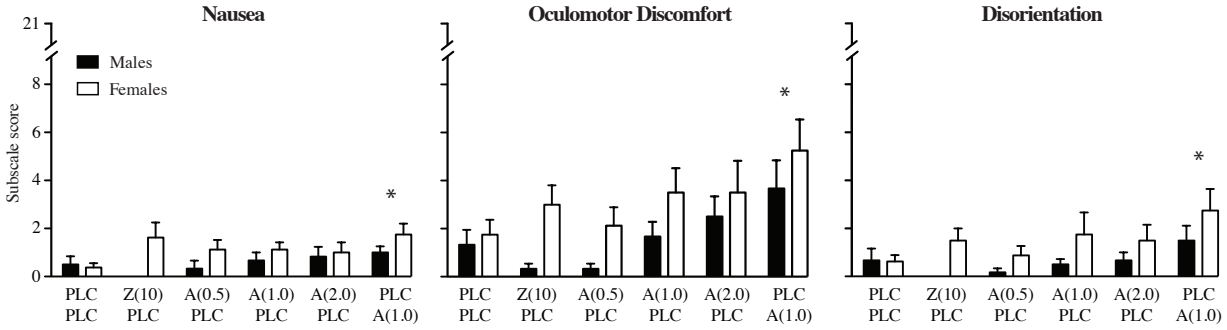

Figure 3.10 Mean Emax simulator sickness questionnaire subscale scores. n = 14 (6 males). Data are means (SEM) and are displayed as a function of dose (mg) and sex. Dose combinations below each graph are shown with the bedtime dose listed above the daytime dose (PLC = placebo, A = alprazolam, Z = zolpidem). Two factor analyses (Sex x Dose) detected a significant main effect of Dose [$F(5,58) = 2.03 – 7.51$, $p < .05$] for oculomotor discomfort and disorientation subscales. * Indicates a significant peak effect of dose compared to placebo (*Tukey's post hoc test*, $p < .05$).

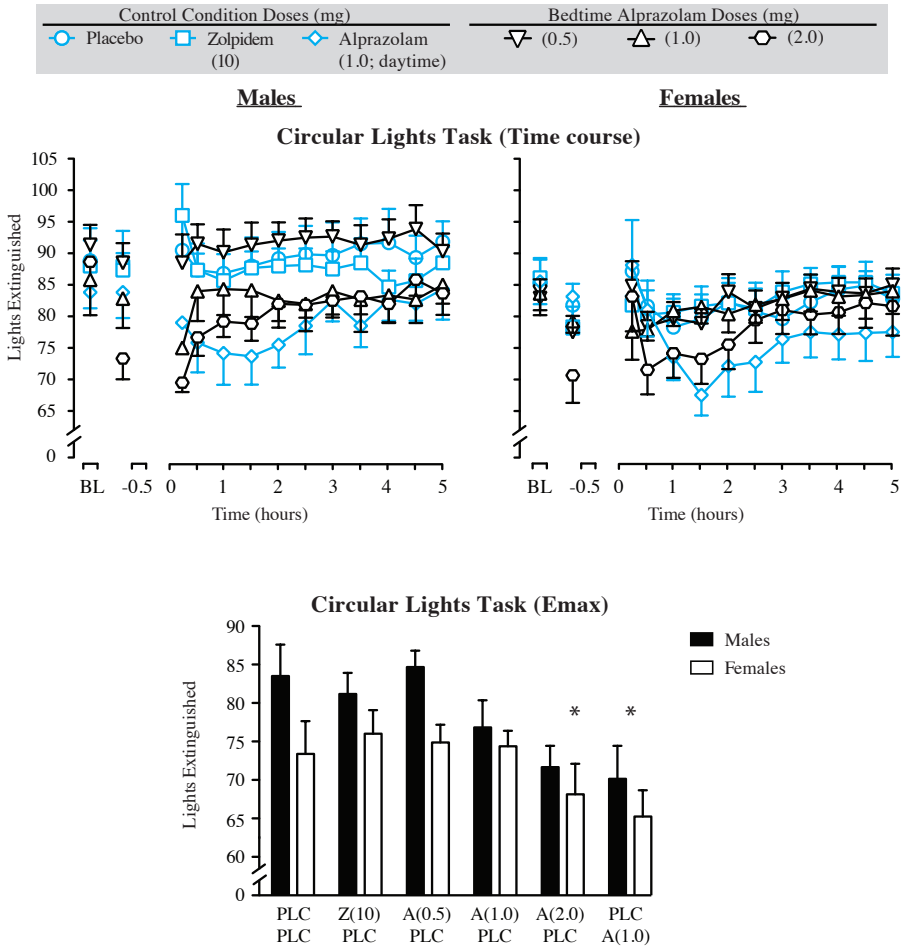

Figure 3.11 Mean time course and Emax outcomes for the circular lights task. n = 14 (6 males). Data are means (SEM) and are displayed as a function of dose (mg), time (top row only), and sex. Time course (top row): Time course analyses indicated main effects of Dose [$F(5,60) = 3.61$, $p = .006$] and Time [$F(12,135) = 5.76$, $p < .0001$] and a Dose x Time interaction [$F(60,674) = 1.64$, $p = .002$] for number of lights extinguished. Emax (bottom row): Emax = fewest lights extinguished during a single session. Dose combinations below the graph are shown with the bedtime dose listed above the daytime dose (PLC = placebo, A = alprazolam, Z = zolpidem). Two factor analyses (Dose x Sex) detected a main effect of Dose [$F(5,58) = 7.48$, $p < .001$]. * Indicates a significant peak effect of dose compared to placebo (*Tukey's post hoc test*, $p < .05$).

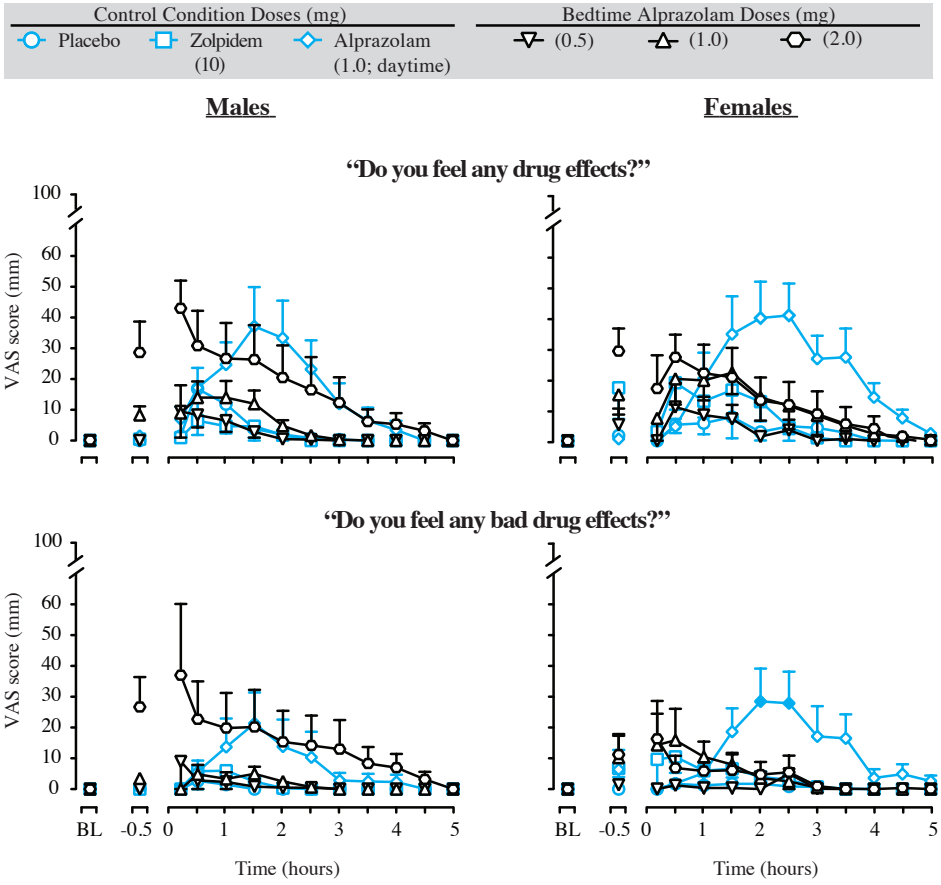

Figure 3.12 Time course of mean visual analog scale (VAS) ratings of the subjective measures 'Do you feel any drug effects?' and 'Do you feel any bad drug effects?' after drug administration, from pre-drug baseline (BL) through the end of the 5hr session. n = 14 (6 males). Data are means (SEM) and are displayed as a function of dose (mg), time, and sex. Time course analyses indicated main effects of Dose [$F(5,60) = 4.11 - 4.76, p < .005$] and Time [$F(12,135) = 5.64 - 11.69, p < .0001$] and a Dose by Time interaction [$F(60,674) = 2.12 - 3.00, p < .0001$] for both measures. Filled symbols indicate mean ratings that were significantly different from placebo at that timepoint within sex (*Tukey's post hoc test*, $p < .05$).

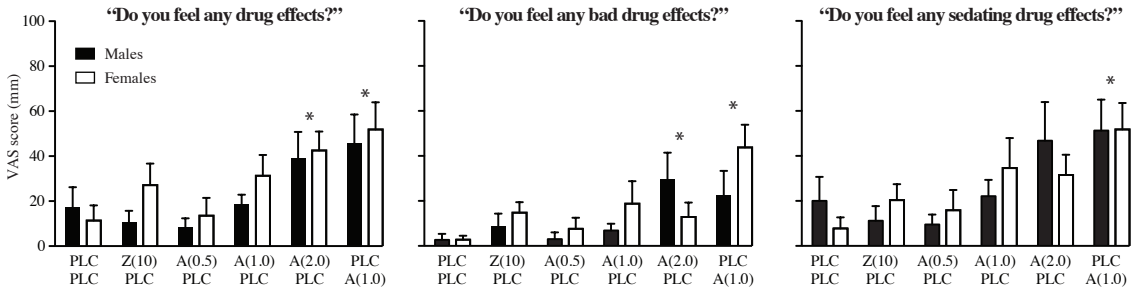

Figure 3.13 Mean peak ratings for representative visual analog scale (VAS) items. n = 14 (6 males). Data are means (SEM) and are displayed as a function of dose (mg) and sex. Dose combinations below each graph are shown with the bedtime dose listed above the daytime dose (PLC = placebo, A = alprazolam, Z = zolpidem). Two factor analyses (Dose x Sex) detected a significant main effect of Dose [$F(5,58) = 6.80 – 8.80$, $p < .0001$] for all three items. * Indicates a significant peak effect of dose compared to placebo (*Tukey's post hoc test*, $p < .05$).

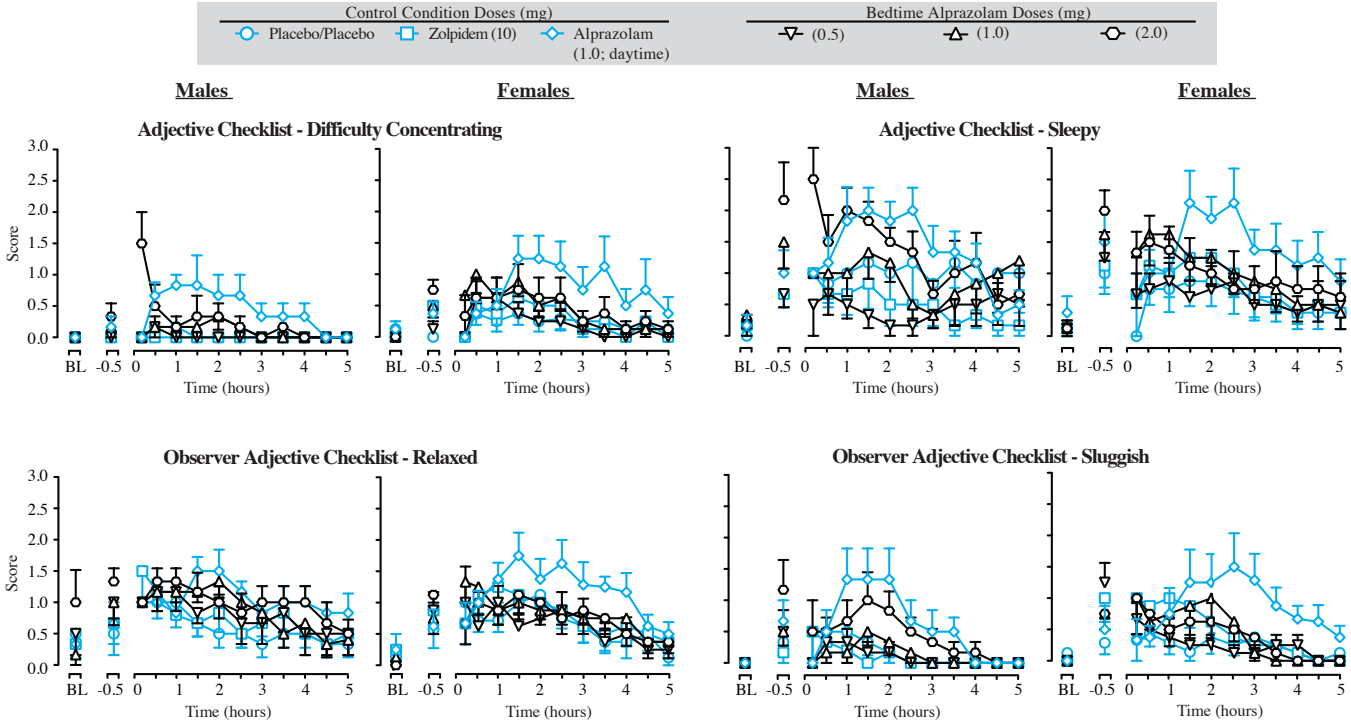

Figure 3.14 Time course of mean adjective and observer adjective checklist ratings for the measures difficulty concentrating, sleepy, relaxed, and sluggish. n = 14 (6 males). Data are means (SEM) and are displayed as a function of dose (mg) and sex. Time course analyses indicated main effects of Dose [$F(5,60)$ = 3.35 - 4.12] and Time [$F(60,674)$ =6.94 - 22.15] for all four items, $p < .05$. A main effect of Sex [$F(1,11)$ = 3.35 - 6.97, $p < .05$] was found for all items except relaxed.

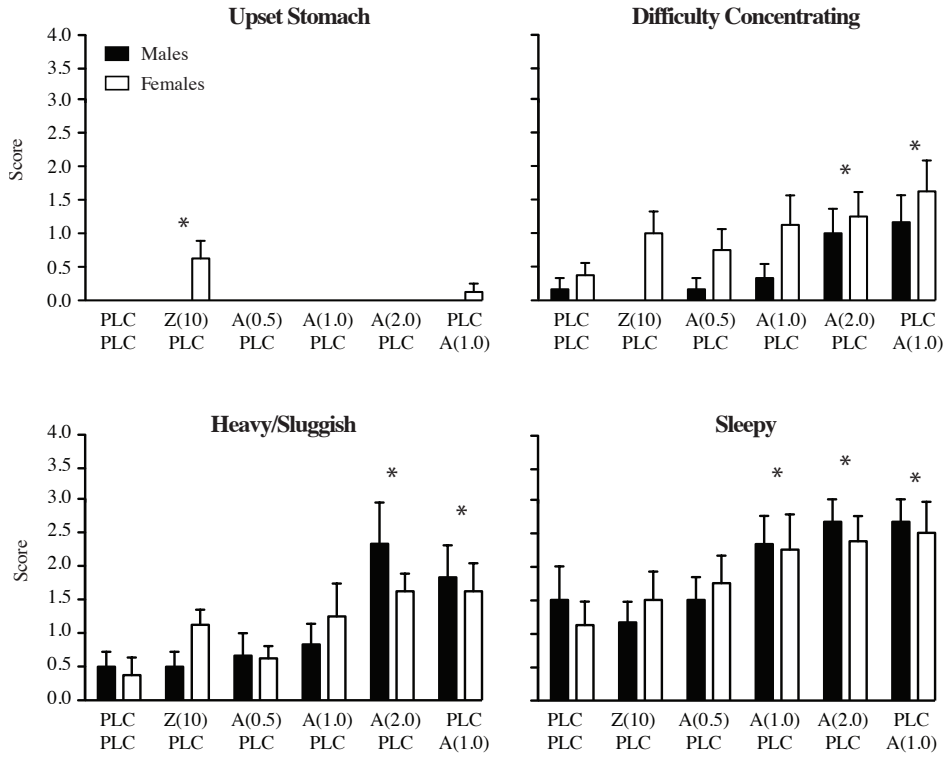

Figure 3.15 Mean peak ratings for representative adjective checklist items upset stomach, difficulty concentrating, heavy/sluggish, and sleepy. n = 14 (6 males). Data are means (SEM) and are displayed as a function of dose (mg) and sex. Dose combinations below each graph are shown with the bedtime dose listed above the daytime dose (PLC = placebo, A = alprazolam, Z = zolpidem). Two factor analyses (Dose x Sex) detected a significant main effect of Dose [$F(5,58) = 3.27 – 8.51$, $p < .02$] for all four items. * Indicates a significant peak effect of dose compared to placebo (*Tukey's post hoc test*, $p < .05$). A Sex x Dose interaction was detected for upset stomach [$F(5,58) = 3.15$, $p = .014$].

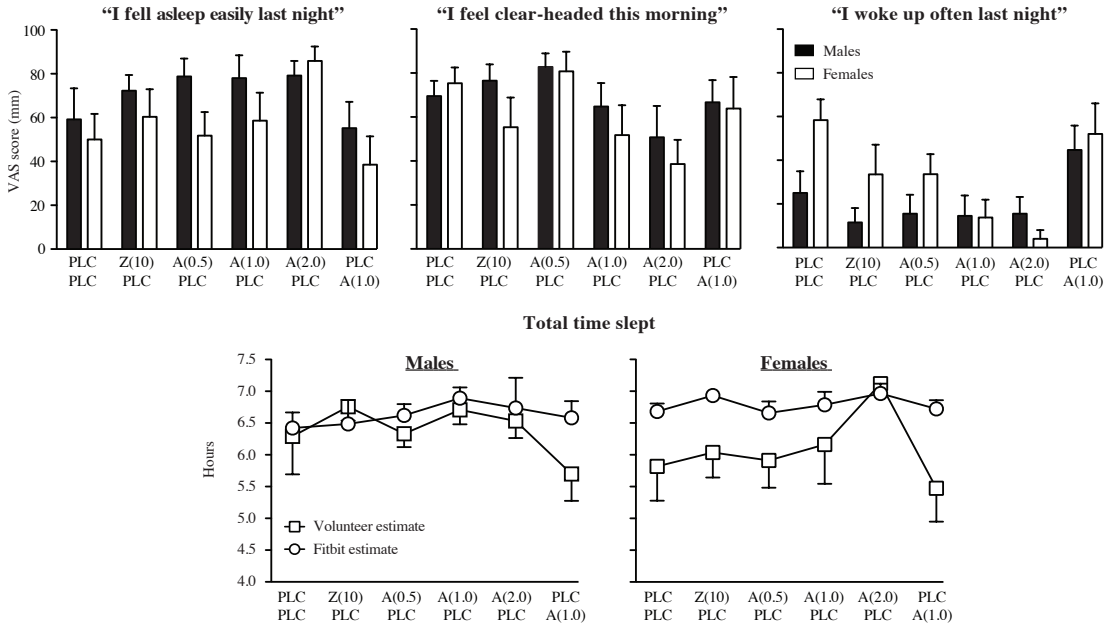

Figure 3.16 Sleep outcomes from visual analog scale (VAS) ratings and fitbit measurement. n = 14 (6 males) for VAS and n = 9 (4 males) for fitbit. Data are means (SEM) and are displayed as a function of dose (mg) and sex. Dose combinations below each graph are shown with the bedtime dose listed above the daytime dose (PLC = placebo, A = alprazolam, Z = zolpidem). Two factor (Dose x Sex) analyses detected a significant main effect of Dose [$F(5,59)$] for the VAS items "I feel asleep easily last night" ($F = 2.89$, $p = .021$), "I woke up clear-headed this morning" ($F = 4.85$, $p = .001$), "I woke up often last night" ($F = 5.47$, $p < .0001$), and total time slept ($F = 3.23$, $p = .012$).

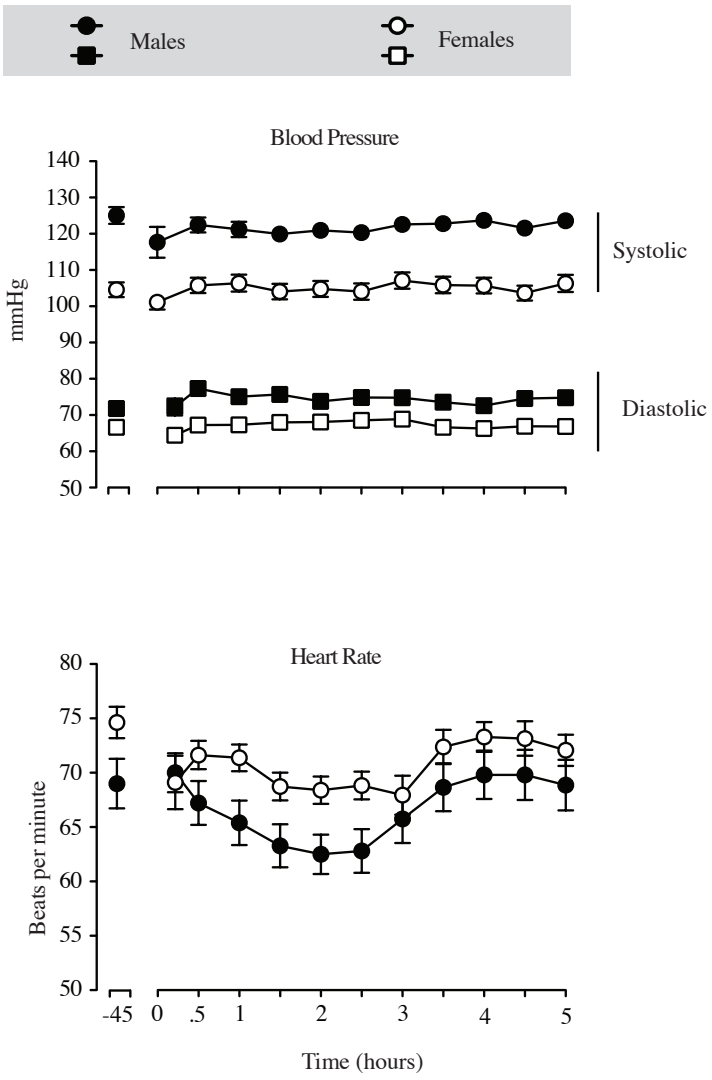

Figure 3.17 Time course of blood pressure and heart rate, from 45min before the daytime drug administration through the end of the 5hr session. n = 14 (6 males). Data are means (SEM) collapsed across the six drug conditions as time course analyses indicated no effect of dose on any physiological outcome measure, $p > .05$. Time course analyses indicated significant main effects of Sex [$F(1,11) = 36.37 – 231.94, p < .0001$] for systolic and diastolic blood pressure, and Time [$F(11,123) = 2.11 – 6.70, p < .05$] for systolic and diastolic blood pressure and heart rate.

CHAPTER V. DISCUSSION

This study used a sophisticated driving simulator to examine the next-day pharmacodynamics (specifically related to driving impairment) of bedtime-administered alprazolam in men and women. The results provided support for the primary study hypothesis. Detrimental effects on simulated driving performance were evident up to 13.5hr after bedtime alprazolam 2mg administration, and the increase in SDLP was equivalent to that exhibited by persons with a 0.08% - 0.10% BAC up to 12.5hr after administration. The objective driving impairment was corroborated by decrements on other psychomotor task performance, including the Circular Lights Task and Digit Symbol Substitution Task. Volunteers were unaware of their own degree of impairment after the bedtime alprazolam 2mg dose and rated their ability to drive safely non-differently than placebo. Even after the positive control, volunteers rated their ability to drive safely around 60 (out of 100) near the time of peak drug effect (1.5hr post drug administration). At that dose, volunteers displayed impairment equivalent to and greater than that seen at a 0.10% BAC, providing support of the hypothesis that persons are unaware of their own degree of impairment.

The profile of effects varied somewhat between the sexes, but not always in a consistent or definitive pattern, and caution should be taken when interpreting these results. Group sizes were small (8 females and 6 males), making this study underpowered to detect and fully characterize possible sex differences in pharmacodynamics. Females did display greater impairment and report more symptoms after administration of the zolpidem 10mg dose, and this is consistent with previous literature (Bocca et al. 1999, Verster et al. 2002a, Leufkens et al. 2009, Verster and Roth 2012b) and with the FDA decision to reduce starting doses of zolpidem in women (FDA 2013c). Females also generally reported more effects related to simulator sickness, which is consistent with previous reports (Park and Hu 1999, Klüver et al. 2015). Males reported more subjective effects after the bedtime alprazolam 2mg dose and also displayed a more consistent pattern of SDLP impairment than females, which was not hypothesized.

The primary outcome measure, SDLP, was sensitive to the impairing effects of the positive control. This confirms that SDLP was a valid measure and provides confidence that the increase in SDLP observed after test doses are indicative of true impairment. Not all driving simulator outcome measures were sensitive to the positive control. These included reaction time measures to incursion events. Because the same simulation was used for each test drive, this could be a function of practice and predictability (e.g., the volunteer knows that a pedestrian will walk out into the road immediately after they pass the red truck, so they can decelerate in anticipation). This anticipatory behavior was demonstrated frequently in the current study. Varying the location of the incursion events would provide a more effective measure of reaction time to unexpected events and would also be a more naturalistic experience. As increased variability is introduced into the simulations, the trade-off between maximizing experimental control and maintaining external validity must be considered.

The driving simulator task appeared to be the most sensitive to drug-induced impairment. The other simple laboratory measures of cognitive and psychomotor impairment tended to show dose effects and significant differences between positive control and placebo, but this was not always the case. One test method that did not appear sensitive to drug effects was the SafeDrive Application. This is unfortunate as it is currently being used by the Italian police force as a sobriety test in cases of suspected drugged driving. The lack of effect seen here could be due to the repeated nature of the study – volunteers performed the task many times. In the field, a suspected drugged driver would perform the task once and would not have the benefit of multiple practices. It should be noted that the pass rate of the four tests ranged from 66% for the simple auditory test to 82% for the complex visual test, and the percentage of volunteers who passed or failed the tests did not significantly differ by dose. This could mean that 34 to 18% of unimpaired drivers may fail one or more tests if this is used as a roadside sobriety measure.

These false-positive rates are lower than what been reported for the battery of field sobriety tests used in the United States. The Standardized Field Sobriety Test (SFST) is a battery of three tests (horizontal gaze nystagmus, the walk-and-turn, and the one-leg stand tests) performed during a traffic stop in order to determine if a driver is impaired. When properly administered, these tests have been found to be over 90% accurate in identifying alcohol-impaired drivers (Stuster and Burns 1998). Given these discrepancies in false positives, a more sensitive and accurate measure of drug-impaired driving is needed.

A finding with possible implications for future drugged driving studies was that SDLP variability in baseline drives was related to age. Older volunteers displayed greater baseline SDLP variability than younger volunteers (i.e. the SDLP measured during the first drive of each session was less consistent for older volunteers from one session to the next). If this association is real (in that it exists in the population as a whole and that older drivers are less consistent in the amount of lateral deviation they engage in while driving), it could have important implications for future results obtained from this type of driving simulator study. There are two possible interpretations to this association. First, it could mean that this type of driving simulator study is not as useful for characterizing drug effects on driving performance in older persons because their baseline and/or placebo drives are not stable. Even in the within-subject design of the study, for a volunteer to serve as their own control, that control condition must be stable and meaningful. Meaningful comparison of SDLP across multiple test conditions and days is most valid if SDLP is stable from one day to the next. The second interpretation is that this type of study may be particularly sensitive in older drivers because it can capture day-to-day differences in baseline SDLP and what impact drugs may have on overall SDLP. A change from baseline analysis may be more appropriate (instead of a comparison to placebo) to account for the larger day-to-day variability in baseline SDLP in older volunteers.

The lack of consistent performance in the driving simulator displayed by older volunteers could be due to their relative inexperience with virtual reality environments (e.g., video games). If that is the case, then additional practice beyond what was provided in the current study may be a solution. Before the baseline drive of the first session, volunteers had used the simulator at least seven times (totaling over an hour of drive time), and a practice drive was performed at the beginning of each session to re-familiarize the volunteer with the simulator prior to data collection. Baseline drive SDLP variability between sessions tended to decrease for the last two sessions compared to the first two sessions in the oldest study volunteers, so there is evidence that practice can reduce SDLP variability. It should be noted that by the start of the fifth session, each volunteer had logged over 6.5hr of drive-time on the simulator. Instead of standardizing the amount of practice each volunteer gets with the simulator, an alternative approach would be to allow the volunteer to practice until a target outcome based on SDLP variability between drives is achieved (or they reach some pre-determined limit on number of attempts and are disqualified).

Major strengths of this study include the double-blind, placebo-controlled design, administration of multiple doses of alprazolam, and inclusion of two positive control doses. Inclusion of body fat percentage as a covariate was another strength of the study design, but body fat was measured once and assumed to remain stable. Because the study took place over a period of weeks (and months in some cases), this may not have been the case. A few volunteers experienced significant weight fluctuations during the study, and this may have confounded the results. A more accurate method would have been to measure body fat percentage at each session.

The profile of effects varied between the sexes, but caution should be taken when interpreting these results. Group sizes were small (8 females and 6 males), making this study underpowered to detect and fully characterize possible sex differences in pharmacodynamics. Sample sizes

were too small to explore effects of birth control method or menstrual cycle phase. This study was performed in a driving simulator, an artificial environment in which there is no real consequence of crashing. Conscientious driving was incentivized in the current study with performance-based bonuses for following the rules of the road during each simulation; nevertheless, the absence of real risk could alter participant decision making. Because the primary outcome measure was not a measure of risk, but of an unconscious and automatic behavior, it is unlikely to have been influenced by altered decision making.

Future studies should seek to further characterize alprazolam effects in other volunteer populations (e.g., older persons, smokers, etc.). Alprazolam clearance has been shown to increase by up to 50% in cigarette smokers (Pfizer 2011) and effects of other nicotine products on alprazolam pharmacokinetics are not well characterized. Although the use of drug concentrations in blood as a marker of impairment is imperfect, future studies could collect blood samples and correlate alprazolam concentrations with driving impairment. Additional field sobriety tests (e.g., walk in a straight line, recite the alphabet backwards, etc.) could also be incorporated into future study designs in order to determine which are most sensitive to drug impairment and possibly inform law enforcement procedures in cases of suspected drugged-driving.

While non-drug-using volunteers are the population expected to be most sensitive to drug effects (and were therefore the most appropriate group for this study), future studies should explore how the development of tolerance affects next-day driving impairment after bedtime alprazolam administration. Characterization of tolerance to drug effects can be methodologically challenging as there are many factors to consider. Multiple types of tolerance can develop in response to repeated drug exposure, and each can have differential effects on drug-induced impairment. Tolerance to benzodiazepine effects (thought to be mediated at the molecular level by adaptations in the $GABA_A$ receptor and/or changes to the glutamatergic system (Bateson 2002, Allison and

Pratt 2003) occur at different rates. Assessment of tolerance effects could be accomplished by enrolling a patient sample or by administering repeated doses of alprazolam to healthy volunteers. The effect of the underlying disease may confound results when studying a patient sample, and this could be particularly salient for a sample of insomniacs because sleep deprivation and sleepiness have significant detrimental effects on driving performance and related skills (Williamson and Feyer 2000, Vakulin et al. 2007, Shekari Soleimanloo et al. 2017). Driving performance of patients with insomnia has been the subject of a few studies.

Perrier and colleagues compared driving performance of 21 untreated insomnia patients and 16 healthy volunteers on a 1hr monotonous highway driving simulator test and reported that insomnia patients had a significantly larger SDLP than healthy controls. Interestingly, total sleep time and self-reported sleepiness scores did not significantly differ between groups, suggesting a separate mechanism of driving impairment in insomniacs (Perrier et al. 2014). This study compared SDLP across groups and not within-subject and enrolled untreated insomnia patients.

In an evaluation of zolpidem effects on driving ability 5.5hr after administration in insomniac women, Partinen and colleagues reported no significant impairment in SDLP compared to placebo (Partinen et al. 2003); however, that result was based on ANOVA testing (known to be insensitive to pharmacodynamic effects). Without access to the raw data, it is impossible to determine if the results would be significant using the more appropriate symmetry analysis. The group SDLP means for the placebo and zolpidem conditions were 34cm and 39cm, respectively (Partinen et al. 2003), suggesting that impairment was present and significant. A SDLP increase of 5cm is consistent with impairment equivalent to a 0.10% BAC.

On-the-road driving performance of 42 insomnia patients prescribed hypnotics (22 frequent users [at least 4 nights per week for more than 3 months] and 20 infrequent users [≤ 3 nights per week])

and 21 healthy, age-matched controls was compared in a single highway driving test (Leufkens et al. 2014a). The night before the test, the frequent hypnotic users were instructed to ingest their own prescribed hypnotic, and the infrequent hypnotic users and controls did not ingest any sleep medication. No differences in SDLP were reported between the three groups, but there was no within-subject comparison to placebo. Volunteers were between the ages of 52 – 71, and results may only be applicable to similar-aged persons. A subsample of these volunteers (16 frequent hypnotic users, 16 infrequent hypnotic users, 16 healthy controls) participated in a double-blind, placebo-controlled, crossover study exploring the effects of bedtime zopiclone 7.5mg on driving performance 10-11hr after administration (Leufkens et al. 2014b). Zopiclone significantly impaired driving in all groups. The SDLP increases from placebo were related to hypnotic experience—1.6cm (frequent users), 2.1cm (infrequent users), and 3.6cm (healthy controls)—and may be explained by the development of tolerance to the impairing effects of zopiclone.

Final Conclusions

This is the first study to provide evidence that alprazolam can produce impairment in driving performance up to 13.5hr after its administration. The only dose to show significant impairment was 2mg, which is a large dose and unlikely to be prescribed to a patient for sleep disturbances. Nevertheless, it provides proof-of-principle that the impairing effects of alprazolam effects can be long-lasting. With a larger sample size and more statistical power, it is possible that impairing effects would become evident for the lower doses.

Appendix 1: FARS Data Query Details

Step 1*: Choose a year to Query (2007 -2016)

Step 2*: Choose the Tables to Query

Option 1 – Crash/Person

Step 3: Choose the Variables to Use

All relevant variables are listed under "Person Fields."

Step 4: Choose Condition Criteria

Step 5: Choose Report Form Options

*Steps 1 and 2 are identical for all queries.

Additional query details for Table 1.2 outcomes:

Step 3: Choose the Variables to Use:

Person Type

Alcohol Test Status

Alcohol Test Results

Step 4: Choose Condition Criteria:

Person Type: (1) Driver of a Motor Vehicle in Transport

Alcohol Test Status: All

Alcohol Test Results: All

Step 5: Cross tabulation of Alcohol Test Status by Alcohol Test Results

Additional query details for Table 1.3 outcomes:

Step 3: Choose the Variables to Use:

Person Type

Drug Test Status

Drug Test Results (1)

Step 4: Choose Condition Criteria:

Person Type: (1) Driver of a Motor Vehicle in Transport

Drug Test Status: All

Drug Test Results (1): All

Step 5: Cross tabulation of Drug Test Status by Drug Test Results (1)

Additional query details for Table 1.4 outcomes:

Step 3: Choose the Variables to Use:

Person Type

Drug Test Status

Drug Test Results (1)

Drug Test Results (2)

Drug Test Results (3)

Step 4: Choose Condition Criteria:

Person Type: (1) Driver of a Motor Vehicle in Transport

Drug Test Status: All

Drug Test Results (1): All

Drug Test Results (2): All

Drug Test Results (3): All

Step 5: Case Listing of Drug Test Results (1), Drug Test Results (2), and Drug Test Results (3) performed to output all drug test results available listed for each driver involved in a fatal crash.

For data specifically relating to drivers killed in an accident, the additional variable "Injury Severity" must be selected under "Person Fields" in Step 3. The condition selected in Step 4 is Injury Severity: (3) Killed.

Appendix 2: Complete Study Inclusion and Exclusion Criteria

Inclusion criteria:

1. Medical clearance by study physician
2. English-speaking and literate
3. Age is 18 to 50 years old
4. BMI approximately less than or approximately equal to 30
5. UDS results on session days negative for all drugs
6. Negative pregnancy test
7. Willing to participate in all study procedures, including 6 overnight sessions (across approx. 3-6 weeks)
8. Valid driver's license
9. Signed screening and main study consent

Exclusion criteria

1. Reports recent use of benzodiazepines or sleeping medications (i.e., past 60 days)
2. Last 30-day history of moderate to severe substance use disorder (excluding caffeine and binge alcohol use)
3. History of hypersensitivity (e.g., allergy, adverse events) to benzodiazepines
4. Score greater than 2 on Fagerstrom Test for Nicotine Dependence
5. Requires ongoing prescription medication (aside from oral birth control pills)
6. History of clinically significant seizures or head trauma
7. Significant ongoing medical problems (e.g., hypertension, diabetes, asthma)
8. Clinically significant abnormal ECG
9. Clinically significant abnormal liver function results
10. History of AIDS-defining illness
11. Women who are pregnant, lactating, or planning to become pregnant
12. Current acute pain conditions (e.g., recent surgery or wounds)
13. History of Acute Narrow Angle Glaucoma
14. Intolerance to simulated driving environment (e.g. vomiting, extreme vertigo)
15. Current ongoing psychiatric disorder (e.g., schizophrenia, bipolar disorder)
16. Recent use of any agent acting as an inhibitor or inducer of P450 3A4 that would affect study session outcomes
17. Physical dependence on benzodiazepines, barbiturates, opioids or alcohol that requires medical detoxification/management

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Professional Positions Held

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Scholastic and Professional Honors

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2017	College of Medicine Graduate School Travel Award FORCE Junior Investigator Travel Award
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2013 - 2016	Daniel R. Reedy Quality Achievement Fellowship Award Predoctoral fellow on NIDA T32 DA016176 "Training in Drug Abuse Related Research"
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Professional Publications

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