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ORIGINAL ARTICLE

Circadian rhythms of saliva melatonin in ADHD, anxious and normal children

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Abstract

BACKGROUND: Attention-deficit/hyperactivity disorder (ADHD) and anxiety disorders are the most frequent psychiatric disorders in children. Changes in rhythms of symptoms during the day may be influenced by genetic, biological and psychological factors. Some changes of melatonin rhythm may hypothetically change the activity of ADHD by changing arousal or in anxiety children by changing their emotional state. In our present study we identify one group of ADHD children combine type without comorbids, one group of anxiety children and a control group. Most changes of melatonin daily rhythm are supposed in the anxiety group, especially in sleeping time, and more prominent change in the ADHD group with prominent hyperactivity and conduct disorder symptoms.
METHODS: Thirty-four ADHD and forty-three control children and eleven anxiety children, all 6–12 years old, participated in the study. The saliva specimens were collected in four different sessions during the school year, around the time of the spring and autumn equinox, when the natural light lasted 11.2 h ± 0.9 h.
RESULTS AND CONCLUSIONS: In our study more symptoms of conduct disorder elevated positive or negative correlations between psychopathology and saliva level of melatonin in ADHD and anxiety samples. We hypothesize that co-morbidity of ADHD or anxiety with impulsivity and conduct disorders might have elevated correlations between psychopathology of ADHD or anxiety and plasma melatonin level.

INTRODUCTION

Biological theory of some child psychiatric disorders is one of the prominent paradigms in the study of etiology and in therapy. The results of these disciplines changed many therapeutic and clinic practices, especially in pharmacotherapy of anxious and hyperactive children. These theoretical implications of clinical practice concern several topics of physiological meaning catecholamins, serotonin and melatonin in these disorders, including arousal, activity and sleeping.

Attention-deficit/hyperactivity disorder (ADHD) is a very frequent childhood neurobehavioral disorder with an estimated prevalence of 5–6% (Polanczyk & Jansen 2008) of all school chil-

dren. Possible differences in prevalence are generated depending on specific research criteria applied in various studies (Polanczyk *et al.* 2007). This disorder is characterized by developmentally inappropriate symptoms of inattention, hyperactivity and impulsivity that begin to be prominent in early childhood, and these symptoms are often detected at the age of 4–6. There are three types (syndromes) of ADHD (inattention, combine and hyperactive-impulsive type).

Anxiety disorders are the most frequent psychiatric disorders (8–10%) in children of various age in middle childhood. Among them are typical generalized anxiety disorders, separation anxiety or fears (review Turk *et al.* 2007). In our study we have selected children with generalized anxiety disorders, which are accompanied by the free floating anxiety syndrome, worries about school competence, future misfortunes, self consciousness, restlessness, irritability, sleep disturbances and other various symptoms.

There is an overlap of psychobiological factors implicating the potential regulations of the day time psychomotor activity (inattention, hyperactivity, impulsivity and anxiety) and sleeping (for instance: restlessness in sleep, insomnia difficulty) in ADHD and anxious children. Typical for ADHD are many changes during behavior activity, variable impulsivity and attention in studying and play. Typical for anxiety disorder is a fluctuation of anxiety symptoms and some psychomotor instability, for instance at school. These changes in rhythms of these symptoms during the day may be influenced by genetic, biological and psychological reasons. The question is if these changes in day activity and sleep of ADHD and anxious children may be influenced by the circadian rhythm of melatonin. Some sleeping problems are typical for ADHD (restlessness in sleep and sleep latency) and also for anxiety disorders (difficulty falling asleep, fears at night, psychosomatic problems, nightmares).

The sleep-wake cycle belongs to circadian, i.e. round 24 hour rhythms. The endogenous circadian time-keeping mechanism is believed to be a pivotal regulator of the sleep-wake cycle, interacting with the homeostatic mechanism in determining the onset and duration of sleep (Borbely 2001, Nováková *et al.* 2011). Some changes of melatonin rhythm may hypothetically change the activity of ADHD by changing arousal or in anxiety children by changing their emotional states (Wagner 1996).

The circadian system involves some genes, for instance the Clock gene; the polymorphisms of this gene might be associated with a greater risk of ADHD (Kissling *et al.* 2008). Circadian rhythms are coordinated by a master coordinator (oscillator) in the suprachiasmatic nucleus of hypothalamus. This nucleus regulates more physiological functions like temperature, sleep wake cycles, REM sleep and hormone secretion in glandula pinealis. Some of these hormones and markers were used for cycling of rhythms (periods and amplitudes). These oscillations and their amplitudes are masked by behavior and environmental influences. One of these markers is the examination of periphery melatonin level. Melatonin production in glandula pinealis is inhibited by light, its secretion is elevated at night.

Several neurotransmitters systems participate in promoting the sleep wake cycle. These neurotransmitter systems include GABA, serotonin and melatonin. The best marker of the human circadian system and of the phase of its central clock, located in the suprachiasmatic nuclei of hypothalamus, is the rhythm of the pineal hormone melatonin (Lewy *et al.* 1999).

In the daily temporal modulation of sleep regulation first articulated by Borbely the coordination of the pacemaker in interaction with the sleep dependent homeostatic processes regulate the onset and offset of sleep. The homeostatically regulated process reflected an increased sleep drive. These processes can be observed when following the slow wave analysis of the electroencephalogram, which increases as wakefulness continues. This process is thought to be largely directed by the circadian onset and offset of REM sleep. There are some methodological problems, for instance full night (dark) condition, seasonal changes of light or postural changes in melatonin secretion (Czeisler & Klerman 1999).

Most of the latest publications on melatonin concentration in body fluids were designed to study only the nocturnal serum and salivary melatonin or urinary sulphatoxymelatonin. Melatonin concentration in body fluids is high at night and undetectable or low during the daytime (reviewed in Arendt 2006; Nováková *et al.* 2011). Our previous studies (Paclt *et al.* 2009; Nováková *et al.* 2011) were to find out whether the circadian system in ADHD – combine type and control children differs. In our studies the salivary melatonin 24 hour daily rhythm was used (a sample of ADHD and control children 6–12 years old, interval of melatonin examination – 2 hours). Our data suggested some differences between melatonin rhythms in ADHD children and controls. The differences were mainly in shortening of the nocturnal melatonin signal and frequent irregularities in melatonin rhythm. A two-peak nighttime melatonin profile was considered when melatonin declined from a high level to an almost basal daytime one and then increased again, or when it fell to less than one half of a preceding high value and then increased anew to the same or higher level. Whereas the ADHD children did not differ in daily melatonin peak to controls, they might differ in daily melatonin profiles expressed as a ratio to the nighttime melatonin maximum (Nováková *et al.* 2011).

In our present study we identify one group of ADHD children combine type without comorbids, one group of anxiety children and a control group. We suppose most changes of melatonin daily rhythm in the anxiety group, especially in sleeping time, and more prominent change in the ADHD group with prominent hyperactivity and conduct disorder symptoms.

METHODS

Patients and control

Thirty-four ADHD and forty-three control children and eleven anxiety children, all 6–12 years old, participated in the study. All ADHD, anxiety and control subjects were of Caucasian origin and non-obese. The ADHD subjects, anxiety disorder children and control subjects were recruited from schools psychology department and suggested by teachers and met the following enrollment criteria: they had not been previously psychotherapeutically treated with pharmaceuticals, nor had they had any medication, either psychotropic or general, without any other psychiatric or somatic disorders at the time of this study. The IQ level was assessed by means of the Wechsler Intelligence Scale for Children ADHD and generalized anxiety disorder were diagnosed by means of a detailed clinical interview, which included a structured psychiatric examination (Children's Psychiatric Rating Scale) (Fish, 1985), and DSM-IV diagnostic criteria for ADHD and anxiety disorders by two independent child psychiatrists.

The DSM-IV criteria for ADHD specify that individuals must have had symptoms of ADHD for at least 6 months, that these symptoms must occur to a degree that is developmentally deviant, and that symptoms producing impairment must have developed by 7 years of age. From the inattention item list, six of nine items must be endorsed as developmentally inappropriate. From the combined hyperactivity and impulsivity items, lists, six of nine items must be endorsed as deviant. The type of ADHD to be diagnosed depends on whether criteria are met for inattention and hyperactivity-impulsivity, Combined Type (ADHD-C) – DSM-IV –TR criteria for ADHD (APA 2000) code 31401 (both criteria A1 and A2 are met for the past 6 months). Parentally reported onset of symptoms was between 4 and 6 years in all children. A score Conner's scale at least 2 SD above the mean on this scale and ADHD index were used to classify the children as having significant ADHD symptoms (When such a stringent criterion as the 97th percentile is applied (2 standard deviations above the mean), it does identify a group of children whose ADHD symptoms are not only seriously deviant but they are also stable over as long a time as 8–10 years and they are highly predictive for later maladjustment, particularly in academic adjustment and attainment (Barkley 1990; Barkley *et al.* 2002; Novakova *et al.* 2011).

For children with diagnosis generalized anxiety disorder we used DSM-IV criteria for Generalized anxiety disorder (DSM-IV-TR 2000; Kaplan & Sadock's 2008) the Children's Manifest Anxiety Scale: Parent Rating (Gittelman & Klein 1985) and Children's Depression Inventory (Kovacs 1985) and Conners scale-30 were employed.

The control children without ADHD and anxiety were identified by teachers from the same classrooms like the ADHD and anxiety children. Conner's scale for parents and teachers <1 sigma). The IQ level was assessed by means of the Wechsler Intelligence Scale for Children. These children were explored using the same procedure as for the ADHD and anxiety children.

The Conner's Parent Rating Scale (Conners *et al.* 1997) was also employed to assess the severity of follow symptoms. Of the norms provided by Conner's scale-3 1997, we identified total score, hyperactivity index, three factors: conducts disorders, anxiety, hyperactivity-impulsivity and items sleeping problems, also do not provide clear clinically significant "cut off" scores and therefore there may be eventual overlaps of the total scores in ADHD and normal population.

Experimental environment and conditions

The study was performed in a country pension near Prague, at 50°N latitude. The saliva specimens were collected in four different sessions during the school year, around the time of the spring and autumn equinox, when the natural light lasted 11.2 h ± 0.9 h. Subjects came to the pension on Friday afternoon and spent one adaptation night there. Saliva sampling started on Saturday morning at 08:00 and continued for 24 hours at 2 h intervals until 08:00 on Sunday morning. On Saturday evening children went to bed at 22:00 and from that time until Sunday morning only dim light of less than 50 lux was occasionally used for saliva sampling. Subjects drank and ate only after saliva collection and avoided brushing teeth and drinking caffeine-containing beverages during the sampling period. Trained members of the medical and research staff were present for consulting and supervising the schedule of the experiments.

Informed consent for all researched apliceted methodes was obtained from parents of all subjects. The study was conducted according to the Declaration of Helsinki II and the Guidelines for Good Clinical Practice. The protocol was approved by the Ethical and Research Committee of 1st Faculty of Medicine and General Teaching Hospital, Prague, Czech Republic. All experiments were conducted under the standards of the Journal (Portaluppi *et al.* 2010).

Radioimmunoassay of saliva melatonin

Saliva samples were collected either directly into the tube or by cotton swabs. The samples were stored at –20 °C until required for assay, when they were defrosted and centrifuged. The Bühlmann Direct Saliva Melatonin Radio Immunoassay test kit (RK-DSM, Bühlman Laboratories AG, Switzerland) was used for measuring melatonin in the supernatant by a double-antibody radio immunoassay based on the Kennaway G 280 anti-melatonin antibody (Vaughan 1993). Briefly, saliva samples and reconstituted standards were incubated with the anti-melatonin antibody and ^{125}I-melatonin. After 20 hours of incubation, solid-phase secondary antibody was added to the mixture to precipitate the

bound antibody. After aspiration of the unbound fraction, the antibody-bound fraction of ^{125}I-melatonin was counted in a gamma counter (Beckmann LS6000 IC). Melatonin was expressed as pg/ml of saliva. The limit of assay detection was 0.2 pg/ml. Daily melatonin profiles of each subject were expressed as the ratio to his/her highest nighttime melatonin concentration.

Statistics methods

T-test non parametric tests

RESULTS

All groups are significantly different in summary symptoms (0–1, 0–2, 1–3). In sleeping symptoms is significant different between control and ADHD group (Table 1). Hyperactivity factor is different significantly in all three groups and conduct disorder is prominent only in ADHD group.

The Children's Manifest Anxiety Scale: Parent Rating (Gittelman & Klein 1985)

Results of children from anxiety group. Mean: 134.6, Minimum 123, Maximum 157, Standard Deviation: 11.2, Standard Error: 3.54, Minimum 123, Maximum 157.

In the Figure 1 the differences between curves of control and ADHD children are not statistically significant, the curve of anxiety group is different but number of children is small.

Comparison of group means showed that the differences are close to statistical significance (F=2,68; d.f.=2; p=0.074), post hoc comaprisons (LSD) showed that the mean of the anxiety group is significantly lower compared to the mean of the control group (p<0.05) (Table 3).

ADHD patients present more sleep problems (p<0.05) then control children and anxious children are not significant different from control (Table 1). ADHD group is significantly different in factors hyperkinetic and conduct disorder. And factor conduct disorders are different in ADHD children and anxiety children. Anxiety children correlate negatively with anxiety symptom in l0 and 12 o'clock (factor conduct disorder) and ADHD children positively correlate (p<0.01) in 18 o'clock (factors hyperactivity and conduct disorder - Table 4).

DISCUSSION

The ADHD subjects exhibited more frequent irregularities in their melatonin profiles, namely two nocturnal melatonin peaks. Such peaks might be due to an underlying circadian clock in the SCN, normally controlling circadian rhythms in the sleep-wake cycle and the melatonin rhythm. The presence of two nocturnal melatonin peaks might suggest a looser coupling of populations of cells in the SCN (suprachiasmatic nucleus). Alternatively, two peaks might signal a still unrecognized underlying ultradian oscillator (Salti *et al.* 2000). Whereas the ADHD children did not differ in daily melatonin peak to controls (Nováková *et al.* 2010), they might differ in daily melatonin profiles expressed as a ratio to the nighttime melatonin maximum. This trend was more apparent with the increasing age (Nováková *et al.* 2011). A significant difference in the melatonin profile was, however, suggested between the 10–12 years-old and 6–7 years-old ADHD children. In the older children, a phase delay of the evening melatonin onset and a phase advance of the morning offset, relative to those in younger children, resulted in a shortening of the nocturnal melatonin signal.

ADHD children exhibit positive correlation (p<0.01), correlation of 0.438 between the melatonin level at 18 hours and hyperactivity score at Conner's scale and correlation (p<0.01), 0.541 for conduct disorder score in Conner's scale. The correlation coefficient between melatonin signal and hyperactivity was lower at 10 hour (r=0.364, p<0.05) compared to the value of r at 18 hour (Tables 4–6).

The Conners' Parent Rating Scale (Conners *et al.* 1997) was also employed to assess the severity of ADHD symptoms. The norms provided by the Conner's scale do not provide clear clinically significant "cut off" scores, and therefore, the total scores in the ADHD and normal populations may eventually overlap. In this study, scores that were at least 2 SD above the mean on this scale and the ADHD index were used to classify

Tab. 1. Psychopathology symptoms (sleeping, hyperactivity and conduct disorder) in control (0), ADHD (1), anxiety disorders (2).

Conner's scale		N	Mean	S.D.	S.E.M.	Min	Max
Sleeping symptom	0	43	.95	1.327	0.202	0	4
	1	34	2.94	2.719	0.466	0	9
	2	11	2.55	1.635	0.493	0	5
	Total	88	1.92	2.209	0.235	0	9
Hyperactivity factor	0	43	1.60	1.650	0.252	0	7
	1	34	10.24	4.573	0.784	2	19
	2	11	4.73	2.005	0.604	2	9
	Total	88	5.33	5.101	0.544	0	19
conduct disorders factor	0	43	1.95	1.951	0.298	0	7
	1	34	6.47	4.077	0.699	1	18
	2	11	3.09	2.343	0.707	0	7
	Total	88	3.84	3.648	0.389	0	18
summary	0	43	18.93	8.827	1.346	3	47
	1	34	69.59	29.003	4.974	34	121
	2	11	45.18	13.600	4.101	33	72
	Total	88	41.78	30.654	3.268	3	121

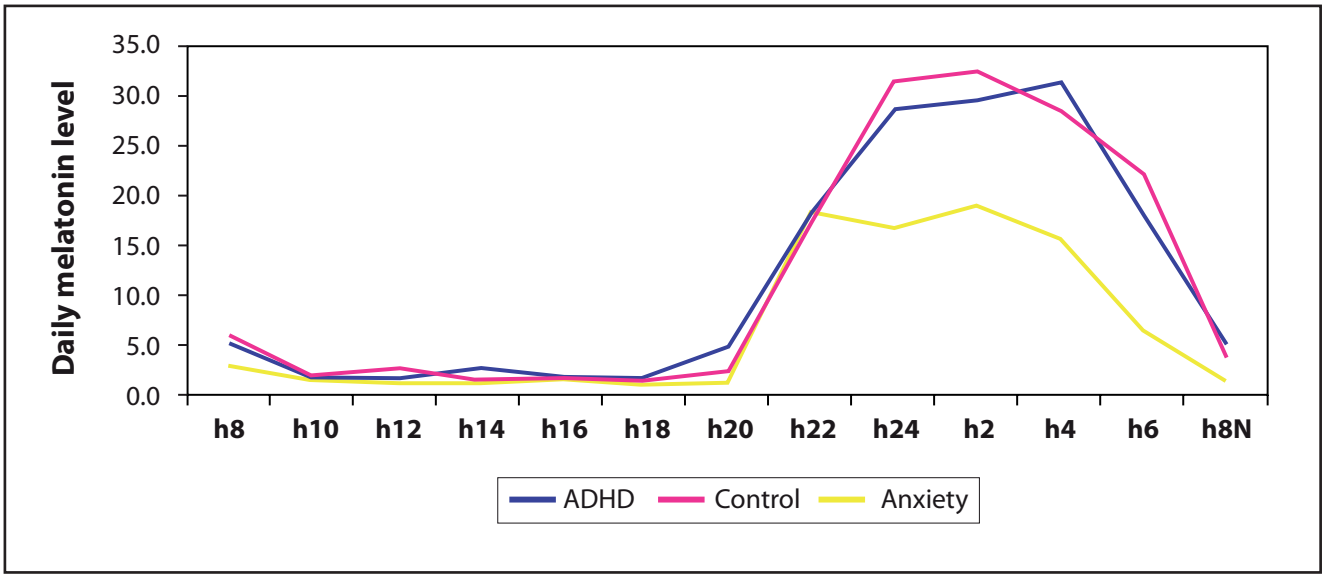

Fig. 1. Daily melatonin level in ADHD children, anxiety children and controls.

Tab. 2. Average melatonine plasma level in 24 hours, ADHD, control and anxiety group.

	h8	h10	h12	h14	h16	h18	h20	h22	h24	h2	h4	h6	h8
ADHD	5.2	1.6	1.7	2.7	1.7	1.6	4.8	18.0	28.6	29.5	31.3	18.3	5.3
control	6.0	1.9	2.7	1.5	1.6	1.3	2.4	17.0	31.3	32.4	28.6	22.2	4.0
anxiety	2.9	1.4	1.3	1.0	1.5	1.1	1.3	18.3	16.7	19.0	15.8	6.5	1.4

the children as having significant ADHD symptoms (Table 1).

Differences in results of Conner's rating scale between the ADHD group, anxious children and controls are significant in hyperactivity factor in ADHD, control and anxiety groups, conduct disorder factor significantly differs between controls and ADHD and ADHD and anxiety. Sleep problems are significantly more prominent ($p<0.01$) in the ADHD group than in the group of controls.

According to reviews and meta-analytic studies, no specific sleep macrostructure change has yet been identified in ADHD children (Příhodová *et al.* 2010). In our previous studies we recorded only restless sleep ($p=0.001$), insomnia ($p=0.047$), difficulties falling asleep and border results in NREM 1% ($p=0.056$). Insomnia in other papers was related to the rating of inattention in ADHD, while noisy sleep was related to the rating of aggression, parasomnias were related to anxiety or depression in children (Stein 2001).

Tab. 3. Mean melatonine plasma levels (24 hour) by study subgroups.

	Mean	S.D.	S.E.M.
ADHD group (N = 34)[a]	11.49	7.70	1.32
Control group (N = 43)[a]	12.46	6.94	1.06
Anx. disord. group (N = 11)[b]	6.79	7.01	2.11

The question of the presented study is whether various profiles of ADHD symptoms (hyperactivity, impulsivity, conduct disorder) and anxiety disorder changed the 24h melatonin cycle. Our results in a sample of anxiety children present we identify low amplitude at night and correlation between very low amplitude in anxious children in the morning with elevated conduct disorder factor of anxiety symptoms ($p<0.05$) at 10 (–0.705) and 12 (–0.750) hours. Some but not all studies support an increased sensitivity to light-induced suppression of melatonin in a group of bipolar patients and it has been hypothesized that this sensitivity might result in circadian face instability between 2–4 a.m. (Whalley *et al.* 1991; Kennedy *et al.* 1996; Nathan *et al.* 1999). But a replication of our data in a larger sample is necessary. In our study of children with generalized anxiety disorder that is in a way related to depressive disorder we identified prominent melatonin suppression at 10 and 12 o'clock.

Less recognized in anxiety research to date is the hypothesis that co-morbid conditions may contribute to the understanding of anxiety mechanisms, especially in childhood. As an example, the occurrence of anxiety and impulsive/inattentive tendencies in children may reflect a common etiologic mechanism. Anxiety may impair the efficient functioning of goal-directed

attentional systems, thereby decreasing attentional control, and it has been suggested that high-trait anxiety is inherently associated with a general inability to maintain attentional focus, rather than exhibiting automatic attentional bias towards threatening information (Fox 1993). The large MTA study confirmed these results.

Tab. 4. Correlation symptoms (sleeping, hyperactivity, conduct disorder, psychosomatics symptoms and anxiety) in ADHD children.

		TOTAL	SLEEP	ADHD	CONDUCT DIS.	ANXIETY	PsO
h8	Pearson Correlation	0.218	0.033	0.200	0.170	0.161	0.172
	Sig. (2-tailed)	0.216	0.854	0.257	0.337	0.362	0.330
	N	34	34	34	34	34	34
h10	Pearson Correlation	0.274	0.195	0.364*	0.305	0.080	0.244
	Sig. (2-tailed)	0.117	0.269	0.034	0.080	0.653	0.164
	N	34	34	34	34	34	34
h12	Pearson Correlation	0.242	0.202	0.336	0.318	0.004	0.157
	Sig. (2-tailed)	0.168	0.251	0.052	0.067	0.983	0.375
	N	34	34	34	34	34	34
h14	Pearson Correlation	–0.035	0.040	0.105	–0.052	–0.154	0.088
	Sig. (2-tailed)	0.843	0.822	0.553	0.770	0.384	0.621
	N	34	34	34	34	34	34
h16	Pearson Correlation	0.237	0.204	0.260	0.321	0.046	0.131
	Sig. (2-tailed)	0.177	0.248	0.138	0.064	0.796	0.462
	N	34	34	34	34	34	34
h20	Pearson Correlation	–0.086	–0.075	–0.121	–0.089	0.055	–0.119
	Sig. (2-tailed)	0.634	0.677	0.501	0.620	0.761	0.509
	N	33	33	33	33	33	33
h22	Pearson Correlation	0.140	0.142	0.122	0.120	–0.001	0.032
	Sig. (2-tailed)	0.438	0.429	0.499	0.505	0.994	0.858
	N	33	33	33	33	33	33
h24	Pearson Correlation	0.213	0.246	0.055	0.082	0.197	0.337
	Sig. (2-tailed)	0.234	0.168	0.761	0.652	0.271	0.055
	N	33	33	33	33	33	33
h2	Pearson Correlation	–0.108	–0.013	–0.042	–0.143	–0.034	0.115
	Sig. (2-tailed)	0.549	0.944	0.816	0.428	0.851	0.524
	N	33	33	33	33	33	33
h4	Pearson Correlation	–0.155	–0.112	–0.088	–0.191	–0.064	0.073
	Sig. (2-tailed)	0.396	0.541	0.632	0.294	0.727	0.692
	N	32	32	32	32	32	32
h6	Pearson Correlation	0.062	0.150	0.070	0.049	–0.056	–0.030
	Sig. (2-tailed)	0.736	0.413	0.705	0.792	0.760	0.870
	N	32	32	32	32	32	32
h8N	Pearson Correlation	0.040	0.093	0.104	–0.021	0.041	0.150
	Sig. (2-tailed)	0.822	0.602	0.559	0.905	0.820	0.396
	N	34	34	34	34	34	34
Total	Pearson Correlation	1	0.635**	0.778**	0.670**	0.658**	0.602**
	Sig. (2-tailed)		0.000	0.000	0.000	0.000	0.000
	N	34	34	34	34	34	34

The data seem to suggest that anxiety in ADHD may be more closely related to ODD and generally disruptive behavior than to fearfulness (March *et al.* 2000). In general, some results suggest that children with ADHD – combined type had different patterns of psychiatric co-morbidity (more anxiety and depression disorders)

Tab. 5. Correlation symptoms (sleeping, hyperactivity, conduct disorder, psychosomatics symptoms and anxiety) in anxiety disorder children.

		TOTAL	SLEEP	ADHD	CONDUCT DIS.	ANXIETY	PsO
h8	Pearson Correlation	–0.125	0.181	–0.242	–0.472	–0.351	0.496
	Sig. (2-tailed)	0.713	0.595	0.474	0.143	0.319	0.145
	N	11	11	11	11	10	10
h10	Pearson Correlation	–0.420	–0.023	–0.443	–0.705*	–0.226	0.087
	Sig. (2-tailed)	0.199	0.946	0.173	0.015	0.529	0.812
	N	11	11	11	11	10	10
h12	Pearson Correlation	–0.604*	–0.082	–0.459	–0.750**	–0.058	–0.317
	Sig. (2-tailed)	0.049	0.810	0.155	0.008	0.875	0.372
	N	11	11	11	11	10	10
h14	Pearson Correlation	–0.719*	–0.385	–0.416	–0.551	–0.069	–0.663*
	Sig. (2-tailed)	0.013	0.243	0.203	0.079	0.850	0.037
	N	11	11	11	11	10	10
h16	Pearson Correlation	–0.040	–0.559	–0.044	–0.303	–0.383	–0.272
	Sig. (2-tailed)	0.906	0.074	0.897	0.365	0.275	0.448
	N	11	11	11	11	10	10
h20	Pearson Correlation	–0.513	0.261	–0.128	–0.281	0.180	0.048
	Sig. (2-tailed)	0.107	0.437	0.707	0.403	0.619	0.895
	N	11	11	11	11	10	10
h22	Pearson Correlation	–0.297	0.073	0.270	–0.232	–0.039	–0.076
	Sig. (2-tailed)	0.376	0.831	0.422	0.492	0.915	0.834
	N	11	11	11	11	10	10
h24	Pearson Correlation	–0.409	–0.146	0.083	–0.339	–0.098	–0.283
	Sig. (2-tailed)	0.211	0.669	0.808	0.307	0.787	0.428
	N	11	11	11	11	10	10
h2	Pearson Correlation	–0.351	–0.095	0.012	–0.327	–0.076	–0.245
	Sig. (2-tailed)	0.290	0.781	0.972	0.326	0.834	0.494
	N	11	11	11	11	10	10
h4	Pearson Correlation	–0.275	0.051	0.035	–0.347	–0.076	–0.136
	Sig. (2-tailed)	0.414	0.883	0.919	0.295	0.836	0.708
	N	11	11	11	11	10	10
h6	Pearson Correlation	–0.101	0.202	–0.076	–0.327	0.149	0.057
	Sig. (2-tailed)	0.767	0.552	0.824	0.326	0.681	0.876
	N	11	11	11	11	10	10
h8N	Pearson Correlation	–0.495	–0.152	–0.115	–0.492	0.035	–0.215
	Sig. (2-tailed)	0.121	0.656	0.736	0.125	0.923	0.552
	N	11	11	11	11	10	10
Total	Pearson Correlation	1	0.463	0.563	0.599	0.063	0.604
	Sig. (2-tailed)		0.152	0.071	0.052	0.862	0.064
	N	11	11	11	11	10	10

from those of children with ADHD – hyperactive, impulsive type (more disruptive behavior – ODD and CD). Our results in anxiety disorders are limited by the small number of children. All data have to be replicated in a larger sample of anxiety children.

In our study more symptoms of conduct disorder elevated positive or negative correlations between psy-

Tab. 6. Correlation symptoms (sleeping, hyperactivity, conduct disorder, psychosomatics symptoms and anxiety) in Controls.

		TOTAL	SLEEP	ADHD	CONDUCT DIS.	ANXIETY	PsO
h8	Pearson Correlation	0.039	0.140	0.169	–0.218	–0.037	–0.010
	Sig. (2-tailed)	0.805	0.372	0.278	0.161	0.818	0.949
	N	43	43	43	43	42	42
h10	Pearson Correlation	–0.172	–0.091	–0.164	–0.212	–0.124	–0.018
	Sig. (2-tailed)	0.275	0.566	0.299	0.177	0.439	0.910
	N	42	42	42	42	41	41
h12	Pearson Correlation	–0.174	–0.041	–0.044	–0.222	–0.043	–0.124
	Sig. (2-tailed)	0.264	0.794	0.779	0.152	0.787	0.433
	N	43	43	43	43	42	42
h14	Pearson Correlation	–0.094	–0.144	–0.161	–0.193	0.003	–0.170
	Sig. (2-tailed)	0.555	0.363	0.308	0.221	0.986	0.288
	N	42	42	42	42	41	41
h16	Pearson Correlation	–0.157	–0.155	–0.225	–0.164	–0.041	–0.081
	Sig. (2-tailed)	0.315	0.321	0.147	0.293	0.795	0.612
	N	43	43	43	43	42	42
h20	Pearson Correlation	0.021	0.144	–0.027	–0.142	0.037	–0.043
	Sig. (2-tailed)	0.894	0.358	0.864	0.363	0.816	0.787
	N	43	43	43	43	42	42
h22	Pearson Correlation	0.036	0.204	0.012	–0.002	0.045	0.090
	Sig. (2-tailed)	0.819	0.189	0.939	0.987	0.776	0.572
	N	43	43	43	43	42	42
h24	Pearson Correlation	–0.069	–0.115	–0.159	–0.233	0.364*	0.020
	Sig. (2-tailed)	0.664	0.467	0.313	0.138	0.019	0.902
	N	42	42	42	42	41	41
h2	Pearson Correlation	–0.139	–0.219	–0.017	–0.162	0.126	–0.059
	Sig. (2-tailed)	0.387	0.168	0.916	0.311	0.439	0.717
	N	41	41	41	41	40	40
h4	Pearson Correlation	–0.040	–0.045	–0.220	0.037	0.254	0.124
	Sig. (2-tailed)	0.801	0.780	0.161	0.817	0.109	0.441
	N	42	42	42	42	41	41
h6	Pearson Correlation	0.020	–0.043	–0.174	–0.214	0.316*	0.186
	Sig. (2-tailed)	0.897	0.783	0.266	0.169	0.041	0.239
	N	43	43	43	43	42	42
h8N	Pearson Correlation	–0.036	0.053	–0.063	–0.164	–0.120	0.014
	Sig. (2-tailed)	0.818	0.737	0.687	0.292	0.450	0.929
	N	43	43	43	43	42	42
Total	Pearson Correlation	1	0.577**	0.454**	0.540**	0.255	0.512**
	Sig. (2-tailed)		0.000	0.002	0.000	0.103	0.001
	N	43	43	43	43	42	42

chopathology and saliva level of melatonin in ADHD and anxiety samples. We hypothesize that co-morbidity of ADHD or anxiety with impulsivity and conduct disorders might have elevated correlations between psychopathology of ADHD or anxiety and plasma melatonin level.

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