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Exogenous Melatonin: Its Relationship to Prolactin and Its Efficacy at Reducing or Alleviating Stress

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Recently we completed a study designed to investigate the effect of melatonin administration (10 mg) at 1300 on menstrual characteristics, prolactin, and premenstrual syndrome-like symptoms during a simulated eastward deployment. Bright lights were utilized to simulate eastward movement across six time zones. During analysis of biochemical results, we made the fortuitous discovery that melatonin appears to alleviate the stress associated with the in-house simulated deployment. The study was double blind and placebo controlled. Melatonin was given to healthy females for 5 consecutive days during the late follicular and early luteal phases of the menstrual cycle. Volunteers spent 24 hours in the hospital before entering the dose administration phase of the study and again on the last dose day. On those 2 days, hourly blood samples were collected from an in-dwelling catheter and were used for analysis of melatonin and prolactin. Volunteers also completed a profile of moods state questionnaire upon waking on each of 8 days which overlapped the in-house dose administration days. The placebo group showed a prolactin peak at 1300 on the last dose day/blood draw, while the melatonin group showed a prolactin

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peak at 1500. It is well known that melatonin stimulates the release of prolactin, and a 2-hour delay between melatonin administration and the prolactin peak is within the normal expected delay. The prolactin peak at 1300 in the placebo group seems to be anticipating the administration of the unknown dose. Since various sources of stress are known to elicit the release of prolactin, we propose that the prolactin peak at 1300 is the result of stress. Since is was not present in the melatonin group, we conclude that melatonin administration reduces or alleviates this form of stress. This conclusion was supported by the results from the profile of moods state questionnaire. The melatonin group consistently demonstrated scores indicative of less stress. Since melatonin (10 mg) previously has been shown to be both safe and effective at maintaining sleep and cognitive performance during deployment conditions, an anti-stress capability would make it a seemingly indispensable addition to the Army's pharmacopeia.

Foreword

Dr. Comperatore is no longer at the U.S. Army Aeromedical Research Laboratory. His current address is:

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Introduction

Stressful conditions are frequently encountered in military operations. Stress can arise from battlefield situations or simply their anticipation; flying aircraft, especially at night; or something seemingly as innocuous as falling behind on normal nighttime sleeping habits. Rapid deployment across multiple time zones and different day-night conditions upon arrival combine with the above to increase stress. Prolonged stress has been shown to result in neuronal cell death in the central nervous system (CNS)(Sapolsky, Krey and McEwen, 1985; Uno et al., 1989). At the very least, stress likely increases the susceptibility of CNS neurons to excitotoxins, antimetabolites, and hypoxia .

Melatonin (N-acetyl-5-methoxytryptamine), a natural hormone which has been shown to resynchronize circadian rhythms and induce sleep in humans (Arendt et al., 1987; Dawson and Encel, 1993; Reiter, 1991; Wurtman, 1986), is currently being marketed widely as a dietary supplement to alleviate desynchronosis (desynchronization of physiological and behavioral rhythms) and assist in obtaining quality sleep. Desynchronosis often results from rapid shifts in work schedules from day to night, or from shifts in the light-dark cycle due to time zone crossing. Symptoms resulting from desynchronosis include fatigue, sleepiness, lethargy, insomnia, gastrointestinal tract disorders, and poor mental performance (for review see Comperatore and Krueger, 1990).

Melatonin is produced by the pineal gland in the absence of bright light. In humans, melatonin synthesis reaches peak levels during the night and lowest levels during the day. Known side effects of melatonin chronobiotic doses (5-10 mg) are limited to sleepiness, fatigue, and reduced alertness shortly after administration, but not upon awakening (Arendt et al., 1987; Comperatore et al., 1996; Petrie et al., 1989).

Melatonin has been implicated in the control of prolactin (PRL) secretion. Prolactin is produced by the anterior lobe of the pituitary gland (adenohypophysis). Its secretion varies predictably during the day, with lowest levels at midday and highest levels at night. Plasma PRL was reported to exhibit a daily rhythm in women showing a nocturnal peak about 1-2 hours after that of melatonin, and remaining consistent throughout the menstrual cycle (Brzezinski et al., 1988). Evening administration of melatonin (4 mg) to women was reported to have a stimulatory effect on PRL release (Terzolo et al., 1993). A dose of 2 mg in the evening stimulated the thyrotropin releasing hormone induced secretion of PRL, especially during the follicular phase of the menstrual cycle (Terzolo et al., 1991). Strongly supporting the interaction between melatonin and PRL, nighttime exposure to bright light, sufficient to induce a decrease in nocturnal melatonin secretion, resulted in a decrease in PRL secretion in women (Bispink et al., 1990). Also, daytime administration of melatonin, when levels of endogenous melatonin are extremely low, stimulated the release of PRL in women (Webley and Lenton, 1987; Okatani and Sagara, 1993). As little as 1 mg melatonin, given to young women at 1300, was enough to induce a significant increase in serum PRL (Okatani and Sagara, 1993; Okatani, Okada and Sagara, 1994).

In addition to melatonin, stress is known to stimulate the release of PRL. Prolactin was reported to increase as much as five times during major surgery with general anesthesia, during gastroscopy, during proctoscopy, and during exercise (Noel et al., 1972). All these events were linked to stress. Other stressful conditions leading to increased release of PRL include parachute jumping (Richter et al., 1996; Schedlowski et al., 1992), flight activity in student pilots (Farrace et al., 1996), and in female transport plane pilots (Dongyun and Yumin, 1990), and thermal stress (Vaha-Eskeli et al., 1991).

We report here PRL results obtained in the course of a study designed to investigate the effects of the afternoon administration of melatonin on menstrual characteristics. These results reinforce the relationship between melatonin and PRL release, and indicate a relationship between melatonin and the alleviation of stress.

Methods

The design of the study was double blind, between subjects, and placebo controlled. Participants were 20 female volunteers between the ages of 18 and 39, meeting specific criteria to assure regular menstrual cycle history and health status (e.g., negative chorionic gonadotropin (β-hCG), no oral contraceptive use for the previous 3 months, regular menstrual rhythms, good general health). After providing their informed consent, participants were assigned randomly to either the melatonin or placebo group. Pregnancy tests were done periodically throughout the study. Also, volunteers were asked to refrain from consuming alcohol, caffeinated beverages, or any type of medication with known CNS effects throughout the in-house dose and testing days.

The total duration of participation consisted of 5 consecutive menstrual cycles, however for this report we only are concerned with the one menstrual cycle in which melatonin or placebo was given, and we collected serum samples from which PRL levels were determined. Menstrual regularity data were used to document the timing of menses and to approximate 7 days during the late follicular and early luteal phase of cycle 4 in which the volunteers lived in the sleep laboratory at the U.S. Army Aeromedical Research Laboratory (USAARL). During the 7 in-house days, participants remained at the USAARL for testing and shifting to a new light-dark cycle designed to mimic the changes corresponding to travelling eastwardly across 6 time zones.

Within 10 days of their scheduled in-house stay and again on in-house day 6, volunteers spent 24 hours in the hospital where they provided hourly blood samples. On each of these days, an intravenous catheter was used for hourly sampling.

Melatonin (10 mg) or placebo was administered daily for 5 consecutive days (days 2-6) at 1300, immediately after collection of saliva and/or blood samples. Blood pressure and pulse were recorded throughout the in-house stay just before dose administration, at bedtime, and upon awakening.

The last dose of melatonin/placebo was given at 1300 on in-house day 6. That also was the day the volunteers were in the hospital providing hourly samples of blood. They returned to the USAARL at about 0800 on day 7, completed that day as scheduled, and were released from the facility at approximately 0800 on day 8 after a brief post-study medical evaluation.

Biochemical assays

Blood levels of β-hCG were determined utilizing an Abbott IMx* immunoassay analyzer, and the values used as a test for pregnancy. Prolactin levels were determined from hourly blood samples drawn on the pre-in-house day and on in-house day 6-7 using the immunoassay analyzer. Blood samples were collected into vaccutainer tubes and centrifuged to separate the serum for analysis. The IMx uses microparticle enzyme immunoassay to determine concentrations, and has a sensitivity of 0.6 ng/ml for PRL.

Blood levels of melatonin were measured by direct radioimmunoassay (RIA) (ALPCO, Inc.*, Windham, NH) from the same blood samples. The melatonin RIA had a sensitivity of 0.3 pg/ml with a intra-assay CV of 6.6% and a inter-assay CV of 7.7%.

Mood testing

A profile of mood states (POMS) questionnaire (McNair, Lorr and Droppleman, 1971) consisting of 65 adjectives, each of which is rated on a 5-point scale, was used to determine the time course of the effects of the melatonin regimen. Factor analysis of the POMS yields the following six factors: tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment. The higher the cumulative score in each factor, the more the subject identified with the mood associated with each factor. This questionnaire was completed upon arising during each in-house day.

Activity monitors

Activity monitors (Precision Control Design, Inc*) were used to study the rest/activity cycles of participants during the 2 weeks just prior to reporting to the USAARL, throughout the week-long in-house stay, and for 2 weeks after leaving the laboratory. Monitoring for disrupted sleep patterns prior to the in-house stay prevented inclusion of participants experiencing sleep-related hormonal anomalies. Also, activity data provided information on the stability of sleep duration both prior to and during the drug regimen.

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* See manufactures' list at Appendix A

Results

Prolactin levels

Hourly prolactin levels were determined for all members of the melatonin and placebo groups for both the pre-in-house (baseline) and day 6 24-hour blood draws, and those values are listed in tables B-1 through B-4 of Appendix B. Using those values, mean hourly baseline and day 6 PRL values then were plotted against time of day for members of the placebo (Figure 1) and

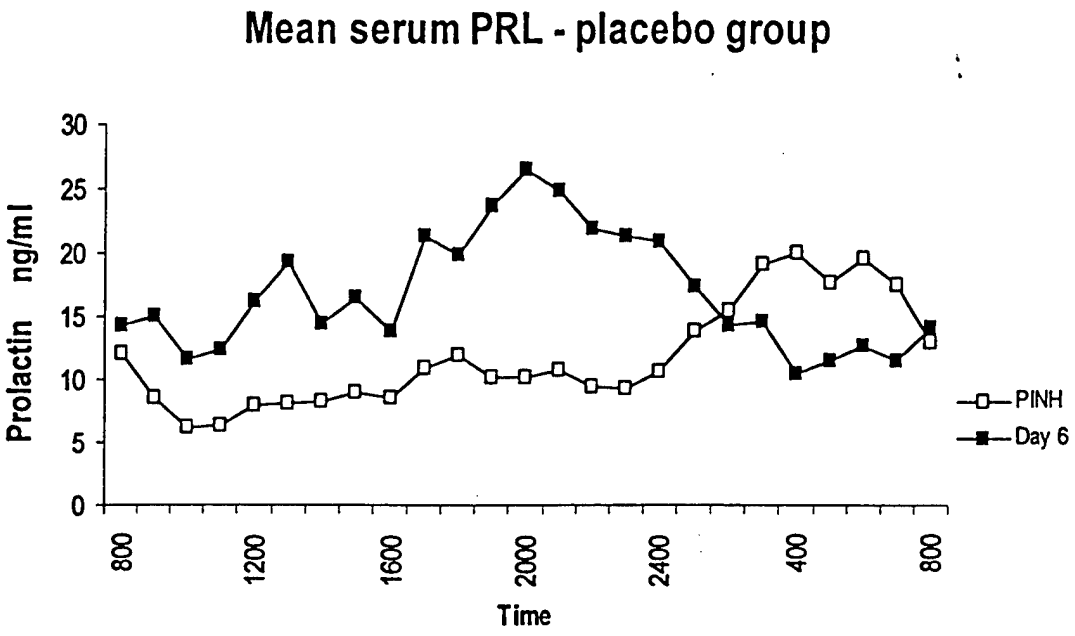

Figure 1. Mean hourly baseline and day 6 serum prolactin levels for the placebo group.

melatonin groups (Figure 2). Note that the nighttime PRL increase begins about 2400 during the pre-in-house sampling, and advances during the in-house stay for both groups. During in-house testing, the placebo group shows an early increase peaking at 1300 (dose time), a decrease, and a gradual climb to the daily peak value by 2000. The melatonin group (Figure 2) shows a similar pattern on day 6, however, the initial peak is at 1500. It is interesting that the daily peak values (after the 1300 and 1500 increases) for the two groups occur at the same time (2000), and the decrease in the daily PRL surge is quite similar in both groups.

Figure 3 shows the day 6 mean serum PRL concentrations plotted together for both the placebo and melatonin groups. Not only is the initial peak difference between the two groups clearly revealed, but note also the higher overall PRL levels in the placebo group throughout the day. The pre-in-house mean serum PRL and melatonin values for both groups combined are

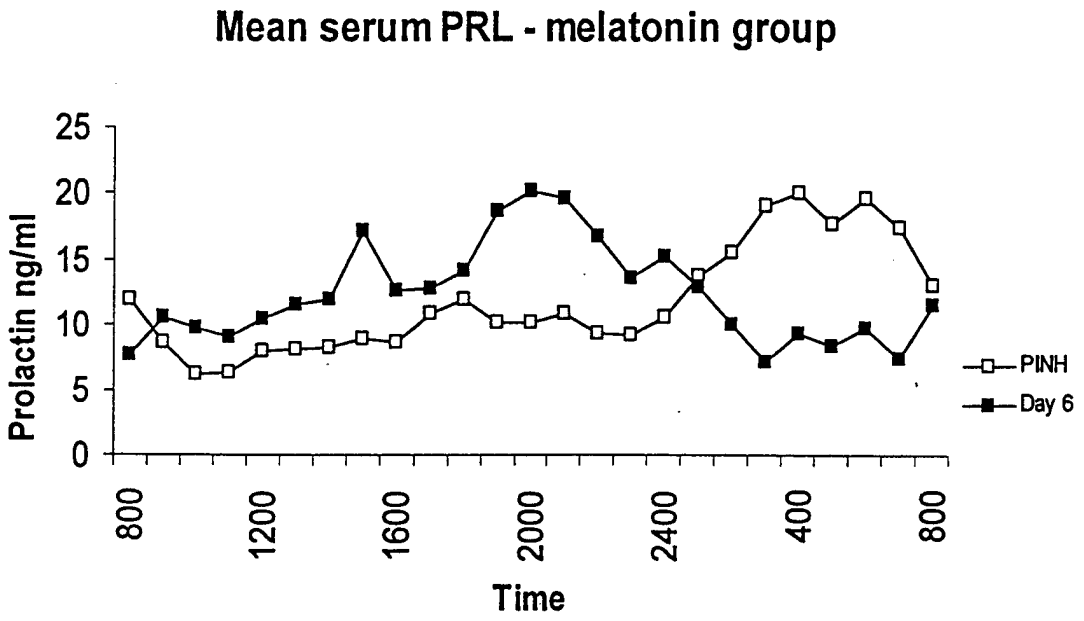

Figure 2. Mean hourly baseline and day 6 serum prolactin levels for the melatonin group.

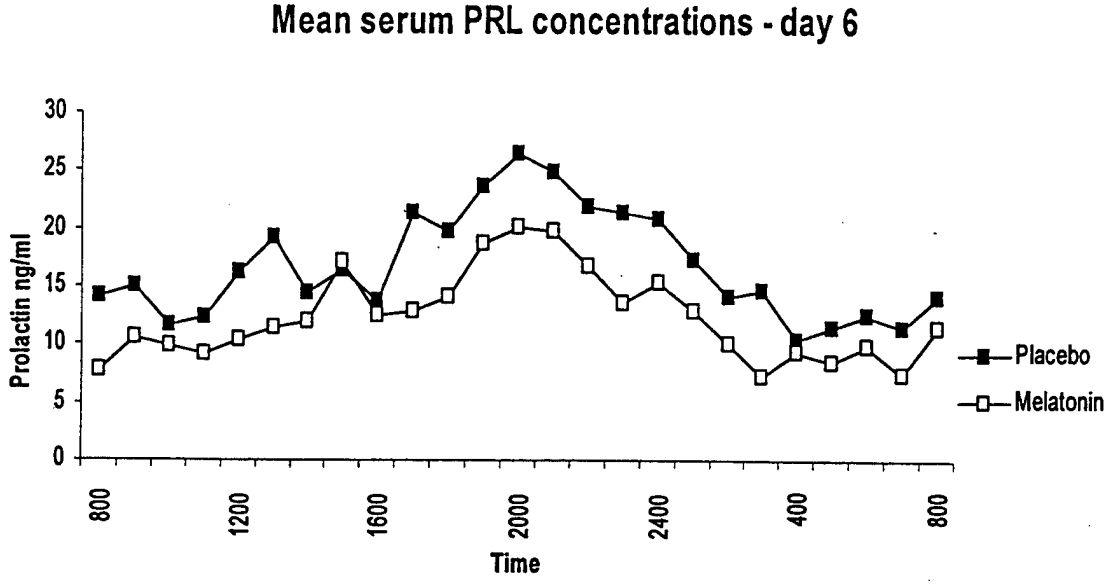

Figure 3. Mean hourly serum prolactin levels for the placebo and melatonin groups on day 6. Note the increase at 1300 for the placebo group and at 1500 for the melatonin group.

plotted together against time in Figure 4. The nightly melatonin increase begins between 2000 and 2200 and precedes the nightly increase in PRL by 3-4 hours. The mean difference between the nightly increase in melatonin and the nightly increase in PRL in all volunteers for which we had sufficient data to determine the time was 3 hours (n=12). The early evening PRL peak occurs about 1800.

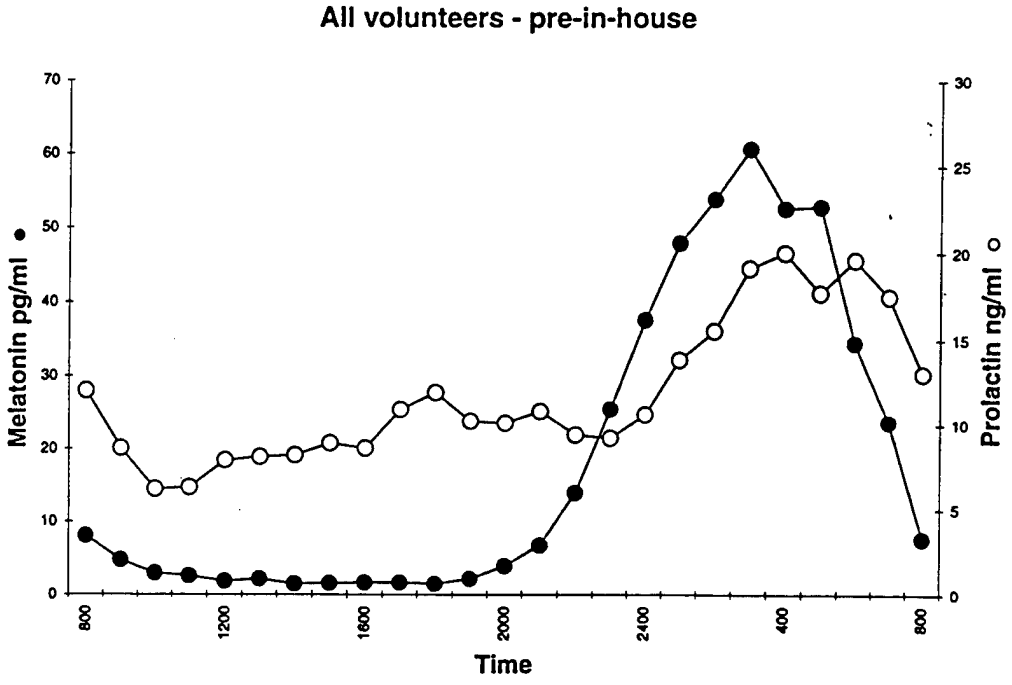

Figure 4. Mean serum prolactin and melatonin for all volunteers collected on the pre-in-house day.

Figure 5 shows mean serum melatonin and PRL concentrations throughout the day on day 6 for the placebo group, and Figure 6 shows the same information for the melatonin group. In addition to the PRL peak at 1300, note in Figure 5 the gradual rise to the daily PRL peak beginning at 1630, before the daily rise in melatonin levels at 1730. A comparison of Figures 4 and 5 shows that the daily increase in melatonin has been advanced about 4 hours. This is attributed to the bright light treatment which was used to simulate an Eastward deployment. Figure 5 also suggests generally higher levels of PRL throughout the day and a more sustained PRL plateau. Coupled with the PRL peak at 1300, we think the changes in PRL are indicative of increased stress in the placebo group.

Changes in the melatonin group are more predictible. As seen in Figure 6, there is a sudden increase in serum melatonin beginning at 1330, agreeing well with the administration of melatonin (10 mg) at 1300. There is then a peak in PRL at 1530, presumably a response to the increased levels of melatonin. That peak quickly begins to fall off, only to begin increasing

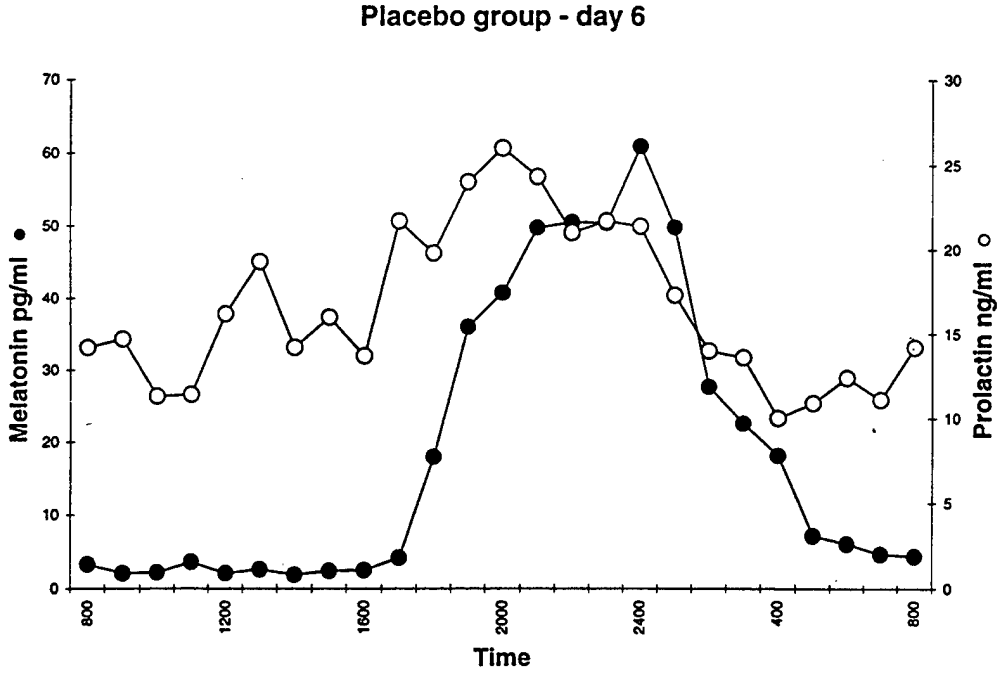

Figure 5. Day 6 mean serum melatonin and prolactin concentrations for the placebo group. Note that the nightly increase in prolactin occurs before the nightly increase in melatonin.

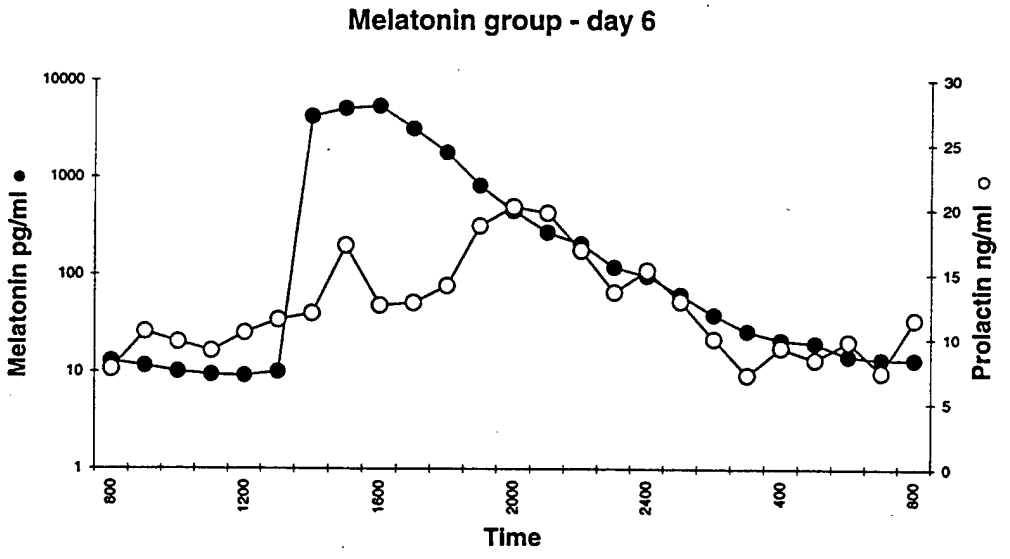

Figure 6. Day 6 mean serum melatonin and prolactin concentrations for the melatonin group.

again at 1730 (4 hours after melatonin). The 1730 increase represents the nightly PRL increase, responding to the "in-house night," advanced from bright light treatment and melatonin administration. Figure 4 reveals a similar difference of about 4 hours between the nightly melatonin increase and the PRL increase during the pre-in-house blood draw.

Results gathered from the POMS questionnaire showed that members of the melatonin group consistently scored lower than their placebo counterparts in all factors suggesting adverse moods (5 of the 6). The sixth factor is vigor and activity (Figure 7), and the melatonin group

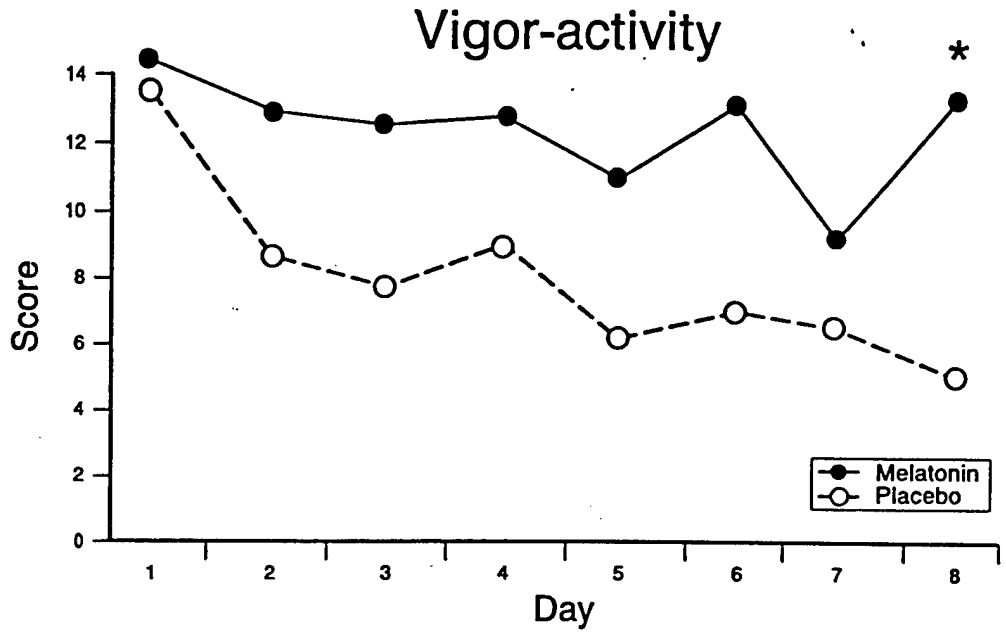

Figure 7. Results for both groups from the profile of moods state questionnaire for the category of vigor-activity. Note that the melatonin group demonstrated higher scores on every day. Statistical significance ($p<0.05$) indicated by *.

consistently demonstrated higher scores, suggesting better rest and an overall better sense of well-being. Even on day 7, when both groups were sleep deprived following their 24 hour blood draw on day 6, the melatonin group showed less fatigue and inertia (Figure 8), anger and hostility (Figure 9), confusion and bewilderment (Figure 10), tension and anxiety (Figure 11), and depression and dejection (Figure 12), and more vigor and activity (Figure 7), than their placebo counterparts. These factors are all plotted against in-house days for both the placebo and melatonin groups in Figures 7-12. Although the trend is there for the melatonin group to be of better mood for all factors on most days, statistical significance ($p<0.05$) was demonstrated only on those days marked with an asterisk in Figures 7-12.

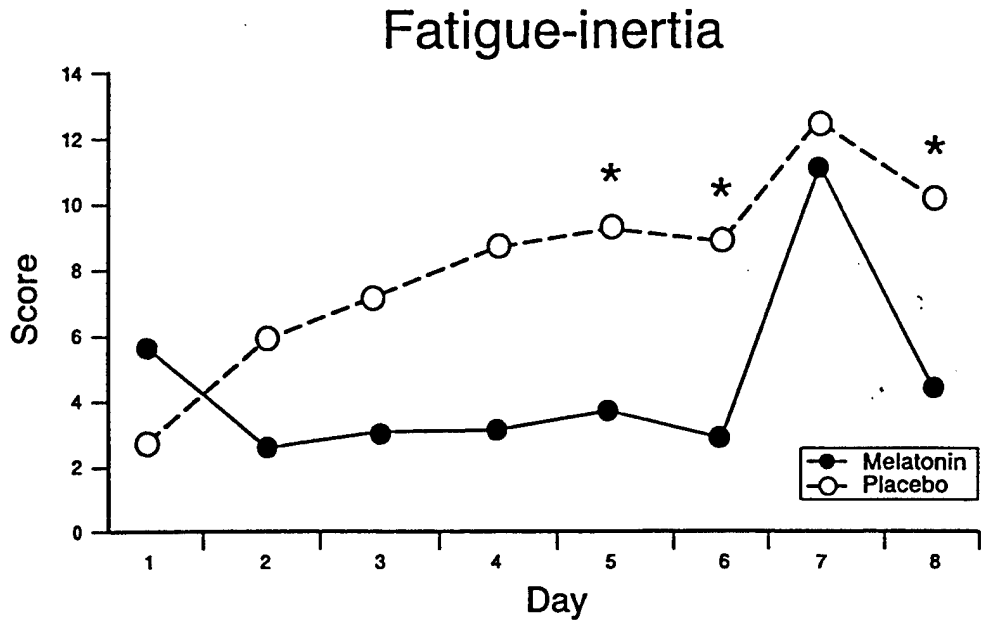

Figure 8. Results for both groups from the profile of moods state questionnaire for the category of fatigue-inertia. Other than day 1, the placebo group demonstrates higher scores. Statistical significance ($p<0.05$) indicated by *.

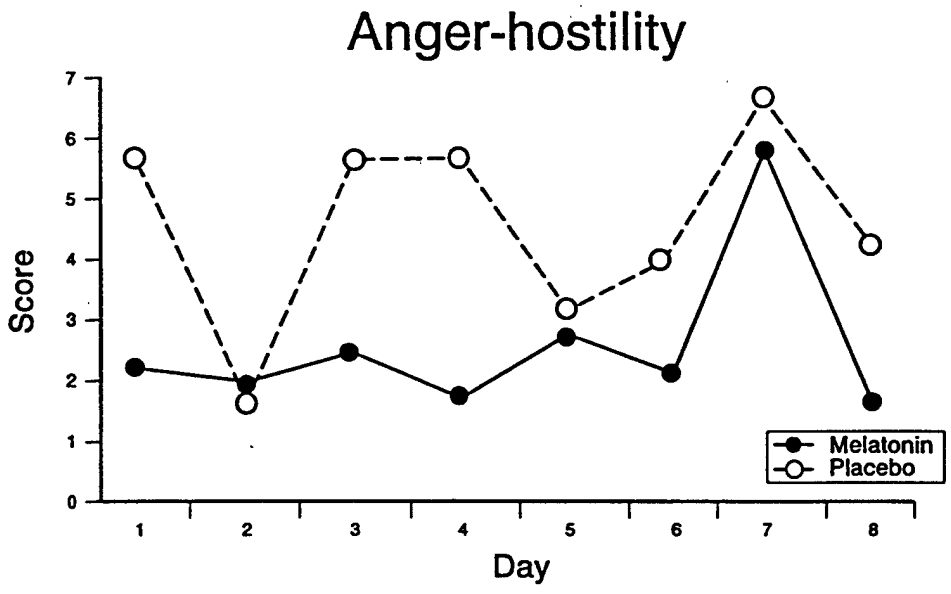

Figure 9. Results for both groups from the profile of moods state questionnaire for the category of anger-hostility. Note that on every day except day 2 the melatonin group demonstrated lower scores.

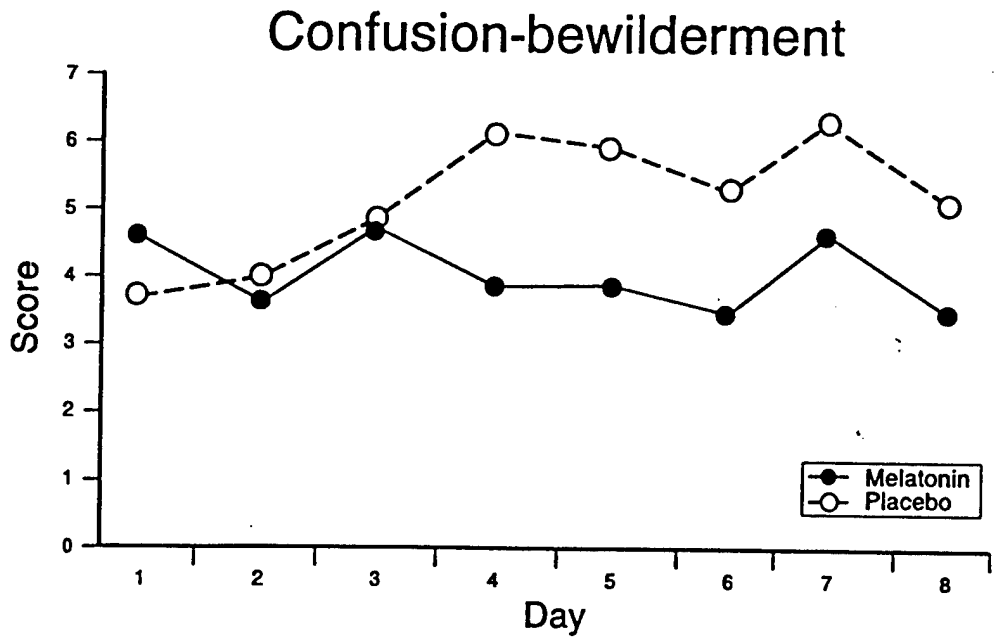

Figure 10. Results for both groups from the profile of moods state questionnaire for the category of confusion-bewilderment. Note that the melatonin group demonstrated lower scores on every day except day 1.

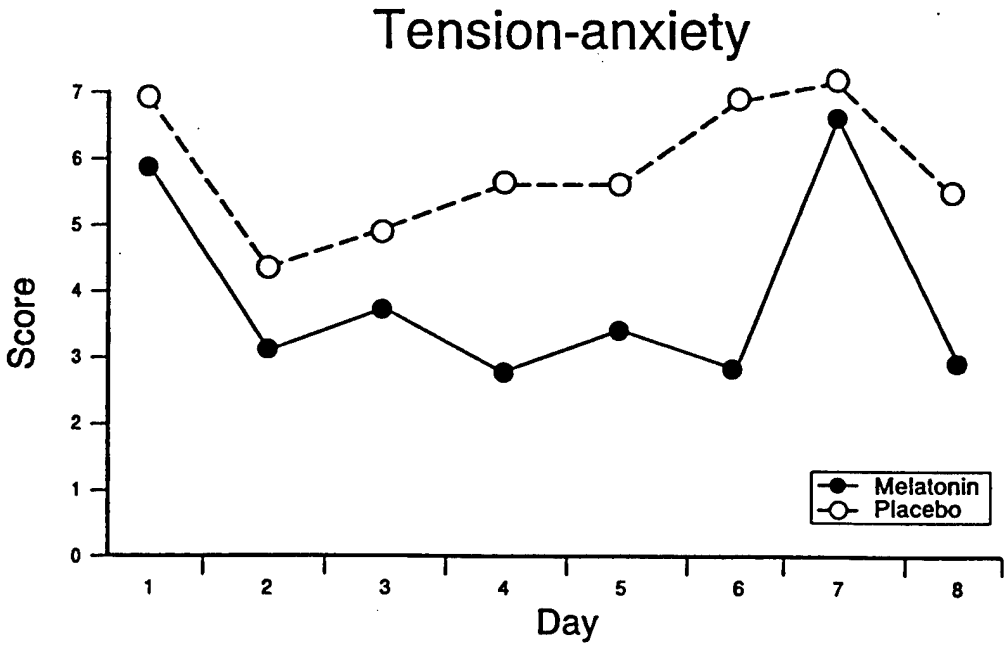

Figure 11. Results for both groups from the profile of moods state questionnaire for the category of tension-anxiety. Note that the melatonin group demonstrated lower scores on each day.

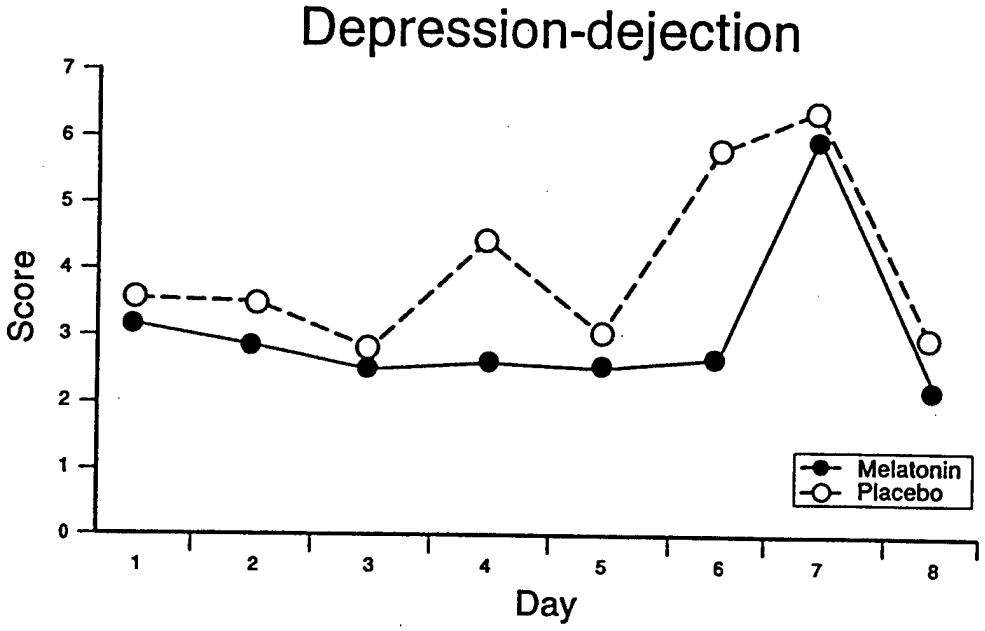

Figure 12. Results for both groups from the profile of moods state questionnaire for the category of depression-dejection. Note that the melatonin group demonstrated lower scores on each day.

In-house sleep

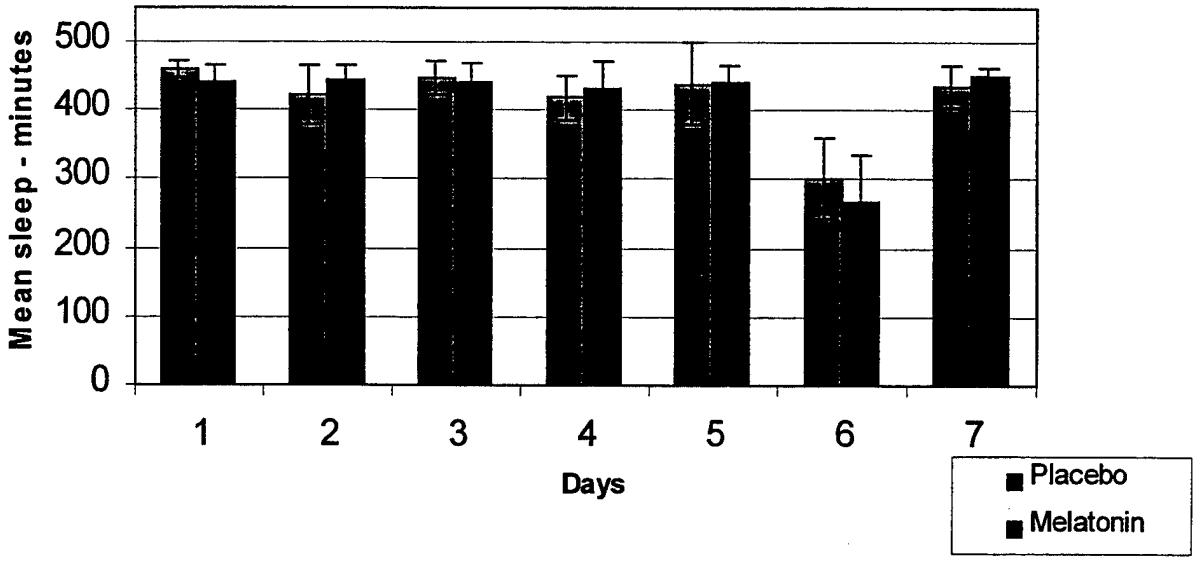

Figure 13. In-house sleep results for the melatonin and placebo groups. Both groups were quite similar and none of the differences reached statistical significance.

Figure 13 shows sleep results for both the melatonin and placebo groups based on information collected by the activity monitors. Mean sleep for each group is plotted for each of the 7 in-house days. There are no statistical differences between the two groups. Sleep remains quite constant for both groups other than on day 6. Day six was the last dose day, and the in-house day that was spent in the hospital providing hourly blood samples. The sleep shown for day 6 represents the sleep that volunteers were able to get between samples.

Discussion

We have shown here an increased release of PRL within 2 hours of melatonin administration. Also, we have documented an increased PRL release immediately prior to dose administration in our placebo group. Other than the actual administration of melatonin, everything was the same for both groups. It is well documented in the literature that human plasma PRL shows a significant rise under various stressful situations. Prolactin was reported to increase as much as five times during major surgery with general anesthesia, during gastroscopy, during proctoscopy, and exercise (Noel et al., 1972). They concluded that the release of PRL in humans was triggered by stress. Other studies have demonstrated that stress is capable of eliciting the release of PRL (Dongyun and Yumin, 1990; Leino et al., 1995; Noel et al., 1972; Schedlowski et al., 1992). Also, elevated levels of plasma PRL have been shown to be a sensitive indicator of psychological workload associated with military flying (Leino et al., 1995). Taken together, this leads us to believe that the PRL peak at 1300 in the placebo group, as well as the generally increased concentrations of PRL, was a result of the stress and anxiety of the impending unknown dose administration in combination with the stress of the simulated deployment conditions and cognitive testing.

There is considerable evidence in the literature that melatonin has a facilitory effect of the secretion of PRL. Most women have a small early evening peak of PRL, followed by a larger nocturnal peak later in the night. The early evening rise in PRL is likely not linked to melatonin, since the evening increase in melatonin concentrations occurs 2-3 hours after the early evening rise of PRL. The early evening rise in PRL appeared in our data as a peak at 1700-1800 (see Figure 4). The nocturnal plasma peak of PRL is reported to occur 1-2 hours after the nightly peak for melatonin (Brzezinski et al., 1988; Lisoni et al., 1986; Okatani and Sagara, 1993), although others report the PRL peak to occur 2-4 hours after the melatonin peak (Okatani, Okada and Sagara, 1994; Webley and Lenton, 1987). The mean difference between the melatonin and PRL peaks observed in this study was 3.0 hours. Allowing for variation between individuals, the consistency of the phase delay between the nocturnal melatonin peak and that of PRL suggests a physiological relationship between the two. Many studies conclude that melatonin increases at least some portion of the release of PRL in both males (Waldhauser et al., 1987; Webley, Bohle and Leidenberger, 1988; Mallo et al., 1988) and females (Bispink et al., 1990; Terzolo et al., 1993; Webley and Lenton, 1987; Okatani and Sagara, 1993; Lisoni et al., 1986; Terzolo et al., 1991).

Although the mechanism by which melatonin affects the release of PRL is not well defined, there seems to be little question of the link between the two. Plasma melatonin has been reported to be high in patients with hyperprolactinemia (Wetterberg, 1979). This is expected if melatonin controls the release of PRL. Finally, the facilitory role of melatonin on the release of PRL was reported to be statistically significant only in the follicular phase of the menstrual cycle (Terzolo et al., 1991). Although there was a similar trend in the luteal phase, it was not significant. This agrees well with the reported effect that melatonin stimulates the release of luteinizing hormone during the follicular but not the luteal phase of the menstrual cycle (Cagnacci, 1996).

Our results showed that the nightly increase in both PRL and melatonin was shifted earlier in both groups (Figures 1 and 2). The nightly production of melatonin also was shifted earlier (Figures 6 and 7). Presumably, the bright light treatment inhibited the normal endogenous nightly production of melatonin and advanced it to an earlier time. Earlier production of melatonin would then advance the production of PRL. In addition to the overall shift to an earlier time of the PRL curve, we find the in-house peaks at 1300 in the placebo group (Figures 1 and 3) and 1500 in the melatonin group (Figures 2 and 3) extremely interesting. Since dose administration was at 1300, the PRL peak at 1300 in the placebo group was too early to be caused by melatonin, and very well could be a result of the stress or anxiety of the impending dose.

Instead of a peak at 1300, the melatonin group demonstrated a PRL peak at 1500. This was 2 hours after administration of melatonin, and agrees well with reports of a 1-2 hour delay between the administration of melatonin and increased release of PRL (Brzezinski et al., 1988; Lisoni et al., 1986; Okatani and Sagara, 1993). That the melatonin group does not demonstrate a peak at 1300 could indicate that they are experiencing much less stress than the placebo group under similar conditions. This conclusion also is supported by results of the profile of moods state questionnaire completed by each volunteer in which the melatonin group demonstrated an improved sense of well-being compared to the placebo group. Taken together, these results suggest that melatonin relieves or lessens anxiety and stress.

During stressful situations, the sympathetic branch of the autonomic nervous system is activated, and the adrenal glands secrete steroid stress hormones. The hormonal cascade, stimulated by stress and ending with the production of stress hormones, begins in the hypothalamus where coticotropin-releasing factor is released and stimulates the anterior pituitary to secrete adrenocorticotropic hormone (ACTH). ACTH, passing through the general circulation, stimulates the adrenal cortex to secrete glucocorticoids. Cortisol is the principle stress-related hormone, and is largely responsible for the glucocorticoid activity of the adrenal glands. Glucocorticoids are so named because of their profound effects on glucose metabolism. Although the short-term effects of glucocorticoids are essential to life, prolonged exposure results in various harmful effects (Munck, Guyre and Holbrook, 1984). Adverse effects include inhibition of inflammatory responses, suppression of the imjune system, acceleration of aging, and destruction of neurons in the hippocampus of the brain. Although physiological concentrations of glucocorticoids have not been shown to be directly toxic to hippocampal

neurons, they apparently induce a state of vulnerability which renders hippocampal neurons less likely to survive additional insults (Sapolsky, 1986). Potential implications of hippocampal damage resulting from glucocorticoid exposure are profound, since the hippocampus plays such an essential role in learning and memory (Squire, 1986).

There are conflicting reports concerning the interactions between melatonin and glucocorticoids, or the control of melatonin over the hypothalamo-pituitary-adrenal (HPA) axis. A recent study (Hajak et al., 1997) investigated those interactions in rats and in man, and concluded that there was no evidence for the existence of mutual influences between melatonin and glucocorticoids. They also found no support for attenuation of glucocorticoid-mediated effects by melatonin on target tissues under physiological conditions. How do such results impact on the conclusions from this study? Since neither the study nor our data collection was designed to investigate stress-related properties of melatonin, we did not collect samples for analysis of cortisol. Since stress is known to stimulate the release of PRL, and a prominent PRL peak was observed at dose time in the placebo but not the melatonin group, we concluded that the most likely explaination was increased stress in the placebo group. Although the stress resulting from this study most likely activated the HPA axis, our melatonin dose (10 mg) was certainly in the pharmacological rather than the physiological range. It therefore is at least possible that our pharmacological dose of melatonin was able to interfere with the HPA axis and reduce stress levels in the melatonin group.

Since melatonin is actively marketed as a sleep aid, members of the melatonin group might have been expected to demonstrate more sleep during the in-house dose days, especially for the transition days following the simulated deployment (bright lights during the night) which began on in-house day 3. There were no statistical differences between the two groups, and the placebo group actually slept longer than the melatonin group on day 3 and the same amount on day 5. However, since we did not utilize polysomnography, we can make no statement about the quality of the sleep. That the two groups demonstrated similar sleep probably reflects the fact that each volunteer was put to bed at 1630 and told to stay in bed until being wakened by a technician at 0030. Also, both groups found the cognitive testing, being completed for another study, very exhausting.

Melatonin has been shown to be useful in resynchronizing circadian rhythms and inducing sleep in humans (Dawson and Encel, 1993; Reiter, 1991; Wurtman, 1986). Also, melatonin therapy has been shown to be effective in preventing sleep loss and maintaining alertness of male aviation personnel after a rapid deployment from the U.S. to the Middle East (Comperatore et al., 1996). When coupled with its antioxidant properties (Cagnacci, 1996; Chan and Tang, 1996; Kelly and Loo, 1997; Reiter, 1995; Reiter, 1996; Reiter et al., 1995) and demonstrated efficacy at preventing sleep loss and maintaining alertness, anti-stress properties would make it a seemingly indispensable pharmacological agent for use in deployment conditions. If these beneficial properties are demonstrated to be gender-independent, the addition of a melatonin regimen during deployments would be of immediate benefit to the Department of Defense. An appropriate melatonin regimen, begun during the early stages of troop deployments, will

effectively eliminate the symptoms of jet lag. Additionally, we hypothesize that maintaining supraphysiological levels of melatonin in serum will reduce or eliminate stress and anxiety. Not only might this enable soldiers to more effectively complete their mission, it also may reduce or eliminate any stress-related component of cell death in the CNS.

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Appendix A.

List of manufacturers.

Abbott Diagnostics
A Division of Abbott Laboratories
One Abbott Park Road
Abbott Park, IL 60064

ALPCO, Inc.
P.O. Box 451
Windham, NH 03087

Precision Control Design, Inc.
135 Eglin Parkway, S.E.
Ft. Walton Beach, FL 32540

Stockgrand Ltd.
School of Biological Sciences
University of Surrey
Guilford, Surrey
GU2 5XH, UK

Whitehall-Robbins Healthcare
American Home Products Corporation
Five Giralda Farms
Madison, NJ 07940

Appendix B.

Prolactin levels for melatonin and placebo groups.

Table B-1.
Placebo volunteers - pre-in-house prolactin levels (ng/ml).

PPIN	05	08	14	17	19	21	25	29	31	32
0800	10.5	7.4	16.1	-	-	16.4	-	5.7	16.4	-
0900	10.0	5.7	11.7	3.8	-	11.3	5.2	4.9	11.3	15.6
1000	6.9	5.4	7.7	3.5	-	10.4	5.6	3.3	10.4	-
1100	6.0	4.7	5.2	2.9	-	7.7	5.3	3.5	7.7	12.4
1200	7.2	5.6	5.5	3.6	-	13.6	7.9	3.0	13.6	15.9
1300	8.5	4.8	4.3	5.9	9.3	13.0	7.5	4.1	13.0	13.6
1400	7.1	6.7	12.1	3.9	8.2	12.6	6.3	3.9	12.6	16.1
1500	8.2	5.3	9.3	4.7	5.6	11.5	7.7	4.7	11.5	16.7
1600	7.3	6.4	5.3	3.3	8.4	11.6	8.9	5.0	11.6	15.3
1700	11.6	11.5	8.9	4.4	7.8	18.9	23.0	5.4	18.9	19.7
1800	-	13.2	5.8	6.4	8.5	14.3	30.5	5.7	14.3	18.6
1900	16.2	8.8	5.5	5.1	10.4	13.5	20.1	4.4	13.5	19.6
2000	10.9	8.6	13.4	4.7	11.4	12.1	10.8	4.6	12.1	23.3
2100	12.1	13.2	13.6	4.1	9.2	12.5	8.3	4.0	12.5	20.9
2200	9.4	9.8	10.2	3.2	6.8	10.7	7.4	-	10.7	15.6
2300	8.9	8.7	8.2	2.9	7.1	13.2	7.2	-	13.2	15.5
2400	10.7	8.2	12.8	2.5	9.6	13.7	6.2	6.9	13.7	20.2
0100	11.2	16.1	17.0	6.8	15.6	12.6	7.8	-	12.6	17.6
0200	10.4	11.5	15.6	15.4	13.3	31.6	9.1	28.8	31.6	21.0
0300	13.3	15.4	12.9	39.3	16.0	21.0	22.1	-	21.0	27.0
0400	13.2	14.2	12.4	21.8	25.7	20.2	26.1	29.2	20.2	25.3
0500	14.0	13.6	18.9	18.2	21.9	21.2	21.0	-	21.2	20.7
0600	10.6	16.1	15.1	16.4	18.3	26.7	22.6	20.4	26.7	22.0
0700	9.5	16.7	17.5	12.6	15.9	18.3	23.2	-	18.3	20.1
0800	9.6	27.6	16.5	6.7	10.9	17.8	11.7	8.9	17.8	18.1

Table B-2.

Melatonin volunteers - pre-in-house prolactin levels (ng/ml).

MPIN	07	09	10	13	20	22	27	33	36	37
0800	11.1	3.9	9.9	25.2	15.0	17.8	12.6	-	4.2	-
0900	8.5	2.8	8.3	20.6	11.2	13.5	6.6	5.4	4.3	5.3
1000	8.9	2.6	7.0	-	10.6	10.9	5.0	4.4	3.0	3.9
1100	8.2	3.3	-	13.7	-	11.6	4.5	4.2	3.3	3.3
1200	6.8	3.7	6.8	12.5	13.1	13.6	6.4	6.5	5.0	3.5
1300	7.9	3.9	-	14.0	9.7	17.3	6.5	5.1	4.1	4.1
1400	7.2	4.8	8.4	9.5	-	15.6	7.1	4.0	4.8	6.2
1500	9.5	4.8	8.1	15.5	10.9	21.3	9.2	6.3	5.2	4.4
1600	8.8	4.8	5.5	21.8	11.7	15.5	6.7	7.5	4.4	5.5
1700	8.1	4.1	10.7	23.0	12.8	16.8	9.0	7.1	5.0	4.5
1800	7.4	-	13.7	20.7	13.1	15.9	8.1	10.5	6.7	6.6
1900	8.1	4.9	12.4	15.7	10.9	16.4	8.9	6.5	4.4	4.5
2000	8.1	4.3	13.5	16.9	13.1	18.2	6.6	5.8	3.8	6.5
2100	10.1	5.0	12.5	19.6	10.3	23.3	-	7.0	4.8	7.1
2200	11.9	5.2	10.7	16.8	10.5	17.5	9.9	6.6	4.8	5.8
2300	9.3	5.8	9.0	17.2	15.2	14.1	8.0	6.5	4.9	6.3
2400	8.4	15.6	9.1	15.0	-	14.0	-	9.9	8.5	12.4
0100	8.3	15.9	9.1	20.1	14.4	17.2	-	13.6	10.5	26.6
0200	8.4	10.3	9.6	20.7	-	18.3	-	12.0	12.2	18.3
0300	10.2	11.7	12.8	29.0	13.2	34.6	-	16.0	11.5	22.1
0400	14.6	13.9	17.3	29.9	-	22.9	-	13.1	12.2	37.5
0500	16.3	7.7	17.7	26.4	23.1	26.2	-	12.9	9.4	20.9
0600	26.7	8.9	42.6	23.1	-	22.1	-	16.0	10.2	23.2
0700	19.7	6.3	23.7	26.6	25.2	22.0	-	11.3	10.2	23.5
0800	16.1	4.2	15.3	19.0	-	13.5	-	9.3	6.0	10.1

Table B-3.
Placebo volunteers - day 6 prolactin levels (ng/ml).

PIN	05	08	14	17	19	21	25	29	31	32
0800	17.1	16.1	13.7	-	15.0	-	-	9.1	-	-
0900	14.6	15.5	17.7	-	16.6	15.6	13.5	9.5	15.6	16.3
1000	13.5	12.9	12.7	3.5	15.4	14.7	9.2	8.2	14.7	-
1100	11.7	14.9	7.7	3.3	18.2	16.2	10.1	8.7	16.2	16.0
1200	12.4	18.6	21.7	3.9	31.3	18.2	12.9	10.5	18.2	14.6
1300	11.9	24.9	31.0	3.8	24.2	23.0	12.2	23.5	23.0	15.4
1400	10.8	18.6	14.1	3.9	25.2	17.8	9.1	14.1	17.8	12.4
1500	12.7	25.7	13.6	4.3	25.8	20.3	10.2	15.5	20.3	-
1600	10.5	17.0	14.1	3.6	24.3	14.9	8.5	16.6	14.9	-
1700	12.1	23.6	31.5	4.2	30.2	19.9	27.4	22.9	19.9	-
1800	8.7	24.5	25.1	3.5	33.9	19.9	15.0	27.7	19.9	-
1900	21.6	37.8	29.2	4.8	22.3	20.8	17.4	38.2	20.8	-
2000	15.1	31.4	20.3	11.2	22.3	31.1	17.1	59.3	31.1	-
2100	18.8	28.7	20.4	11.6	29.1	28.9	18.2	38.4	28.9	-
2200	17.8	22.3	23.5	5.7	34.2	29.6	13.8	20.8	29.6	-
2300	28.2	20.0	24.6	10.7	30.1	18.0	17.7	24.5	18.0	-
2400	17.1	19.6	21.0	10.9	27.3	17.0	24.6	33.6	17.0	-
0100	16.3	14.9	23.6	5.8	23.4	18.1	17.2	18.7	18.1	-
0200	10.4	9.9	34.1	5.2	14.9	15.5	10.7	11.3	15.5	-
0300	9.8	16.7	23.3	3.9	13.8	22.2	9.0	10.1	22.2	-
0400	8.6	8.6	15.5	3.1	11.4	14.6	9.1	8.9	14.6	-
0500	9.3	8.2	15.1	3.1	14.8	15.3	9.5	11.7	15.3	-
0600	10.4	6.2	13.1	6.3	21.2	14.3	10.1	17.5	14.3	-
0700	8.3	6.3	15.7	4.4	11.1	13.6	11.5	17.8	13.6	-
0800	12.1	13.3	17.7	4.8	10.5	13.7	16.5	24.8	13.7	-

Table B-4.
Melatonin volunteers - day 6 prolactin levels (ng/ml).

MIN	07	09	10	13	20	22	27	33	36	37
0800	9.6	4.3	7.0	-	-	10.0	-	-	-	-
0900	15.7	3.8	7.2	17.1	17.5	13.9	5.6	10.0	-	4.2
1000	13.4	4.8	8.1	15.9	10.4	14.0	5.2	7.5	-	8.8
1100	13.5	6.2	7.3	16.5	13.3	8.9	5.9	7.0	6.6	6.0
1200	12.9	5.6	16.9	19.0	11.0	11.5	5.9	7.0	8.7	6.2
1300	17.2	9.3	9.4	18.2	17.3	14.0	5.0	7.0	8.3	9.7
1400	17.5	12.4	9.5	20.9	13.5	14.5	5.6	11.2	7.0	7.6
1500	28.2	15.9	15.3	27.7	15.7	23.4	7.7	11.3	7.7	19.3
1600	17.3	13.5	11.6	17.6	12.9	18.4	5.1	10.3	6.2	12.7
1700	18.1	9.7	10.2	22.3	10.1	12.8	5.5	11.7	7.3	20.0
1800	19.0	11.6	9.4	36.1	-	9.5	6.1	-	8.2	12.8
1900	15.6	6.3	32.8	34.4	-	19.4	7.5	17.2	9.2	25.5
2000	21.9	5.9	20.7	40.9	-	21.0	13.5	29.6	11.2	17.0
2100	25.3	9.1	33.1	34.1	29.3	13.8	12.3	15.7	10.6	14.1
2200	21.4	12.6	21.2	19.8	-	21.5	13.7	12.5	-	11.9
2300	18.1	8.9	25.3	-	-	13.2	9.0	9.6	-	11.3
2400	18.9	12.5	16.3	23.4	21.9	23.6	8.2	10.0	12.2	6.0
0100	10.2	11.9	15.5	-	-	22.6	14.7	9.9	8.7	9.3
0200	13.5	6.4	10.3	16.1	13.2	17.3	5.9	6.0	5.7	5.8
0300	10.3	5.4	8.2	-	-	12.1	5.0	5.9	5.2	5.5
0400	12.5	4.6	7.8	15.1	12.6	11.0	-	6.1	5.1	9.0
0500	12.5	4.6	7.7	-	-	15.4	7.7	6.3	6.0	6.6
0600	9.2	3.8	9.7	20.1	11.6	15.8	-	6.4	4.8	6.7
0700	10.4	3.9	7.8	-	-	13.1	5.1	6.1	5.0	7.7
0800	13.1	5.3	12.3	19.0	19.9	10.6	7.4	7.8	6.6	12.7