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The effects of adenosine antagonists on vigilant attention in sleep restricted rats

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The Effects of Adenosine Antagonists on Vigilant Attention in Sleep Restricted Rats

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Abstract

The relation between chronic sleep restriction and performance on the Psychomotor Vigilance Task (PVT) have been well documented in the human literature, with chronic sleep restriction as little as 7 hours per night resulting in significant impairment in sustained attention performance measured via the PVT. Recently, an analogous version of the human PVT has been developed for use with rodent models (rPVT). Recent studies have measured the effects of sleep restriction on rPVT performance, citing similar results found in the human literature. However, few studies to date have directly examined the role of adenosine accumulation during sleep deprivation in producing deficits in rPVT performance. The purpose of the current study was to extend previous rPVT research to include two adenosine antagonists as potential countermeasures for the effects of sleep restriction on rPVT performance in Sprague Dawley rats. After meeting baseline criterion, animals experienced two conditions for four days each: 6hr/day sleep restriction and 6hr/day sleep restriction followed by i.p. administration of an adenosine antagonist. Half of the animals received 30mg/kg caffeine, a non-selective adenosine antagonist, and half of the animals received 3mg/kg CPT, an adenosine A1 receptor antagonist. Performance on the rPVT was measured daily at 15:00. Mixed ANOVAs were conducted to analyze the effects of each condition on rPVT performance. No significant differences were found. Six hours per day of sleep restriction or administration of either adenosine antagonist had no effect on rPVT performance. Subsequently, a small group of animals underwent an additional 18hr/day sleep restriction condition. Visual analysis of this data showed that 18hr/day of sleep restriction had no effect on rPVT performance. The results of this study contradict

previous studies using the rPVT, including a pilot study conducted in our lab. Potential explanations for these contradictory results are discussed, highlighting the potential for strain differences in rPVT performance and response to sleep restriction. Future research should seek to explore the effects of sleep restriction on rPVT performance across rat strains. Additionally, the role of adenosine accumulation during sleep deprivation in producing deficits in rPVT performance is still not well understood, and requires further investigation.

Introduction

Sleep is defined as a state of consciousness characterized by low motor activity, decreased sensory reactivity, and reduced interactions with the environment. However, this definition does not adequately convey the importance of sleep in physical and mental and well-being. Lack of sleep results in a multitude of poor outcomes. In a practical sense, chronic sleep restriction leads to an increased risk of motor vehicle accidents (Jongen, Perrier, Vuurman, Ramaekers, & Vermeeren, 2015), decreased productivity at work (Meyers, 2015), and increased risk of adverse health events such as high blood pressure and stroke (Tobaldini et al., 2017).

Neurocognitive impacts of sleep restriction are vast and include impaired decision making (Harrison & Horne, 2000), reduced cognitive speed (Goel, Rao, Durmer, & Dinges, 2009), deficits in long-term memory (Lowe, Safati, & Hall, 2017), and most noticeably decreases in vigilance (Boonstra, Stins, Daffertshofer, & Beek, 2007; Van Dongen, Maislin, Mullington, & Dinges, 2003). Vigilance, or "sustained attention," is often identified as a fundamental component of attention and higher-order cognitive processes. Vigilance is characterized by a subject's readiness to detect randomly-presented signals over prolonged periods of time (Sarter, Given, & Bruno, 2001). Although several previous studies have documented the effectiveness of sleep restriction in decreasing behavioral vigilance (Lim & Dinges, 2008; Oken, Salinsky, & Elsas, 2006; Van Dongen et al., 2003), the neural mechanisms underlying the relationship between sleep and vigilance remain elusive. Additionally, few accessible methods for counteracting the effects of sleep restriction on vigilance have been proposed. Tasks of behavioral vigilance in rodent models have recently been developed to allow more

invasive investigations into potential mechanisms and mediations for the relationship between sleep and vigilance performance. The current study will measure vigilant attention after chronic sleep restriction in rats.

Sleep Restriction in Rats

Rats are a commonly used animal model in sleep restriction research due to several notable similarities in responses to sleep restriction between rats and humans. During sleep restriction, both rats and humans display similar increases in extracellular adenosine (Elmenhorst et al., 2007; Basheer et al., 2007). Following sleep restriction, both rats and humans display the same initial increase in slow save sleep (SWS) followed by an increase in REM during subsequent recovery sleep (Borbély & Neuhaus, 1979). Rats are frequently used in comparative pharmacology research to study the role of various drugs that effect adenosine, dopamine, and serotonin on sleep (Higgins et al., 2007; Nakazawa, Nakamichi, Imai, & Ichihara, 2015; Thomas et al., 2003).

However, there are also several differences between rat and human sleep. Unlike humans, rats do not typically sleep in one, consolidated session, and require significantly more sleep than humans. Rats typically sleep in shorter sleep bouts of 20-30 minutes, with most of these bouts occurring during daylight hours, and spend slightly more than 50% of their day asleep (Mendelson & Bergmann, 1999). Additionally, the method of sleep restriction differs considerably between humans and rats. One of the most common methods for inducing sleep deprivation in rodents is the use of forced exercise wheels. Forced exercise sleep deprivation consists of placing the animal in a slow-moving exercise wheel for the duration of the sleep deprivation period. The movement of the wheel requires the animal to move every few seconds or fall over, thereby keeping the

animal awake. This method of sleep deprivation is one of the most common, well validated methods of automated sleep deprivation in rats (Christie et al., 2008a; Deurveilher et al., 2015; Kim et al., 2015; McCoy et al., 2013).

Neurobiology of Sleep and Sleep Restriction

Sleep can be divided into two main systems, sleep-promoting and wake-promoting. These systems were proposed by von Economo in 1926 when he discovered that sleep and wakefulness are located in distinct areas of the brain using deceased patients with various sleep dysfunctions as his subjects (Triarhou, 2006). The wake-promoting functions reside in a collection of areas known as the Ascending Reticular Activating System (ARAS). These brain regions receive input from the brainstem and send mostly excitatory projections to the cortex to maintain behavioral arousal and consciousness (McCarley & Sinton, 2008). However, the ARAS does send inhibitory projections to the Ventrolateral Preoptic Area (VLPO), which serves as a center of the sleep-promoting system. In contrast to the excitatory ARAS projections, the VLPO sends inhibitory projections to many of the same structures targeted by the ARAS, including the tuberomammillary nucleus (TMN), the raphe nuclei (RN), and the locus coeruleus (LC; Saper, Chou, & Scammell, 2001). Through communication with various cortical structures, the sleep-promoting system works by inhibiting the wake-promoting system.

The activity of the sleep-promoting and wake-promoting systems is not random but rather controlled by a sleep-wake regulation process with numerous moving parts. According to Borbély's two-process model of sleep-wake regulation, there are two main processes that regulate sleep-wake cycles: the circadian process and the homeostatic process (Borbléy, 1982; Borbléy, Daan, Wirz-Justice, & Deboer, 2016). The circadian

process, also referred to as Process C or circadian rhythm, is a rhythmic variation in sleep propensity. The circadian process in most animals follows a 24-hour cycle and is considered a "sleep independent" process because it is typically unaffected by moderate day-to-day changes in sleep patterns. The homeostatic process, also referred to as Process S or the homeostatic sleep drive, is a non-rhythmic, sleep dependent process. The level of the homeostatic process at any given time is a function of the duration of prior wakefulness. Sleep propensity associated with the homeostatic process builds at an exponential rate with prolonged wakefulness and declines during periods of sleep. Process S and process C interact to result in a global sleep propensity experienced by the individual. However, in laboratory settings they are typically studied independently of one another. In the case of sleep restriction such as the current study, the majority of research has focused on the role of the homeostatic sleep drive while controlling circadian rhythms.

One brain area shared by the sleep-promoting and wake-promoting systems that is implicated in the homeostatic sleep drive is the basal forebrain. The most frequently studied function of the basal forebrain is its maintenance of arousal via widespread, excitatory cholinergic projections to the cortex (Baxter & Chiba, 1999; Detari, Rasmusson, & Semba, 1999). These cortical projections can be inhibited by activation of adenosine receptors located at pre-synaptic terminals on cholinergic neurons in the basal forebrain (Strecker et al., 2000). Extracellular adenosine has been shown to accumulate in the basal forebrain during periods of prolonged wakefulness or sleep restriction, inhibiting excitatory cholinergic projections, and resulting in decreased behavioral arousal (Basheer, Bauer, Elmenhorst, Ramesh, & McCarley, 2007; Basheer, Strecker,

Thakkar, & McCarley, 2004; Blanco-Centurion et al., 2006). However, general arousal is not the only function maintained by these cholinergic projections. Previous research has shown that cortical acetylcholine is necessary for performance on attention tasks, but not necessarily required for tasks that do not explicitly tax attention (Arnold, Burk, Hodgson, Sarter, & Bruno, 2002).

Sleep and Attention

Although there are multiple definitions and types of attention, research on the effects of sleep restriction on attention has focused on one of the most basic types of attention: sustained or vigilant attention. Vigilant attention following sleep restriction in humans is most frequently measured using the well-validated Psychomotor Vigilance Task (PVT). The PVT was developed and validated by David Dinges in 1985 as a method for measuring the dynamic effects of cumulative sleep deprivation on vigilant attention (Dinges & Powell, 1985). The computer-based task requires an individual to respond to a randomly presented visual stimulus, usually a light or time counter, by pressing a response button as soon as the stimulus appears. The PVT has been used in numerous studies in the last 30 years, and the effects of sleep deprivation on PVT performance are well documented. Three consistent findings from research on the effects of sleep deprivation on PVT performance have emerged. Chronic sleep restriction as little as 6hrs in bed per night results in an overall slowing of responses, an increase in response variability characterized by increased false starts and lengthy lapses of attention, and increases in fatigue throughout the duration of the task (Urry & Landolt, 2014). The validity of the PVT is further evidenced by its ability to distinguish the dose-response effects of multiple levels of sleep restriction, accurately distinguishing the effects of 4hrs,

6hrs, and 8hrs time in bed per night from one another in multiple studies (Belenky et al., 2001; Drake et al., 2001; Van Dongen et al., 2003). However, the neural mechanisms underlying the observable behavioral changes are not as well documented, as it is more difficult to observe or manipulate neurochemical changes in the active human brain.

To further investigate these underlying neural mechanisms, researchers have recently developed an analogous version of the PVT for use with rodent models termed the rat Psychomotor Vigilance Task (rPVT). Instead of using a computer, rats are placed inside operant boxes to complete the PVT procedure. Rats are required to respond to a centrally-mounted stimulus light by pressing a lever as soon as the stimulus appears. Preliminary research utilizing the rPVT has found similar results as human studies. Two studies by Christie et al. showed that pharmacological elevation of adenosine as well as 24-hour sleep deprivation resulted in longer response times and increases in false starts and prolonged lapses of attention (Christie et al., 2008a; Christie et al., 2008b). Since the introduction of the rPVT by Christie et al. (2008a), these sleep deprivation results have been replicated in various other studies (Davis, Roma, & Heins, 2016; Deurveilher, Bush, Rusak, Eskes, & Semba, 2015; Oonk, Davis, Krueger, Wisor, & Van Dongen, 2015). Additionally, a pilot study was conducted in our laboratory to investigate the relationship between sleep deprivation, caffeine, and attention (Crewe, Holt, & Dyche, 2019). We concluded from this study that 6 hours/day of sleep deprivation was sufficient to produce impairment on the rPVT, consistent with the results of previous studies. However, 10mg/kg caffeine administered via intraperitoneal (i.p.) injections was not sufficient to counteract the effects of sleep deprivation on rPVT performance.

Adenosine

Adenosine is a neuromodulatory neurotransmitter whose main role in the brain is inhibitory modulation of the homeostatic sleep drive via inhibition of cholinergic neurons in the basal forebrain (Basheer et al., 2004). Unlike other neurotransmitters that are synthesized within specialized cells, the large majority of endogenous adenosine comes from the breakdown of adenosine tri-phosphate (ATP), the body's main energy source. When energy in the form of ATP is used by the cell, it becomes the de-phosphorylated adenosine monophosphate (AMP) which is then further broken down into adenosine and transported to the extracellular space via an adenosine transporter (Cunha, 2001). Since adenosine is essentially a by-product of cellular metabolism, it is easy to understand how extracellular concentrations of adenosine build throughout the day.

The ability of adenosine to decrease arousal is accomplished by inhibition of cholinergic neurons via two sub-types of adenosine receptors: A1 and A2A. Both A1 and A2A function as inhibitory receptors located on the pre-synaptic terminals of acetylcholine neurons in the basal forebrain (Basheer et al., 2004). Specifically, when extracellular adenosine attaches to an A1 or A2A receptor, the amount of acetylcholine being released into the cortex is decreased. However, it is unclear whether A1 and A2A receptors serve similar or differential functions.

To investigate the role of different adenosine receptors, many researchers turn to both selective and non-selective pharmacological manipulations. Caffeine, the most widely used psychostimulant in the world, works as a non-selective adenosine antagonist. Caffeine blocks the ability of extracellular adenosine to utilize both A1 and A2A receptors, reducing the amount of cholinergic inhibition and resulting in increased arousal

(Fredholm, Battig, Holmen, Nehlig, & Zvartau, 1999; Urry & Landolt, 2014). While the use of caffeine as an experimental manipulation provides information about the role of the adenosine system as a whole, the use of more selective adenosine antagonists can give further insight to the role of each specific receptor. One commonly used selective adenosine antagonist is 8-cyclopentyltheophylline (CPT). CPT, a psychostimulant from the same xanthine class as caffeine, is a selective A1 receptor antagonist that blocks the ability of endogenous adenosine to utilize A1 receptors while still allowing the utilization of A2A receptors (Virus, Tiche, Pilditch, & Radulovacki, 1990). When non-selective antagonists such as caffeine and selective antagonists such as CPT are used to evaluate the role of adenosine receptors within the same task, more specific conclusions can be drawn about the role each adenosine receptor plays in task performance.

The majority of research on the homeostatic sleep drive has pointed to A1 receptors as the main source of inhibition of arousal-promoting cholinergic projections in the basal forebrain, citing little impact of A2A receptors (Basheer et al., 2004; Greene, Bjorness, & Suzuki, 2017; Porkka-Heiskanen et al., 1997). Nonetheless, the role of basal forebrain A2A receptors is not well established as research on A1 and A2A receptors have proposed conflicting results. A study conducted by Solinas et al. (2002) on the role of basal forebrain adenosine receptors in vigilant attention point to A2A receptors as the main source of inhibition of attention-promoting cholinergic projections, citing little impact of A1 receptors (Solinas et al., 2002). However, a study conducted by Christie et al. (2008a) found that the effects of increased adenosine on attention could be attributed to A1 receptors rather than A2A receptors. Only one study has been conducted utilizing non-selective caffeine, the selective A1 antagonist CPT, and the selective A2A antagonist

SCH 412348 on attentional performance in rats using the 5-Choice Serial Reaction Time Task (5CSRTT; Higgins et al., 2007). This study concluded that the ability of caffeine to increase reaction times on the 5CCRTT could be replicated by the A2A antagonist but not by the A1 antagonist, suggesting A2A receptors play a more prominent role in mediating attention than A1 receptors. Taken collectively, the majority of these studies would suggest that cholinergic neurons with presynaptic A1 receptors are responsible for maintaining arousal while cholinergic neurons with presynaptic A2A receptors are responsible for maintaining vigilant attention. However, very few studies have been conducted on the relationship between basal forebrain adenosine, acetylcholine, and attention—some with conflicting results—to allow these kinds of definitive conclusions.

Overview of the Current Study

The purpose of the current study was to investigate the role of adenosine receptors in maintaining vigilant attention by looking at the effects of a non-selective adenosine antagonist and a selective A1 adenosine antagonist on rPVT performance following chronic sleep restriction. The non-selective adenosine antagonist chosen for this study was caffeine, and the selective A1 adenosine antagonist chosen for this study was 8-cyclopentyltheophylline (CPT). Investigating the role of specific adenosine receptors in vigilant attention has many important implications. A more thorough understanding of the role of adenosine in sleep will contribute to the understanding of the neurobiology of sleep as a whole, as well as contribute to research on potential countermeasures for the effects of sleep restriction on neural functioning. Since the rPVT is not as well-validated as the human PVT or other tasks of attention in rodents, a second goal of this study was to validate the effects of chronic sleep restriction on rPVT performance observed in

previous studies. Continuing to validate the rPVT will improve confidence in the generalization of rPVT results to humans and comparisons of rPVT and PVT results. It was hypothesized that 6 hours/day of sleep restriction would result in significant decreases in rPVT performance, and administration of 30 mg/kg caffeine following sleep restriction would result in near-baseline performance on the rPVT. However, no specific hypotheses were made concerning the effects of CPT on rPVT performance following sleep restriction due to conflicting results in the few studies conducted using this drug.

Methodology

Subjects

Twenty four adult Sprague-Dawley (SD) rats weighing between 300-350 grams were utilized for this study. A pilot study in our lab was conducted using Wistar rats. However, the rats in this study displayed additional undesirable behaviors such as excessive biting, and appeared to be particularly reactive to adverse events such as i.p. injections. Therefore, the decision was made to switch to SD rats for this study. SD rats are commonly utilized as a general normative strain for behavioral testing. Animals were housed on a 12-hour light/dark cycle (lights on at 8:00). Necessary sample sizes were determined via power analysis (G*Power) using data from a pilot study conducted for this project, and the minimal sample size for this study was set at 18. However, only 85% of animals reached the final performance criterion during the pilot study. Therefore, 24 animals were trained on the attention task in hopes of achieving a final sample size of 18.

Food Restriction

During this study, animals were subjected to sleep deprivation prior to completing a behavioral task. Therefore, the need to sleep and the need to eat were competing reinforces for the animal. To increase motivation for behavioral task performance, rats were maintained at 85-90% of their free-feeding weight for the duration of the experiment. This weight range is consistent with previous behavioral studies utilizing food restriction, and is considered a standard weight range for behavioral motivation. Upon arrival, all rats were given ad libitum access to food until they reached a minimum of 300 grams, considered a healthy adult weight for Sprague-Dawley rats. Once they reached this weight, an 85-90% weight range was calculated for each animal. Each animal was weighed each morning, and given a selected amount of food to increase, decrease, or maintain their weight based on the specified weight range. Each animal was given a minimum of 6 grams and a maximum of 12 grams daily throughout the food restriction process. Any animal that fell below the 85% boundary of the weight range was given ad libitum access to food until they returned to the specified weight range.

rPVT Training

The rat Psychomotor Vigilance Task (rPVT) is a rodent adaptation of the widely used human Psychomotor Vigilance Task (PVT). However, unlike the verbal instructions given to human PVT participants, the rPVT requires an intensive training procedure and performance criterion before it can be used to assess vigilant attention. All rPVT training and testing sessions were conducted in standard rat operant chambers (Med-Associates, Burlington, VT) at 15:00 daily. Each chamber contained the target stimulus, a centrally mounted stimulus light panel, located below a singular response lever and above a pellet dispenser magazine. During the final procedure, a single response on the lever during the

stimulus light (0.5 seconds) or during the subsequent response interval (2.5 seconds) was rewarded with a singular 45mg sucrose pellet. See Figure 1 for a graphical depiction of the rPVT task.

Training procedures were adapted from Christie et al. (2008a), with two main adjustments. A lever was used for responding rather than a nose poke, and animals received food rewards rather than water rewards. Rats were first trained via autoshaping to lever press on a continuous reinforcement schedule (CRF), in which the target stimulus light was constantly illuminated, and one press on the response lever resulted in the delivery of one sucrose pellet. Each rat obtained a minimum of 120 responses on the CRF procedure to demonstrate acquisition of lever-pressing behavior before advancing to the rPVT training phase. In each phase of rPVT training, rats were required to elicit a single lever response when a central stimulus light panel was illuminated or during the subsequent response interval. A response that occurred before the stimulus light was illuminated was considered a premature response, and failure to respond within the given response interval was considered an omission. Both premature responses and omissions resulted in a 10-second time-out period in which no reward was dispensed, the house light was extinguished, and the lever was retracted to remove access to the reinforcer from the animal. In the training procedures outlined by Christie et al. (2008a), the first phase of rPVT training consisted of a 30-second stimulus light duration with no subsequent response interval or time-out period following premature responses or omissions. However, during the first phase of pilot study training, we saw extremely variable performance and the animals were unable to achieve greater than 50% response accuracy, indicating they were unable to discriminate between stimulus and no-stimulus

conditions. (Crewe et al., 2019). We were able to improve response accuracy by progressing the animals on to the 15-second stimulus light duration which includes the time-out period, making the difference between stimulus and no-stimulus conditions more salient for the animals. Therefore, the first phase of rPVT training for this study consisted of a 15-second stimulus light illumination with no subsequent response interval and included a time-out period following premature responses or omissions. The stimulus light duration was then be progressively decreased (10s, 5s, 2.5s, 1s, 0.5s) until the final criterion was met. In order to progress through each phase of training, rats were required to maintain a minimum of 60% correct responses and less than 20% omissions for two consecutive days to move on to the next task. In order to progress to the experimental phase of the project, rats were required to maintain a minimum of 60% correct responses and less than 20% omissions for three consecutive days. All rPVT training phases and necessary criterion are displayed in Table 1. Any rats were unable to meet this criterion at any phase of the training will be removed from the protocol.

Sleep Restriction

All rats who met the final performance criterion (see rPVT training) were subjected to 6 hours of sleep restriction for 4 days at a time. The 6 hour sleep restriction duration was chosen based on previous research showing 6 hours of sleep restriction produced significant increases in concentrations of extracellular adenosine (Basheer et al., 2001), and pilot study results from our laboratory showing 6 hours of sleep restriction produced significant impairment in rPVT performance (Crewe et al., 2019). All sleep deprivation sessions occurred in three forced exercise wheels (Lafayette Inc., Lafayette, LA). Sleep deprivation via forced exercise wheels or similar forced exercise apparatuses

are among the most common methods of sleep deprivation currently in use. During the sleep deprivation condition, animals were placed into the sleep deprivation wheels at 9:00 and removed from the wheels at 15:00. Following sleep deprivation, animals were either immediately transported to the pharmacy to receive intraperitoneal injections of adenosine antagonists or immediately transported to the behavioral testing room for rPVT testing depending on the experimental condition (see procedures for a detailed description of conditions).

Adenosine Antagonists

Caffeine and 8-cyclopentyltheophylline (CPT) was purchased from Sigma-Aldrich (Sigma-Aldrich, Inc., St. Louis, MO). Caffeine was dissolved in warm saline to a concentration of 15mg/ml and stored at 4 degrees Celsius for up to 4 weeks at a time. Caffeine was administered via intraperitoneal injection to one group of rats at a volume of 2ml/kg for a total dose of 30mg/kg. For the pilot study conducted in our laboratory, a dosage of 10mg/kg caffeine was chosen as a theoretical equivalent of two cups of coffee for an adult male based on previous research suggesting the metabolism of caffeine in rodents is much faster than the metabolism of caffeine in humans (Deurveilher, Lo, Murphy, Burns, & Semba, 2006; Fredholm et al., 1999). However, the 10mg/kg dosage was not sufficient to counteract the effects of sleep deprivation on rPVT performance (Crewe et al., 2019). Therefore, a higher dosage of 30mg/kg was chosen for this study based on previous research on the effects of caffeine on motor behavior in rodents (El Yacoubi et al., 2000; Gasior, Jaszyna, Peters, & Goldberg, 2000) and dosages used by Higgins et al. (2007) in their study on the effects of adenosine antagonists on attention performance using the 5CCRT task.

CPT was dissolved in warm saline with minimal amounts of NaCl (to improve solubility) to a concentration of 3mg/ml. CPT was administered via intraperitoneal injection to a second group of rats at a volume of 1ml/kg. This dosage of CPT was chosen based on the dosage used by Higgins et al. (2007) in their study on the effects of adenosine antagonists on attention performance. Caffeine or CPT was administered at 15:00 daily during drug conditions, 15 minutes prior to rPVT testing.

Procedure

Ten rats who meet the final rPVT training performance criterion (see rPVT training) progressed to the experimental phase of the study. After the final criterion was met, data for four days of rPVT performance was collected as a baseline measurement. Since our laboratory currently has access to three sleep deprivation chambers, groups of three rats at a time progressed through two four-day experimental conditions following baseline. The first condition was a sleep deprivation only condition consisting of 6hr/day sleep deprivation in forced exercise wheels immediately followed by rPVT testing. The second condition was a combined sleep deprivation and drug administration condition. Animals underwent 6 hr/day sleep deprivation followed by administration of an adenosine antagonist before rPVT testing. One group of rats (n=5) received a 30mg/kg intraperitoneal injection of caffeine, and one group of rats (n=5) received a 3mg/kg intraperitoneal injection of CPT. One week of recovery time occurred between the sleep deprivation only and the combined sleep deprivation and drug administration conditions to minimize potential carry over effects. See Figure 2 for a graphical depiction of the study timeline.

Statistical Analyses

Statistical analyses were conducted in two ways. First, data were analyzed in accordance with previously published rPVT studies (Christie et al., 2008a; Christie et al., 2008b; Deurveilher et al., 2015). Five different metrics were analyzed: average inter-response time (IRT), percent correct responses, percent omissions, percent incorrect responses, and percent lapses. Average IRT was calculated by taking the mean of the response time distribution for correct responses. Percent correct, percent omissions, and percent incorrect responses was calculated by dividing each response type by the total number of trials. Lastly, percent lapses was calculated by dividing the number of lapses by the total number of correct responses. Lapses were defined for each animal as any correct response that occurred at greater than two times that animal's average IRT at baseline. All variables were analyzed using separate 3x2 (condition x drug) mixed ANOVAs.

Although this analysis approach has been used in several previous studies, there are some clear issues that make this type of analysis ineffective for reaction time data like that obtained from the rPVT (Whelan, 2008). Specifically, conducting an ANOVA on average IRT, the main dependent outcome of the rPVT, assumes that the underlying reaction time distribution is normally distributed. However, reaction times do not follow a Gaussian (normal) distribution. Reaction times more closely approximate an ex-Gaussian distribution where the data are positively skewed with a long, exponential-like decay on the right. In addition to violating the underlying assumptions of the ANOVA, using the mean of a non-normal distribution severely reduces the ability to detect the actual difference in response times. Additionally, one of the observable changes in PVT

or rPVT performance due to sleep deprivation is an increase in response variability. However, changes in response variability are not accurately captured by separately analyzing percent correct, percent omissions, and percent incorrect responses.

The ex-Gaussian distribution is a convolution of a Gaussian and an exponential distribution. Unlike the two-parameter (mean and standard deviation) Gaussian distribution, the ex-Gaussian distribution has three parameters that describe the shape of the data: the mean of the Gaussian half of the distribution (Mu), the standard deviation of the Gaussian half of the distribution (Sigma), and the rate of decay of the exponential tail of the distribution (Tau; Dawson, 1988; Sternberg, 2014). The three-parameter distinction of this distribution influences the accuracy of interpretations made from reaction time data in several meaningful ways. First, by estimating the mean and standard deviation of just the Gaussian half of the distribution, the effects of skew on the mean are eliminated. Therefore, the mean of the data is a more accurate representation of the average response time and will be more accurate in detecting actual changes in response times. Additionally, by calculating and analyzing the standard deviation of the Gaussian half of the distribution as well as the rate of exponential decay, the changes in response variability as a result of sleep deprivation can be directly examined.

This ex-Gaussian approach to the analysis of reaction time data has been used in various studies of attention using the Continuous Performance Test (Hervey et al., 2006; Hwang Gu, Gau, Tzang, & Hsu, 2013), and more recently the PVT (Jollie, 2016; Yehayes, 2017). Mu, sigma, and tau were estimated for each condition using the Reaction Times Analysis (retimes; Massidda, 2013) package in the statistical software R (Version

2.4; R Core Team, 2018), which is based on the RTSYS DOS application (Heathcote, 1996). Separate 3x2 mixed ANOVAs were conducted for each parameter.

Results

At the end of the study, only 10 of the 24 animals used for this study were able to meet baseline criterion. One animal was excluded from the study due to health issues while the other 13 animals were not able to meet baseline criterion. Therefore, data from a total of 10 animals were utilized for the following analyses.

Traditional Analyses

Data were first analyzed in accordance with previously published studies utilizing the rPVT. Variables of interest included in these "traditional analyses" included the percentage of total trials that were correct responses (%Correct), incorrect responses (%Incorrect), omissions (%Omissions), and lapses (%Lapses), as well as average response time (MeanRT). A series of 3x2 mixed ANOVAs were conducted to examine the effects of condition (baseline, 6hr sleep deprivation, 6hr sleep deprivation + drug) and drug (caffeine, CPT) on each of these variables. An alpha level of .01 was used to control for familywise error. All assumptions, including sphericity, HOV, and normality were met with the exception of sphericity for %Omissions, %Lapses, and MeanRT. In cases where sphericity was violated, a Greenhouse-Geisser correction was used.

Descriptive statistics for each variable across each condition can be found in Table 2. Results of the 3x2 mixed ANOVAs showed there were no significant differences for any two levels of condition or drug for any of these variables (all $p > .01$). Neither six hours of sleep deprivation nor the combination of six hours of sleep deprivation and

adenosine antagonist had an effect on any of these traditional variables when compared to baseline performance.

Ex-Gaussian Analyses

In addition to the traditional analyses, data were analyzed using an ex-Gaussian approach which assumes the data is approximately normal with the exception of a long, exponential-like tail on the right half of the distribution. Variables of interest for this approach included the mean of the Gaussian half of the distribution (Mu), the standard deviation of the Gaussian half of the distribution (Sigma), and the rate of decay of the exponential tail of the distribution (Tau). Prior to analyzing data, a chi-square test of misfit was conducted to determine if the data fit an ex-Gaussian distribution. A significant result for this test would indicate data were significantly different from an ex-Gaussian distribution. All chi-square tests were significant ($p < .05$). However, this chi-square test is very sensitive to sample size, and a visual analysis of the reaction time data showed data did seem to approximate an ex-Gaussian distribution **(Figure 3).**

A series of 3x2 mixed ANOVAs were conducted to examine the effects of condition (baseline, 6hr sleep deprivation, 6hr sleep deprivation + drug) and drug (caffeine, CPT) on each of these variables. An alpha level of .01 was used to control for familywise error. All assumptions, including sphericity, HOV, and normality were met with the exception of sphericity for Mu and Sigma. In cases where sphericity was violated, a Greenhouse-Geisser correction was used.

Descriptive statistics for each variable across each condition can be found in Table 2. As expected due to the skewness of the data, the ex-Gaussian means (Mu) were

considerably lower than the traditional means based on a normal distribution (MeanRT). Results of the 3x2 mixed ANOVAs showed there were no significant differences for any two levels of condition or drug for any of these variables (all $p > .01$). Neither six hours of sleep deprivation nor the combination of six hours of sleep deprivation and adenosine antagonist had an effect on any of the ex-Gaussian variables when compared to baseline performance.

18-Hour Sleep Deprivation

In an attempt to produce deficits in rPVT performance not seen with six hours of sleep deprivation, a small number of animals ($n = 6$) underwent 18 hours of sleep deprivation. Due to the small sample size for this condition, results were analyzed descriptively and visually rather than using inferential tests. Descriptive statistics for each condition for the animals who underwent 18 hour sleep deprivation (excluding the four animals who did not) can be found in Table 3. During the 18 hour sleep deprivation condition, percentage of correct responses were eight percent lower when compared to baseline, but only two percent lower when compared to the 6 hour sleep deprivation condition. Percentage of omissions during the 18 hour sleep deprivation condition were almost twice as high compared to both baseline and 6 hour sleep deprivation performance. Differences in average response times both calculated as traditional means (MeanRT) and as ex-Gaussian means (Mu) were approximately 50 milliseconds slower during the 18 hour sleep deprivation condition when compared to baseline. However, each of the metrics discussed above display high variability across each condition **(Table 3)**.

The variability observed through descriptive statistics was investigated through a series of spaghetti plots depicting performance trends for each animal across all four conditions (baseline, 6hr sleep deprivation, 6hr sleep deprivation + drug, 18hr sleep deprivation) for each variable **(Figure 4-Figure 11).** In examining the spaghetti plots, few patterns emerged. Eighteen hours of sleep deprivation did not appear to have a clear effect on any of the variables measured compared to baseline measures. Only one variable, percentage of incorrect responses, appeared to have a clear pattern across all four conditions **(Figure 5)**. With the exception of one animal, percentage of incorrect responses increased slightly from baseline to 6 hour sleep deprivation, decreased to below baseline levels for 6 hour sleep deprivation + drug, and increased to slightly above baseline levels for the 18 hour sleep deprivation condition. However, the difference between the percentage of correct responses at baseline and during 18 hour sleep deprivation was less than two percent **(Table 3).**

Discussion

The current study sought to accomplish two goals. The first goal was to validate the use of the rPVT for detecting the effects of sleep restriction on vigilant attention in rodents. We were unable to detect deficits in vigilant attention due to both 6 hours and 18 hours of sleep restriction using the rPVT. The second goal was to examine the efficacy of two adenosine antagonists as countermeasures to the deficits in rPVT performance produced by sleep loss. Due to the lack of deficits in rPVT performance following both 6 and 18 hours of sleep restriction, we were unable to assess the effects of the adenosine antagnoists on vigilant attention. Caffeine or CPT administration did not have an effect on rPVT performance compared to baseline or sleep restricted performance.

These results are not consistent with previous studies using the rPVT (Christie et al., 2008a; Christie et al., 2008b; Deurveilher et al., 2015; Oonk et al., 2015), including pilot study results conducted in our lab (Crewe et al., 2019) that showed 6hr/day of sleep restriction was sufficient to produce deficits in rPVT performance. Considering the results of several previous studies, it is unlikely that the rPVT is simply not sensitive enough to detect deficits produced by sleep loss. Below, we discuss alternative explanations for the results observed such as possible strain differences in reactivity to stress and task performance.

Strain Differences in Reactivity to Stress

Animals who met baseline criterion and were included in the study did not display deficits in rPVT performance observed in previous studies (Christie et al., 2008a; Christie et al., 2008b; Crewe et al., 2019; Deurveilher et al., 2015; Oonk et al., 2015). Wistar and Long-Evans rats are the most common strains that have been used in previous rPVT research, including a pilot study conducted in our lab using Wistar rats. However, the Wistar rats in our pilot study displayed additional undesirable behaviors such as excessive biting, and appeared to be particularly reactive to adverse events such as i.p. injections. Therefore, the decision was made to switch to Sprague Dawley rats for this study, a common behavioral strain used in several sleep restriction studies conducted in our lab.

Sprague Dawley (SD) rats have not been utilized for previous rPVT studies. A possible explanation for the lack of deficits in rPVT performance following sleep restriction in SD rats is potential differences in reactivity to stress, as sleep restriction is an inherently stressful experience. There is little research that directly examines

differential effects of sleep restriction on different rat strains. However, studies examining the effects of chronic mild stressors on different rat strains consistently report that Wistar and Long-Evans rats are among the most easily and profoundly stressed strains while SD rats show little to no stress response and faster habituation to stress under the same conditions (Bekris, Antoniou, Dakas, & Papadopoulou-Daifoti, 2005; Dhabhar, McEwen, & Spencer, 1997; Pardon et al., 2002).

Although more research is needed to determine if the strain differences in reactivity to stress contribute to strain differences in responses to sleep restriction, a growing body of research suggests there is not one uniform response to sleep restriction. Several studies in both humans (Dennis, Wohl, Selame, & Goel, 2017; Van Dongen & Belenky, 2009; Van Dongen, Baynard, Maislin, & Dinges, 2004) and animals (Dissel et al., 2015; Frolinger et al., 2018) have demonstrated individual differences in resiliency to sleep loss, with some individuals displaying little to no observable deficits following prolonged sleep restriction. Specifically, there appears to be trait-like individual differences in the effects of sleep loss on PVT performance in humans, with some individuals' performance remaining relatively unaffected by both chronic and acute sleep deprivation (Dennis et al., 2017; Jung, Lee, & Shin, 2017; Rupp, Wesensten, & Balkin, 2012).

The behavioral differences mentioned above are hypothesized to have specific genetic underpinnings that make an individual more resilient or more vulnerable to the effects of sleep restriction and other stressors (Goel & Dinges, 2012; Satterfield, Wisor, Field, Schmidt, & Van Dongen, 2015). Considering there are genetic differences between rat strains, it is possible that SD rats are more resilient to sleep loss than other strains.

Additionally, Wistar and Long-Evans rats are inbred rats, meaning there are very small differences in the genetic makeup of any two Wistar or Long-Evans rats. In contrast, SD rats are outbred rats, meaning there are measurable differences in the genetic makeup of any two SD rats. If genetic differences do contribute to resiliency to sleep loss, these differences may be more pronounced within a group of SD rats, leading to more variability in their responses to sleep restriction.

Observed Differences in Task Performance

Fewer animals met the baseline criterion for the rPVT than expected. Interestingly, other labs have not reported similar difficulties. Christie et al. (2008a) reported that training success rates for the rPVT are as high as 95% while Deurveilher et al. (2015) and Oonk et al. (2015) reported that 81% and 70% of animals met baseline criterion, respectively. During a pilot study in our lab, 58% of animals met baseline criterion (Crewe et al., 2019). In the current study, only 43% of SD rats were able to meet the baseline criterion, which was set at >60% correct responses and <20% omissions for three consecutive days. Considering the majority of previous studies have used Wistar or Long-Evans rats rather than SD rats, it is possible that this strain simply does not perform as well on the rPVT as other strains. One previous study testing Wistar rats and SD rats on another task of attention, the 5-Choice Serial Reaction Time Task (5CSRTT), found that SD rats had consistently worse performance than Wistar rats (Semenova, Stolerman, & Markou, 2007). However, the Wistar rats used in our pilot study also performed worse than previous studies have reported (Crewe et al., 2019).

Limitations

A potential design limitation that may have contributed to the lack of effects is the specification of the baseline criterion. In this study, the baseline criterion was set as >60% correct responses and <20% omissions for three consecutive days. This criterion was chosen as a "middle ground" between previously used criterion we believed to be too lenient (>100 reinforced responses; Christie et al., 2008a) and criterion we believed to be too strict based on pilot data (>70% correct responses, <10% omissions; Deurveilher et al., 2015). However, after animals met baseline criterion performance tended to be extremely variable. After demonstrating a minimum of 60% correct responses for three consecutive days, several animal's performance continued to trend upward during baseline data collection, with several animals reaching >70% correct responses during the start of the sleep deprivation period. While some animals did display decreases in performance toward the end of the sleep deprivation period, the averages for the baseline period and the sleep deprivation period were ultimately very similar **(Table 2).**

These trends suggest an additional measure of variability should be added to the baseline criterion to ensure rPVT performance has stabilized before any experimental manipulations are added. For example, researchers using a differential reinforcement of low rates of responding task (DRL) frequently require animals to display stable responding at baseline, defined as less than 10% variation in response times across three consecutive sessions (Anastasio et al., 2011; Chiang, Cheng, & Liao, 2015). An additional variability criterion would ensure animals have achieved stable responding at baseline, and increase confidence that the subsequent changes in response variability are due to the experimental manipulations.

A smaller number of animals than desired met baseline criterion for the rPVT and were included in the study. Based on a power analysis conducted prior to the start of the study, a minimum sample size of 18 was needed to detect differences in rPVT performance due to sleep restriction. This sample size was not achieved, which raises concerns about statistical power. However, due to the lack of obvious mean differences **(Table 2)** and the lack of patterns observed in the spaghetti plots **(Figure 4-Figure 11)**, it is not likely that statistical power is the sole cause of the null results observed.

Finally, we must consider the possibility that technical difficulties may have contributed to the difficulties with task acquisition. The operant box apparatuses in our lab were purchased in 2008 and have been moved, disassembled, and reassembled several times, undergoing normal wear and tear in the process. Although box check sessions were implemented prior to each rPVT session, the reliability of the operant chambers was not perfect. These technical difficulties are unlikely to have masked any effects of sleep restriction on rPVT performance for animals who did meet baseline criterion. However, inconsistencies or errors in the pattern of reinforcement and punishment due to equipment failure could easily have contributed to difficulties with task acquisition. In the future, professional maintenance and possible replacement of the existing operant box equipment is strongly suggested.

Suggestions for Future Research

In future research, two avenues should be explored in response to the results of this study. A growing body of research suggests different rat strains may have different responses to stress, but it is unclear if there are strain differences in response to sleep restriction as well. Therefore, additional research should explore the dose-response

effects of chronic sleep restriction on rPVT performance in several commonly used rat strains including Sprague Dawley, Wistar, and Long-Evans rats. If these strains do differ in their responses to sleep restriction, researchers should seek to understand the physiological underpinnings of these differences, as they have important implications for research on the neurobiology of sleep and sleep restriction. Second, the role of adenosine in maintaining vigilant attention following chronic sleep restriction is still not well understood. In the future, this study or a similar study should be replicated with a level of sleep restriction that has been shown to produce deficits in rPVT performance to further investigate the role of specific subtypes of adenosine receptors in vigilant attention.

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Table 1.

Phases of rPVT training. Animals are required to meet the performance criterion before progressing on to the next task.

Task	Stimulus Duration	Response Interval Duration	Performance Criterion
CRF	Constant	N/A	> 120 responses
PVT15	15s	0s	>60% correct responses, <20% omissions for 2 consecutive days
PVT10	10s	0s	>60% correct responses, <20% omissions for 2 consecutive days
PVT5	5s	0s	>60% correct responses, <20% omissions for 2 consecutive days
PVT2	2.5s	0.5s	>60% correct responses, <20% omissions for 2 consecutive days
PVT1	1s	2s	>60% correct responses, <20% omissions for 2 consecutive days
PVTFinal	0.5s	2.5s	>60% correct responses, <20% omissions for 3 consecutive days

Table 2.

Descriptive statistics for all ten animals included in analyses.

		Baseline		6hr SD		6hr SD + Drug	
Variable	*N*	*M*	*SD*	*M*	*SD*	*M*	*SD*
%Correct*	10	66.27	5.55	62	10.25	66.02	7.66
%Incorrect*	10	26.2	5.63	29.82	9.84	25.10	7.28
%Omissions*	10	7.54	3.72	7.60	2.97	8.88	4.82
%Lapses*	10	7.18	1.86	8.30	4.19	8.06	8.02
MeanRT*	10	986.60	286.35	997.84	241.85	976.90	268.32
Mu**	10	578.33	264.89	532.13	227.12	513.77	202.47
Sigma**	10	308.12	229.20	299.53	192.94	252.71	136.87
Tau**	10	408.32	155.77	487.43	126.73	474.43	203.12

*Traditional analysis variable
**Ex-Gaussian analysis variable

Table 3.

Descriptive statistics for all six animals that underwent 18 hours of sleep restriction. Animals that did not undergo 18 hours of sleep restriction were excluded.

		Baseline		6hr SD		6hr SD + Drug		18hr SD	
Variable	*N*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
%Correct*	6	64.65	2.78	58.99	8.86	66.6	8.84	56.91	5.18
%Incorrect*	6	26.71	3.16	31.58	8.67	23.19	8.35	28.22	8.78
%Omissions*	6	8.64	3.58	8.45	3.13	10.19	5.42	14.85	8.49
%Lapses*	6	7.34	2.42	8.81	5.19	8.58	10.51	9.31	3.27
MeanRT*	6	997.87	330.82	1017.79	274.39	1014.76	289.61	1056.88	297.10
Mu**	6	559.58	283.01	506.95	263.32	555.5	254.53	597.97	366.30
Sigma**	6	300.43	211.61	263.38	158.95	323.46	247.29	330.04	243.97
Tau**	6	543.35	195.13	510.92	158.57	459.18	197.34	447.77	173.13

*Traditional analysis variable
**Ex-Gaussian analysis variable

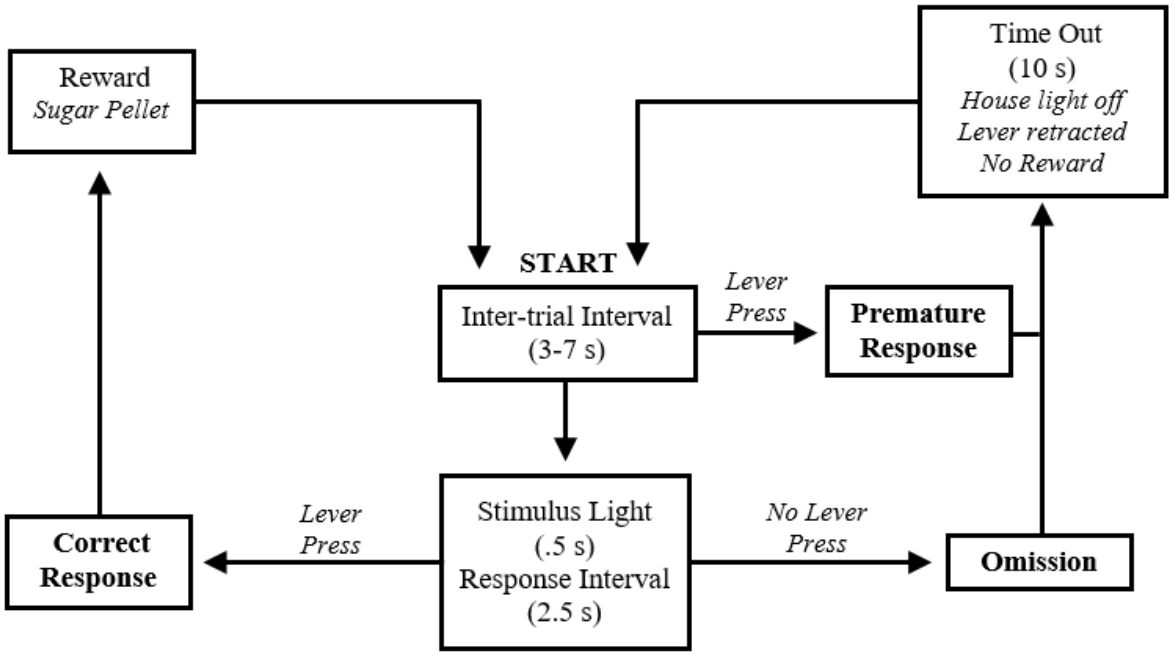

Figure 1. Graphical depiction of the final rPVT task used for data collection.

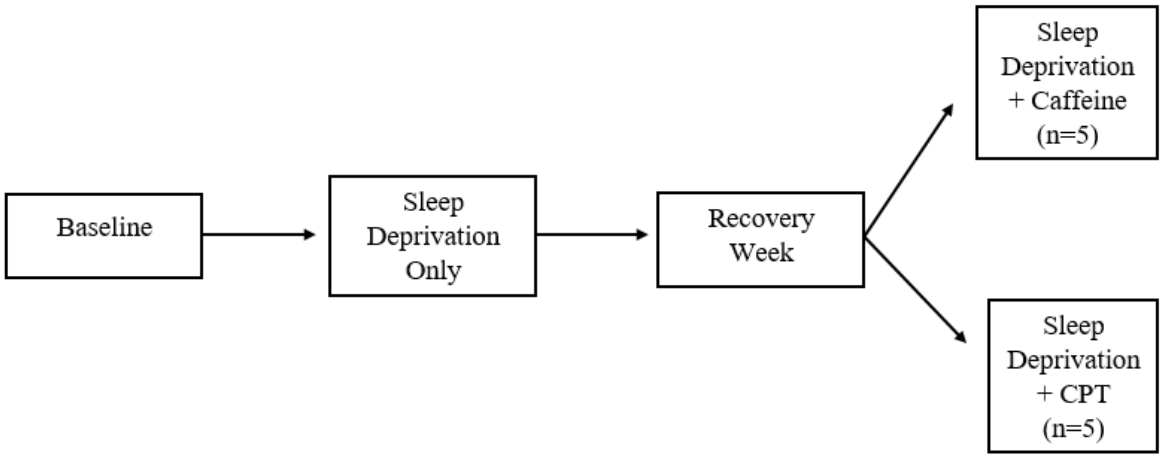

Figure 2. Graphical depiction of the study timeline. The duration of each condition depicted above is seven days.

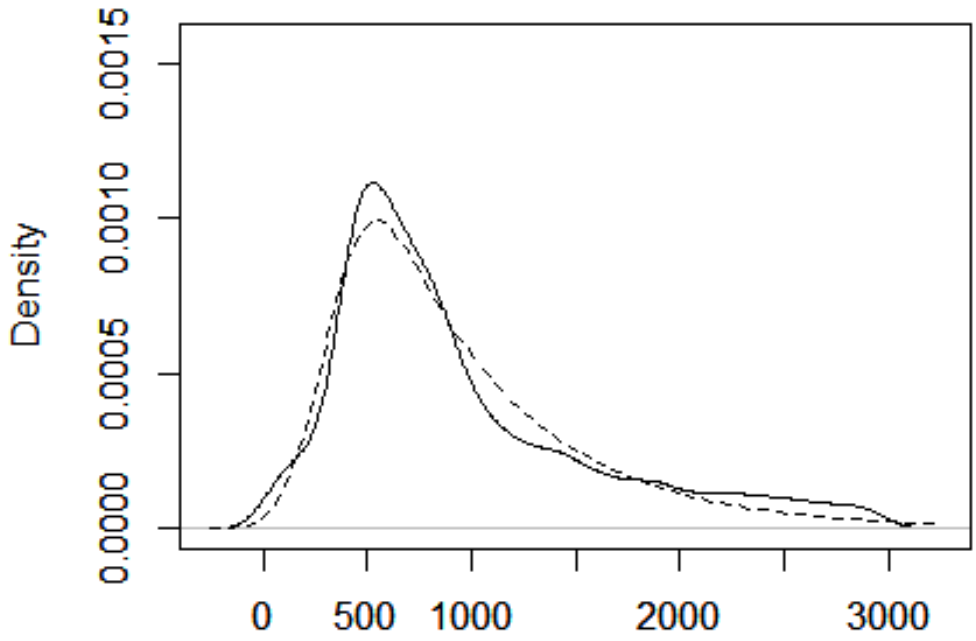

Figure 3. Graphical depiction of the ex-Gaussian distribution produced by the baseline reaction time data. The solid black line represents the observed baseline data while the dashed line represents the ex-Gaussian distribution produced by the parameters estimated from the baseline data.

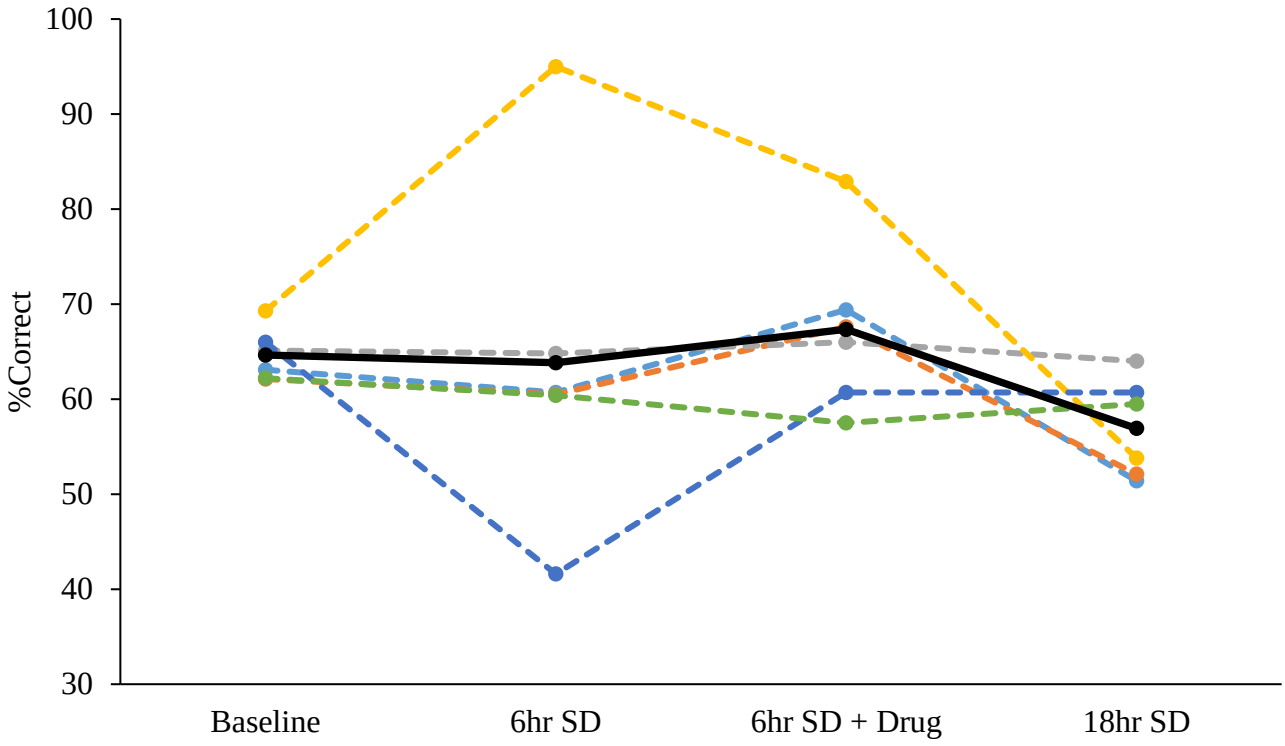

Figure 4. Percentage of correct responses for each animal that experienced all four experimental conditions. Dark black line represents average percentage of correct responses for each condition.

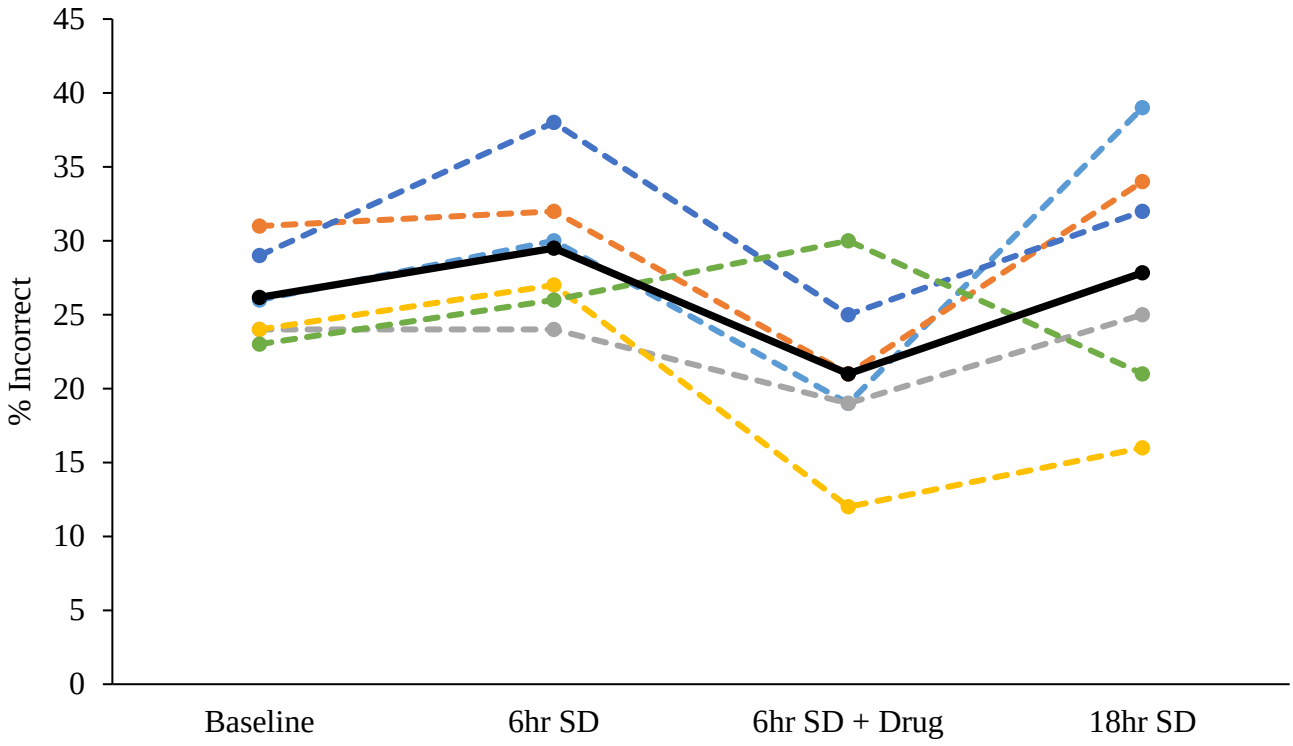

Figure 5. Percentage of incorrect responses for each animal that experienced all four experimental conditions. Dark black line represents average percentage of incorrect responses for each condition.

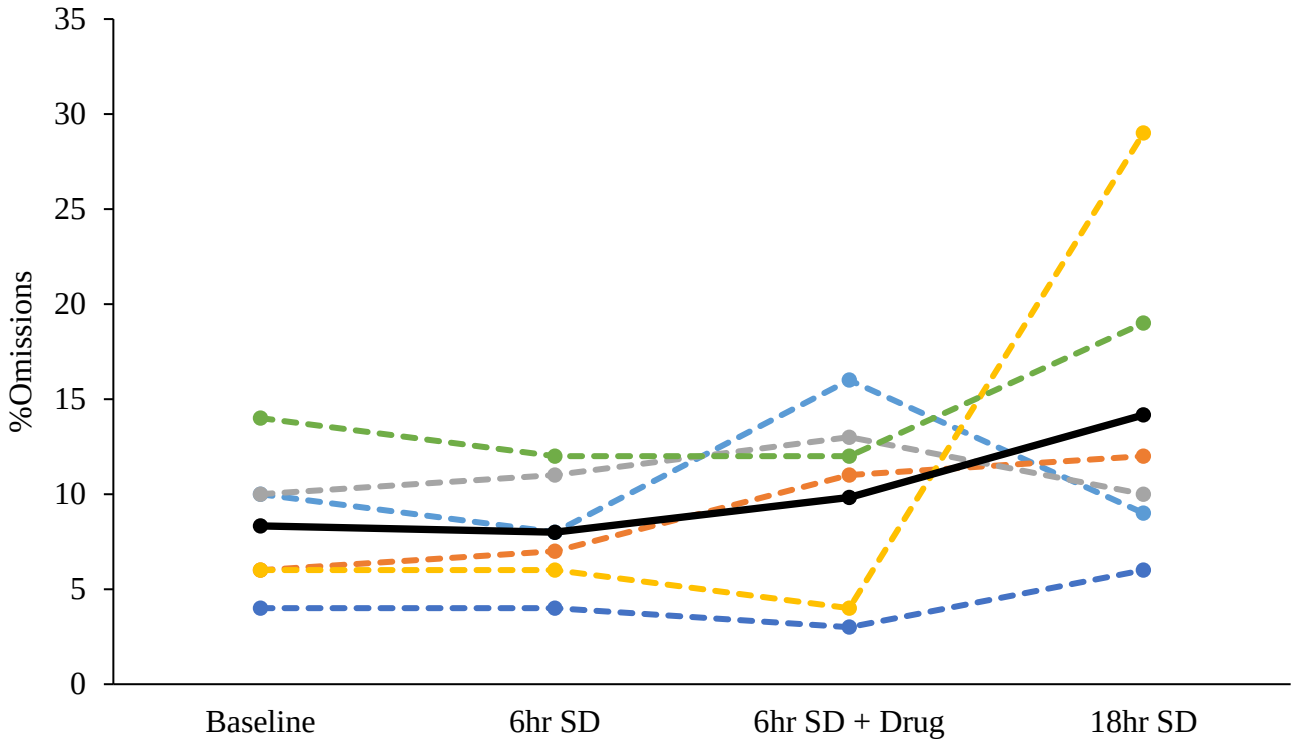

Figure 6. Percentage of omissions for each animal that experienced all four experimental conditions. Dark black line represents average percentage of omissions for each condition.

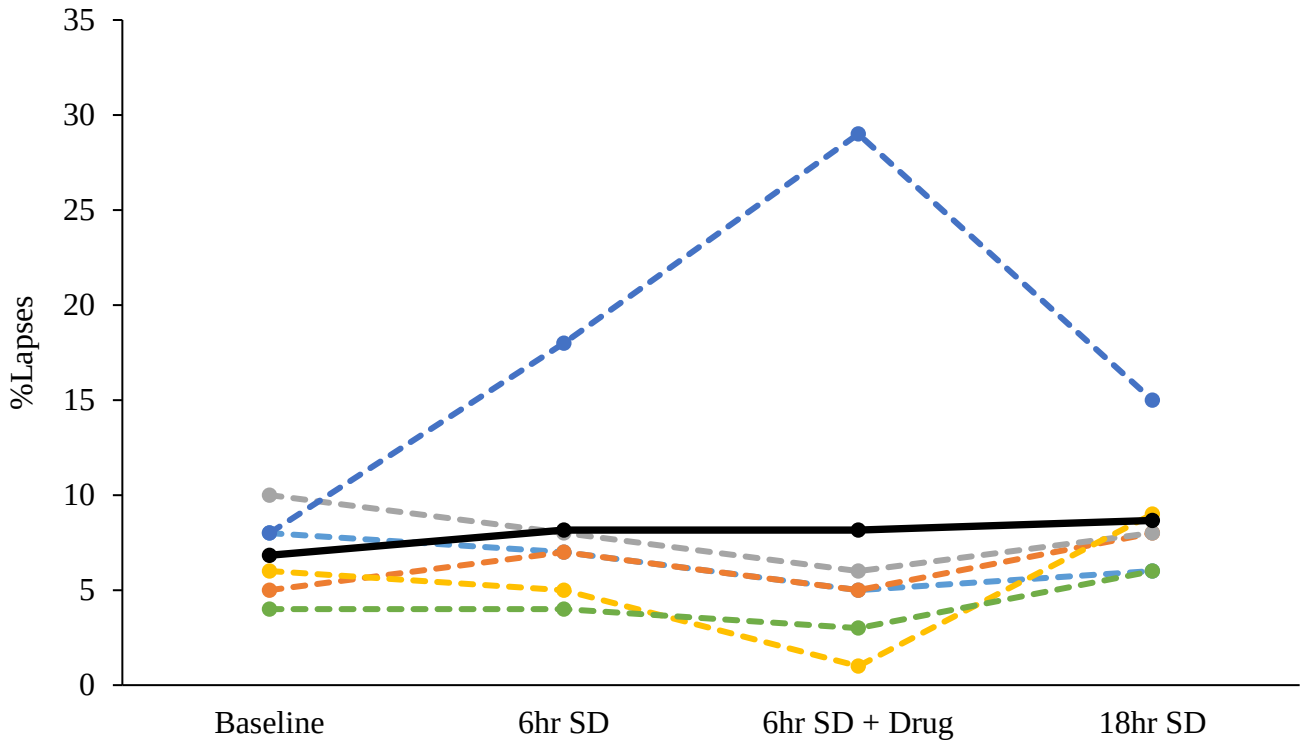

Figure 7. Percentage of lapses for each animal that experienced all four experimental conditions. Dark black line represents average percentage of lapses for each condition.

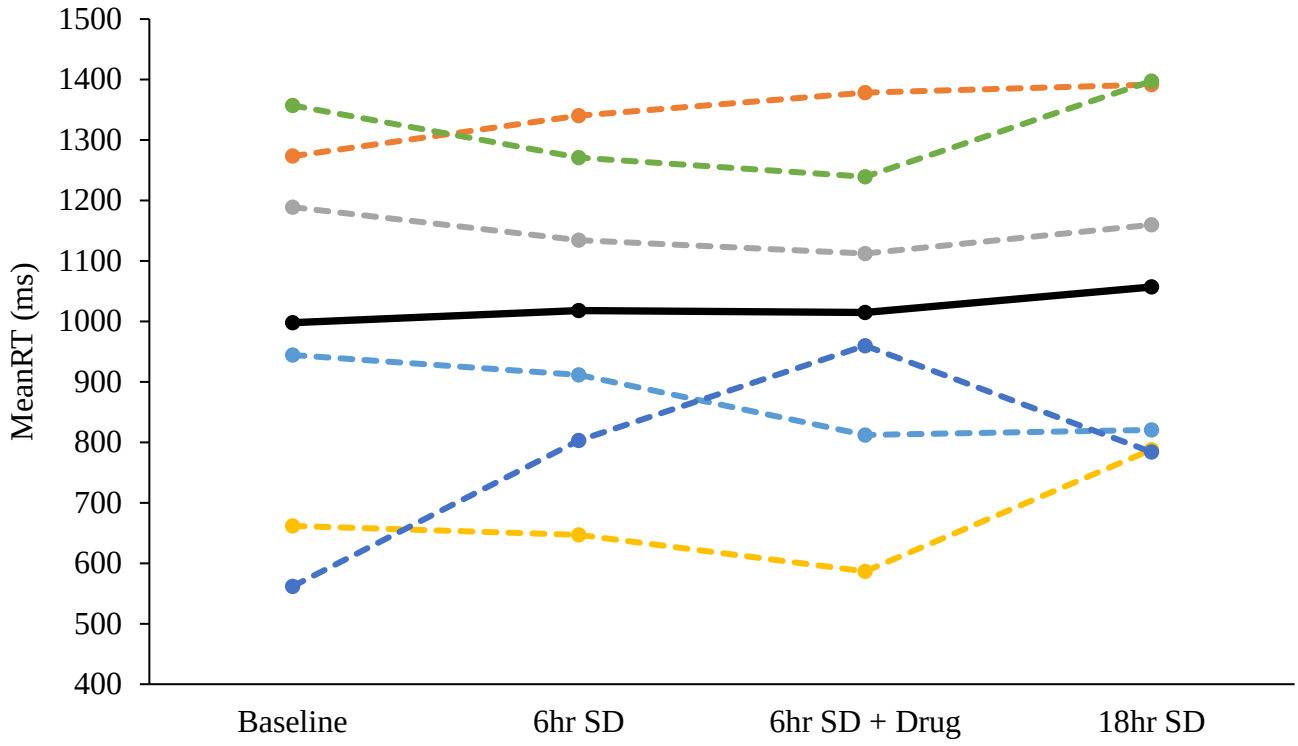

Figure 8. Average response time in milliseconds for each animal that experienced all four experimental conditions. Dark black line represents average response time for each condition.

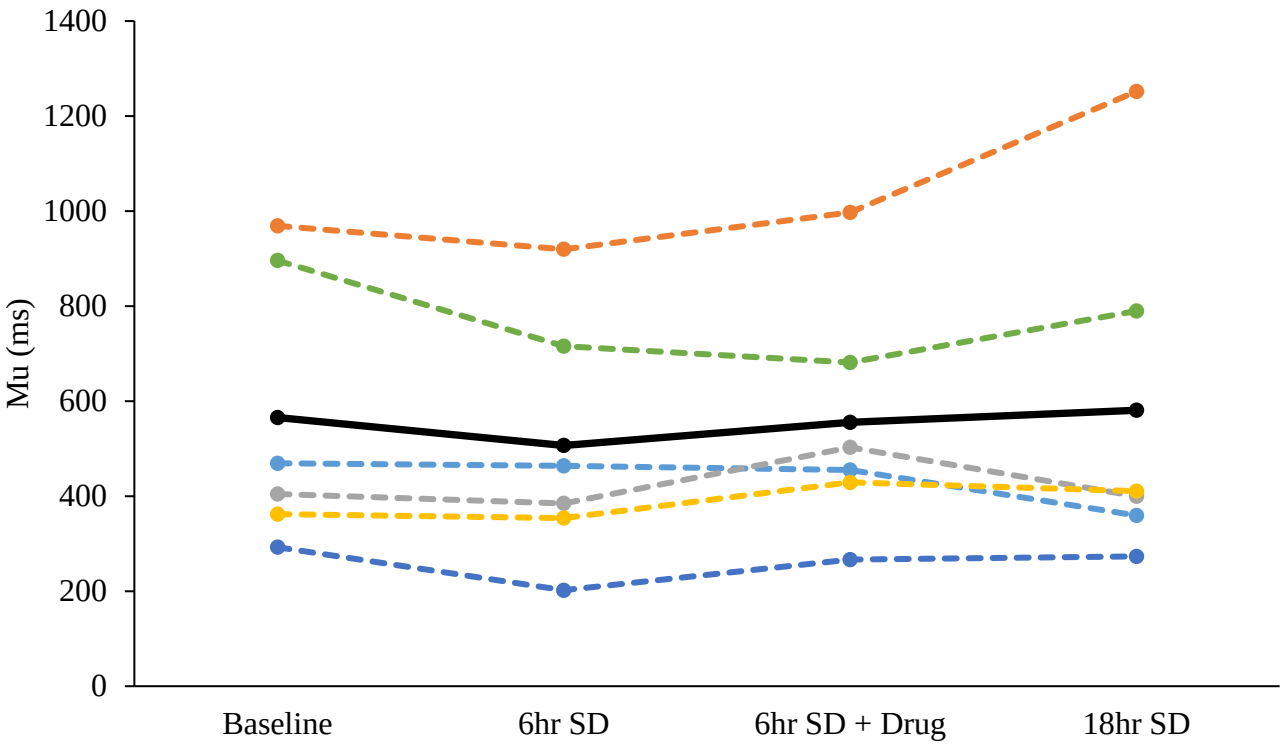

Figure 9. Mu (average response time in milliseconds based on an ex-Gaussian distribution) for each animal that experienced all four experimental conditions. Dark black line represents average Mu for each condition.

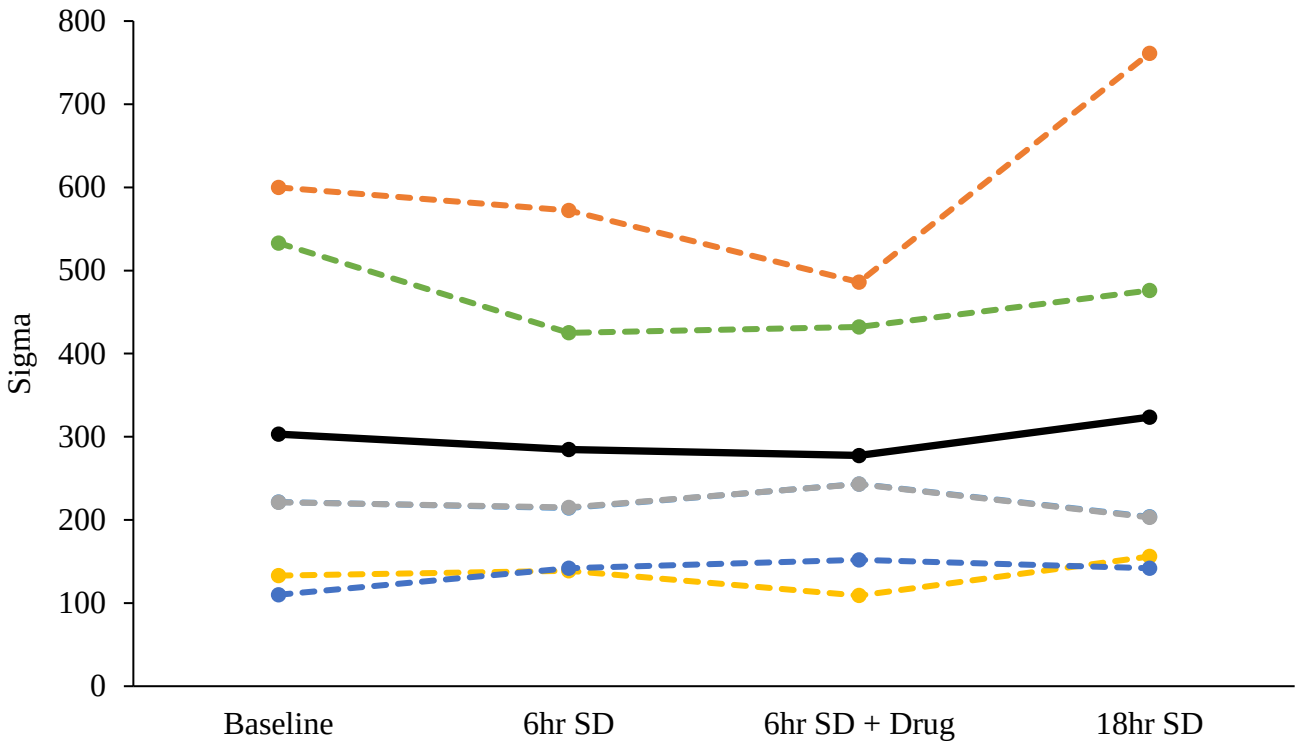

Figure 10. Sigma (standard deviation of response times based on an ex-Gaussian distribution) for each animal that experienced all four experimental conditions. Dark black line represents average Sigma for each condition.

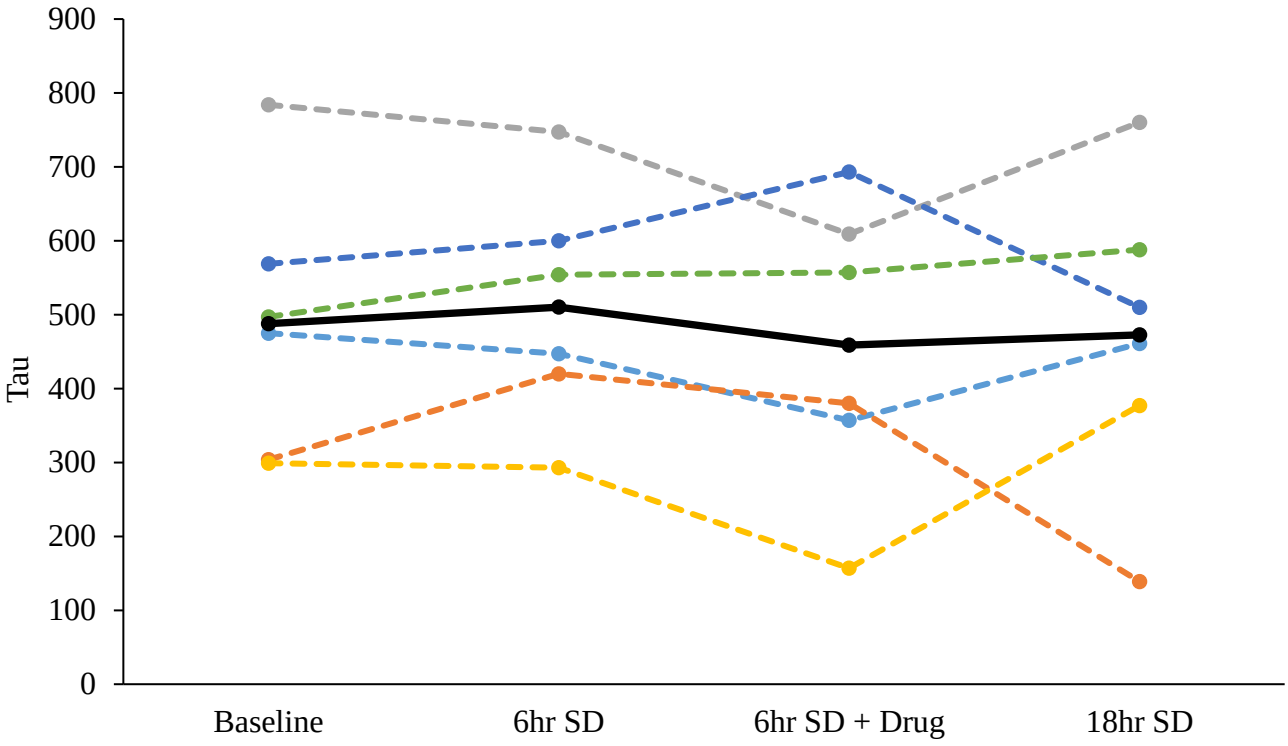

Figure 11. Tau (rate of exponential decay of response times based on an ex-Gaussian distribution) for each animal that experienced all four experimental conditions. Dark black line represents average Tau for each condition.