

# Timing (Not Just Amount) of Sleep Makes the Difference: Event-related Potential Correlates of Delayed Sleep Phase in Adolescent Female Students

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## Abstract

Adolescents tend to experience alterations in their sleep cycles inducing them to go to sleep and wake up at later times than they typically would at younger age (*Delayed Sleep Phase, DSP*). We asked a relatively homogeneous sample ( $N = 22$ ) of female high-school students to maintain an exhaustive sleep log during two weeks. Within this period, we randomly assigned each participant to a morning or afternoon testing condition (morning vs. afternoon group), in which we measured performance data along with ERPs during a standard Stroop task. The sleep log data confirmed a two-hour DSP in both groups. Participants in the afternoon group performed significantly better (less Stroop interference) than those in the morning group and showed a differential ERP pattern to incongruent and congruent stimuli, not shown by the morning group. We conclude that DSP can be associated with an attentional impairment in the morning.

**Keywords:** Delayed Phase Preference; Sleep & Circadian Rhythms; Attention; Stroop; Adolescence.

As children transition to adolescence, they tend to experience alterations in their sleep cycles (*Delayed Sleep Phase, DSP*) which may induce them to go to sleep and wake up at a later time than they typically would (Crowley, Acebo, & Carskadon, 2007; Fischer, Radosevic-Vidacek, Koscec, Teixeira, Moreno, & Lowden, 2008; Mitru, Millrood, & Mateika, 2002). We investigated the influence of sleep cycles on the performance of adolescent females during mornings and afternoons of ordinary school days by examining neural correlates of cognitive performance using Event-Related Potentials (ERPs), along with behavioral data. Our results support previous research which has found that early school start times, which do not accommodate the DSP observed in adolescents, result in poor academic performance (Epstein, Chillag, & Lavie, 1998; Fischer, Radosevic-Vidacek, Koscec, Teixeira, Moreno, & Lowden, 2008; Hansen et al., 2005; Wallstrom, Wrobel, & Kubow, 1998). The findings of our study show that adolescents tested in the morning are cognitively performing at a low level in comparison to their counterparts tested in the afternoon. Furthermore, the poor cognitive performance observed in the morning was associated with significantly different ERP patterns which appear to be evidence of a deficit in attentional resources and an impeded ability to respond when competing stimuli are present.

## Method

### *Participants*

Twenty two female adolescents, ranging in age from 16 to 18, were recruited from grades 11 and 12 psychology classes at a high school in Central Southern, British Columbia. Participants took part in the study for course credit in their psychology class.

### *Measures*

**Stroop Task.** A standard version of the Stroop Colour-Word Task, selected from STIM (Neuroscan, Compumedics Ltd.). Stimulus duration was set at 200 ms with an inter-trial interval of 1000 ms. Participants' response time and accuracy were measured through a hand-held response pad. To counterbalance any possible novelty effects incongruent stimuli were presented at a higher frequency than congruent stimuli (Macloed, 1991) at a ratio of approximately 3:1, 224 trials and 76 trials respectively, for a total of 300 trials.

**Event-Related Potentials (ERPs).** EEG was recorded concurrently with the Stroop task. Electrodes were placed at: Pz, P3, P4, Cz, C3, C4, Fz, F3, and F4 (International System) with reference electrode at nose tip. An electro-oculogram (EOG) was vertically placed above and below. Using the standard eye movement reduction algorithm devised by Semlitsch, Anderer, Schuster and Presslich (1986), the EOG was used to filter out artifacts due to eye movement from the relevant EEG signal. Impedance was kept below 5 $\Omega$ . High and low pass filters were set at 0.5Hz and 30Hz respectively. Time locking was implemented through an internal serial port trigger synchronized with stimulus presentation.

**2-week sleep-log questionnaire.** The sleep log questionnaire was adapted from the Thompson Rivers University Respiratory Therapy Program Sleep Questionnaire. The questionnaire was used to assess the sleeping schedules of participants over a two week period including weekends.

**Pre- and Post-Stroop affective questionnaires.** Before and after the Stroop task, participants completed a 5-point rating scale measuring affective states, adapted from an affective questionnaire (Usala & Hertzog, 1989), and a task appraisal questionnaire (Tomaka et al., 1999).

### *Procedure*

The participants began recording their sleeping schedule over a two week period beginning on the Thursday

preceding the first testing phase. They were then randomly assigned to the AM or PM testing group. Test and retest sessions were conducted from 8:30 – 10:30 a.m. (AM testing group), and 12:15 – 12:55 p.m. (PM testing group); each participant was tested at the same time on test and retest. On the day of testing, each participant was first administered the pre-Stroop questionnaire, then the EEG cap with embedded electrodes was placed on the participant and the Stroop task testing phase was conducted. Upon conclusion of the Stroop task, the EEG cap was removed and the post-Stroop questionnaire was administered.

## Results

### Sleep Logs

It was found that participants' total sleep per night ranged between  $M = 7$  hr 35 min ( $SD = 1$  hr 45 min) and  $M = 8$  hr 2 min ( $SD = 2$  hr 18 min) regardless of whether it was the weekend or a school night (Figure 1).

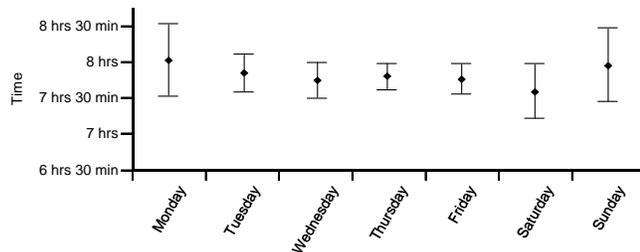


Figure 1. Mean hours of sleep per night by day of the week (with +/- 1 SE).

During the school week participants reported mean falling asleep times between  $M = 11:04$  p.m. ( $SD = 1$  hr 12 min) and  $M = 11:53$  p.m. ( $SD = 1$  hr 9 min), and mean wake times between  $M = 6:55$  a.m. ( $SD = 49$  min), and  $M = 7:55$  a.m. ( $SD = 2$  hr 13 min). In contrast, the weekend participants reported mean falling asleep times of  $M = 1:30$  a.m. ( $SD = 1$  hr 46 min) for Friday night, and  $M = 1:29$  a.m. ( $SD = 2$  hr 19 min) for Saturday night, and mean wake times of  $M = 9:05$  a.m. ( $SD = 1$  hr 21 min) for Saturday morning, and  $M = 9:26$  a.m. ( $SD = 2$  hr 16 min) for Sunday morning. It is noteworthy that the mean fall asleep time of Sunday night, and the mean wake time of Monday morning was slightly later than other school nights/days. In sum, we found sleep patterns indicating a DSP of about 2 hours which clearly manifested itself during the weekend. Mean fall asleep and wake up times are displayed in figure 2.

### Stroop Task Data

Because in this paper we were interested in relating sleep patterns to optimal performance we defined stroop interference in relation to accuracy differences in the congruent vs. incongruent trials. Although currently Stroop interference is more frequently defined in terms of RT differences, our interference measurement is not at all unusual in Stroop research (see MacLeod, 1991),

The participants' mean level of correct responses was used to determine accuracy. For Test 1, morning participants had an average accuracy of  $M = 67\%$  ( $SD = 16$ ) and  $M = 72\%$  ( $SD = 17$ ) for Test 2. Afternoon participants

showed an increase in their level of accuracy; Test 1 and Test 2 yielded  $M = 75\%$  ( $SD = 13$ ) and  $M = 83\%$  ( $SD = 15$ ), respectively. None of these means were significantly different. Each participant's mean accuracy was then used to determine their individual mean level of Stroop interference (computed as  $M_{\text{congruent}} - M_{\text{incongruent}}$ ). For Test 1, morning and afternoon participants showed an average Stroop interference of  $M = 23\%$  ( $SD = 12$ ) and  $M = 13\%$  ( $SD = 9$ ) respectively. For Test 2, participants showed an average Stroop interference of  $M = 20\%$  ( $SD = 16$ ) in the morning, and  $M = 10\%$  ( $SD = 19$ ) in the afternoon. Stroop interference changed significantly according to the time of day, morning

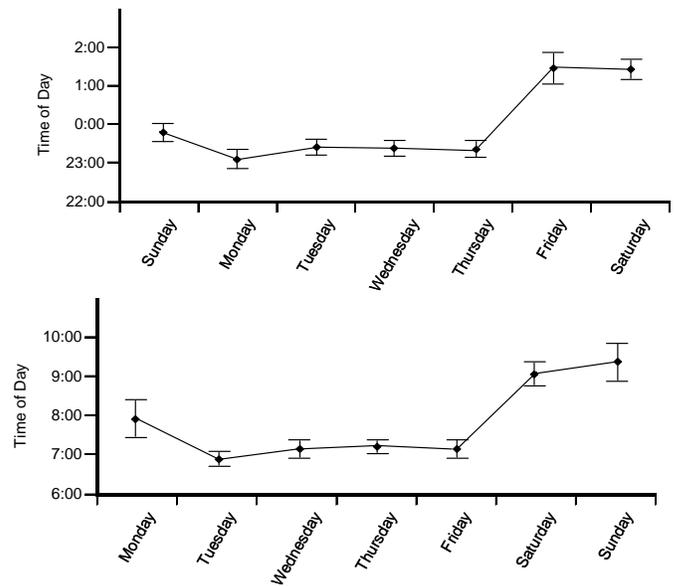


Figure 2. Average fall asleep time by day of week (with +/- 1 SE) displayed above average wake time by day of week (with +/- 1 SE).

or afternoon, for both Test 1 ( $t(19) = 2.83$ ;  $p < .02$ ), and Test 2 ( $t(19) = 2.63$ ;  $p < .02$ ). These results show that participants tested in the morning experienced significantly greater levels of Stroop interference than participants tested in the afternoon.

The mean reaction times (RT) for Test 1 were  $M = 815.91$ ms ( $SD = 96.58$ ) in the morning, and  $M = 902.97$ ms ( $SD = 98.89$ ) in the afternoon. The results for Test 2 show RTs of  $M = 726.95$ ms ( $SD = 118.88$ ) in the morning, and  $M = 782.49$ ms ( $SD = 111.35$ ) in the afternoon. Even after outlier removal ( $\pm 3SD$  from mean RT) there were no significant and reliable differences in mean RTs between morning and afternoon groups for both Test 1 and Test 2. However, there was a significant difference between the overall reaction times of Test 1 ( $M = 865.66$ ms,  $SD = 105.16$ ms) and Test 2 ( $M = 758.49$ ms,  $SD = 115.14$ ms) indicating that both groups responded faster on the second day of testing ( $t(19) = 4.68$ ,  $p < .001$ ).

### Pre- and Post-Questionnaires

The questionnaires revealed that the mean level of perceived task performance was significantly higher in the afternoon

group than it was in the morning group ( $M_{rank} = 7.75$  and  $M_{rank} = 3.90$  respectively; Wilcoxon matched-pairs signed-ranks test,  $z = -2.063$ ,  $p < .05$ ). It was also found that the mean level of boredom was significantly higher in the AM test group ( $M_{rank} = 8.42$ ) than it was in the afternoon group ( $M_{rank} = 4.58$ ;  $z = -1.895$ ,  $p < .05$ ). There were no significant differences found between AM and PM conditions in all other control variables.

### ERP Data

Our analyses focused on the critical window typically involved in Stroop ERPs (300-600ms). We conducted planned focused  $t$ -tests with Bonferroni confidence interval adjustment. In what follows we summarize the most relevant results, although we note that the effects are evident by visual inspection in the example shown below (similar patterns with smaller sizes were observed on across sites).

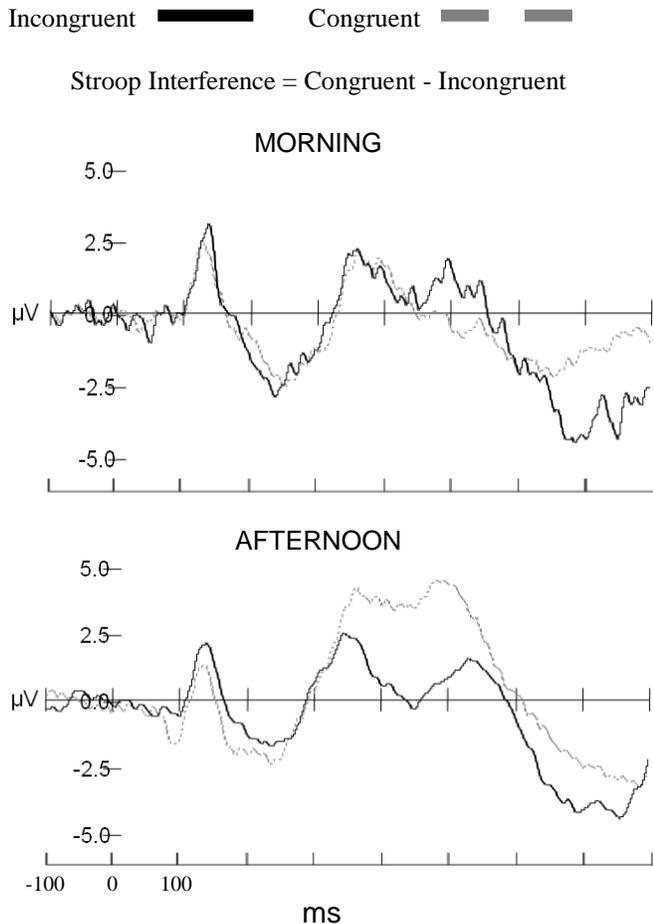


Figure 3. Grand mean ERP signals for congruent and incongruent Stroop correct trials for morning and afternoon groups at central parietal electrode site (Pz). Baseline correction was done on 100 ms prestimulus.

There was a significant difference in the level of ERP amplitudes relative to congruent and incongruent stimuli between 350 and 550ms ( $p < .05$ , Bonferroni  $t$ -interval) in

the afternoon but not in the morning group. Figure 3 shows the best example at the central parietal electrode site. In the afternoon, the processing of congruent stimuli was accompanied with a higher level of ERP amplitudes. There was a significant positive difference occurring between 500 and 800 ms. Congruent stimuli peaked at close to 500 amplitudes and remained fairly constant between 500 and 800 ms, while incongruent stimuli reached maximum peak amplitudes at 500 ms and tapered off gradually. In contrast, the morning group showed no significant differences between congruent and incongruent trials.

### Discussion

In the present study, the total hours of sleep obtained by adolescent students did not significantly vary from weekdays to weekends; however, these participants tended to go to sleep and wake up approximately two hours later on weekends than they did during the week. These results support previous research that has shown that adolescents have a natural preference for waking that is at odds with early school start times (Wolfson, & Carskadon, 1998; Wolfson, & Carskadon, 2003). This conflict seems to be associated with relatively poor performance and lower observed neural activity during complex cognitive tasks. Because previous research has shown no reliable differences between genders for the age range studied in the present investigation we suggest that likely the present findings can be generalized to both male and female adolescent students.

Consistent with the present findings, previous studies have shown that adolescents' cognitive performance significantly improves in the afternoon as compared to the morning (Wolfson, & Carskadon, 2003; Wright Jr., Hull, Hughes, Ronda, & Czeisler, 2006; Yoon, May, & Hasher, 1999). The increase in cognitive performance of adolescents as the day progresses may be attributed to the advent of the wakeful stage of the circadian rhythm. When tested in the afternoon, participants are naturally in a wakened stage allowing them to cognitively perform at their optimal level (Hasher, Zacks, & Rahhal, 1999; Wright Jr., Hull, Hughes, Ronda, & Czeisler, 2006). Participants tested in the morning appear to be experiencing symptoms of a misaligned circadian rhythm which impair performance reflected in a significantly greater level of observed Stroop interference (May, & Hasher, 1998).

The differences in cognitive performance between individuals tested in the afternoon and those tested in the morning were also accompanied by substantial ERP differences. P300 amplitudes, which have been found to reflect the amount of attentional resources being employed (Wickens, Kramer, Vanasse, & Donchin, 1983), were significantly greater in the afternoon. These results invite the interpretation that participants tested in the morning had (or allocated) less attentional resources as compared to participants tested in the afternoon. This difference may be directly related to the misalignment of the adolescent circadian rhythm as it correlates with the pattern reflected in the self-reports from the sleep logs, and it would be

compatible with the possibility that adolescents did not enter into a wakeful stage until later in the early afternoon. Once they were in a wakened stage, they were more attentive (Doran, Van Dongen, & Dinges, 2001), and therefore showed greater brain wave activity differentials concomitant with the Stroop task.

The disparity in ERP amplitudes between congruent and incongruent conditions occurring between 500ms and 800ms in the P.M. group could also be explained by *slow-route processing*, which has been proposed to occur during the incongruent condition of a Stroop task (Frith, & Done, 1986). *Slow-route processing* is characterized by a late positivity following the initial P300 peak (Ilan, & Polich, 1999), which may also be the manifestation of other subcomponents of the complex labeled the P300 (Falkenstein, Hohnsbein, & Hoormann, 1994; Rosler, Borgstedt, & Sojka, 1985). Whether the late positivity reflects *slow-route processing* or response selection processes associated with the Stroop task, the lack of ERP amplitude differences between the congruent and incongruent conditions in the AM group seems to signify the omission or attenuation of processes related to stimulus evaluation and response selection.

Previous research has concluded that N200 amplitudes either reflect a top-down inhibitory mechanism (Eimer, 1993) or conflict monitoring processes (Nieuwenhuis, Yeung, Van Den Wildenberg, & Ridderinkhof, 2003) that facilitate responses when competing stimuli are present. Therefore, based on either of these assumptions, our results suggest that the comparatively less pronounced negative peaks observed in the morning group at 200 ms are associated with a diminished ability to appropriately respond during a task involving competing stimuli.

The negative waves that occurred around 800 ms in both morning and afternoon groups may represent the contingent negative variation (CNV) that occurs between a warning signal and a second stimulus that demands a response (Gomez, Marco, & Grau, 2003; Rohrbaugh, Syndulko, & Lindsley, 1976). Therefore, the observed pattern could be explained by participants preparing for the next stimuli to be presented on the screen. The fact that CNVs did not differ between the two groups is consistent with the lack of differences between groups in the RT data.

Although there were no statistically significant differences in RTs between morning and afternoon groups, the overall mean RT of Test 1 was significantly greater than that of Test 2, indicating that participants in both groups responded faster during the Test 2 trials. It is reasonable to suggest that as familiarity with the cognitive task increased, the mean RT decreased; however, it is important to note that this practice effect was not associated with any significant change in accuracy or ERPs. These results suggest that motor response confounds were not a major determinant of the observed ERP patterns; rather, the observed ERP patterns were likely related to actual latent cognitive processes.

In addition to the P300 amplitude differences, another possible indicator of an attentional impairment is the elevated mean level of boredom observed in morning participants. Recent research has found a robust relationship between attention related deficits and perceived boredom. More specifically, an individual's tendency to be inattentive is a likely cause of proneness to boredom (Carriere, Cheyne, & Smilek, 2008). In our study, morning participants had a significantly elevated mean level of boredom but no other significant differences in other affective states; given this, it is possible that the elevated mean level of boredom is a sign of an attentional impairment related to poor cognitive performance.

The differences in cognitive performance between morning and afternoon conditions were accompanied by a significantly different pattern of ERP results that suggest a cognitive deficiency in the morning condition. That is, adolescents tested in the afternoon showed an appropriately elevated attentional waveform, associated with better Stroop performance, in response to incongruent stimuli. In comparison, adolescents tested in the morning showed no significant ERP differences in response to congruent or incongruent stimuli. Lack of ERP differences between congruent and incongruent stimuli was found to be associated with relatively poor Stroop performance (higher interference). The observed relationship between morning and afternoon test times, Stroop task performance, and ERPs is further evidence that suggests adolescents are cognitively performing at a sub-optimal level in the morning.

The implications of our findings are particularly relevant as they seem to illustrate the impact of early school start times on cognitive performance. Previous studies have found that students beginning school later in the day tended to sleep longer and perform better academically than students beginning school in the morning (Epstein, Chillag, & Lavie, 1998; Wahlstrom, Wrobel, & Kubow, 1998). However, the present study shows that there is a significant difference in the cognitive performance of students from morning to afternoon that is not exclusively dependent on duration of sleep, as there were no significant differences in the duration of sleep observed between our groups and the range of sleep time per night of our sample was within the range of the average healthy sleep (7 hrs 30 min to 9 hrs) (Mitru, Millrood, & Mateika, 2002). These results suggest that it is not necessarily the amount of sleep but the time of day according to the natural circadian rhythm that is relevant in determining, presumably, the quality of sleep (the "good sleep") and the optimal cognitive performance. In contrast with what is suggested by some researchers and educators, a counterintuitive implication of our findings is that having students go to bed earlier in the evening – so that they can obtain adequate amounts of sleep – will not offset the cognitive impairment of a misaligned circadian rhythm.

The results of this study, as well as previous research, suggest that conventional school schedules could

accommodate the shifted circadian rhythm of adolescents for the purpose of increasing academic success. Based on the difference between weekday and weekend rise time, a delay in school start times by as little as 2 hours may be enough to account for DSP; still, there are sociocultural and logistic factors that prohibit widespread change in school timing and scheduling. An alternative to changing school operation times could be to rearrange students' class schedules to appropriately account for lower levels of cognitive functioning in the morning. Having adolescents attend gym class in the morning and mathematics in the afternoon is an example of such an arrangement. Future research should be conducted with regard to the impact of possible interventions in this direction. Another related issue deserving further study is the fact that the present findings may be explained by the fact that the activities done in weekends are different from those done in the schooldays and that forces adolescents to stay awake later and then compensate by also waking up later. On this interpretation, school DSP may be related to the more complex systemic interdependence between school starting times and weekend sleep times.

In conclusion, conflict between the naturally delayed circadian rhythm of adolescents and early school start times may explain poor performance of adolescents on some cognitive activities carried out in the morning. Furthermore, the poor cognitive performance of adolescents observed in the morning is associated with distinctive ERP patterns that suggest an attentional deficiency and a tendency to process congruent and incongruent stimuli in a similar manner. Therefore, the ERPs observed in this study may be indicative of the cognitive impairment that adolescents struggle with when performing mentally demanding school activities in the morning.

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