Title: Rotating night shifts too fast may cause anxiety, decreased attention performance and may impact prolactin levels during the daytime after night shifts- a case control study

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Abstract

**Background:** We investigated the circadian changes and effects on mood and cognitive performance after working consecutive night shifts in a rapidly rotating shift system. Cognitive function, sleep propensity and sleep-related hormones (growth hormone, cortisol, prolactin, thyrotropin) in the daytime after working two and four consecutive night shifts were compared. **Methods:** Twenty-three off-duty nurses, 20 nurses working two consecutive night shifts, and 16 nurses working four consecutive night shifts were enrolled. All of the nurses completed the Maintenance of Wakefulness Test, State-Trait Anxiety Inventory, Stanford Sleepiness Scale, visual attention tasks (VAT), Wisconsin Card Sorting Test, modified Multiple Sleep Latency Test, and hormone levels were measured four times throughout the daytime at 2-hour intervals. **Results:** During the daytime, the subjects in the night shift groups were less able to maintain wakefulness, and had poor performance on VAT than that in the off-duty group. The subjects in the two night shifts group were more able to maintain wakefulness, had higher anxiety scale scores, poorer performance on VAT with a lack of learning effect, and higher prolactin levels compared to the four night shifts group. However, the cortisol level did not consist with anxiety state in the two night shifts group during the daytime after night shifts. **Conclusion:** It is suggested rotating night shifts too fast may cause anxiety, decreased attention
performance and may impact prolactin levels during the daytime after night shifts.

The impact on sleep-related hormones during the daytime after working different lengths of consecutive night shifts would be an interesting topic for future studies.

Keywords: anxiety, cognitive function, nurse, shift work, sleep-related hormone
Background

A three-shift system with faster rotation (shifting every three to five days) is common in the medical nursing field in Taiwan, where it is assumed that nurses will maintain a constant circadian rhythm in coordination with the environment [1]. The night shift work schedule of most nursing staff at our hospital consists of two to four consecutive night shifts followed by at least one day off. During the off day, it would be expected that the nurses adjust their circadian rhythms in preparation for the next day shift. Sleep deprivation studies [2] have shown a diverse impact on mood and cognitive performance, and many studies have investigated the influence of night shifts on the performance of work at night. However, little is known about the impact of night shifts on daytime performance after consecutive night shifts. The impact of night shift work on daytime functioning is an interest area of research in rapidly rotating shift systems that can provide valuable information regarding circadian changes and effects on mood and cognitive performance. In this study, therefore, we compared changes in cognitive function and anxiety state, and objectively measured sleep propensity in the daytime after two consecutive night shifts and after four consecutive night shifts. We also measured levels of sleep-related hormones (growth hormone, GH; cortisol; prolactin, PRL; thyrotropin, TSH) during the daytime.
Method

Participants and procedures

The participants included 23 off-duty female nurses (mean age, 26.1 ± 1.9 years; mean years of education, 15.3 ± 1.0), 20 female nurses (mean age, 26.0 ± 2.0 years; mean years of education, 14.7 ± 1.0) working two consecutive night shifts, and 16 female nurses (mean age, 27.1 ± 2.0 years; mean years of education, 15.4 ± 1.0) working four consecutive night shifts from the acute ward of Kaohsiung Municipal Kai-Syuan Psychiatric Hospital in southern Taiwan. We excluded those who were currently using hypnotics, regularly drinking coffee, with any psychiatric illnesses, major systemic diseases, and sleep disorders according to a questionnaire. All of the nurses had worked either day time shifts or been free of duty for at least three days before entering the study. Those in the night shift groups were asked to sleep prophylactically during 7-11 p.m. while working nights. The demographic data included age, years of education, and mean self-reported total sleep time (TST, including daytime sleep and prophylactic sleep) for the night shift groups, and the sleep time at night the day before the study for the off-duty group. This study was approved by the Ethics Committee of Kaohsiung Municipal Kai-Suan Psychiatric Hospital (KSPH-2011-15) and all subjects gave written consent after being fully informed of the nature and procedures of the study.
All subjects arrived at the sleep laboratory at about 9:00 a.m. at the end of
consecutive night shifts and on the off-duty day, respectively and spent about 8
hours in the laboratory and performed the following tests four times every 2 hours
starting at 9:20 a.m. The measurements included the Maintenance of Wakefulness
Test (MWT), State-Trait Anxiety Inventory (STAI) [3], Stanford Sleepiness Scale
(SSS) [4], Wisconsin Card Sorting Test (WCST) [5], Digit Symbol Substitution Test
(DSST), Symbol Searching Test (SST) [6], and the modified Multiple Sleep Latency
Test (modified MSLT) (Figure 1). Blood samples were collected at the end of the
MSLT at the bedside and were tested for sleep-related hormones. All participants
were required to remain awake during the test day, and all of the tests were given
individually in an equivalent experimental setting.

**Measurements**

The STAI is a self-reported measure of both state and trait anxiety which
contains 20 self-reported items, respectively. All items are rated on a 4-point scale
with a higher score indicating a higher anxiety. The STAI has been shown to have
good validity as well as good internal consistency (Cronbach’s $\alpha > 0.85$) and
test-retest reliability ($\geq 0.75$) [7]. The SSS is a 7-point self-rating scale which is used
to quantify progressive steps in sleepiness, from one (alert) to seven (no longer
fighting sleep).
The computerized WCST consists of four stimulus cards and 128 response cards. The nurses were asked to match each response card from the deck with one of the four stimulus cards that she thought was most appropriate. The dependent variables included the number of perseverative errors, number of total errors, number of categories, percent of conceptual level responses, and failure to maintain set. Both the DSST and SST of visual attention tasks (VAT) are subsets of the Wechsler Adult Intelligence Scale [6]. For the DSST, the nurses were asked to enter appropriate symbols into empty squares beneath digits. In the SST, the nurses were asked to respond to either one of two target symbols from four selective symbols. The raw scores of the DSST and SST were determined by the number of items correctly completed in 120 seconds, and the raw scores were then converted to a scale score according to age. The information process index (IPI) was obtained after transforming the sum of the scale scores of the SST and DSST.

The modified MSLT was performed by partial-montage polysomnography consisting of electroencephalography (at F3/A2, F4/A1, C4/A1, C3/A2, O2/A1, O1/A2), electrooculography, and submental electromyography. We scored sleep records visually according to the American Academic of Sleep Medicine criteria [8]. The subjects lay in a quiet, dark bedroom and attempted to fall sleep. They were awoken immediately after three consecutive 30-second epochs of stage one sleep or
any epoch of another sleep stage. The sleep latency was taken as the first epoch of any stage of sleep. If sleep onset did not occur, a latency of 20 minutes (the end of the test) was used for data analysis. The MWT was administered as per the modified MSLT, except the subjects were instructed to remain awake and the trials lasted 40 minutes.

The serum levels of cortisol, PRL, GH, and TSH were measured by a paramagnetic particle, chemiluminescent immunometric assay using a Beckman Access system (Beckman Coulter Inc. Fullerton, CA, USA), Siemens DPC Immulite 2000 analyzer (Siemens Healthcare Diagnostic Products Ltd. Llanberis, Gwynedd, United Kingdom), Siemens DPC Immulite 2000 analyzer (Siemens Healthcare Diagnostic Products Ltd. Llanberis, Gwynedd, United Kingdom) and Abbott I2000 (Abbott Ireland Diagnostics Division, Longford, Ireland), respectively. The intra-assay coefficient of variation averaged 5% in each item.

Statistical analysis

One-way analysis of variance (one-way ANOVA) was used to compare continuous variables among the three groups. Repeated measures ANOVA was performed with groups as between factors and time series data as within factors. A $p$-value less than 0.05 was considered to be statistically significant.
Results

There were no differences in age ($F(2, 56) = 1.77, p = 0.179$), years of education ($F(2, 56) = 2.58, p = 0.085$), trait anxiety scores ($43.9 \pm 8.1$ vs. $43.0 \pm 7.6$ vs. $43.8 \pm 8.0$; $F(2, 56) = 0.08, p = 0.927$), mean self-reported TST ($6.9 \pm 1.1$ h vs. $6.7 \pm 1.0$ h; $F(2, 56) = 0.18, p = 0.833$), mean sleep latency (MSL) of the MSLT ($p = 0.314$), SSS scores ($p = 0.451$), and GH ($p = 0.697$) and cortisol ($p = 0.884$) levels among the three groups (Table 1). There were no significant differences among the groups in all parameters of the WCST. However, there were significant differences among the groups in MSL of the MWT ($p < 0.001$), state anxiety scores ($p = 0.009$), IPI ($p = 0.002$), DSST ($p = 0.013$) and SST ($p = 0.003$) scores, and PRL ($p = 0.003$) and TSH ($p = 0.003$) levels (Table 1).

The subjects in the off-duty group had a significantly longer MSL of the MWT compared to the night shifts groups. The MSL of the MWT in the two night shifts group was longer than that in the four night shifts group, however, there was no significant time-of-day effect in either group (Figure 2). The subjects in the two night shifts group had higher anxiety scale scores than those in the off-duty and four night shifts groups, and both the off-duty and four night shifts groups also showed significantly decreased anxiety throughout the daytime (Figure 3). The subjects in the off-duty group had better performances on the SST and DSST than both night
shift groups. All groups showed a trend of improved performance on the IPI, SST and DSST during the daytime, however the learning effect in the off-duty group and four night shifts group were more prominent than that in the two night shifts group (Figure 4). Regarding sleep-related hormones, the PRL level in the two night shifts group was significantly higher than in the other two groups. TSH levels in both night shift groups were significantly higher compared to the off-duty group, and were significantly elevated throughout the daytime (Figure 5).
Discussion

The results suggest that there were no significant differences in sleep propensity as measured by the MSLT and in subjective sleepiness as measured by the SSS among the three groups. The capacity to maintain wakefulness was better in the off-duty group, followed by the two and four night shifts groups, respectively. The subjects in the two night shifts group had higher state anxiety scale scores than those in the other two groups, and also showed a persistent anxiety state throughout the daytime. Neuropsychological assessments revealed no significant differences among the groups in WCST performance. The performances on perceptual and motor abilities, as measured by the DSST and SST, were better in the off-duty group than in the night shift groups. Although all groups showed a trend of improved perceptual and motor abilities during the daytime, the improvements were more significant in the off-duty group and four night shifts group than in the two night shifts group.

Latency in the MSLT reflects the propensity to fall asleep during the daytime. Healthy subjects without emotional or sleep disturbances allowed to fall sleep in appropriate conditions can often achieve stage one sleep within 10 minutes according to the MSLT [9], and accordingly there were no statistically significant differences in MSL of the MSLT among the three groups in the study. However, the
ability to maintain wakefulness decreased with longer periods of consecutive night work. This may be explained by a phase shift in the circadian rhythms of the nurses, as a gradually decreasing latency in the MWT during the daytime after two to four consecutive night shifts and a significant improvement in cognitive performance in the nighttime on the fourth day when working consecutive night shifts have been reported in previous studies [10-12]. But we did not evaluate the sleep quality in the daytime when working night shifts. Although there was no statistically significant difference in the average self-reported TST among the three groups, it is possible that poor sleep quality in the daytime when working consecutive night shifts caused sleep debt which then affected the ability to maintain wakefulness during the daytime of the off day after consecutive night shifts.

We found no differences in subjective sleepiness among the three groups, even though the nurses who worked more consecutive night shifts had a significantly decreased capacity of maintaining wakefulness as measured by the MWT during the daytime compared to those not working as many consecutive night shifts. Night shift work has been reported to lead to chronic partial sleep deprivation [13], and it has been reported that subjects with chronic partial sleep deprivation frequently have the subjective impression that they have adapted to this because they do not feel particularly sleepy [14]. In addition, the performances on VAT in the night shift
groups were also worse than those in the off-duty group which is compatible with previous studies [15, 16] reported people frequently underestimated the impact of sleep restriction on cognitive functions. Sleep deprivation studies [2] have shown a diverse impact on cognitive performance as well as on mood due to destabilization of the wake state. The effects of sleep restriction on cognitive performance in healthy adults are consistent with the effects of sleep restriction on physiological sleep propensity measures (MWT, MSLT) [14, 17]. It is reasonable, therefore, to assume that the cognitive performance in the two night shifts group following sleep restriction in the daytime would be better than that in the four night shifts group. However, the results of performance on perceptual and motor abilities in the two night shifts group were not statistically different from the four night shifts group. Although a time-of-day effect on improvements in the performance of perceptual motor coordination was also observed in the two night shifts group, this effect was not as prominent as in the other two groups. Besides, the subjects in the two night shifts group had higher state anxiety scale scores than the other two groups and showed a persistent throughout the daytime. That is nurses in two consecutive night shifts following sleep restriction is more emotional stress than that in four consecutive night shifts following sleep restriction. This may be explained by two consecutive night shifts following sleep restriction in the daytime causing
persisted emotional stress which resulted in a state of hyperarousal, and that may have contributed to the decreases both in performance on attention tasks and learning effect [15, 18].

The WCST can be considered a measure of “executive function” [19]. In this study, there was no difference in the ability to perform the WCST among the three groups. That is, the ability to shift cognitive strategies in response to changing environmental contingencies was not affected during the daytime of sleep restriction.

Regarding the sleep-related hormones, the levels of TSH in the night shift groups were higher than those in the off-duty group which were also elevated throughout the daytime. The level of PRL in the two night shifts group was higher than in the other two groups, however there were no significant changes in GH and cortisol levels during the daytime among the three groups.

Cortisol release is mainly controlled by the circadian rhythm, and TSH is regulated by both sleep and circadian rhythm [20]. Sleep deprivation would not affect cortisol release, however it would cause persistent elevation of TSH during the daytime because of the prolonged half-life of this hormone [21]. In this study, the subjects in the night shift groups experienced sleep deprivation before the test day which would not affect circadian rhythm-related cortisol release. However, it is
known stress has been associated with the activation of the hypothalamic-pituitary-adrenal axis, which cause to arousal and sleeplessness [22]. It was supposed the two night shifts group had higher cortisol level than that in the other two groups because of their higher anxiety scale scores. The negative finding may be due to small sample size, and it would be interesting to further study the impact of consecutive night shifts on mood and cortisol level during the daytime of sleep restriction.

The TSH levels in the night shift groups were higher than that in the off-duty group and were also elevated throughout the daytime. There was no difference in TSH level between the night shift groups, which possibly indicates a comparable effect on TSH level during the daytime of sleep restriction after working a different number of consecutive night shifts. GH and PRL secretion are only triggered by sleep onset [20], and therefore there was no significant change in GH during the daytime among the three groups. However, the PRL level in the two night shifts group was higher than that in the four night shifts group. This suggests that rotating night shifts too fast may have an impact on PRL level during the daytime of sleep restriction, and this would also be an interesting topic for future research.

There are some limitations to this study. First, because the tasks were performed in an experimental setting, the results of the neuropsychological findings cannot be
generalized to real life practice. Second, we excluded the nurses who used hypnotics
and regularly drank coffee and who were least tolerant to shift work, and this may
have overestimated the daytime results. Third, we did not collect data on when the
nurses slept while working nights, which may have been related to how sleepy they
were during the 8-hour daytime test period. Fourth, there was a small sample size in
each group. Fifth, we did not use indwelling catheters to avoid discomfort when
collecting blood samples, nor did we record the stage of menstrual cycle which is
related to the PRL level [23]. These may have been confounding factors for the
cortisol and PRL levels. Sixth, not all subjects had the same shift work schedule
before entering the study, which may also have confounded the results. Finally, we
did not evaluate chronotype, depression, or psychosocial work characteristics, which
may have confounded the results.

In conclusion, the nurses who worked two consecutive night shifts were
higher emotional stress than those worked four consecutive night shifts during the
daytime after night shifts, which may in turn to a decrease both in performance on
attention tasks and learning effect, and possibly an impact on PRL level. However,
the cortisol level did not consist with anxiety state in the two night shifts group
during the daytime after night shifts. It is suggested rotating night shifts too fast may
cause anxiety, decreased attention performance and may impact prolactin levels
during the daytime after night shifts. The impact on sleep-related hormones during the daytime after working different lengths of consecutive night shifts would be an interesting topic for future studies.
Abbreviations

DSST: Digit Symbol Substitution Test; IPI: information process index; GH: growth hormone; MSLT: Multiple Sleep Latency Test; MWT: Maintenance of Wakefulness Test; PRL: prolactin; SSS: Stanford Sleepiness Scale; SST: Symbol Searching Test; STAI: State-Trait Anxiety Inventory; TSH: thyrotropin; TST: total sleep time; WCST: Wisconsin Card Sorting Test; VAT: visual attention tasks

Competing interests

The authors declare that they have no competing interest.

Authors’ contributions

YSC: Study design, Data analysis, interpretation of results, and preparation of the manuscript; YHW: Data collection; HLC: Data collection; CYH: interpretation of results; CKL: Study design; CH: study design

All authors read and approved the final manuscript.

Acknowledgments

This work was supported by Kaohsiung Municipal Kai-Syuan Psychiatric Hospital.


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Table 1-1. Comparison of time series data among the off duty, two night shifts and four night shifts groups

<table>
<thead>
<tr>
<th>Variables (mean±SD)</th>
<th>Group</th>
<th>Morning time 1 (1)</th>
<th>Morning time 2 (2)</th>
<th>Afternoon time 1 (3)</th>
<th>Afternoon time 2 (4)</th>
<th>F(2,56)</th>
<th>P-value</th>
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<td>Off-duty</td>
<td>24.3±7.6</td>
<td>26.1±7.0</td>
<td>23.4±9.4</td>
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<td>16.3±14.8</td>
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<td>Four night shifts</td>
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<td>11.0±12.2</td>
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<td>Four night shifts</td>
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<td>Sleep latency of MSLT</td>
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<td>Four night shifts</td>
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<td>State anxiety scale</td>
<td>Off-duty</td>
<td>3.2±0.2</td>
<td>3.5±0.2</td>
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<td>Stanford Sleepiness Scale</td>
<td>Off-duty</td>
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<td>Number of total errors</td>
<td>Off-duty</td>
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<td>16.8±4.9</td>
<td>15.8±4.2</td>
<td>1.44</td>
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<td>Four night shifts</td>
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<td>18.2±4.5</td>
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Abbreviations: MWT, Maintenance of Wakefulness Test; MSLT, Multiple Sleep Latency Test

Scheffe’s post-hoc test: a off-duty group > two night shifts, off-duty group > four night shifts, two night shifts > four night shifts;
b off-duty group < two night shifts, four night shifts < two night shifts.
Table 1-2. Comparison of time series data among the off duty, two night shifts and four night shifts groups

<table>
<thead>
<tr>
<th>Variables (mean±SD)</th>
<th>Group</th>
<th>Morning time 1 (1)</th>
<th>Morning time 2 (2)</th>
<th>Afternoon time 1 (3)</th>
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<tr>
<td>Number of categories</td>
<td>Off-duty</td>
<td>7.6±1.9</td>
<td>8.0±1.5</td>
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<td>Two night shifts</td>
<td>7.9±2.3</td>
<td>8.4±2.2</td>
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<td>8.1±2.2</td>
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<td>Four night shifts</td>
<td>8.4±1.4</td>
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<td>8.3±1.3</td>
<td>8.3±1.1</td>
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<td>% of conceptual level responses</td>
<td>Off-duty</td>
<td>73.7±14.5</td>
<td>79.4±8.9</td>
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<td>84.7±5.9</td>
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<td>76.7±15.7</td>
<td>79.4±13.1</td>
<td>78.8±15.0</td>
<td>77.6±14.8</td>
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<td>Four night shifts</td>
<td>81.2±7.1</td>
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<td>81.6±6.0</td>
<td>83.8±3.1</td>
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<td>Failure to maintain set</td>
<td>Off-duty</td>
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<td>1.8±1.4</td>
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<td>Four night shifts</td>
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<td>1.7±1.3</td>
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<td>Digit Symbol Substitution Test</td>
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<td>Scoring scale</td>
<td>Off-duty</td>
<td>14.7±1.6</td>
<td>15.2±1.9</td>
<td>15.6±1.9</td>
<td>16.5±2.1</td>
<td>4.72</td>
<td>0.013</td>
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<td>Two night shifts</td>
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<td>14.0±2.7</td>
<td>14.4±2.8</td>
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<td>Four night shifts</td>
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<td>13.4±2.9</td>
<td>14.0±2.8</td>
<td>14.2±2.0</td>
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<td>Scoring scale</td>
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<td>Four night shifts</td>
<td>11.7±4.1</td>
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<td>12.7±3.5</td>
<td>14.1±2.8</td>
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<tr>
<td>Information process index</td>
<td>Off-duty</td>
<td>126.0±7.7</td>
<td>130.3±10.7</td>
<td>131.5±9.2</td>
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<td>7.21</td>
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<td>118.0±14.6</td>
<td>121.6±13.2</td>
<td>124.6±14.7</td>
<td>126.0±13.7</td>
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<td>Four night shifts</td>
<td>110.9±18.1</td>
<td>117.1±17.2</td>
<td>116.1±18.8</td>
<td>123.9±12.6</td>
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</table>

Scheffe’s post-hoc test: a off duty group > two night shifts, off duty group > four night shifts.
Table 1-3. Comparison of time series data among the off duty, two night shifts and four night shifts groups

<table>
<thead>
<tr>
<th>Variables (mean±SD)</th>
<th>Group</th>
<th>Morning time 1 (1)</th>
<th>Morning time 2 (2)</th>
<th>Afternoon time 1 (3)</th>
<th>Afternoon time 2 (4)</th>
<th>F(1,41)</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Sleep related hormone</td>
<td>Off-duty</td>
<td>9.7±3.0</td>
<td>8.5±3.5</td>
<td>8.6±2.9</td>
<td>9.8±3.7</td>
<td>6.31</td>
<td>0.003a</td>
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<td>Two night shifts</td>
<td>11.8±6.0</td>
<td>12.5±7.0</td>
<td>13.4±7.0</td>
<td>14.9±6.6</td>
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<td></td>
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<tr>
<td></td>
<td>Four night shifts</td>
<td>8.1±2.2</td>
<td>8.2±3.0</td>
<td>9.6±2.9</td>
<td>11.1±3.5</td>
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<td>Growth hormone (ng/ml)</td>
<td>Off-duty</td>
<td>1.3±4.0</td>
<td>1.3±2.0</td>
<td>0.5±1.3</td>
<td>1.2±2.6</td>
<td>0.36</td>
<td>0.697</td>
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<td>Two night shifts</td>
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<td>Four night shifts</td>
<td>1.1±0.8</td>
<td>0.9±0.5</td>
<td>0.6±0.9</td>
<td>1.1±1.1</td>
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<tr>
<td>Thyrotropin (mIU/L)</td>
<td>Off-duty</td>
<td>1.0±0.6</td>
<td>1.0±0.6</td>
<td>1.0±0.6</td>
<td>1.1±0.6</td>
<td>6.64</td>
<td>0.003b</td>
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<td>Two night shifts</td>
<td>1.3±0.7</td>
<td>1.5±0.7</td>
<td>1.9±0.9</td>
<td>2.0±0.7</td>
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<td></td>
<td>Four night shifts</td>
<td>1.0±0.4</td>
<td>1.4±0.5</td>
<td>1.7±0.7</td>
<td>2.1±0.8</td>
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<tr>
<td>Cortisol (ug/dl)</td>
<td>Off-duty</td>
<td>5.7±3.4</td>
<td>6.0±2.8</td>
<td>4.9±2.8</td>
<td>5.2±2.9</td>
<td>0.12</td>
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<td>Two night shifts</td>
<td>5.5±2.2</td>
<td>5.9±2.3</td>
<td>4.5±3.0</td>
<td>4.4±2.1</td>
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<td>Four night shifts</td>
<td>5.4±3.1</td>
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<td>5.2±2.5</td>
<td>5.1±2.5</td>
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</tr>
</tbody>
</table>

Scheffe’s post-hoc test: a off duty group < two night shifts, four night shifts < two night shifts; b off duty group < two night shifts, off duty group < four night shifts.
Maintenance of Wakefulness Test (maximum 40 minutes)
  ↓  10 minutes interval
State-Trait Anxiety Inventory (1-2 minutes)
  ↓  3 minutes interval
Stanford Sleepiness Scale (1 minute)
  ↓  3 minutes interval
Wisconsin Card Sorting Test (15-20 minutes)
  ↓  3 minutes interval
Digit Symbol Substitution Test (2 minutes)
  ↓  3 minutes interval
Symbol Searching Test (2 minutes)
  ↓  10 minutes interval
Modified Multiple Sleep Latency Test (maximum 20 minutes)

Figure 1. Procedure of measurements
Figure 2. Mean sleep latency of MWT on trend for time-of-day effect (F (2, 56) = 11.03, p < 0.001)

Abbreviation: MWT, Maintenance of Wakefulness Test
Scheffe’s post-hoc test: A > B, A > C, B > C
Within group A: F(3, 66) = 2.00, p = 0.126; within group B: F(3, 57) = 1.18, p = 0.326; within group C: F(3, 45) = 1.17, p = 0.331
Figure 3. Mean scores of State Anxiety Scale on trend for time-of-day effect (F(2, 56)=5.18, p=0.009)

Scheffe’s post-hoc test: A < B, C < B
Within A group A: F(3, 66) = 5.92, p = 0.001 (1 > 3, 1 > 4, 2 > 4);
within group B: F(3, 57) = 0.61, p = 0.610; within group C: F(3, 45) = 7.83, p < 0.001 (1 > 3, 1 > 4, 2 > 4)
Figure 4. DSST (F(2, 56) = 4.72, p = 0.013), SST (F(2, 56) = 6.28, p = 0.003) and IPI (F(2, 56) = 7.21, p = 0.002) scoring scale on trend for time-of-day effect.

Abbreviations: DSST, Digit Symbol Substitution Test; SST, Symbol Searching Test; IPI, information process index.

Scheffe’s post-hoc test: A > B, A > C

Within group A: F(3, 66) = 10.11, p < 0.001 (1 < 3, 1 < 4, 2 < 4, 3 < 4);
within group B: F(3, 57) = 2.96, p = 0.040 (1 < 2, 1 < 3); within group C: F(3,45) = 5.63, p = 0.002 (1 < 2, 1 < 3, 1 < 4)
Abbreviation: SST, Symbol Searching Test

Scheffe’s post-hoc test: A > B, A > C

Within group A: $F(3, 66) = 8.85, p < 0.001$ (1 < 2, 1 < 3, 1 < 4, 2 < 4, 3 < 4); within group B: $F(3, 57) = 4.52, p = 0.007$ (1 < 3, 1 < 4); within group C: $F(3, 45) = 4.23, p = 0.010$ (1 < 2, 1 < 4, 3 < 4)
Abbreviation: IPI, Information process index

Scheffe’s post-hoc test: A > B, A > C

Within group A: F(3, 66) = 15.11, p < 0.001 (1 < 2, 1 < 3, 1 < 4, 2 < 4, 3 < 4); within group B: F(3, 57) = 6.26, p < 0.001 (1 < 3, 1 < 4); within group C: F(3,45) = 7.25, p < 0.001 (1 < 2, 1 < 4, 2 < 4, 3 < 4)
Figure 5. Mean TSH (F(2, 56) = 6.64, \( p = 0.003 \)) and prolactin (F(2, 56) = 6.31, \( p = 0.003 \)) level on trend for time-of-day effect.

Abbreviation: TSH, Thyrotropin

Scheffe’s post-hoc test: A < B, A < C

Within group A: F(3, 66) = 0.55, \( p = 0.652 \); within group B: F(3, 57) = 9.97, \( p < 0.001 \) (1 < 2, 1 < 3, 1 < 4, 2 < 3, 2 < 4); within group C: (3, 54) = 30.77, \( p < 0.001 \) (1 < 2, 1 < 3, 1 < 4, 2 < 3, 2 < 4, 3 < 4)
Scheffe’s post-hoc test: A < B, C < B
Within group A: F(3, 66) = 3.33, p = 0.025 (1 > 3, 3 < 4);
within group B: F(3, 57) = 2.44, p = 0.073; within group C:
F(3, 45) = 8.68, p < 0.001 (1 < 4, 2 < 4, 3 < 4)