



Attachment and dream emotions: The mediating role of trait anxiety and depression

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Abstract

Although associations of attachment insecurity and sleep quality have gained major interest in recent literature, little attention has been given to dream content in this regard. The aim of this study was twofold: 1) to examine whether a relationship exists between attachment and the emotional tone of dreams (positive vs. negative); and 2) to investigate the potential mediator role of trait anxiety and depression between attachment and dream emotions. The sample consisted of 1661 university students (68.5% male; M_{age} : 20.48 years). Attachment characteristics were assessed by the Relationship Scales Questionnaire, dream emotions were measured by the Dream Quality Questionnaire, while trait anxiety and depression were assessed using the Depression and Anxiety facets of the Revised NEO Personality Inventory. The results indicated that attachment anxiety and attachment style (secure, preoccupied, dismissing, and fearful) were associated with both negative and positive emotions in dreams even when controlling for sociodemographics and that these relationships were mainly mediated by general anxiety and depression proneness. However, attachment avoidance was not related with either dream quality indicator and dream recall frequency was unrelated with categorical attachment style. Major limitations of the study include the low ratio of females in the sample and the use of retrospective assessment methods in the measurement of dream content and dream recall frequency. Nevertheless, the results could provide new perspectives on the role of attachment in dreaming and might suggest that the consideration of attachment could help us better understand not only attachment- but nightmare-, mood- and anxiety disorders as well.

Keywords Attachment · Dream emotions · Sleep · Anxiety · Depression

Introduction

Attachment theory encompasses a model on the development of the relationship with a primary caregiver in infancy and its consequences on the patterns of close interpersonal relationships throughout the lifespan (Bowlby 1988/2005; Bretherton 1985; Main et al. 1985). One of the most impactful typologies describing different adult attachment patterns was developed

by Bartholomew and colleagues (1991). This model proposes one secure and three insecure distinct attachment styles and two underlying factors based on the internal representation of the self (worthy vs. unworthy of love and attention) and others (trustworthy, caring and available vs. rejecting and distant).

Individuals with secure attachment style can be characterized by a sense of self-worth and an expectation that others are usually responsive and caring, while those with insecure attachment are in doubt regarding the value of their own self and/or the trustworthiness of others. Individuals with ‘preoccupied’ attachment – one of the three insecure attachment styles – attempt to improve their low self-esteem by gaining the approval of valued others, while those with ‘fearful’ attachment also have a low level of perceived self-worth but in their case, it is combined with the expectation that others will reject them, which in turn leads to a pattern of overall avoidance of close relationships. Finally, individuals with ‘dismissing’ attachment style possess a positive self-image combined with an expectation that others will reject them, which leads them to reject others while valuing their own independence (Wilhelm et al. 2016).

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It is worthy of note though, that many researchers prefer the use of a dimensional over a categorical approach when describing adult attachment. The two underlying dimensions in these models are attachment avoidance (discomfort with closeness and being dependent on others) and attachment anxiety (fear of rejection and abandonment) (Brennan et al. 1998).

Research has shown that attachment characteristics modulate many biopsychosocial phenomena including stress response (Diamond 2001; Gunnar et al. 1996), psychological well-being (Bódizs et al. 2008; Maunder and Hunter 2001; Troxel et al. 2007), health behaviors (Maunder et al. 2011), or social functioning (Bretherton 1992; Main et al. 1985). Further, a systematic review on the relationship between individual attachment style and sleep characteristics concludes that the literature suggests that secure attachment is associated with better sleep quality than insecure attachment styles (Adams et al. 2014). Empirical research in the field has also revealed that sleep difficulties both in children (Keller and El-Sheikh 2011) and adults (Maunder et al. 2011; Troxel et al. 2007), as well as in the elderly (Niko Verdecias et al. 2009), are associated with insecure attachment. For instance, insecure/ambivalent infants (equivalent to the preoccupied style in adults) at six months of age showed the most sleep disturbances (measured by maternally-reported night awakening times and durations of night waking episodes), while their avoidant counterparts were reported to have the least sleep problems (McNamara et al. 2003). In a study of US adults, researchers found that attachment anxiety was associated with higher levels of self-reported sleep difficulties for both men and women (Carmichael and Reis 2005). Results of two other studies also showed that preoccupied attachment was related to self-reported impairment of sleep (Scharfe and Eldredge 2001; Niko Verdecias et al. 2009). Objective measures of sleep quality were also found to be in connection with attachment security; more specifically, alpha intrusion in NREM sleep – which is thought to be a marker of hyperarousal – was positively associated with attachment anxiety (Sloan et al. 2007).

The content of dreams has also been linked to sleep quality: studies have shown that those reporting more negative dream content were also more likely to struggle with sleep difficulties (Schredl et al. 1998; Schredl 1998; Simor et al. 2012). In addition, some of the examinations of dream content and narratives suggest that daytime emotional states and relationship patterns appear vividly and frequently in the dreams of adults (Cartwright et al. 2006; Hartmann 2010; Nielsen et al. 2006). Although, the relationship between adult romantic bonding and dreaming was investigated in many settings (Selterman et al. 2012; Selterman et al. 2014), we are only aware of a very limited number of studies directly examining the relationship between general (not specific to intimate relationships) attachment characteristics and dream content.

Mikulincer and colleagues found that attachment anxiety was associated with more closeness-related wishes in dreams (Mikulincer et al. 2011), while findings of another study revealed that those scoring higher on indicators of avoidant and anxious-ambivalent attachment (equivalent to the preoccupied style) reported more stress, conflict, and anxiety in dreams related to their romantic partner (Selterman and Drigotas 2009). Finally, McNamara and colleagues found that intimacy content (not limited to romantic partners) in dreams was lower in individuals with avoidant attachment style than in their counterparts with secure attachment (McNamara et al. 2014).

Keller reviews four potential models to explain the relationship between attachment and sleep characteristics, namely: 1) sleep disruptions considered as manifestations of attachment behavior, 2) attachment insecurity considered as a predictor of sleep disruptions, 3) sleep disruptions considered as predictors of attachment insecurity, and 4) both sleep and attachment characteristics understood as consequences of parenting (Keller 2011). Other researchers examined potential mediators between attachment and sleep characteristics and proposed daytime emotional states as links between the two constructs (Lee et al. 2007; Gunnar et al. 1996; Kirschbaum et al. 1995). A Canadian study found for instance that attachment avoidance and attachment anxiety were associated with both subjective measures of sleep and depressive symptomatology (Maunder et al. 2011). Anxiety with its physiological correlates is also a good candidate to at least partially explain the link between attachment and sleep difficulties. Cortisol reactivity, a key element of the operation of the hypothalamic-pituitary-adrenal axis, for instance is an important element of both sleep modulation (Buckley and Schatzberg 2005; Rodenbeck et al. 2002) and the psychophysiological correlates of anxiety (Yehuda et al. 1996; O'Donovan et al. 2010). Moreover, animal research in rhesus monkeys suggests that higher levels of cortisol during the day are associated with poorer sleep quality in nursery-raised animals compared with maternally-raised ones (Barrett et al. 2009). Similar findings were reported regarding sleep difficulties and increased cortisol levels in human children (El-Sheikh et al. 2008).

As depression and anxiety were also linked to both attachment (Besser and Priel 2008; Marganska et al. 2013; Tasca et al. 2009) and dream characteristics such as frequency of nightmares, nightmare distress (trait-like level of distress in waking life caused by nightmares) or negative dream affect (Levin and Fireman 2002; Nielsen et al. 2000), it is feasible to assume that daytime anxiety and depression might mediate the attachment-dream quality relationship as well (not only the one between attachment and sleep quality). Therefore, the aim of the present study was two-fold: 1) to explore the interrelationships between the overall emotional tone of dreams (positive vs. negative) and attachment characteristics, an area

that is largely neglected in the literature, and 2) to investigate whether trait anxiety and depression mediate the potential relationship between dream emotions and attachment.

Methods

Sample and Procedure

The study protocol has been approved by the Research Ethics Board of the Department of Cognitive Sciences, Budapest University of Technology and Economics. Informed consent was obtained from participants, all of whom were informed that the aim of the study was to investigate the interrelationships between sleep, dreaming, and personality. Respondents ($N = 1661$; 68.5% males) were recruited from different undergraduate introductory psychology courses and received partial credit points for completing the online questionnaires. Participant age varied between 17 and 40 years ($M = 20.48$, $SD = 1.97$). Most respondents were single (57.0%) or in an intimate relationship with or without cohabitation (37.8%) and only a small minority reported to be married (1.1%) or divorced (4.1%).

Measures

Attachment was assessed by the Relationship Scales Questionnaire [RSQ; (Bartholomew and Horowitz 1991)], which considers two main dimensions: attachment anxiety (negative sense of self) and attachment avoidance (negative sense of others). Attachment anxiety is associated with an expectation of abandonment, insufficient love, preoccupation with the responsiveness of others, and hyperactivation of attachment behaviors. Attachment avoidance can be described as the devaluation of close relationships, the low toleration of intimacy and dependence, and the relative deactivation of attachment behaviors. Since the different cultural adaptations of the scale (and the empirical data related to their psychometric properties) indicate that the item content and reference values vary largely (Andersen et al. 2017; Siegert et al. 1995), the procedures and standards developed for the Hungarian population were used (Csóka et al. 2007). According to these empirically derived guidelines, four items were used to assess attachment avoidance (Cronbach's alpha: 0.81) and three questions were considered when calculating attachment anxiety (Cronbach's alpha: 0.69).

A categorical conceptualization of attachment based on attachment anxiety and attachment avoidance has also been proposed by the original authors of the RSQ (Bartholomew and Horowitz 1991). Therefore, the two scale scores in the present study were also transformed into four attachment categories: preoccupied (above-median anxiety and below-median avoidance), secure (below-median anxiety and

avoidance), dismissing (below-median anxiety and above-median avoidance), and fearful (above-median anxiety and avoidance).

The Dream Quality Questionnaire [DQQ; (Bódizs et al. 2008)] was used to assess the general emotional load of dreams. This assessment tool also captures the tendency to have recurrent and non-recurrent nightmares, night terror-like symptoms, the vividness and bizarreness of dreams, and the effect of dreams on daytime mood. Response options vary across items and for space considerations these are not described in detail here; instead, the interested reader should consult the original publication on the instrument, which is freely available on the publisher's website (www.ejmh.eu/mellekletek/2008_1_035_Bodizs_etal.pdf). In the present sample, internal consistency (Cronbach's alpha) of the Positive (2 items) and the Negative Dream Affect (6 items) Subscale was 0.73 and 0.59, respectively. Dream recall frequency was assessed by the question "On average, how often do you remember your dreams?" The seven answer options ranged from never (=1) to almost every day (=7).

As the attachment variables are conceptualized as trait-rather than state-like characteristics, similar trait-type depression and anxiety measures have been chosen for the purposes of our study. To assess general (non-attachment-specific) anxiety and depression proneness, facets from the Neuroticism scale of the NEO-PI-R personality questionnaire (Costa and MacCrae 1992; Fruyt et al. 2004) were used. In the present sample, both eight-item scales proved to have good internal consistency (Cronbach's alpha for both scales: 0.77).

Statistical Analyses

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows version 23. Given the non-normal distribution of the continuous variables according to the Shapiro-Wilk test, non-parametric statistical methods were used to investigate their relationship with each other (Spearman correlation coefficients) or with categorical study variables (Kruskal-Wallis and Mann-Whitney U test) when conducting the bivariate analyses.

Given the interrelationships between sex, age, marital status, and dream emotions (Nielsen et al. 2000; Nielsen et al. 2006; Schredl 2001), these sociodemographic variables were controlled for during the multivariate analyses. First, the interrelationships between the predictors (attachment indicators) and the potential mediators (trait anxiety and depression) as well as the connections between the outcome variables (dream emotions) and the potential mediators were tested using the general linear model procedure to investigate the prerequisites of a formal mediation analysis (Baron and Kenny 1986). In the next phase, the analytic plan included separate general linear models for each dream emotion indicator. In each case, two models were tested: in the first one, only the attachment

indicators were entered into the model along with the sociodemographic covariates. In the second step – to investigate mediation – trait anxiety and depression were entered into the models.

Results

Bivariate analyses revealed that attachment anxiety was significantly related to both negative ($r = 0.180$,

$p < 0.001$) and positive ($r = -0.077$, $p = 0.002$) dream affect in the expected directions. In contrast, attachment avoidance was not associated with any of the dream affect indicators ($r_{\text{negative dream affect}} = 0.012$, $p = 0.624$; $r_{\text{positive dream affect}} = -0.011$, $p = 0.654$); therefore, this variable was excluded from the multivariate analyses. When investigating the relationship between dream emotions and distinct attachment categories, the data indicated that both negative (Kruskal Wallis $\chi^2 = 40.165$, $p < 0.001$) and positive (Kruskal Wallis $\chi^2 = 12.137$, $p = 0.007$)

Fig. 1 Negative and positive dream affect as functions of attachment categories (means with 95% confidence intervals)

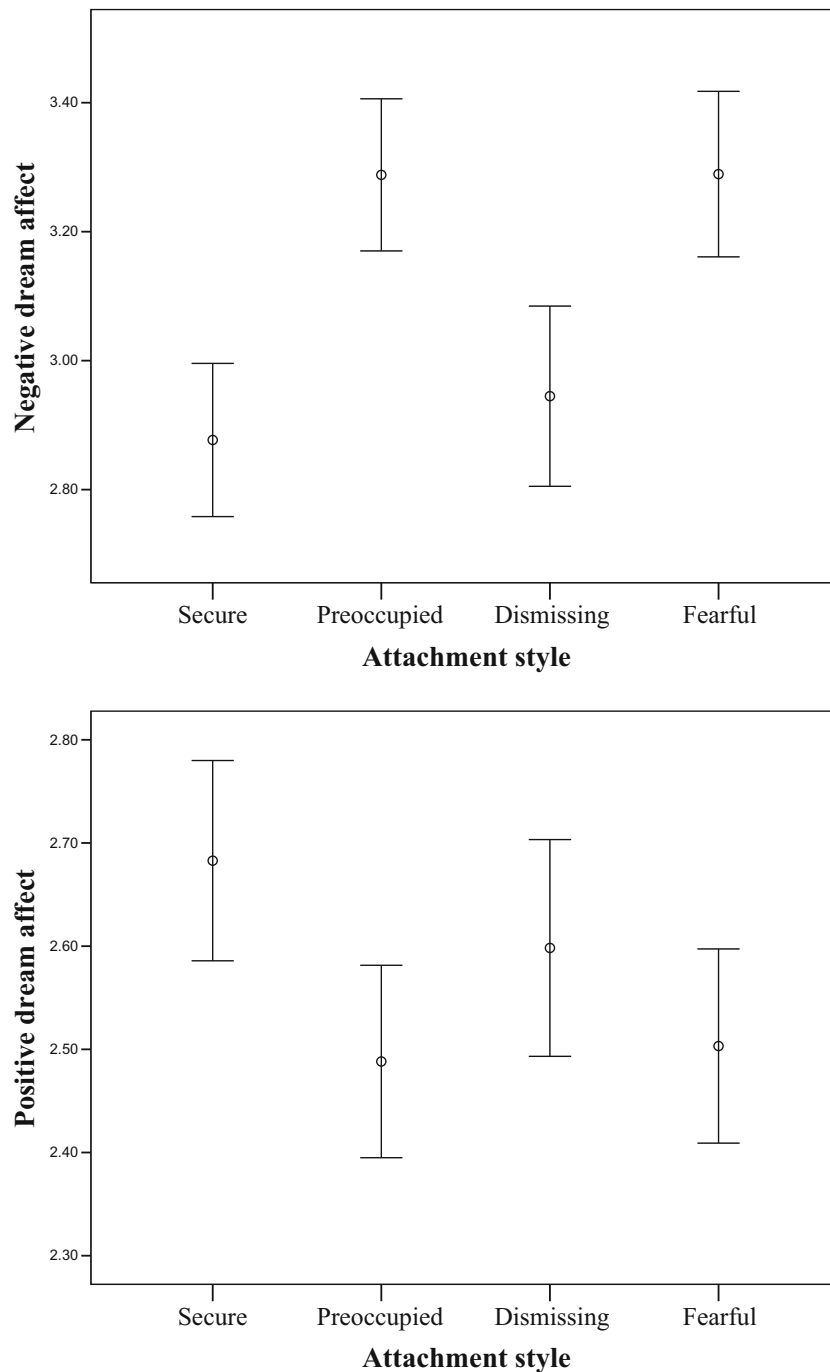


Table 1 Results of the post hoc analyses regarding the relationships between dream affect and attachment categories

Negative dream affect		Positive dream affect	
Secure vs.	Preoccupied: $U = 74,637, p < 0.001$ Dismissing: $U = 75,303, p = 0.732$ Fearful: $U = 69,701, p < 0.001$	Secure vs.	Preoccupied: $U = 82,399.5, p = 0.004$ Dismissing: $U = 74,125, p = 0.462$ Fearful: $U = 76,093.5, p = 0.006$
Preoccupied vs.	Dismissing: $U = 72,349, p < 0.001$ Fearful: $U = 96,406.5, p = 0.726$	Preoccupied vs.	Dismissing: $U = 80,724.5, p = 0.044$ Fearful: $U = 97,176, p = 0.880$
Dismissing vs.	Fearful: $U = 67,609, p < 0.001$	Dismissing vs.	Fearful: $U = 74,513.5, p = 0.058$

dream emotions were significantly related to attachment style. In line with the theoretically plausible assumptions, secure attachment style was associated with the highest level of positive dream emotions, followed by the other categories; namely fearful, dismissing, and preoccupied attachment style (Fig. 1). Similarly, secure attachment style was associated with the lowest level of negative dream emotions, followed by the other categories of fearful, dismissing, and preoccupied attachment. Detailed pairwise comparisons of the attachment categories along dream affect are displayed in Table 1. In terms of dream recall frequency, no significant relationship (Kruskal Wallis $\chi^2 = 2.358, p = 0.502$) was observed between this and the categorical attachment variable (Fig. 2).

When investigating the prerequisites of a formal mediation analysis, attachment anxiety – when controlling for sex, age, and marital status – was predictive of both trait anxiety ($F = 228.02, p < 0.001$) and depression ($F = 511.89, p < 0.001$). The same was true for categorical attachment style (anxiety: $F = 62.39, p < 0.001$; depression: $F = 103.11, p < 0.001$). In terms of the relationship

between the outcome variables and general anxiety and depression proneness, both negative (anxiety: $F = 248.77, p < 0.001$; depression: $F = 190.03, p < 0.001$) and positive dream emotions (anxiety: $F = 66.39, p < 0.001$; depression: $F = 74.89, p < 0.001$) were significantly predicted by the potential mediators when controlling their relationship for sex, age, and marital status.

The next phase of the analyses showed (Table 2) that attachment anxiety was a significant predictor of both positive and negative dream emotions when controlling only for the sociodemographic characteristics (Model 1). However, when entering trait anxiety and depression into the model, these predictors were significant, while attachment anxiety lost its predictive power for both dream affect indicators. A similar pattern could be observed when investigating the role of distinct attachment categories in predicting dream emotions (Tables 3 and 4). Namely, attachment style was significantly associated with both negative and positive dream emotions when controlling only for the sociodemographic covariates but it failed to significantly predict the outcome variables

Fig. 2 Dream recall frequency as a function of attachment categories (means with 95% confidence intervals)

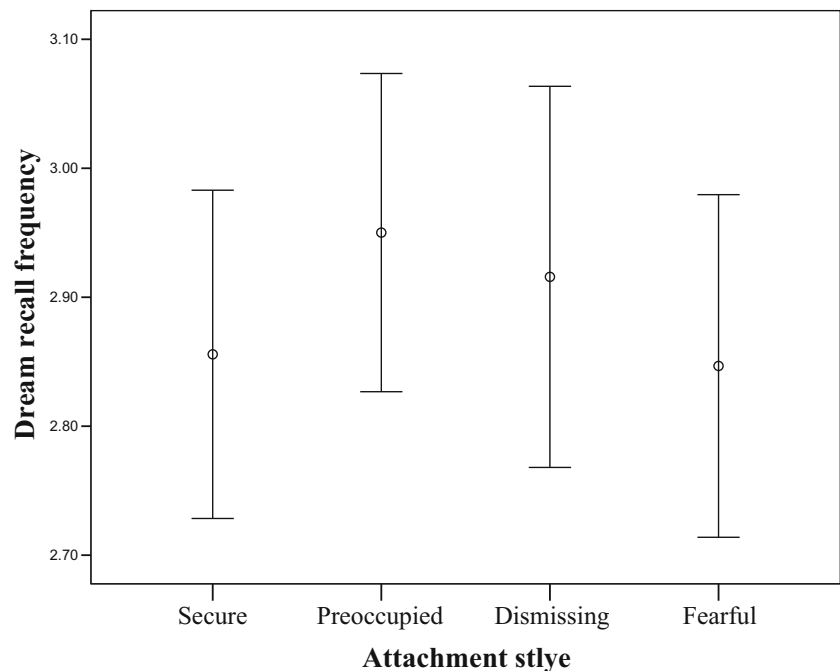


Table 2 The role of attachment anxiety in predicting negative and positive dream emotions

	Model 1			Model 2		
	F	<i>p</i>	Partial eta squared	F	<i>p</i>	Partial eta squared
Negative dream affect						
Sex	114.88	<0.001	0.065	44.14	<0.001	0.026
Age	5.43	0.020	0.003	3.73	0.054	0.002
Marital status	0.40	0.755	0.001	0.80	0.495	0.001
Attachment anxiety	54.07	<0.001	0.032	0.16	0.685	<0.001
Trait anxiety				83.97	<0.001	0.048
Trait depression				25.59	<0.001	0.015
Model	31.99	<0.001	0.104	55.13	<0.001	0.211
Positive Dream Affect						
Sex	0.02	0.884	<0.001	3.77	0.052	0.002
Age	7.41	0.007	0.004	6.01	0.014	0.004
Marital status	2.15	0.092	0.004	2.11	0.097	0.004
Attachment anxiety	8.11	0.004	0.005	3.17	0.075	0.002
Trait anxiety				14.43	<0.001	0.009
Trait depression				24.91	<0.001	0.015
Model	3.83	<0.001	0.014	13.48	<0.001	0.061

when entering trait anxiety and depression into the model.

Discussion

McNamara and colleagues suggest that REM sleep and dreams may influence and promote attachment, especially among insecurely attached individuals (McNamara 1996; McNamara et al. 2002). These authors reported reduced REM latency in individuals with insecure attachment styles (McNamara et al. 2011) and more frequent dream recall, and more intense dream emotions in insecurely attached university

students, but did not find differences in directly attachment-related dream content between securely and insecurely attached groups (McNamara et al. 2001). The aim of the present study was to examine whether a relationship exists between attachment characteristics and general (non-attachment-related) dream emotions, and to test whether this association was mediated by trait anxiety and depression.

Bivariate results showed that while attachment anxiety was significantly associated with dream emotions, attachment avoidance was not, which falls in line with previous findings indicating, for instance, that some objective measures of sleep quality (e.g., alpha intrusion in NREM sleep, a marker of hyperarousal during sleep) were associated with attachment

Table 3 The role of distinct attachment styles in predicting negative dream emotions

	Model 1				Model 2			
	F	t	<i>p</i>	Partial eta squared	F	t	<i>p</i>	Partial eta squared
Sex	115.29	–	<0.001	0.065	42.90	–	<0.001	0.025
Age	5.27	–	0.022	0.003	3.29	–	0.070	0.002
Marital status	0.45	–	0.718	0.001	0.71	–	0.545	0.001
Attachment style (omnibus)	12.86	–	<0.001	0.023	1.32	–	0.265	0.002
Fearful vs. secure	–	4.45	<0.001	0.012	–	1.14	0.166	0.001
Preoccupied vs. secure	–	4.94	<0.001	0.015	–	0.89	0.375	<0.001
Dismissing vs. secure	–	0.59	0.558	<0.001	–	1.89	0.059	0.002
Trait anxiety	–				85.83	–	<0.001	0.049
Trait depression	–				28.52	–	<0.001	0.017
Model	21.88	–	<0.001	0.096	44.54	–	<0.001	0.213

Table 4 The role of distinct attachment styles in predicting positive dream emotions

	Model 1				Model 2			
	F	t	<i>p</i>	Partial eta squared	F	t	<i>p</i>	Partial eta squared
Sex	.02	–	0.878	<0.001	3.83	–	0.050	0.002
Age	7.40	–	0.007	0.004	5.84	–	0.016	0.004
Marital status	2.08	–	0.101	0.004	2.06	–	0.104	0.004
Attachment style (omnibus)	3.15	–	0.024	0.006	1.14	–	0.333	0.002
Fearful vs. secure	–	–2.28	0.023	0.003	–	–0.20	0.845	<0.001
Preoccupied vs. secure	–	–2.28	0.005	0.005	–	–0.14	0.892	<0.001
Dismissing vs. secure	–	–0.94	0.349	0.001	–	–1.66	0.098	0.002
Trait anxiety	–	–	–	–	14.59	–	<0.001	0.009
Trait depression	–	–	–	–	22.40	–	<0.001	0.013
Model	3.04	–	0.002	0.015	10.79	–	<0.001	0.061

anxiety but not with attachment avoidance (Sloan et al. 2007). This pattern may suggest that attachment anxiety (negative sense of self) is a more robust correlate of negative emotional states influencing sleeping / dreaming characteristics than attachment avoidance (negative sense of others).

In line with the predictions of attachment theory, most data in the present study showed that the distinct attachment styles were also related to dream emotions: secure attachment style was associated with less negative and more positive dream affect than preoccupied and dismissing attachment. These data, showing a link between attachment and dream characteristics, are also in line with previous data indicating that attachment characteristics are mirrored in dream narratives as well: for instance, attachment anxiety was associated with more closeness-related wishes and negative views of self in dreams, while dismissive attachment style was linked with more avoidance-related wishes and negative views of others (Mikulincer et al. 2011).

Interestingly however, participants of the present study with secure versus dismissing attachment did not differ significantly from each other in terms of either positive or negative dream emotions. Given the exploratory nature of the present study, we can only speculate on the reasons for this somewhat counterintuitive finding. We can reasonably assume though that the low level of attachment anxiety in both of these attachment styles is responsible for the lack of difference in the emotional tone of dreams; that is, these data may suggest that the emotional tone of dreams (or at least their later recollection; cf. emotional tone of dreams was assessed by a few questionnaire items) is rather influenced by attachment anxiety than attachment avoidance. This hypothesis is also supported by the fact that participants with fearful and preoccupied attachment styles – both characterized by high attachment anxiety – did not differ significantly in terms of the emotional tone of their dreams.

Results from the multivariate analysis in the present study revealed that attachment anxiety and the distinct attachment styles were significant – although weak – predictors of dream emotions even when controlling for sex, age, and relationship status. These significant connections disappeared however, when entering trait anxiety and depression into the models, indicating that the latter variables fully mediate the relationship between attachment and dream emotions. This phenomenon could be easily interpreted when considering on one hand that attachment quality has a noticeable effect on the development of emotion and stress response regulation (Diamond 2001), and the continuity hypothesis on the other hand, i.e. the assumption that general (awake) emotional and health states are reflected in dreams and dream emotions (Bódizs et al. 2008; Domhoff 2003; Németh and Bányai 2011).

The mediating role of trait anxiety and depression between attachment and dream emotions found here is also in line with the findings of a study investigating the mediators of the childhood adversity–adult sleep quality relationship (Ramsawh et al. 2011). Authors of this study found that neuroticism mediated the association between poor sleep quality and adverse childhood experiences, the latter being previously linked to insecure attachment (Fonagy et al. 1993) and negative dream experiences (Germain and Nielsen 2003; Krakow et al. 2001). Thus, both these data and findings of the present study indicate that the effect of attachment variables on dream characteristics might occur through more general (non-attachment specific) emotional states.

Limitations of the present study need to be acknowledged as well. First, despite the relatively large sample, participants' variability in terms of age and educational attainment was narrow. Further, even though the high ratio of males is a rare and thus valuable characteristic of psychology research using non-probability samples, it still has the disadvantage of the

other sex' underrepresentation. In addition, self-administered paper-and-pencil tests have considerable limitations in assessing complex constructs such as attachment whose valid evaluation requires considerable insight and self-reflection. Since they focus on the conscious attitudes towards, or awareness of, behaviors regarding separation, loss, intimacy, dependence, and trust, they are less valid when defense mechanisms distort responses. Further, self-administered attachment questionnaires are criticized for being passive (not activating the attachment system they are meant to measure) and focusing mainly on current relationships (Ravitz et al. 2010). Therefore, a more sophisticated measurement of attachment (e.g., Adult Attachment Interview) when investigating its relationship with dream content, dream recall frequency, and sleep quality would be a valuable direction for further research.

An additional limitation of the study is that attachment categories were created based on the medians of the attachment anxiety and avoidance variables (RSQ) in the present study and not from an independent, large, representative, and culture-specific sample (for which data are unfortunately not yet available in Hungary). This ad-hoc categorization may limit the sensitivity / specificity of the attachment categories created. Further, suboptimal internal consistency coefficients for several scales of the present study (attachment anxiety, negative dream affect) also indicate that the results should be interpreted with caution. Finally, assessing dream emotions (and dream recall frequency) based on dream logs could provide more reliable data by overcoming recall bias, which would be a significant improvement for purposes similar to that of the present study as it is also possible that anxiety and depression proneness rather / also influence dream recall processes than / not only actual dream emotions.

Despite these limitations, results of the present study showed that attachment characteristics were associated with the emotional tone of dreams and that these associations were mediated by trait depression and anxiety. Although we cannot establish a causal relationship among the variables observed, these results could provide new perspectives on the role of attachment in the neurocognitive understanding of dreaming. Findings of the present study may also suggest that attachment style deserves more attention in the treatment of not only attachment- but nightmare-, mood- and anxiety disorders.

Acknowledgments The authors are grateful to Dr. Péter Simor for sharing the data set the analyses were based on. The authors also wish to thank Lauren Wright for her valuable comments on an earlier version of the manuscript and Audree Francis for the proofreading of the paper.

Compliance with Ethical Standards

Conflicts of Interest The authors declare not having any conflicts of interest.

Research Involving Human Participants All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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