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*J Biol Rhythms* 2009; 24; 232
DOI: 10.1177/0748730409335546

The online version of this article can be found at: http://jbr.sagepub.com/cgi/content/abstract/24/3/232

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Subjective Well-Being Is Modulated by Circadian Phase, Sleep Pressure, Age, and Gender

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Abstract  Subjective well-being largely depends on mood, which shows circadian rhythmicity and can be linked to rhythms in many physiological circadian markers, such as melatonin and cortisol. In healthy young volunteers mood is influenced by an interaction of circadian phase and the duration of time awake. The authors analyzed this interaction under differential sleep pressure conditions to investigate age and gender effects on subjective well-being. Sixteen healthy young (8 women, 8 men; 20-35 years) and 16 older volunteers (8 women, 8 men; 55-75 years) underwent a 40-h sleep deprivation (high sleep pressure) and a 40-h nap protocol (low sleep pressure) in a balanced crossover design under constant routine conditions. Mood, tension, and physical comfort were assessed by visual analogue scales during scheduled wakefulness, and their average formed a composite score of well-being. Significant variations in well-being were determined by the factors “age,” “sleep pressure,” and “circadian phase.” Well-being was generally worse under high than low sleep pressure. Older volunteers felt significantly worse than the young under both experimental conditions. Significant interactions were found between “sleep pressure” and “age,” and between “sleep pressure” and “gender.” This indicated that older volunteers and women responded with a greater impairment in well-being under high compared with low sleep pressure. The time course of well-being displayed a significant circadian modulation, particularly in women under high sleep pressure conditions. The results demonstrate age- and/or gender-related modifications of well-being related to sleep deprivation and circadian phase and thus point to specific biological components of mood vulnerability.

Key words  mood, constant routine, sleep deprivation, sleepiness, melatonin, cortisol, sleep-wake homeostat, circadian rhythm

CIRCADIAN AND HOMEOSTATIC INFLUENCES

Subjective well-being largely depends on current mood, which is determined by both psychological and physical state. Under controlled laboratory conditions, subjective mood, assessed by a visual analogue scale (VAS), exhibits circadian rhythmicity (Boivin et al., 1997; Koorengevel et al., 2003) similar to that of subjective sleepiness and cognitive performance (Van Dongen and Dinges, 2005) and can be linked to rhythms in many physiological circadian markers such as core...
body temperature, heart rate, or the hormones melatonin and cortisol. In addition to the circadian component, manipulations of the sleep-wake cycle also have a strong impact on mood regulation such that subjective mood dramatically changes by altering the duration and timing of sleep episodes, thus suggesting that the duration of sleep and its position in the circadian cycle is critical for mood regulation (Monk et al., 1992; Taub and Berger, 1974; Taub and Berger, 1973; Wood and Magnello, 1992). Lack of sleep per se leads to mood deterioration in healthy subjects (Brendel et al., 1990; Scott et al., 2006) to such an extent that the timing of sleep, in sleep displacement studies, can significantly impact daily mean values of mood (Totterdell et al., 1994). Taken together, both circadian phase and the amount of prior wakefulness play a key role in the regulation of subjective mood.

Quantification of the differential influence of these 2 important factors in a forced desynchrony study design has revealed that subjective mood is modulated by a complex and nonadditive interaction of circadian phase and duration of prior wakefulness (Boivin et al., 1997). The nature of this interaction was such that even moderate changes in the timing of the sleep-wake cycle led to profound effects on mood (Boivin et al., 1997). Similarly, after advancing the sleep-wake cycle daily by 20 min for a week, mood ratings fell strongly during the biological night compared with the stable sleep control group (Danilenko et al., 2003).

The Profile of Mood States questionnaire (POMS; McNair et al., 1971), a self-report inventory, is commonly used for measuring distinct mood states. Another frequently used instrument is the PANAS, which measures 2 broad dimensions of positive affect (PA) and negative affect (NA) (Watson and Clark, 1997). NA did not exhibit a clear circadian component, whereas PA did, and its 24-h rhythm correlated with the circadian rhythm of rectal temperature (Murray et al., 2002). Affective state, as measured by various mood and subjective activation scales (e.g., Covi et al., 1977; Monk, 1989), is also sensitive to sleep loss (Bonnet, 1989; Reynolds et al., 1986). Another interesting aspect is that the characteristics of morningness (M) or evenness (E) in healthy subjects significantly impact on their mood states (measured by POMS): E chronotypes improved mood and decreased anger-hostility after partial and total sleep deprivation, and activity was decreased after total sleep deprivation. For M chronotypes partial sleep deprivation did not modify mood, whereas total sleep deprivation worsened depressive mood and tiredness, and decreased vigor/activity (Selvi et al., 2007).

**AGE- AND GENDER-RELATED INFLUENCES**

Younger subjects consistently rated themselves lower on global measures of vigor and affect than older subjects, with a sharper decrease of vigor on the day following sleep deprivation (Brendel et al., 1990). This suggests that acute sleep deprivation may actually be more disruptive for younger than for older adults, who may have greater mood stability and less rhythmic changes (Monk et al., 1992). However, another study under 36 h of bed rest conditions revealed no differences in temporal profiles between older and young volunteers (Buysse et al., 1993).

No significant gender effect on mood was found in a study with pilots (Caldwell and LeDuc, 1998), although another study showed that the diurnal rhythm of mood in women peaked 2 h earlier than men (Adan and Sanchez-Turet, 2001). However, to our knowledge there are no studies on age- and gender-related differences in circadian and homeostatic mood regulation so far.

**STUDY AIM**

We investigated the time course of subjective mood, tension, and physical comfort ratings in young and older healthy subjects under differential sleep pressure conditions to quantify circadian and homeostatic contributions to these ratings. Because the 40-h protocol assesses subjective well-being at a high sampling frequency (in addition to collecting physiological variables and carrying out performance and memory tests), we could not implement a large questionnaire battery, but selected the previously validated, readily comprehensible, and fast VAS technique to maintain subjects’ motivation. A significant concern in the present study was that physical discomfort and tension arising in the course of the demanding protocol (64 h of bed rest) may impact subjective mood ratings, particularly in the older cohort. Thus, we decided to combine tension and physical comfort together with subjective mood into a composite score and defined this score as an index of subjective well-being.

We utilized a very strictly controlled constant routine protocol to minimize the majority of confounding
chronotype-specific differences in circadian phase and 8 men; age range, 20-31 years; mean age, 25.0 ± 5.5 years) were selected. The mean body mass index (BMI) was 21.5 ± 5.7. Sixteen healthy young volunteers (8 women and 8 men; age range, 20-31 years; mean age, 25.0 ± 3.3 years [SD]) and 16 healthy older volunteers (8 women and 8 men; age range, 57-74 years; mean age, 65.0 ± 5.5 years) were selected. The mean body mass index (BMI) was 21.5 ± 1.6 SD for the young and 23.3 ± 2.1 SD for the older volunteers (t test: p < 0.05). Each study volunteer underwent a physical examination, an interview about sleep quality, life habits, and health state, a neuropsychological test battery (CANTAB® test battery and the Stroop Test [only for the older group]). All were free of medical, psychiatric, neurological, and sleep disorders (as per Pittsburgh Sleep Quality Index [PSQI] score ≤ 5 [Buysse et al., 1993], and a polysomnographically [PSG] recorded screening night). The mean PSQI value was 2.1 ± 1.3 SD for the young and 3.4 ± 1.7 SD (t test: p < 0.05) for the older volunteers. Volunteers were included if their clinical sleep EEG scoring had no pathological findings (apnea/hypopnea-index [AHI] < 10/h; periodic leg movements [PLM] index < 10/h). To exclude chronotype-specific differences in circadian phase preference we selected only moderate chronotypes (morning-evening type [M/E] questionnaire ratings between 14 and 21 points) (Torsvall and Akerstedt, 1990). Nevertheless, M/E scores were slightly higher in the older than in the younger group (mean ± SEM: 18.8 ± 0.8 vs. 16.4 ± 0.8; t test: p < 0.05), corresponding to an earlier chronotype. All participants were non-smokers without any drug abuse. This was verified in the young group by urinary toxicological analysis, sensitive for amphetamines, benzodiazepines, opiates, and tetrahydrocannabinol (Drug-Screen Card Multi-6®, von Minden GmbH, Moers, Germany). Participants were also required to abstain from excessive caffeine and alcohol consumption as well as heavy physical exercise. Other exclusion criteria were: shift work within 3 months and transmeridian flights within 1 month prior to the study, and excessive caffeine and alcohol. The young women started the study on days 1 to 5 after menses onset to complete the entire study block within the follicular phase, with the exception of 5 young women taking oral contraceptives. All procedures conformed to the Declaration of Helsinki. The local ethical committee approved the study protocol, screening questionnaires, and consent form (for details, see Münch et al., 2004; Münch et al., 2007), and all study participants gave signed informed consent.

Protocol and Study Design

Each participant was instructed to maintain a regular sleep-wake cycle (bed and wake times within ± 30 min of self-selected target time), which was verified by wrist activity monitors (Cambridge Neurotechnology®, Cambridge, Cambridgeshire, UK) and sleep logs during 1 week prior to study begin. Habitual bedtimes did not vary significantly between groups (young: 2334 h ± 56 min vs. older: 2311 h ± 40 min; mean ± SD; p = 0.2, t test). The study entailed a balanced and gender-matched crossover design, each block lasting 3.5 days (details in Fig. 1), and started with an 8-h PSG night in the laboratory. During day 1 subjects adjusted to the experimental dim light condition (<8 lux), and a morning blood sample was taken from the older participants to verify both a normal hemogram and physiological coagulation; they received a low-dose heparin injection on the 3 consecutive days of each study block (Fragmin® 0.2 ml, 2500 IE/UL; Pharmacia AG, Dübendorf, Switzerland) to prevent any venous thrombosis. After a 2nd 8-h sleep episode, all subjects participated in a 40-h “constant routine” (CR) protocol (as detailed in Cajochen...
et al., 2001; Knoblauch et al., 2005; Münch et al., 2004; Münch et al., 2007), which was followed by a recovery night.

The subjects underwent 2 CR conditions spaced 1 to 3 weeks apart: a high sleep pressure (40-h sleep deprivation [SD] protocol) and a low sleep pressure (10 cycles of 150 min scheduled wake/75 min scheduled sleep; NAP protocol). The 8-h sleep episode was calculated with respect to the midpoint of each individual’s habitual sleep episode as assessed by actigraphy and sleep logs during the baseline week. All wake episodes were spent under semirecumbent CR conditions (<8 lux) during wakefulness with a minor shift to supine posture during scheduled sleep episodes (0 lux).

Subjective Rating Scales

Subjective well-being was a composite score averaged over the 3 items “mood, tension, and physical comfort,” each assessed by a 100-mm bipolar VAS at 30-min intervals. The participants were asked to indicate how he or she felt “at that moment” by placing a vertical mark on the VAS ranging from 0 (“worst ever”) to 100 mm (“best ever”). Since the direction of the extremes was not the same for all the 3 items the formula was as follows: subjective well-being = [VASmood + (100 – VASTension) + VASphysicalcomfort]/3. In addition, VAS estimates of alertness, hunger, and subjective thermal comfort were collected. Although the reliability and validity of VAS, especially in measuring emotions, have been confirmed in many studies (Aitken, 1969; Folstein and Luria, 1973), our index of subjective well-being has not yet been validated. However, we have first evidence from an ongoing constant routine study in our laboratory that the dimension of positive affect in the PANAS correlate rather well with the composite score of subjective well-being ($r = 0.66; p < 0.025$) and even more so for the specific items VASmood and the item “happiness” on the PANAS ($r = 0.74; p < 0.01$).

Subjective sleepiness was assessed by the composite score of the Karolinska Sleepiness Scale (KSS) and the Karolinska Sleepiness Symptoms Check List (KSSCL) at 30-min intervals.

Salivary Assays

Saliva collections for hormonal assays were scheduled during wakefulness at the same 30-min intervals as subjective ratings.

Melatonin. A direct double-antibody radioimmunoassay (RIA) was used for the melatonin assay (validated by gas chromatography–mass spectroscopy with an analytical least detectable dose of 0.65 pm/ml; Bühmann Laboratories, Schönenbuch, Switzerland; Weber et al., 1997).

For mean melatonin levels, values of all samples between the upward- and downward-mean crossing points were averaged per subject and age group. A nap was classified as occurring during the biological night if the melatonin concentration of the last saliva sample prior to the nap was above the individual mean, otherwise it was classified as a nap during the biological day (Knoblauch et al., 2005; Münch et al., 2005).

Cortisol. Cortisol was measured by RIA (Ciba Corning Diagnostics, Halstead, Essex, UK) with a detection limit of 0.2 nmol/l. The intra-assay coefficient of variations was 4.0% above 0.4 nmol/l and 10.0% for levels below.
Data Analyses and Statistics

For data reduction, all values were collapsed into 3.75-h bins per subject before averaging over subjects. For all analyses, the statistical packages SAS® (Version 6.12; SAS Institute Inc., Cary, NC) and Statistica® (Stat-Soft Inc., 2000-2004, STATISTICA for Windows, Tulsa, OK) were used. Four-way repeated measures ANOVA (rANOVA) with the factors “age” (young vs. older), “gender” (women vs. men), and the repeated factors “sleep pressure” (high vs. low sleep pressure condition) and “time of day” (11 time points) were performed. All p values derived from rANOVAs were based on Huynh-Feldt’s (H-F) corrected degrees of freedom (significance level: p < 0.05).

At some time points the data for different variables (e.g., subjective well-being, melatonin, etc.) were not normally distributed, and thus a nonparametric test was used for post hoc comparisons (Mann-Whitney U test). Backward stepwise regression analysis was performed to identify the important predictor variables among subjective sleepiness, cortisol and melatonin for subjective well-being.

RESULTS

Subjective Well-Being

Mean subjective well-being ratings in the course of the high (SD) and low sleep pressure (NAP) protocol for the young and older women and men are illustrated in Figures 2 and 3. In general, all participants assessed their subjective well-being as better than average with an initial score above 50 (0 = worst ever and 100 = best ever). The rANOVA yielded significance for the main factors “age,” “sleep pressure,” and “time of day” (Table 1). Average well-being was significantly lower in older participants than the young (56.9 ± 2.2 vs. 65.3 ± 2.1), and lower during SD than NAP conditions (59.7 ± 1.9 vs. 62.4 ± 1.6). A circadian modulation revealed lower ratings during the biological night compared with the biological day. Well-being of older participants was more impaired under SD conditions than the young (−5.5 ± 2.4 vs. −0.1 ± 2.2; interaction “age × sleep pressure”; Table 1). The significant 2-way interaction “gender × sleep pressure” emphasizes a clear decrement in subjective well-being in women but not in men under the SD condition (−6.0 ± 2.4 vs. 0.4 ± 2.6). Young men did not seem to be affected by rising sleepiness under SD (Fig. 3), and show a rather flat curve throughout. In contrast, subjective well-being declined in both young and older women during the evening (significant difference between young women and men between 1730 h and 0430 h, p at least 0.04, Mann-Whitney U test). In the NAP protocol we only found a significant time-of-day effect. This protocol has the characteristic of revealing the underlying circadian rhythm since sleep pressure does not rise to mask it.

Subjective Sleepiness

The time course of subjective sleepiness is the second panel in Figures 2 and 3. The rANOVA yielded significance for the main factors “age,” “sleep pressure,” and “time of day” and was at near significance for the factor “gender” (p = 0.053; Table 1). Older volunteers were on average sleepier than the
There was a marked circadian modulation of sleepiness in all sleep pressure conditions and age groups (previously reported for the NAP protocol; Münch et al., 2005).

**Melatonin**

The time course of salivary melatonin concentration is illustrated in the 3rd panel of Figures 2 and 3. The rANOVA yielded significance for the main factors “age,” “sleep pressure,” and “time of day” (Table 1). Older volunteers had significantly lower mean melatonin levels than the young (3.8 ± 0.5 vs. 6.4 ± 1.1 pg/ml), and all volunteers had slightly but significantly higher melatonin levels during the low (NAP) than the high sleep pressure (SD) protocol (5.4 ± 0.7 vs. 4.8 ± 0.6 pg/ml). The significant 2-way interaction “age” × “sleep pressure” indicated that elevated melatonin levels under low compared with high sleep pressure conditions were only seen in the young but not the older volunteers. Furthermore, the significant 2-way interaction “sleep pressure” × “time of day” and a significant 3-way interaction “age” × “sleep pressure” × “time of day” was observed. Post hoc comparisons revealed significantly higher melatonin levels in young women compared with young men between 0245 h and 0630 h (SD) during high sleep pressure (SD) and 1700 h and 1000 h the next day during low sleep pressure (NAP) (p at least 0.04, Mann-Whitney U test).

Although melatonin secretion was diminished in older volunteers, there were no significant differences compared with the young in circadian phase position or timing of the sleep-wake cycle, nor did the phase angle between them differ (results
The time course of salivary cortisol concentration is illustrated in the bottom panel of Figures 2 and 3. Only the main factor “time of day” yielded significance (Table 1). However, the 4-way interaction (“age” × “gender” × “sleep pressure” × “time of day”) as well as the 2-way interactions “age” × “time of day” and “gender” × “time of day” were significant. The former most likely reflects a reduced circadian profile in cortisol secretion in the older subjects. The latter reflects higher cortisol levels in the evening in young women than young men between 2300 h and 0630 h, and lower cortisol levels from 1400 h to 1745 h on the 2nd day (p < 0.05, Mann-Whitney U test).

Subjective Sleepiness and Cortisol Levels as Predictors for Subjective Well-Being

To investigate possible relationships between subjective well-being, subjective sleepiness, and the circadian marker cortisol, a backward stepwise regression analysis was calculated (Table 2). Subjective well-being showed the highest correlations (r = −0.45) with subjective sleepiness followed by cortisol (r = −0.15), while melatonin was excluded by the regression model. Thus, subjective sleepiness can explain, in general, about 20% of the variation in the subjective well-being ratings of our data pool. To further test whether this association depended on circadian phase and sleep pressure conditions, subjective well-being and sleepiness were correlated at the 11 different time points throughout the high and low sleep pressure protocol separately (Fig. 4). For time points exceeding the usual 16 h of wakefulness, we found a significant correlation between subjective well-being and sleepiness. Up to 50% of the variation in subjective well-being could be explained by subjective sleepiness at times when high sleep pressure coincided with the circadian trough (0400 h). Under low sleep pressure conditions, however, there was...
never a significant correlation observed, despite the evidence for a clear circadian impact on both subjective well-being and sleepiness ratings.

**DISCUSSION**

Despite the very demanding constant posture conditions (64 h in bed), our healthy study participants rated their subjective well-being in general as good and never attained low levels. As predicted, well-being was modulated by time of day (worse during the biological night than during the biological day) and by sleep pressure (worse during SD than NAP). This confirms an important role of the circadian and the sleep-wake homeostatic system on subjective well-being, modified by age and gender. In the following section we will discuss the impact of the different factors.

**Circadian Modulation and Sleep Pressure**

The time of day modulation of subjective well-being was prominent in both protocols, indicating that circadian phase plays a pivotal role in well-being. This is in accordance with forced desynchrony data in which a significant circadian component of mood regulation could be educed (Boivin et al., 1997; Kooorengevel et al., 2003). Not clear is the role of wakefulness duration on mood, visible in our CR and in the short forced desynchrony protocol by Kooorengevel et al., 2003, but not in the classic study (Boivin et al., 1997). All our participants in the SD protocol showed deterioration of subjective well-being following 24 h of wakefulness, declining about 4 h earlier in women than in men and improving again in young women already after 22 h of prior wakefulness. Could women have had a higher motivation to overcome sleepiness? More plausible is the higher stress (see increased cortisol levels in the early evening; Fig. 3) in our young female participants when they faced having to stay awake all night. The circadian component again was visible in the improved well-being that returned on day 2, despite very high sleepiness levels.

Are women more vulnerable to circadian and sleep homeostatic influences, or do they “sense” these changes better than men? Are men less “sensitive,” less attentive of their well-being, or are they more socially conditioned to not show negative emotions? Men had smaller circadian amplitude in well-being during high sleep pressure conditions, suggesting more stability. Indeed, well-being in men did not decline under high sleep pressure compared with low sleep pressure conditions, whereas it did in women. The near-linear pattern in young men was remarkable. Is this male insensitivity to circadian and sleep homeostatic alterations in well-being and sleepiness related to higher risk-taking behaviors in young men, particularly when sleep deprived (e.g., their greater accident rate; Horne and Reyner, 1995; Pack et al., 1995)? Of course, there are major differences between real and simulated environments (Philip et al., 2005), and cognitive performance is highly influenced by motivation (Hull et al., 2003).

**Age Effects**

Subjective well-being was significantly lower in our healthy older cohort than in the young, whether due to a decrease in subjective perception or diminishing physiological and/or psychological constitution.
The demanding study protocol could have been experienced more negatively in the older group. Surprisingly, the older subjects did not show a reduced circadian modulation of well-being, as originally hypothesized, but a tendency to an even more prominent circadian rhythm in subjective well-being than young subjects.

However, the older group responded to high sleep pressure with a significant greater deterioration in well-being than the young. This implies a greater vulnerability to changes in circadian phase and challenges of sleep pressure with age. In contrast, other measures such as psychomotor vigilance performance appear less vulnerable to sleep debt in older individuals, not only in our study (Adam et al., 2006; Blatter et al., 2006). Even middle-aged subjects are less vulnerable than the young in this aspect of behavior (Bliese et al., 2006; Philip et al., 2004). Thus, we could only partially confirm previous findings (Brendel et al., 1990) of greater mood and performance disturbances after sleep loss in older subjects.

Other Circadian Parameters

It is interesting to compare subjective well-being with other circadian parameters, such as subjective sleepiness, melatonin (data already partially published; Cajochen et al., 2001, 2004, 2006; Knoblauch et al., 2003, 2005; Münch et al., 2005), and cortisol concentrations. Although our results indicate that subjective sleepiness and cortisol levels were significantly correlated with subjective well-being, these variables had dissimilar time courses under both protocols. Increasing sleepiness under high sleep pressure exhibited significant repercussions on subjective well-being in the early morning when increased homeostatic sleep load coincided with maximal circadian drive for sleep (corresponding time of day: 0400 h; Fig. 4), but not when highest sleep pressure (38-40 h) coincided with the maximal circadian drive for wakefulness in the evening (corresponding time of day: 2200 h to midnight the 2nd day of the protocol; Fig. 4). In contrast, sleepiness under low sleep pressure manifested a circadian rhythm similar to subjective well-being, but no significant correlation between these measures was observed at any given time point. Thus, the relationship between subjective sleepiness and well-being is not trivial but depends on a complex interaction between the circadian pacemaker and the sleep homeostat.

The circadian melatonin and cortisol profiles followed the well-known temporal dynamics under CR conditions (reviewed in Arendt, 2006). The cortisol rhythm did not change with age, but nocturnal melatonin was significantly attenuated. We do not have any explanation for the lower nocturnal melatonin levels in young men—apart from possible chance differences in this particular group (there are much greater interindividual differences in melatonin than there are differences between men and women (Arendt, 2006). The elevated cortisol levels around the evening nadir in young women may have reflected their more “stressful” reaction to the SD protocol, and this was correlated with well-being in a backward regression analysis. However, other studies have shown no or only minimal stimulation of cortisol secretion by sleep deprivation (Brun et al., 1998; Scheen et al., 1996).

Limitations of the Study

Measuring behavior under highly controlled laboratory conditions is important to assess contributions of circadian and homeostatic processes to a subjective variable such as well-being. However, the results are not directly applicable to understanding sequelae of a chronic sleep deficit caused by sleep problems or shift work in real life. Chronic partial sleep restriction shows a different dynamic profile than acute total sleep deprivation (Banks and Dinges, 2007), and a much smaller change in subjective ratings (Belenky et al., 2003; Brunner et al., 1993).

Our results demonstrate clear age- and gender-related modification of circadian and sleep-wake-homeostatic contributions to subjective well-being. In general, both older adults and women were more affected by sleep deprivation, showing a tendency to lower subjective well-being and a prominent circadian trough. Given that circadian and sleep homeostatic processes regulate mood in healthy subjects, it is not surprising that the circadian dysregulation and sleep disturbances associated with depression may have profound detrimental effects on mood in depressed patients, thus further perpetuating the disorder.

ACKNOWLEDGMENTS

We are very grateful to our technicians Claudia Renz, Marie-France Dattler, and Giovanni Balestrieri and the student shift workers for their help in data acquisition. We thank Gabrielle Brandenberger for coordinating the analysis of cortisol samples and Sarah Chellappa for her linguistic editing support.
This research was supported by Swiss National Science Foundation grants START # 3100-055385.98, and 3130-0544991.98 and 320000-108108 to CC, the Velux Foundation (Switzerland), and Bühllmann Laboratories, Allschwil (Switzerland).

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Birkle-Pedross et al. / MODULATION OF SUBJECTIVE WELL-BEING 241

Birchler-Pedross et al. / MODULATION OF SUBJECTIVE WELL-BEING 241

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