Title: Selective REM-Sleep Suppression Increases Next-Day Negative Affect and Amygdala Responses to Social Exclusion

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Short Title: REM & Affect

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Abstract

Healthy sleep, positive general affect, and the ability to regulate emotional experiences are fundamental for well-being. In contrast, various mental disorders are associated with altered rapid eye movement (REM) sleep, negative affect, and diminished emotion regulation abilities. However, the neural processes mediating the relationship between these different phenomena are still not fully understood. In the present study of 42 healthy volunteers, we investigated the effects of selective REM sleep suppression (REMS) on general affect, as well as on feelings of social exclusion, emotion regulation, and their neural underpinnings. Using functional magnetic resonance imaging we show that REMS increases amygdala responses to experimental social exclusion, as well as negative affect on the morning following sleep deprivation. There was no evidence that emotional responses to experimentally induced social exclusion or their regulation using cognitive reappraisal were impacted by diminished REM sleep. Our findings indicate that general affect and amygdala activity depend on REM sleep, while specific emotional experiences possibly rely on additional psychological processes and neural systems that are less readily influenced by REMS.

62 **Introduction**

63 Sleep is fundamental for general well-being [1,2]. Healthy sleep consists of two repeatedly cycling types of
64 sleep: non-rapid eye movement sleep (NREM) including slow-wave sleep (SWS) phases of deep sleep and
65 rapid eye movement sleep (REM). Over the course of the night, the percentage of SWS decreases while
66 REM sleep percentage increases towards the morning [3]. In many occasions however, sleep is
67 fragmented or shortened (e.g. due to stress, early school or work starts and late bedtimes) and even
68 minor sleep deprivation can have broad, short-term as well as long-term consequences for health and
69 well-being. Sleep disturbances affect health on the level of immune regulation [4–6] and metabolic
70 markers [7,8]. Furthermore, sleep loss negatively impacts cognitive processing and emotional reactivity
71 [9,10], as well as social behavior, leading to withdrawal from social interaction and feelings of loneliness
72 [11].

73 Interestingly, most mental disorders are associated with sleep peculiarities. Insomnia is
74 comorbid in 85-90% of individuals diagnosed with major depression [12–14]. Alterations in REM sleep
75 architecture (REM sleep latency, density and distribution) are particularly prominent in most mental
76 disorders such as major depression, bipolar disorder and Posttraumatic Stress Disorder (PTSD) [15,16].
77 Given that these disorders are mainly characterized by altered affective experience during daytime, it is
78 interesting to note that inadequate sleep itself has been shown to impact next day emotionality. Sleep
79 loss has been shown to reduce the ability to regulate emotions, leading to exaggerated responses to
80 aversive or even neutral experiences [17,18]. Recent studies even suggest sleep abnormalities as being
81 causal agents in mood disorders rather than just a symptom [13,19].

82 Although single sleep stages seem to be differentially involved in mood disorders, the role of
83 these sleep stages for cognitive and emotional functioning is not well understood. Increasing evidence
84 suggests that SWS and associated neurophysiological processes support the consolidation of newly
85 encoded declarative memories [20,21]. REM sleep, on the other hand, is particularly important for

86 emotional processing, including emotional reactivity [22,23] and the formation of emotional memories [24,25].
87 REM-sleep dreaming was also found to attenuate residual emotional load from the day before [26,27].
88 However, only very few studies have examined the effects of selective suppression of REM sleep during
89 an otherwise normal night of sleep. The existing evidence suggests that the selective deprivation of REM
90 sleep mainly disturbs the consolidation of emotional memories [28–30], whereas the selective suppression
91 of SWS mainly impairs emotionally-neutral declarative memory encoding and consolidation [31,32]. These
92 experimental findings are in line with clinical observations suggesting that REM sleep in particular is
93 closely tied with emotional functioning [19].

94 In order to better understand the processes underlying the interaction of sleep and emotion,
95 research increasingly addresses the neural mechanisms associated with REM sleep-dependent emotional
96 functioning. At the neural level, a reduction of emotional reactivity in response to previously learned
97 emotional events was found to be accompanied by an overnight decrease of amygdala reactivity, a
98 cluster of nuclei in the temporal lobes and part of the limbic system [27,33]. The limbic system has been
99 widely associated with the processing of affectively laden stimuli and plays an important role in the
100 guidance of behavioral responses to such stimuli [34,35]. Under conditions of total sleep deprivation, next-
101 day negative affect is accompanied by increased amygdala reactivity and decreased functional coupling
102 of the medial prefrontal cortex (MPFC) with limbic structures [36]. Since the MPFC is thought to exert
103 inhibitory control on the amygdala [37], this finding has been interpreted to reflect a failure of top-down
104 control in the regulation of appropriate emotional responsivity [36]. Simon and colleagues directly
105 examined the effect of sleep deprivation on the neural correlates of next day emotion reactivity [17].
106 Following sleep deprivation, there was no indication for valence-specific processing of affective pictures
107 in the amygdala, however in the sleep-rested control night, a low amount of REM sleep was associated
108 with a decline in anterior cingulate cortex (ACC)-amygdala connectivity, possibly reflecting a specific
109 effect of REM sleep on cognitive control of emotions.

110 Successful cognitive control of emotions is regarded to be an essential prerequisite of mental
111 health [38,39]. In daily life, emotions are constantly regulated either implicitly or explicitly by applying
112 specific cognitive strategies like suppression (e.g. distracting the attention away from unpleasant
113 emotional experiences) or reappraisal (e.g. reinterpreting an unpleasant emotional situation). Emotion
114 regulation thus refers to the ability to "influence which emotions we have, when we have them, and how
115 we experience and express these emotions" (p.497 [40]). Interestingly, correlational evidence indicates
116 that the success of emotion regulation is associated with sleep quality [41]. In this study, participants were
117 asked to engage in cognitive reappraisal (CRA) [42], in this case applying a previously learned cognitive
118 strategy to "redirect the spontaneous flow of emotions" (p. 6 [43]), while watching a sadness-inducing
119 film. The ability to decrease self-reported sadness using CRA compared to baseline was lower in
120 participants who reported poorer sleep quality during the preceding week [41].

121 Despite their substantial clinical significance, the neural mechanisms of the effect of REM sleep
122 on the efficacy of regulation strategies in ameliorating unpleasant affect remain unknown. To fill this
123 gap, it is important to experimentally induce negative affect that may then be attenuated by applying
124 CRA strategies. In order to induce negative affect in the present study, we simulated social exclusion in a
125 laboratory setting using the so-called *Cyberball* [44]. *Cyberball* is a virtual ball-tossing paradigm, where
126 participants are playing with a preset computer program while believing that they are playing with two
127 other human participants. By manipulating the number of ball-tosses towards the participant, the degree
128 of social inclusion can be controlled experimentally. Evidence suggests that the distressing experience of
129 social exclusion might share neural regions and mechanisms with the affective processing of physical
130 pain [45–47]. In a neuroimaging study using the *Cyberball* game, participants showed greater activations in
131 cingulate and prefrontal regions involved in the processing of the affective pain component when
132 excluded compared to being included [48]. Although behavioral consequences and neural activation

133 patterns associated with social exclusion have been studied quite intensively, there is scarce research on
134 intervening cognitive appraisals and coping mechanisms regarding feelings of social exclusion [49].

135 The aim of the present study was to examine how REM sleep suppression impacts the following
136 day general affect, emotional reactivity, and associated neural mechanisms of emotion regulation during
137 the acute experience of social exclusion. In a between-subjects design we invited participants to a
138 combined polysomnography and fMRI study. After a habituation night allowing regular sleep, for the
139 second, experimental night participants were randomly allocated to either a REM sleep suppression
140 (REMS) group or one of two control groups: a non-suppression control group with regular sleep (CTL) and
141 a high-level control group with similar amounts of awakenings, but where suppression targeted phases
142 of slow wave sleep (SWSS). To assess the impact of REMS on general affect, subjects repeatedly filled in
143 the Positive and Negative Affect Schedule (PANAS) [50]. In the morning after the experimental night,
144 subjects participated in the *Cyberball* during fMRI scanning to induce feelings of social exclusion. All
145 participants engaged in two sessions of the game. In the first session, participants played the game
146 without any instructions. In the second session, participants were instructed to actively regulate their
147 emotions by applying the previously learned CRA.

148 We hypothesized that selective REMS (vs. SWSS and regular sleep) generally reduces positive
149 and increases negative affect. Furthermore, we expected that selective REMS (vs. SWSS and regular
150 sleep) increases emotional reactivity during social exclusion and dampens the effect of CRA on emotional
151 reactivity. On the level of neural systems we explored whether REMS leads to altered functional activity
152 in (para-)limbic areas such as the amygdala, ACC, insula, and hippocampus during social exclusion and
153 whether neural activity of these regions is modulated by targeted REMS during cognitive reappraisal.

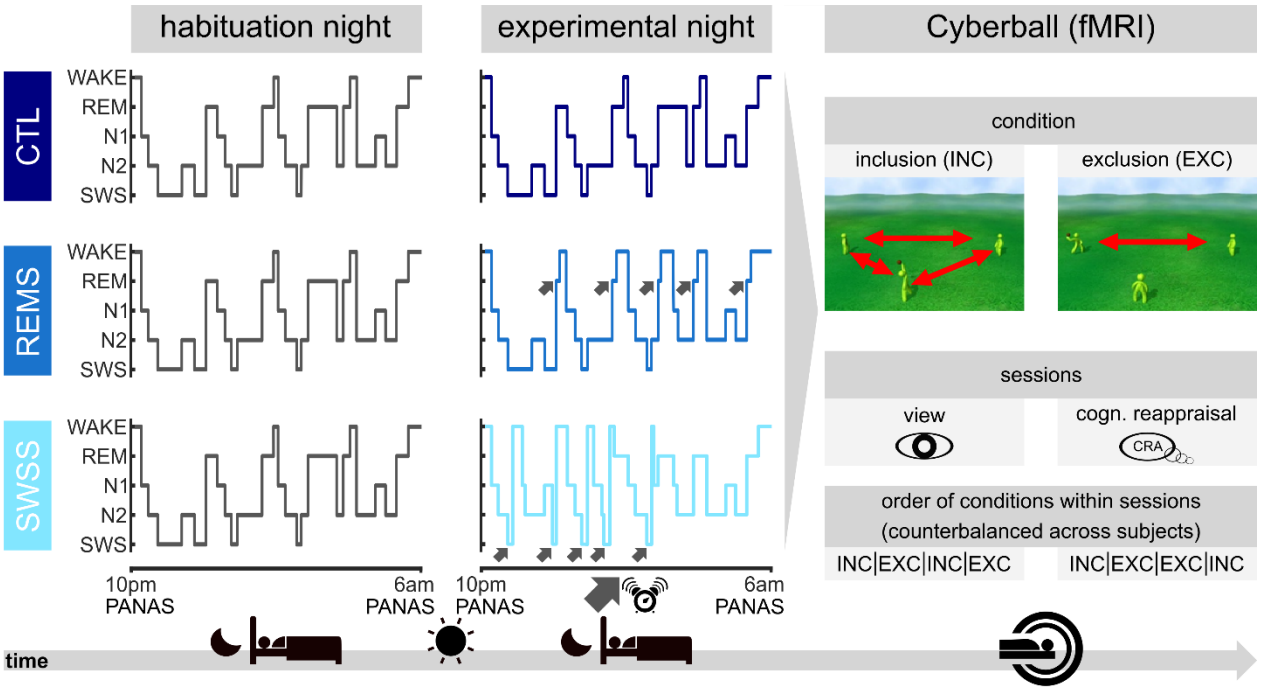
habituation night
experimental night
Cyberball (fMRI)
CTL
REMS
SWSS
WAKE
REM
N1
N2
SWS
10pm
PANAS
6am
PANAS
time
condition
inclusion (INC)
exclusion (EXC)
sessions
view
cogn. reappraisal
CRA
order of conditions within sessions
(counterbalanced across subjects)
INC|EXC|INC|EXC
INC|EXC|EXC|INC

Figure 1.

154

Results

Experimental Sleep Manipulation Selectively Reduces REM Sleep Percentage

The experimental sleep manipulation (see figure 1) successfully suppressed REM sleep in the REMS group during the experimental night (REMS score = %REM sleep in habituation – %REM sleep in experimental night; effect of group: $F(2,37)=13.21$, $p<.001$, $\eta^2=.42$; planned contrast REMS > others: $t(38)=5.17$, one-sided $p<.001$). The SWSS and CTL groups were similar with regard to REMS ($t=0.52$, two-sided $p=.608$; see table 1 and figure 2 for details).

REM Sleep Predicts General Negative Affect

General affect ratings in the PANAS obtained in the morning differed significantly between habituation and experimental night (effect of night: positive affect (PA): $F(1,37)=4.55$, $p=.040$, $\eta_p^2=.11$; negative affect (NA): $F(1,37)=9.33$, $p=.004$, $\eta_p^2=.20$), while type of suppression had no significant effect (PA: $F(2,37)=0.74$, $p=.484$, $\eta_p^2=.04$; NA: $F(2,37)=2.21$, $p=.124$, $\eta_p^2=.11$). The interaction effect was not significant for positive affect (PA: $F(2,37)=0.34$, $p=.713$, $\eta_p^2=.02$) but approached significance for negative affect (NA: $F(2,37)=3.08$, $p=.058$, $\eta_p^2=.14$). Across groups, post-hoc comparisons demonstrated that positive affect was lower and negative affect was higher after the experimental night as compared to the habituation night (PA: $t(39)=2.20$, two-sided $p=.034$, $d=0.35$, 95% $CI=[0.03;0.67]$; NA: ($t(39)=-3.23$, two-sided $p=.003$, Cohen's $d=-0.51$, 95% $CI=[-0.84;-0.18]$), with the increase in negative affect being most pronounced in the REMS group ($t(14)=4.47$, $p=.001$, Cohen's $d=1.15$, 95% $CI=[0.48;1.80]$; see figure 2). A planned contrast showed that this increase in negative affect was stronger in the REMS group as compared to the other groups ($t(38)=2.47$, two-sided $p=.037$, Cohen's $d=.81$, 95% $CI=[0.14;1.47]$), whereas no group difference could be observed for change in positive affect ($t(38)=-0.72$, two-sided $p=.950$, Cohen's $d=.24$, 95% $CI=[-0.88;0.41]$, all p-values Bonferroni-corrected).

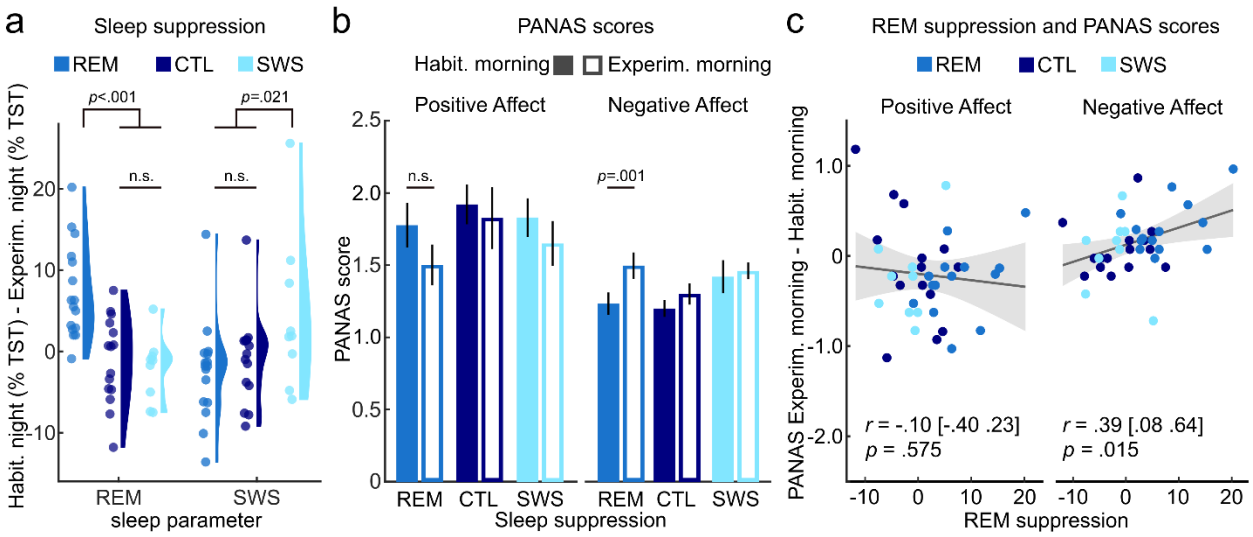

Figure 2.

Regarding the evening ratings, type of sleep suppression did not have any significant effects or interactions, neither for PA nor for NA (all *ps*>.110; see supplementary information for details). Across groups, increases in morning negative affect from habituation to experimental night could be predicted by the REMS score (Pearson's *r*=.39, one-sided *p*=.015, 95% *CI*=[0.08;0.63]; figure 2), even after controlling for changes in SWS, total sleep time (TST) and wakefulness after sleep onset in a multiple linear regression model (see table 2).

No Effect of Selective REM Sleep Suppression on Emotional Responses to Social Exclusion

By means of the *Cyberball*, we successfully induced the unpleasant feeling of social exclusion (main effect of INC/EXC: $F(1,39)=131.65$, $p<.001$, $\eta_p^2=.771$; see table 3 for descriptive statistics), thereby replicating earlier findings [48]. Moreover, if participants were asked to engage in emotion regulation using cognitive reappraisal, feelings of being excluded could be toned down significantly (main effect of VIEW/CRA: $F(1,39)=66.99$, $p<.001$, $\eta_p^2=.632$), with this effect being most strongly pronounced during trials of social exclusion (interaction effect INC/EXC x VIEW/CRA: $F(1,39)=44.97$, $p<.001$, $\eta_p^2=.536$).

192

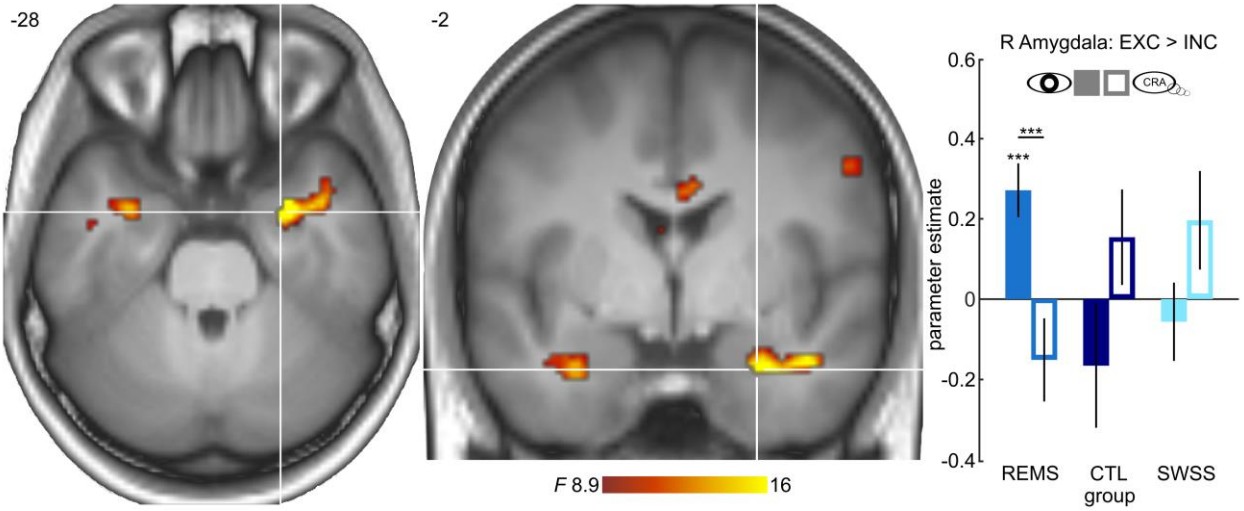

Figure 3.

193 However, regarding our second hypothesis, type of sleep suppression neither had a significant
194 impact on emotions after social exclusion (INC/EXC * group interaction: $F(2,39)=1.47$, $p=.243$, $\eta_p^2=.070$)
195 nor on the effect of emotion regulation (VIEW/CRA * group interaction: $F(2,39)=.027$, $p=.973$, $\eta_p^2=.001$).
196 Last, we did not find a statistically significant three-way interaction on the self-reported emotions after
197 social exclusion (INC/EXC * VIEW/CRA * group interaction: $F(2,39)=.45$, $p=.639$, $\eta_p^2=.023$). Similarly, no
198 significant main effect of or interactions with type of sleep suppression were found when contrasting
199 REMS against the other two groups (all *p*-values >. 41).

200 **REM Sleep Suppression Alters Amygdala Activity during Social Exclusion**

201 Similar to earlier studies, we found that during social exclusion participants' neural activity in the left and
202 right hippocampus (left: x,y,z (mm): -36,-40,-6; $T=7.15$, $k=89$, $p<.001$, FWE-corrected; right: 36,-32,-8;
203 $T=4.88$, $k=4$, $p=.022$, FWE-corrected) as well as the right insula (34,-10,22; $T=5.07$, $k=13$, $p=.014$, FWE-
204 corrected) was significantly increased compared to the inclusion condition [51,52]. In addition, across
205 groups, neural activity in the right anterior insula was significantly increased during VIEW as compared to
206 CRA blocks (main effect VIEW>CRA: 30,24,-4; $T=4.58$, $k=1$, $p=.048$, FWE-corrected). Testing whether
207 these two main effects interacted or whether they were modulated by type of sleep suppression did not

208 yield any significant effects. This held both when testing for differences between any of the three
209 experimental groups and when comparing the REMS group against the other two groups combined.

210 Last, we tested the three-way interaction of EXC/INC, VIEW/CRA, and type of sleep suppression.
211 A test of differences between any of the three groups for the two-way interaction contrast
212 [EXC/VIEW>INC/VIEW] > [EXC/CRA>INC/CRA] was not significant. However, comparing the REMS group
213 to the other two groups indicated differential neural responses in the right amygdala for the REMS group
214 (26, -2, -28; F=23.70, k=1, p=.048, FWE-corrected inside the a priori mask; see figure 3). Precisely, the
215 contrast EXC>INC was positive during VIEW in the REMS group, but did not differ from zero in the other
216 groups (REMS: t=4.04, two-sided p=.005; CTL: t=-1.07, two-sided p=1.000; SWSS: t=-0.58, two-sided
217 p=1.000; see figure 3). In addition, EXC>INC was more positive during VIEW than during CRA in the REM
218 group (t=5.27, two-sided p<.001), but not in the other groups (CTL: t=-2.18, two-sided p=.141; SWSS: t=-
219 1.70, two-sided p=.369; all p-values Bonferroni-corrected). This pattern of results suggests that the
220 signaling of information that is relevant to the individual's social well-being and mediated by amygdala
221 activity depends on intact REM-sleep.

Discussion

Nearly everyone can relate to the devastating effects of a sleepless or interrupted night on one's next day mood. While the effect of total sleep deprivation on emotional reactivity has been investigated intensively in the past [53,54] the present study focused on the specific impact of selective REM sleep suppression on general affect, as well as emotion regulation and its neural correlates under conditions of social exclusion.

We found that lower amounts of REM sleep across all participants were associated with higher levels of general negative affect in the next morning, a finding that is in line with previous literature implicating REM sleep in emotional functioning [55]. Despite this general effect, however, our findings do not provide evidence for a direct link of REMS with the subjective emotional response to experimentally induced social exclusion. The ability to regulate one's negative emotions during social exclusion was also not affected by prior REMS, which was an unexpected finding. Interestingly though, despite no changes in subjectively reported emotions, neural activity in the limbic system was altered after REMS when participants experienced social exclusion. Precisely, neural responses to passively experienced ostracism were specifically increased in right amygdala after REM sleep was selectively suppressed. The amygdala is strongly associated with emotional processing [56,57] and has anatomical connections to the anterior insula and the anterior cingulate cortex [58,59]. As part of this so-called salience network [60], the amygdala's assumed function of signaling the relevance of information is central for the domain of affective experiences [57,61]. Previous studies showed that amygdala responses to viewing negative emotional stimuli increased after total sleep deprivation [62], depended on intact REM sleep in particular [33], and correlated with autonomic responses to psychosocial stress [63]. Our study connects these previous findings by demonstrating that amygdala activity tracks information relevant to the subjects' social well-being in dependence of REM sleep.

245 We can only speculate why these alterations in brain function after REMS did not manifest on
246 the behavioral level in form of increased emotional reactivity to the experience of social exclusion.
247 Previous research provides several explanations that might be helpful to understand this dissociation.
248 First, the effects of experimental short-term sleep manipulations might be strongest immediately after
249 awakening and may be readily washed out thereafter [64], and may thus not have lasted until the fMRI
250 session in the present study. This may hold in particular for selective suppression of specific sleep stages
251 rather than total sleep deprivation, which produces stronger and more long-lasting effects [64]. The
252 efficacy of REMS in the present study may have been too small to surface on the behavioral level later in
253 the morning. However, the effects of REMS nonetheless persisted on the level of brain systems. The
254 altered brain activity in the amygdala could indicate that even small changes in REM sleep can influence
255 brain systems implicated in regulating responses to affectively salient stimuli [56,57], while they are too
256 small to penetrate the level of subjective experiences, which are possibly processed further downstream
257 [63,65]. Yet, what speaks against this explanation are findings by Wiesner and colleagues [30] or
258 Morgenthaler and colleagues [66] who applied even more rigorous REM sleep deprivation, achieving a
259 mean REM sleep percentage of around one percent of TST, but nevertheless could not find REM sleep
260 related behavioral effects during emotion recognition tasks. Similarly, Liu and colleagues compared the
261 effect of a 24h sleep deprivation to regular sleep on the experience of distress following social exclusion
262 in the *Cyberball* game [67]. As in the present study, the authors did not find an effect of sleep deprivation
263 on the subjective experience of social rejection.

264 Regardless of the timing and potential washout during the day, the lack of significant findings for
265 the experience of social exclusion might also relate to the conceptual breadth and the ecological validity
266 of the affective assessment. While the underlying brain systems show increased reactivity towards
267 potentially threatening conditions and signal greater homeostatic imbalance, the distinct assessment of
268 emotional responses to ostracism, such as feelings of exclusion, might touch a different facet of affective

269 construal. That is, the affective salience of information tracked by amygdala activity [57,61] may be
270 modulated by REMS. However, the construal of emotional experience is assumed not to rely on the
271 activity in single regions, but on the dynamic interaction of various neural systems supporting multiple
272 psychological processes of emotional experience apart from affective salience [68,69]. Hence, amygdala
273 responses to psychosocial stress do not necessarily influence the construal of subjective emotion ratings,
274 that may depend on additional networks involving prefrontal cortical regions, as suggested by previous
275 studies [63,70,71]. In addition, we did not find evidence that REM sleep deprivation modulated the ability to
276 apply cognitive reappraisal on the behavioral level. Therefore, REM sleep deprived subjects may have
277 been able to counteract the impact of increased amygdala responses during social exclusion. A potential
278 factor diminishing the severity of experimental social exclusion is that despite our attempts to create a
279 socially immersive context [72], laboratory studies per se have limited relevance beyond the experimental
280 situation itself. Thus, even without explicit reappraisal strategies, this limited relevance might have
281 entered into the construal of emotional responses during passive social exclusion, and reduced the
282 influence of altered amygdala responses in the REMS group.

283 A related explanation for the brain-behavior dissociation is that healthy participants have
284 resources for compensation. As the neuroimaging findings advocate that REMS impacts limbic circuit
285 activity during emotional experiences, the question arises whether participants with a priori (sub)clinical
286 peculiarities in reaction to social exclusion, ostracism or labile sense of belonging would also report
287 altered subjective experiences. It has been shown that inter-individual dispositions such as differences in
288 rejection sensitivity [73], social anxiety [74], trait self-esteem, depression [75], attachment style [76] and
289 situational factors of the exclusion experience moderate the effect of social exclusion. Last, it is possible
290 that experimental REM-sleep suppression becomes effective on the emotional level only when applied
291 repeatedly, simulating chronic sleep disturbances that are associated with psychiatric disorders in a
292 more realistic manner.

293 Speculatively, a further explanation could relate to the psychological task used to examine
294 emotional reactivity. Earlier research indicated that sleep deprivation effects might differ depending on
295 whether the focus was on general state-like morning affect (e.g. as measured using the PANAS), the
296 processing and responding to affective material (e.g. emotion recognition, pain processing, reactivity to
297 threatening stimuli) or on the direct induction of emotional states (e.g. inducing the unpleasant
298 experience of social exclusion). One assumption derived from the present data could be that direct
299 emotion-induction tasks are so powerful that a single night of sleep deprivation or selective REMS might
300 not suffice to exert effects on the psychological level. To our knowledge, however, apart from the
301 present study there is no further work that directly examined the effect of selective REMS on
302 experimentally induced emotional states. Second, general unspecific affect may function differently than
303 emotional reactions to specific elicitors [77], potentially moderating the effect of REMS on these different
304 affective processes. However, at least one study found that general affect was not influenced by REMS,
305 which contradicts our findings [30]. Last, we are not aware of any study systematically comparing the
306 extent to which the different psychological aspects of affective experience are susceptible to selective
307 suppression of sleep stages. Taken together, the specific interaction of selective REMS with general
308 affect, in contrast to more confined emotional responses, demands more in-depths analyses of different
309 kinds of experimental designs and dependent variables.

310 Our findings of increased next morning general negative affect as well as increased limbic activity
311 after REMS are well in line with findings in patients with mental disorders. A wide range of mental
312 disorders, including mood and anxiety disorders, are not only accompanied by profound disturbances of
313 REM sleep [78,79] but also by deficits in generating and controlling emotions in an appropriate way.
314 Posttraumatic Stress Disorder (PTSD) is one of these mental disorders, where a link between REM sleep
315 and emotion regulation has been frequently discussed [80,81]. In these patients, deficits in emotion
316 processing and regulation on the behavioral level are coincided by alterations in brain networks

317 implicated in emotion regulation such as the amygdala, hippocampus, insula, and anterior cingulate [82].
318 Apart from that, PTSD patients as compared to traumatized individuals who did not develop a PTSD,
319 show a profound REM sleep fragmentation and alterations in REM density accompanied by higher
320 sympathetic drive during this sleep stage [78,79]. Importantly, these REM sleep disturbances early after
321 trauma predict later PTSD development [83,84], which let researchers speculate that REM sleep alterations
322 are not only a secondary symptom but represent one mechanistic factor contributing to the
323 development and/or the exacerbation of PTSD symptoms [80,81]. However, empirical evidence for such a
324 mechanistic link is scarce.

325 Our findings of altered negative affect and changed brain responses to social exclusion after one
326 night of REMS in healthy subjects resemble patterns typically seen in PTSD patients. Hence, the present
327 data provide additional evidence for a role of REM sleep in emotion processing and regulation and they
328 further suggest a mechanistic link between REM sleep disturbances and psychopathology. Future
329 research should investigate the effect of experimental REM sleep manipulation (i.e. REM sleep increase
330 as well deprivation/fragmentation) through pharmacological and/or psychological treatments (i.e.
331 cognitive behavioral therapy for insomnia, CBT-I[85,86]) on emotion regulation in healthy subjects and
332 patients suffering from mental disorders. Conducting more research in this field gains additional
333 importance from the fact that sleep deprivation does not have negative effects only, but can also have
334 positive effects, as evident in its anti-depressant potentials [87].

335 **Limitations**

336 Although in the present study, the use of cognitive reappraisal (CRA) strategies was effective in
337 toning down feelings of social exclusion [88], CRA was always applied in the second session. Therefore, we
338 cannot rule out that habituation to the task during the second session influenced the regulation of
339 emotionality by CRA. However, counterbalancing the view and reappraisal session would have
340 introduced even stronger confounds, considering that participants would have most likely also engaged

341 in CRA in the second session if they had learned about this strategy in the first session. In order to better
342 discriminate between CRA and habituation effects one could apply a three-session-design and randomly
343 instruct participants to use CRA either in the second or third session. In a larger sample than ours, Mauss
344 and colleagues applied such a design to show that poorer sleep quality over the course of the last week
345 was linked to decreased abilities in engaging in CRA [41].

346 A second limitation may be the presence of social desirability effects. While CRA is regarded as
347 the most efficient emotion regulation technique [89], the experimenters' intention may have been too
348 obvious in the present social exclusion context, which might have increased social desirability effects,
349 rendering this emotion regulation strategy suboptimal. Alternatively, in a different experimental setup,
350 one could implicitly offer subjects an opportunity for diverting their attention away from the unpleasant
351 emotional experience in one session and thereby apply another emotion regulation technique (i.e.
352 suppression) without an explicit instruction and the danger of social desirability effects. Future studies
353 will need to specifically target the effect of different emotion regulation strategies and the impact that
354 selective REMS might exert on such techniques.

355 **Conclusion**

356 In conclusion, the present study examined the effect of selective REMS on general affect as well
357 as the neural correlates of task-induced negative emotionality and the ability to regulate emotional
358 experiences by cognitive reappraisal during experimentally induced social exclusion. While we found that
359 REMS predicted general negative affect in the next morning, we did not find experimental evidence for
360 task-induced changes of negative emotionality during the experience of social exclusion. However, limbic
361 system activity during social exclusion was significantly increased in REM sleep suppressed participants
362 pointing to a possible neural mechanism linking REM sleep disturbances and affect. Further
363 investigations of the underlying mechanisms of REM sleep and emotional dysregulation in social

364 situations will pave the way for a better understanding of the role of sleep disturbance in psychiatric

365 disorders.

366 **Methods**

367 **Participants**

368 A total of 45 participants were initially invited to take part in the experiment (29 female, age (years):
369 *M*=23.69, *SD*=2.67), gave written informed consent prior to participation in the study and received
370 financial compensation for their participation. All participants were recruited at Philipps-University
371 Marburg, were fluent in German, and had normal or corrected-to-normal vision. None of them were
372 diagnosed with neurological or psychiatric disorders (present and past), current alcohol or drug abuse,
373 use of psychiatric medications (present and past), anatomical brain abnormalities (e.g. lesions, strokes
374 etc.), or sleep disturbances. Due to technical issues with the polysomnographic recording in the
375 experimental night (n=1) or insufficient quality of the fMRI data (n=2), 3 subjects were not included in
376 the final analyses. The final sample thus consisted of 42 participants (27 female, age (years): *M*=23.76,
377 *SD*=2.74). Participants were randomly assigned to one of three groups, which differed regarding to the
378 sleep protocol in the experimental night (i.e. either REMS, SWSS or CTL, details below). Groups did not
379 differ in age (CTL: *M*=24.00 years, *SD*=3.12; REMS: *M*=23.06, *SD*=2.22; SWSS: *M*=24.60, *SD*=2.91;
380 $F(2,39)=1.09$, $p=.346$) or gender (CTL: 8 female, 7 male; REMS: 12 female, 5 male; SWSS: 7 female, 3
381 male; $X^2(2,42)=1.22$, $p=.543$).

382 **Sleep Manipulation Procedure**

383 Participants came to the laboratory for two consecutive nights – one habituation night and a subsequent
384 experimental night (see figure 1). The purpose of the habituation night was to exclude sleep disorders,
385 make participants familiar with the polysomnography (PSG) recording procedure and adapt to the
386 environment in the sleep laboratory. The morning after the experimental night, participants were taken
387 to the fMRI facility where they completed the experimental task in the scanner (see Experimental task
388 below). During both nights PSG was recorded to monitor and identify sleep phases (see table 1). A

389 maximum of three participants was invited to the sleep laboratory on each night. Upon arrival on the
390 habituation night, participants were informed about the procedure of the study and gave written
391 informed consent. After the habituation night participants could spend the day as usual but were asked
392 to refrain from napping, smoking and consuming stimulating foods and drinks (e.g. coffee, tea, energy
393 drinks). On the subsequent night, participants entered a fully equipped single sleep room for PSG (Embla
394 N7000 PSG, TNI-Medical, Würzburg, Germany) consisting of electroencephalography (EEG),
395 electrooculogram (EOG), electromyogram of the mentalis muscle (EMG) and pulse oximetry using EMBLA
396 N7000 (TNI-Medical). The EEG system had 9 electrodes positioned according to the 10-20 system,
397 adhering to the guidelines of the American Academy Of Sleep Medicine (AASM)[90], and placed on the
398 following positions: F3, F4, C3, Cz, C4, O1, O2, M1 and M2. Limb movements were videotaped with
399 infrared camera. The experimenter turned off the lights at 10 pm and asked participants to try to fall
400 asleep. In case a deterioration of the PSG recordings was observed, the experimenter entered the sleep
401 room to improve the signal by reattaching the respective electrodes. At 6 am, lights were turned on,
402 participants were woken up and the electrodes were removed.

403 For those participants in the REMS and SWSS group, during the second night (i.e. the
404 experimental night), the experimenter started an acoustic beep (80 dB, 500 Hz, 500ms) [91] once the
405 polysomnographic trajectories indicated that the participant had entered the target sleep phase. After
406 an awakening, participants were kept awake for 90 seconds. In case the participant did not wake up, the
407 volume of the acoustic beep was increased, and ultimately the experimenter entered the participant's
408 room to turn on the lights to make sure the target sleep phase was interrupted. To control for the
409 number and lengths of awakenings, both suppression groups were disturbed similarly often during the
410 experimental night. In the REMS group, participants' sleep was disturbed as soon as they entered the
411 REM sleep stage. Awakenings for the SWSS group were selectively carried out during the non-REM sleep
412 stage N3, defined according to AASM guidelines [90]. The control subjects were not awakened at all. PSG

413 recordings were scored by an experienced sleep technician (C.L.) at the sleep laboratory at the
414 Department of Otorhinolaryngology at the University of Lübeck according to AASM guidelines [90] and
415 using Somnologica 3.3.1 (Build 1529). The technician was blinded for group assignments and hypotheses.

416 **General Affect Ratings**

417 The PANAS [50] was completed shortly before going to bed and after waking up on both nights by all
418 participants to assess the effect of sleep manipulation on general positive and negative affect.

419 **Experimental Task**

420 Upon waking up after the experimental night, participants were accompanied to the functional magnetic
421 resonance imaging (fMRI) facility. In the MRI, participants were instructed via a screen that they were
422 about to play a ball-tossing game, i.e. the *Cyberball* task, allegedly with two other persons (see Figure 1;
423 a second paradigm was presented to the subjects afterwards, which involved IAPS pictures to study
424 regulation of basic emotions but is not further described in the present manuscript). All three players,
425 including the participant, were represented by avatars, i.e. green, 3D-animated stick-figures standing in a
426 triangle on a lawn (for a detailed description of the animation see [92]). Participants were able to control
427 the avatar on the bottom edge of the screen, while the other two avatars were placed on the left and
428 right of the horizontal midline of the screen. The names of the participant and of the other two players
429 were displayed next to the respective avatar. For each throw, a short sentence on the bottom of the
430 screen indicated who threw the ball to whom. When one of the computer avatars had the ball, they
431 waited a random amount of time between 1000 and 2000 ms before tossing the ball. When the
432 participant had the ball, they had 2000 ms to decide where to toss the ball. In case they responded too
433 slowly, the computer randomly selected which of the other avatars would receive the ball. The duration
434 of each throw was 750 ms and was visualized by a series of 40 frames, showing an avatar doing a
435 throwing movement and the red ball flying from one avatar to the catching avatar, with the latter
436 moving its arm to catch the ball. While participants believed that the other avatars were controlled by

437 two other anonymous persons sitting in adjacent rooms, their behavior in fact followed a predefined
438 script (see below). Following the instructions, a short sequence of lines of text built up on the screen,
439 making the subjects believe that the computer was being connected to a gaming server of the university
440 on which the experiment was run. This included the request of entering an IP-address, as well as lines
441 stating how many players were logged into the game. Subsequently, participants performed a short
442 training session consisting of seven trials in which they received and threw the ball three times.

443 The task then consisted of two sessions, each of which consisted of four experimental blocks.
444 Before each of the experimental blocks, a line of text was presented on-screen for 1500 ms telling the
445 participant that a new round would start. In every block, a maximum number of 24 ball tosses were
446 completed. In two blocks of every session, the participant was included in the game and repeatedly
447 received the ball throughout the entire block (inclusion blocks, INC), having the possibility to toss the ball
448 to one of the other avatars eight or nine times by clicking a button using the index finger (left player) or
449 middle finger (right player). In the other two blocks (exclusion blocks, EXC), after the participant had
450 received the ball three or four times, the paradigm was programmed so that the two avatars only tossed
451 the ball between one another, thereby effectively excluding the participant from the game.

452 After having completed four blocks during which subjects simply participated in the task without
453 additional instructions (VIEW session), a second session of the task was played. For the second session, a
454 short on-screen text instructed participants to use cognitive reappraisal to regulate their emotions
455 during the game (CRA session; see Fig. 1). Participants were specifically asked to reappraise the situation,
456 in case any negative emotions should arise ("In case negative emotions arise, please try to re-evaluate
457 the situation"). This was accompanied by the instruction to "partake in the game and try to visualize the
458 situation as vividly as possible", which was also presented before the first session. Subjects were asked
459 to press the left button as soon as they were ready, and then a short break varying randomly between

1500 and 2500 ms was presented, instructing participants to wait until the other two players had indicated that they were ready. After presentation of a fixation cross for one second, the block started.

In each session, two inclusion and two exclusion blocks were presented to the subjects, with the first block of each session always being an inclusion block. In one session, inclusion and exclusion blocks were alternating, while in the other session, the initial inclusion block was followed by two consecutive exclusion blocks and a final inclusion block. The assignment of block order to the VIEW and the CRA sessions was counterbalanced across subjects. On average, EXC blocks lasted 50.93 seconds (*SD*=0.62), and INC blocks lasted 45.87 seconds (*SD*=2.21).

Between blocks, participants were asked to rate how they felt about the preceding block regarding the extent to which they felt socially excluded. The low end of the scale was labelled as rejected/despised and the high end was labelled as accepted/familiar. In addition, subjects rated their sadness, anger, and shame, but these ratings were intended to distract from the purpose of the study and not analyzed (sadness was described by: sad, downcast, gloomy; for anger: angry, irritated, furious, mad; for shame: abashed, embarrassed). Each rating was displayed using a 9-point Likert-type scale ranging from 0 (not at all) to 8 (very much). Every rating was initialized at 4 (i.e. a neutral rating), and participants used presses of the right or left response buttons to move the rating to higher or lower values, respectively. The time for ratings was limited to 4 seconds. After each rating and the start of the next block within a session there was a short pause, jittered between 4.3 to 5.3 seconds. Emotion ratings for each condition (EXC, INC) and emotion regulation session (VIEW, CRA) were averaged for each participant and analyzed using repeated measures analyses of variance (rmANOVA).

Statistical Analysis

Average ratings of feeling excluded for each condition (EXC, INC) and session (VIEW, CRA), as well as the percentages of the different sleep stages for the habituation and experimental nights were analyzed

483 using analyses of variance (ANOVA). Significant interactions and main effects were followed up using
484 paired comparisons. Since we expected that selective REMS would specifically alter affective experience
485 and associated neural responses [22,23], we furthermore performed an a priori planned contrast of the
486 REMS group against both control groups, the CTL and SWSS. The alpha-level was set to .05, and was
487 adjusted using Bonferroni-correction, in case multiple tests were performed.

488 **FMRI Data Acquisition and Preprocessing**

489 For each of the two experimental sessions, 130 functional volumes were recorded at 3T (Siemens Trio,
490 Erlangen), of which the first three were discarded to allow for equilibration of T1 saturation effects.
491 Functional volumes consisted of 36 ascending near-axial slices (voxel size=3*3*3 mm, 10% interslice gap,
492 FOV=192 mm) and were recorded with TR=2200 ms, TE=30 ms, FA=90°. In addition, a high-resolution
493 anatomical T1 image was recorded consisting of 176 slices (voxel size=1*1*1 mm, FOV=256 mm,
494 TR=1900 ms, TE=2.52 ms, 9° FA).

495 The MRI data were analyzed using SPM12 in Matlab 2019b. Functional MRI images from each
496 session of the *Cyberball* paradigm were slice-time corrected to the middle slice and spatially realigned.
497 Subsequently, spatial normalization to MNI space was performed using unified segmentation [93] by
498 estimating the forward deformation fields from the mean functional image and applying these to the
499 realigned functional images. These spatially normalized images were then resliced to a voxel size of
500 2*2*2 mm and smoothed with an 8 mm full-width at half-maximum isotropic Gaussian kernel and high-
501 pass filtered at 1/256 Hz.

502 **FMRI Data Analysis**

503 The preprocessed functional images were statistically analyzed by a two-level mixed effects GLM
504 procedure. For each participant, a statistical model was specified, including data from both experimental
505 sessions (VIEW, CRA). For each session, the INC, EXC and rating phases were modelled as regressors of

interest and the six realignment parameters estimated during spatial realignment were included to account for variance in the functional data that was due to head motion. The contrast images obtained from the individual participants were then aggregated in a random effects model on the second level to test for effects of condition and session, and to test for differences between the three experimental groups. To disentangle significant interaction effects, we ran a series of post-hoc tests on the parameter estimates extracted from the peak activation, applying Bonferroni-correction for multiple comparisons.

Regions of Interest (ROI) for FMRI Analysis

In order to focus our analyses on the limbic system as well as the insula, regions commonly associated with affective processing [94], we constructed an a priori mask using the Wake Forest University Pickatlas (v. 2.4)[95]. This mask comprised the anterior cingulate cortex, bilateral insula, bilateral hippocampus, as well as left and right amygdala.

Author Contributions

Prepared and conceived the study: W.C., K.K., U.K., S.K., S.W.; Collected the data: S.K., F.M.P., S.W; Analyzed the fMRI and behavioral data: R.G., F.M.P., L.M.P., S.K., D.S.S.; Analyzed the PSG data: A.S.; Interpreted the data and wrote the manuscript: S.B., S.D., R.G., S.K., F.M.P., L.M.P., D.S.S., I.W.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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720 **Figures captions**

721 **Figure 1.** Summary of the experimental procedure. Three groups of subjects spent two consecutive nights in the sleep
722 laboratory. During both nights, polysomnography was recorded. In the second night, two groups were woken up as
723 soon as they entered REM sleep or SWS (groups REMS and SWSS, respectively), while the control group was not
724 woken up (CTL). After waking up on the second morning, all subjects performed two sessions of the *Cyberball* game
725 while inside the fMRI scanner [92]. The game included a total of eight blocks, with four inclusion (INC) and four
726 exclusion (EXC) blocks. During inclusion, subjects received the ball continuously throughout the block, while during
727 exclusion, the other two players stopped throwing the ball to the participant soon after the beginning of the block,
728 effectively excluding the participant from the game. Additionally, during the first four blocks (half INC, half EXC),
729 subjects simply performed the task (VIEW), while during the second four blocks (half INC, half EXC) they were
730 instructed to use cognitive reappraisal (CRA) to regulate their emotions.

731

732 **Figure 2.** Selective sleep suppression and general affect. **a** REM sleep was significantly more reduced in the REMS
733 group than in the other two groups, whereas SWS suppression was significantly stronger in the SWSS group than in
734 the other two groups. Suppression scores for REM (SWS) sleep are the difference of percentage REM (SWS) of TST
735 between the habituation night minus the experimental night. **b** Ratings of general positive and negative affect,
736 measured with the Positive And Negative Affect Scale (PANAS)[50] on the mornings after the habituation night and
737 after the experimental night, separately for the three groups. **c** Changes in negative (right) but not positive affect
738 (left) from the habituation night to the experimental night correlate significantly with REM sleep suppression scores
739 across all groups. Shaded areas represent 95% confidence intervals for simple bivariate regression. Error bars
740 represent standard error of the mean. n.s.=not significant.

741

742 **Figure 3.** Neural responses to ostracism are altered by selective REM sleep suppression. **Left** Neural responses for
743 the contrast [EXC/VIEW>INC/VIEW] > [EXC/CRA>INC/CRA] differed significantly between the REMS group and the
744 other two groups in the right amygdala, (peak MNI-coordinates: x=26, y=-2, z=-28, surviving FWE-correction at $p<.05$
745 inside the a priori mask). Displayed at $p<.005$, uncorrected, for visualization purposes. **Right** Parameter estimates for
746 the contrast EXC>INC in the VIEW session were positive in the REMS group, but did not deviate from 0 in the other
747 two groups, nor in the CRA session for any of the three groups. In addition, parameter estimates for EXC>INC were
748 significantly more positive in the REMS group during VIEW than during CRA, while sessions did not differ in the other
749 two groups. See main text for details. ***: $p<.001$. Error bars represent standard error of the mean.

750

751

Tables

Table 1. Sleep Measures

	CTL				REMS				SWSS			
	Habituation night		Experimental night		Habituation night		Experimental night		Habituation night		Experimental night	
n subjects with valid PSG dataset	15		15		16		17		9		10	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
TST (min)	369.94	78.06	414.59	26.08	410.86	40.24	399.05	39.02	384.63	54.15	402.96	31.28
N1 (% TST)	1.87	2.76	0.58	0.96	0.60	0.65	1.72	2.54	2.47	2.57	1.18	1.39
N2 (% TST)	61.82	7.27	61.11	8.53	64.83	4.69	68.66	7.62	65.68	8.44	69.33	8.29
SWS (% TST)	17.11	6.21	18.04	7.92	14.18	6.88	16.86	5.62	13.98	7.43	9.83	4.82
REM (% TST)	19.21	6.33	20.27	4.39	20.39	5.86	12.76	6.16	17.42	6.56	19.65	8.87
WASO (min)	67.16	50.46	51.06	29.00	47.01	40.18	71.10	42.66	57.57	38.50	61.63	37.96
WASO/(TST+WASO)	0.16	0.13	0.11	0.06	0.10	0.09	0.15	0.09	0.13	0.10	0.13	0.08
Awakenings	8.13	3.09	9.00	4.21	7.75	3.66	11.47	4.90	11.11	6.64	15.00	6.68
REM latency (min)	142.97	68.32	106.10	51.71	101.19	43.15	154.12	93.11	137.78	86.27	123.35	82.01

Note. In both the REMS group and the SWSS group, PSG data quality from one subject was not suitable for analysis in the habituation night. CTL=Control group with undisturbed sleep; REMS=rapid eye movement sleep suppression group; SWSS=slow wave sleep suppression group; TST=total sleep time (time from sleep onset to morning awakening after the time awake during the night is subtracted); N1=Sleep stage 1, N2=Sleep stage 2, SWS=Sleep stage N3; REM=rapid eye movement sleep; WASO=wakefulness after sleep onset to morning awakening; *M*=Mean; *SD*=Standard Deviation

Table 2. Regression of affective changes on sleep deprivation parameters (dependent variable: change in negative affect (Δ Experimental morning – Habituation morning))

	B	*SE*	*β*	*t*	*p*	sign.
(Intercept)	0.098	0.060		1.64	.110	n.s.
REM sleep suppression	0.020	0.008	0.406	2.60	.014	*
SWS sleep suppression	-0.000	0.008	-0.002	-0.01	.989	n.s.
TST suppression	0.002	0.002	0.404	1.60	.118	n.s.
WASO increase	0.005	0.002	0.638	2.95	.006	**

Note. n.s.: not significant; *: $p<.05$; **: $p<.01$

754

Table 3. Feelings of being excluded, separately for groups and conditions

	EXC/VIEW		INC/VIEW		EXC/CRA		INC/CRA	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
CTL	6.67	1.92	2.20	1.25	*3.67*	*2.50*	1.50	0.78
REMS	6.38	2.90	2.00	1.21	*2.82*	*2.33*	1.62	0.86
SWSS	5.90	2.49	2.25	0.98	*2.60*	*1.51*	1.75	1.46