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Melatonin reduces arousal and startle responsiveness without influencing startle habituation or affective startle modulation in young women

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ABSTRACT

Melatonin has been suggested to affect human emotion, but conflicting evidence exists. Therefore, we tested the effect of a single dose of a 4 mg prolonged release formulation of melatonin on a biologically based model of emotional processing. Affective modulation of acoustic white noise startle (103 dB) by emotional slides selected from the International Affective Picture System (IAPS) was assessed in 16 healthy young women twice, in a double-blind, placebo-controlled, balanced cross-over design. Melatonin significantly reduced startle responsiveness, but did not impact affective startle modulation, nor startle habituation. Melatonin significantly reduced arousal ratings and induced a parasympathetically dominated heart rate variability pattern indicative of a non-aroused state. We conclude that melatonin reduces arousal and startle responsiveness. However, no evidence for a direct emotion-modulating effect of melatonin was found in this healthy cohort.

Introduction

In all mammalian species the release of melatonin follows an endogenous circadian rhythm, such that it is highest during the nighttime. Its release is suppressed (masked) by light and synchronized to the environmental 24-hour light–dark cycle, via suprachiasmatic nucleus (SCN)-hosted mechanisms. In humans, it has been shown that endogenous melatonin secretion in the evening, as well as exogenous administration of melatonin during daytime, increases sleepiness and sleep propensity (Krauchi et al., 2006). This effect is especially strong in situations of disturbed awake/sleep rhythms, such as working during night shift or experiencing jet lag (Macchi and Bruce, 2004). Melatonin has also been implicated in immune and reproductive system function, as well as cardiovascular and thermoregulatory control (for reviews see Macchi and Bruce, 2004; Krauchi et al., 2006). Recent work suggested melatonin to play a role in human seasonal affective disorder (Wehr et al., 2001), and major depression (Crasson et al., 2004). Furthermore, melatonin administration has been shown to reduce preoperative anxiety in adults (Naguib and Samarkandi, 2000). Melatonin is thought to play a role in rodent depression-like (Weil et al., 2006) and anxious (Nava and Carta, 2001; Krskova et al., 2007) behavior. However, this issue remains controversial since other authors reported a failure to find a reduction in human anxiety (Capuzzo et al., 2006).

Human affective processing may be explored psychophysiologically with the startle response. The acoustic startle eye blink response is modulated by the organism's motivational state, as has been shown repeatedly in animals and humans (Lang et al., 2000). It has been shown that startle can be enhanced when the eliciting acoustic stimulus is presented during an unpleasant slide, due to a match in the valence, or affective impact, of the unpleasant slide adding to that of the unpleasant startle stimulus (Vrana et al., 1988). In this research design, startle responses are measured while participants look at slides that vary in subjective ratings of valence (pleasant, neutral, unpleasant). This affective modulation of startle has been demonstrated for a wide variety of stimuli, including imagined situations (Robinson and Vrana, 2000; Bradley et al., 2006). The valence effect is dependent upon a certain level of arousal inherent in the slide (determined by having participants rate the arousal level of the slide) (Cuthbert et al., 1996). Slides that are not sufficiently arousing do not result in the valence modulation of startle. The above described methodology offers the potential to non-verbally assess pre-attentive activity of brain circuitry involved in emotion, approach, avoidance, and fight-flight operations (Lang et al., 2000), thereby supplementing methods based

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on self report of emotional states, which may be biased by expectation and social factors.

Melatonin has been shown to impact rodent startle (Datta et al., 1981) in that melatonin inhibited the motor movements and significantly decreased the heart rate responses to intense acoustic stimuli of rats, but comparable data in humans are missing. However, it has been shown that melatonin decreases arousal in humans (Cajochen et al., 2003b). Given the sensitivity of the human startle response to emotional content of background stimuli (Bradley et al., 2006) and the dependence of affective startle modulation upon a sufficient level of arousal inherent in the background stimuli (Cuthbert et al., 1996), we reasoned that melatonin may influence affective startle modulation in humans.

Although melatonin profiles are highly consistent and reproducible within subjects (Cajochen et al., 2003a), melatonin effects on startle cannot conclusively be studied by non-experimental 'time of day' studies. This is because of the confounding effects of cortisol and other variables (e.g. sleepiness, motor activity, eating patterns, etc.). Cortisol is a circadian hormone that may also impact emotion (Barak, 2006) and startle (Buchanan et al., 2001). Cortisol increases shortly after awakening, gradually declining over the day. Similarly to melatonin, its circadian pattern is also driven by the SCN (Buijs et al., 2003), whereas a lack of effect of light on cortisol secretion may be the due to masking, since no effect of light is found in mildly stressful laboratory conditions, while a clear effect is present in a less stressful home condition in the early morning (Scheer et al., 2002). Interestingly, cortisol and startle are both increased by anxiety (Grillon and Baas, 2003). The impact of cortisol administration on startle in humans appears to be dose-dependent, with low doses (5 mg) increasing acoustic startle reactivity and higher doses (20 mg) decreasing startle reactivity (Buchanan et al., 2001), and different types of central corticosteroid receptors (mineralocorticoid and glucocorticoid) may be involved (Buchanan et al., 2001). It was shown that startle and cortisol are inversely related when measured at different times of day, such that baseline startle responses are larger in the evening than in the morning, but cortisol levels are higher in the morning than in the evening (Miller and Gronfier, 2006). It cannot be excluded that corticotrophin releasing hormone (CRH), which is in negative feedback with circulating cortisol, may increase startle via nucleus reticularis pontis caudalis (nRPC)-based mechanisms (Birnbaum and Davis, 1998).

Melatonin effects are best studied in pharmacological intervention studies. Because of the potential impact of cortisol, the optimal time for an intervention with melatonin is in the afternoon, when cortisol levels are low. Exogenous melatonin is readily absorbed after oral administration. However, melatonin has a short half-life, so that plasma melatonin levels decrease rapidly. This can be avoided by prolonged release melatonin formulations (Grossman et al., 2006) which have the advantage of long plateaus, thereby stabilizing melatonin effects during steady state conditions.

The aim of the current study was to assess whether melatonin influences human affective processing. We predicted that exogenous melatonin would attenuate valence effects on startle responding, to the extent that melatonin reduces arousal. We assessed subjective sleepiness ratings, as well as more objectively describing arousal levels by measuring autonomic function, in the form of heart rate and heart rate variability (HRV). High frequency HRV indices are considered valid noninvasive estimates of cardiac vagal control (Malik, 1996). Decreases in high frequency HRV during stress and arousal (Buchholz et al., 2003; Schachinger et al., 2004) have been explained by vagal withdrawal; increases in high frequency HRV have been found during reduced arousal and relaxation (Sakakibara et al., 1994; Nava et al., 2004) and under the influence of medication known to reflexively increase parasympathetic cardiac control (Schachinger et al., 2001). Therefore, the parasympathetically dominated high frequency component of heart rate variability is a good index of physiological arousal.

Methods

Participants

The study was approved by the local Ethical Committee, and all study procedures conformed to the Declaration of Helsinki. Participants gave written informed consent before participation. Sixteen healthy young women were recruited via local poster announcements posted at prominent places of the University of Basel campus. After a brief telephone screening, subjects were invited for an initial appointment where they received detailed study information, and medical history and physical status were assessed. Only healthy subjects were included. Exclusion criteria were: any acute or chronic mental or physical disease, regular smoking, regular medication except vitamins or oral contraceptives, history of illicit drug abuse, shift work within 3 months prior to the examination days, or trans-continental travel within 1 month prior to the study. Eight females were taking low dose oral contraceptives (0.02–0.05 mg ethinylestradiol and 0.075–2 mg gestagens). The remaining females were tested during their luteal phase, with timing based on self-reported menstrual data. Mean age of the participants was 24.75±0.63 (SEM) years, and body mass index was 20.59±0.51 kg/m^2. During the 3 days preceding the study, subjects were instructed to maintain a regular sleep–wake schedule (lights off and bedtime at about 23:00; total sleep duration of about 7 to 9 h). They were instructed to refrain from excessive physical activity, and from caffeine and alcohol consumption, starting after noon preceding the study days. Three subjects had to be excluded from the final statistical analysis. One woman was regularly wearing contact lenses because of heavy myopia. Two other subjects had missing data due to complete startle habituation on at least one of the two study days.

Procedures

Subjects were studied twice, within an interval of 1 week, in a placebo-controlled, double-blind, balanced cross-over design. Subjects entered the psychophysiological laboratory at 11:00 am. At 13:00 pm a prolonged release formulation of melatonin (4 mg melatonin prolonged release over a 9 hour period, Circadin™, Neurim, Israel) or placebo was administered orally, together with 50 mL of water. The sequence of application was randomized and given in a double-blind fashion. Subjects remained in a semi-recumbent position (armchair) under controlled environmental conditions (<30 lx light intensity, room temperature 24 °C) from 11:30 until the end of the study.

One set of slides was presented on both study days, containing 27 slides selected from the International Affective Picture System, which provides standardized valence and arousal ratings (Lang et al., 1995). It has been documented that the impact of slide valence is present even after repeated presentation of the same slides (Bradley et al., 1993). No sexually explicit slides were used in this study. Slides were chosen to be low, medium, or high in arousal, crossed with low, medium, or high in valence (we speak of 'low valence' in the sense of 'unpleasantness', and 'high valence' in the sense of pleasantness), with three slides in each combination.[1] We chose slides at three levels of arousal (based on standard arousal ratings (Lang et al., 1995)) within each valence category for the specific purpose of evaluating the interaction between arousal and valence, and the impact of melatonin on this interaction. It has been shown that startle is affected by valence only at high levels of arousal (Cuthbert et al., 1996), and one of our goals was to explore the potential action of melatonin as a modulator of this arousal by valence interaction. Average valence and arousal ratings within each valence category were similar those found in other studies (Bradley et al., 2006). The distance from subject to screen was 2 m, the diameter of the presented pictures was 1.4 m. Slides were presented (VGA presentation) for 6 s, with 12 second inter-slide-intervals. No specific instructions other than 'Sit quietly and watch all slides' were given.

Startle testing commenced at 16:00 pm. Acoustic startle probes (broadband noise, 103 dB(A), instantaneous rise time, 50 ms duration) were delivered binaurally by headphones. The first three startle trials prior to IAPS stimulation were assessed to index early startle habituation. After that, another 3 startle stimuli were presented to assure that early startle habituation had developed. During IAPS presentation, the occurrence of startle probes was made unpredictable by randomly varying their timing between 3 and 5 s after slide onset. Furthermore, only two thirds of the slides (18) were startled. Startle stimuli were also presented 6 times during inter-slide-intervals (ISI).

Solid gel Ag-AgCl electrodes (ARBO Co., Brunswick, Germany) were placed on the face below the right eye to record electromyographic (EMG) responses from the orbicularis oculi muscle, using a bio-amplifier with a 20 to 500 Hz band pass and a 50 Hz notch filter. The raw EMG signal was then rectified by true root mean square conversion, integrated (time constant 20 ms), and sampled by analogue/digital conversion (1000 Hz, 12 bit) for offline analysis. EMG responses were identified by computer-assisted manual scoring as the EMG maximum following 20 to 150 ms after noise onset. If no startle eye blink

[1] The IAPS slide numbers were (valence category/arousal category): (high/high) 8030, 8200, 4572; (high/medium) 7270, 2150, 1710; (high/low) 7580, 2530, 5200; (medium/high) 2220, 7820, 7190; (medium/medium) 7550, 7130, 7050; (medium/low) 7000, 7100, 5530; (low/high) 3170, 3140, 3010; (low/medium) 9050, 9250, 1300; and (low/low) 3220, 9000, 9180. The standardized female IAPS picture ratings (mean± SEM) were (valence/arousal): (high/high) 7.57±0.15/6.68±0.35); (high/medium) 8.22± 0.24/5.48±0.18); (high/low) 7.84±0.20/3.66±0.36); (medium/high) 5.22±0.21/4.20± 0.32); (medium/medium) 4.99±0.12/3.20±0.18); (medium/low) 5.23±0.11/2.58±0.22); (low/high) 1.33±0.09/7.30±0.18); (low/medium) 2.55±0.45/6.67±0.02); and (low/low) 2.64±0.27/5.02±0.48).

occurred, the response magnitude was scored as zero. Individual startle response magnitude data were log-transformed and *t*-scored (mean = 50, standard deviation = 10), with both sessions pooled for each participant.

Standard electrocardiogram (ECG) was recorded at 12:30, prior to drug intake, as well as 15:30, 30 min before startle testing, with analog to digital conversion at 1000 Hz. Mean respiratory frequency is a strong confounder of heart rate variability (HRV). Thus, subjects adjusted their respiratory rhythm to 0.2 Hz following breathing instructions delivered from tape by headphones. Interbeat intervals (IBI) and heart rate were determined offline. The hardware and software algorithms used are highly sensitive to R-waves (coefficient of variation less than 0.2 %, internal laboratory protocol). Each IBI series was plotted on screen and manually examined for artifacts, and adjusted when appropriate. Only artifact-free periods of pure cardiac sine rhythms were analyzed. The duration of each individual time series was 5 min. IBI series were linearly de-trended, and power spectral densities were derived for each experimental period by using algorithms based on Fast Fourier Transformations (FFT) as previously described (Schachinger et al., 2004). HRV was assessed in the frequency domain, separately for the parasympathetically dominated high frequency band (0.15–0.4 Hz), as well as for the sympathetically and parasympathetically dominated low frequency band (0.07–0.14 Hz). Data on very low frequency (0.02–0.06 Hz) variations are provided for completeness. As frequency based HRV measures tend to be skewed, data were expressed as natural logarithms.

One half-hour before (12:30 pm) and three and one-half hours after (16:30 pm) administration of the pill (placebo or melatonin), subjects rated their sleepiness on the 9-point Karolinska Sleepiness Scale (KSS: Akerstedt and Gillberg, 1990), and saliva samples were collected for measurement of melatonin. This measurement used a highly specific direct double-antibody RIA (Weber et al., 1997).

Statistical testing was performed by repeated measures ANOVA with drug (placebo vs. melatonin) and time (pre- vs. post-treatment) being within subject factors. The order of treatment ('placebo-first, melatonin-second' vs. 'melatonin-first, placebo-second') did not have any effect on the dependent variables. We did not expect such an effect 'a priori'. Therefore, and for the reason to preserve statistical power, we did not include the between subject factor 'order of treatment' into the statistical model. For comparison of the startle responses during the inter-slide-interval trials, which are considered to represent naive control periods, a paired *t*-test was employed. Statistical significance was considered at a level of $p<0.05$. All data reported in text, tables and figures represent means and standard errors.

Results

As expected, the administration of melatonin predicted saliva melatonin levels ($F(1,15)=198.00$, $p<.0001$), with melatonin levels increasing from pre-test to post-test in the melatonin group (from 1.1 ± 0.2 to 81.2 ± 6.0 pg/mL) but not in the placebo group (from 1.3 ± 0.3 to 1.2 ± 0.3 pg/mL).

ANOVA was performed on the startle response magnitude data from the habituation trials, with trial and drug being within-participant factors. As expected, startle eye blink reactivity habituated and response magnitudes declined with time ($F(2,22)=10.30$, $p<0.001$), and startle eye blink response magnitude was reduced by melatonin during early habituation trials ($F(1,11)=4.50$, $p<0.05$) (see Fig. 1). However, melatonin did not affect the startle habituation curve (no significant drug by trial interaction).

During slide presentation, melatonin caused a non-significant reduction of startle reactivity during ISI trials ($t(5)=1.90$, $p=0.08$).

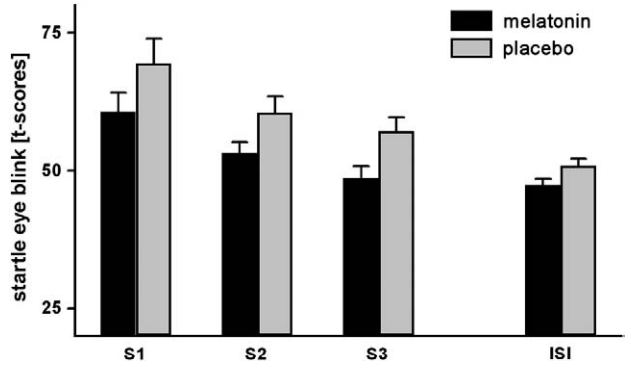

Fig. 1. Eye blink magnitude (±SEM) in response to initial startle stimulation (S1–S3), and during inter-slide-intervals (ISI), 3 h after double-blind oral placebo (open bars) or a 4 mg prolonged release formulation of melatonin (closed bars) administration (cross-over design), indicating that startle eye blink response magnitude is reduced after melatonin administration ($p<0.05$).

Table 1
Standardized eye blink responses (mean ± SEM) to acoustic startle while watching IAPS slides of different valence and arousal categories

		Arousal		
		High	Mid	Low
Valence high	Melatonin	43.5 ± 0.9	45.1 ± 1.0	45.5 ± 1.1
	Placebo	47.6 ± 2.2	48.3 ± 1.2	46.2 ± 0.9
Valence mid	Melatonin	47.7 ± 1.6	49.4 ± 2.3	48.5 ± 1.7
	Placebo	51.0 ± 1.9	49.7 ± 1.9	53.1 ± 1.3
Valence low	Melatonin	48.4 ± 1.1	44.9 ± 1.4	47.4 ± 1.4
	Placebo	55.4 ± 2.1	49.2 ± 2.1	49.8 ± 1.3

ANOVA was performed on the startle response magnitude data from the slide presentation trials, with slide valence, slide arousal, and drug being within-participant factors. During the affective modulation trials, startle eye blink response magnitude was affected by slide valence, $F(2,22)=9.30$, $p<0.01$, and a marginal valence by slide arousal interaction was found, $F(4,44)=2.30$, $p=0.07$ (see Table 1). Because slides that are not sufficiently arousing may not result in the valence modulation of startle (Cuthbert et al., 1996), a second ANOVA was based on the startle response magnitude data from the slide presentation trials with highest arousal values (left column of Table 1), with slide valence, and drug being within-participant factors. During the affective modulation trials, startle eye blink response magnitude was affected by slide valence, $F(2,24)=8.32$, $p<0.002$ (see Table 1). Melatonin decreased startle reactivity during slide presentation, $F(1,12)=7.57$, $p<0.02$, but melatonin did not influence affective startle modulation (no interaction of slide valence or arousal with drug, $F(2,24)=0.8$, $p=0.46$).

ANOVA was performed on the heart rate, and heart rate variability data (for all three frequency bands) measured before and after drug administration, with time of testing (pre-, post-intervention) and drug being within-participant factors. Melatonin reduced physiological arousal, as seen in a significant interaction between time of testing and drug for heart rate ($F(1,15)=6.70$, $p<0.02$), and both low ($F(1,15)=5.90$, $p<0.03$) and high ($F(1,15)=7.50$, $p<0.01$) frequency band heart rate variability (see Fig. 2). Melatonin decreased heart rate, and increased low frequency heart rate variability. High frequency heart rate variability decreased in the placebo condition but was maintained in the melatonin condition, indicating enhanced parasympathetic cardiac control after melatonin administration as compared to placebo. Subjective ratings of sleepiness decreased in the placebo condition (from 4.1 ± 0.3 to 3.7 ± 0.3), but increased in the melatonin condition (from 4.1 ± 0.3 to 5.4 ± 0.4), showing a significant interaction between drug condition and pre–post-test, $F(1,15)=15.00$, $p<.002$.

Discussion

This human placebo-controlled double-blind study addressed melatonin effects on startle eye blink reactivity, and affective startle modulation. Our main finding is that melatonin (4 mg) reduces startle eye blink and arousal, but does not influence either affective startle modulation or habituation of startle eye blink. Melatonin administration was shown to increase saliva melatonin concentration, and caused a decrease in arousal, as indicated by measures of arousal at different levels of the central and peripheral nervous systems: self-reported sleepiness increased, heart rate and high and low frequency heart rate variability data were shifted towards more parasympathetic heart rate control, a pattern typically exhibited in non-aroused states. Although previous studies in rat have shown that melatonin may reduce startle responsiveness (Datta et al., 1981), this is, to the best of our knowledge, the first report indicating that melatonin inhibits the human somatic eye blink response to acoustic startling stimuli, as well. The reduction of startle eye blink amplitude during the very first stimulations reflects habituation, which represents a very basic form of learning, and melatonin has been shown to affect learning and memory processes (Rawashdeh et al., 2007). However, melatonin did not affect the

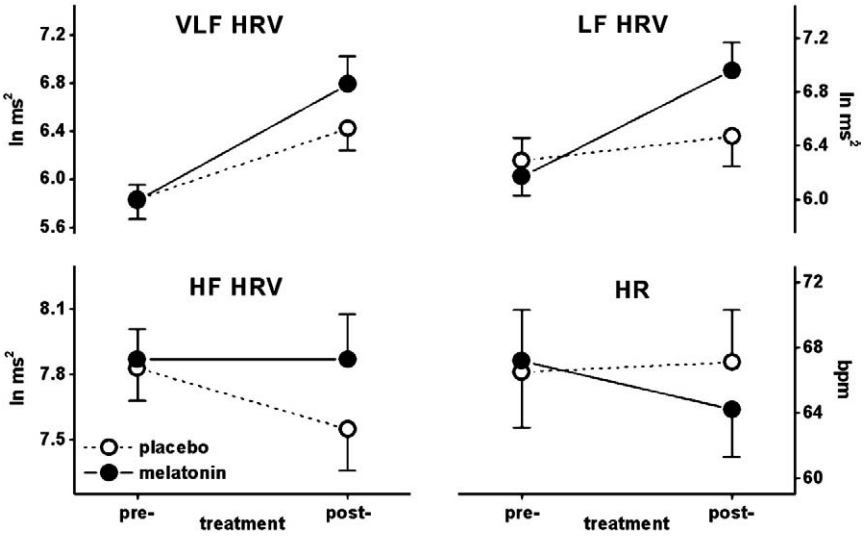

Fig. 2. Heart rate, and heart rate variability (±SEM) before and 3 h after double-blind oral placebo (open circles) or a 4 mg prolonged release formulation of melatonin (closed circles) administration (cross-over design), indicating that melatonin reduces heart rate (HR; $p<0.02$), and increases low frequency (LF, 0.07–0.14 Hz; $p<0.03$) and high frequency (HF, 0.15–0.4 Hz; $p<0.01$) heart rate variability in comparison to placebo (drug×time interaction), but does not affect very low frequency (VLF) heart rate variability.

habituation curve of startle eye blink in our sample of healthy subjects, suggesting that melatonin does not play a role in this type of learning process. Previous studies have already shown that melatonin increases vagus-dependent heart rate variability (Nishiyama et al., 2001). We could replicate this finding, and can now conclude that in the presence of effectively reduced arousal, melatonin did not specifically influence affective processing.

Given the fact that melatonin has a demonstrated effect in decreasing arousal (Szabadi, 2006), and given the fact that startle potentiation by unpleasant backgrounds has been shown to depend upon a sufficient level of arousal (Cuthbert et al., 1996), we expected melatonin to interfere with the valence modulation of startle. However, no significant effect of melatonin was found on valence-modulated startle, with comparable startle potentiation by unpleasant slides being found in both melatonin and placebo conditions. That is, melatonin reduced startle reactivity and arousal, but did not influence affective startle modulation, much like the absence of an effect of exogenous cortisol on affectively modulated startle (Buchanan et al., 2001). Since melatonin decreased arousal without eliminating the valence effect of the slides, the arousal requirement in the slide paradigm may be based on slide ratings but not on actual arousal of the participant at the time of testing. That is, the valence modulation of startle is eliminated when the slides are rated as less arousing (Cuthbert et al., 1996), but not when the physiological arousal level of the participant is reduced (as in the present study).

Our present data do not provide information about the central structures that may mediate the effect of melatonin on the startle reflex. For example, it is not known whether melatonin directly influences the brainstem startle center (nucleus reticularis pontis caudalis: nRPC) (Lee et al., 1996), and there are no publications on histological data regarding the presence or absence of melatonin receptors in the nRPC. Also, low arousal is known to impair attention (Coull, 1998). Thus, by reducing the arousal level of the participants (Szabadi, 2006), high levels of melatonin might result in less attentional resources available for the processing of an external stimulus, such as the acoustic startle stimulus. Both physiological and psychological measures of arousal (heart rate, heart rate variability, self report measures) were indeed reduced after administration of melatonin, indicating a non-aroused state.

The high frequency component of HRV is under parasympathetic control, and it was shown that parasympathetic blockade extinguishes the high frequency component of HRV (Akselrod et al., 1981). The low frequency component (around 0.1 Hz) of HRV is considered to reflect sympathetic nervous system influences (e.g. Schachinger et al., 2001). However, this frequency component is also substantially affected by parasympathetic mechanisms. It was shown that parasympathetic blockade largely reduced the low frequency component of HRV (Akselrod et al., 1981). Therefore, the observed relative increase of the low frequency component of HRV after melatonin treatment rather reflects increased parasympathetic cardiac control than sympathetic influences, especially since heart rate was reduced by melatonin.

Several limitations of the present study should be considered. This study was done in young females, only. Furthermore, the alterations in arousal occurred 3 h after the administration of prolonged release formulation of melatonin, and were not assessed earlier. Thus, we cannot exclude the possibility that such differences appeared late, nor that affective processing might have been influenced earlier after melatonin administration. This, however, is unlikely since we employed a prolonged release formulation of melatonin, which guaranteed steady state plasma melatonin levels throughout several hours. It is unlikely that cortisol had any effect on this study outcome, since this study was placebo-controlled and any effects of cortisol or time-of-day would have been present in both the drug and placebo conditions. In any case, cortisol effects would be expected to be in a direction opposite that of melatonin in these response systems. Small alterations in the placement of the electrodes or changes in electrode quality between days might unsystematically affect the data. However, a high test–retest correlation of eye blink response magnitude has been demonstrated in previous studies of our group (Luthy et al., 2003). The melatonin effect has only been studied during daytime, which is a rather non-physiological time of day to investigate them; normally melatonin is not present during the day. It remains to be investigated whether melatonin moderates the affective modulation of startle responsiveness during the night, when it is normally secreted, e.g. by implementing a study including a limb with light to suppress, one with light and melatonin to restore melatonin levels and one without light as a control for the effect of light. No data is available on whether melatonin simply alters auditory processing and thresholds. From an evolutionary viewpoint it seems to be unwise to impair hearing during nighttime when visual modality suffers from darkness. However, this does not rule out a detrimental effect of melatonin on hearing, and future studies will have to clarify this aspect.

We conclude that melatonin reduces arousal and acoustic startle eye blink responsiveness. Our data, however, do not support the assumption that melatonin impacts emotional processing.

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