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Does sex hormone treatment reverse the sex-dependent stress regulation? A longitudinal study on hypothalamus-pituitary-adrenal (HPA) axis activity in transgender individuals

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ABSTRACT

Background: Studies in mammals indicate a role for sex hormones in the regulation of hypothalamic–pituitary–adrenal (HPA)-axis reactivity. However, in humans, experimental paradigms investigating long-term exposure to sex hormones are sparse, limiting the understanding of the influence of sex hormones on HPA-axis activity. Gender-affirming hormone therapy (GAHT) in transgender persons enables to study the physiological role of sex steroids partially uncoupled from the distinct genetic background of men and women.

Methods: Ten transwomen (male genotype and female gender identity) and 15 transmen (female genotype and male gender identity) were investigated at baseline and following three months of GAHT by means of the combined dexamethasone (dex)/CRH-test. Linear mixed-effects model analysis was used to assess changes over time and to identify determinants of HPA-axis reactivity.

Results: In response to CRH, overall ACTH (+18%) as well as cortisol (+15%) output were increased in transwomen after 3-months of estrogen and antiandrogen treatment, while the opposite was the case for transmen after testosterone treatment (-15% and -58%, respectively). The ACTH/Cortisol-ratio indicated that testosterone attenuated sensitivity for ACTH at the adrenal level in transmen. Interestingly, copeptin levels before CRH administration were a strong predictor of overall cortisol secretion.

Conclusions: This is the first study demonstrating long-term effects of a complete reversal of the sex-hormonal milieu on HPA-axis activity in humans. Our findings hereby expand the current knowledge of the physiology of HPA-axis regulation. and may be particularly relevant for transgender and cisgender people undergoing hormonal suppression or substitution therapies.

1. Introduction

The influence of sex hormones on hypothalamic–pituitary–adrenal (HPA)-axis reactivity is complex, and findings are partially conflicting between humans and other mammals (Kudielka and Kirschbaum, 2005). Studies in rodents have shown an increased stress response caused by estradiol (E2) in females (Turner, 1990) and testosterone (T) withdrawal in males (Handa et al., 2013). In humans, significant effects of sex steroids on HPA-axis functioning have also been repeatedly shown (Kirschbaum et al., 1996; Roelfsema et al., 2016). Short-term E2 application exaggerates physiological stress response to a psychological stressor in men (Kirschbaum et al., 1996; Sharma et al., 2014), and T withdrawal due to Gonadotropin-releasing hormone (GnRH)-analogue treatment results in an increased cortisol but not adrenocorticotropic hormone (ACTH)-secretion following Corticotropin-releasing hormone (CRH)-administration (Kirschbaum et al., 1996). In women, spontaneous ACTH secretion is attenuated by E2 (Sharma et al., 2014) and progesterone exerts an inhibitory effect on HPA-axis reactivity

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(Stephens et al., 2016). Moreover, low-dose T may mitigate the physiological stress response in women (Hermans et al., 2007). Importantly, however, these findings have mostly failed to fully explain the observed sex differences in HPA-axis activity between women and men, indicating also a critical role for genetic effects. Moreover, findings such as men showing higher ACTH- and cortisol responses to psychological stressors (Kelly et al., 2008; Stephens et al., 2016) seem to contradict the abovementioned findings on the role of sex hormones.

So far, a major limitation of studies in humans was that effects of sex hormones on stress response were investigated either by "withdraw and replace"-approaches (Roelfsema et al., 2016; Rubinow et al., 2005; Sharma et al., 2014) or by short-term (Kirschbaum et al., 1996) or low-dose (Hermans et al., 2007) hormone administration in a cross-sex fashion. However, these approaches do not provide a realistic picture on the long-term effects of sex hormones. In contrast, hormonal therapy in transgender individuals provides a unique situation in helping to understand sex hormone driven sex differences. Transgender people present with incongruence between gender identity and external sexual anatomy. During medical transition, gender-affirming hormonal therapy (GAHT) is often applied to mitigate gender dysphoria (GD). GAHT in transwomen (male genotype and female gender identity) is achieved by the application or intake of E2, mimicking premenopausal hormone substitution therapy, while T injections or gels are the treatment of choice in transmen (female genotype and male gender identity). In transwomen, the usually administered dosages of E2 are not enough to completely suppress endogenous hormonal production and thus treatment is complemented by either antiandrogenic progestins or GnRH-analogues. GAHT in transgender persons enables therefore, at least to some extent, to investigate the physiological role of long-term sex steroid exposure partially uncoupled from the different genetic background of men and women through within subject measurements before and after initiation of hormonal therapy.

Strikingly, studies on HPA-axis functioning in transgender subjects are sparse. One study showed that one year of GAHT treatment resulted in a lower cortisol awakening response in both sexes in accordance with a reduction in perceived stress levels (Colizzi et al., 2013). In another study, basal serum cortisol levels in transwomen were reduced after one year of GAHT by up to 50%; however, the authors did not account for expected hormone-induced changes in corticosteroid binding globulin (CBG) and mental health (Mueller et al., 2006). In transgender people it is important to disentangle "direct" from "indirect" hormonal effects on HPA-axis activity, as a general increase in wellbeing has been repeatedly reported following initiation of GAHT (Fisher et al., 2016; Gómez-Gil et al., 2012). This could influence HPA-axis activity as a relation between HPA-axis activity and mental health has been repeatedly shown (Paslakis et al., 2011; Schmider et al., 1995; Weber et al., 2000). Some (Gómez-Gil et al., 2012), but not all studies (Colizzi et al., 2013; Fisher et al., 2016) have reported that depressive and anxiety symptoms tend to improve particularly in transmen under treatment (Auer et al., 2013). The mechanisms by which T may affect mood in men and women are incompletely understood, but an interesting study from Kranz et al. (2015) demonstrated that T treatment in transmen is capable of upregulating serotonin reuptake transporter binding in mood-critical brain regions, while GAHT in transwomen had the opposite effect.

A recent meta-analysis including 27 randomized controlled trials (RCTs) with 1890 men, has shown that T treatment might be effective in reducing depressive symptoms in men (Walther et al., 2019), particularly when higher-dosage regimens were applied in carefully selected samples. Therefore, mental health has to be considered as a potential covariate in longitudinal analyses. The combined dexamethasone (dex)/CRH-test is a suitable tool to assess HPA-axis reactivity (Deuschle et al., 1998; Heuser et al., 1994b). It provides a comprehensive analysis regarding glucocorticoid-receptor sensitivity as well as adrenal responsiveness and allows for drawing conclusions on central CRH- and vasopressin tone/secretion. The idea behind the dex/CRH test is that although dexamethasone is a potent suppressor of ACTH-secretion by glucocorticoid receptor (GR) activation at the pituitary level, it only hardly passes the blood brain barrier, in contrast to cortisol. It is assumed that suppression of endogenous cortisol levels by dexamethasone reduces the negative feedback at the hypothalamic level, while subsequent CRH administration demasks increased chronic vasopressin secretion, the second important stimulant of ACTH secretion (Rivier and Vale, 1983a). It has been shown before that women show a stronger response in the dex/CRH test (Heuser et al., 1994a; Kunugi et al., 2006; Rampp et al., 2018), although this is not a consistent finding (Hatzinger et al., 2011). As vasopressin itself - due to its small molecular size and short half-life - is hard to measure reliably in vivo, copeptin as a surrogate marker has gained growing attention in the last years. Copeptin is a product of the vasopressin precursor and therefore released in equimolar amounts into the circulation from the axon terminals of magnocellular hypothalamic neurons of the paraventricular and supraoptic nucleus that project to the posterior pituitary. Due to its significantly larger molecular mass, it is however much easier to measure than vasopressin. It has not been conclusively studied to which extent, systemic copeptin levels might also reflect dendritic release from the same nuclei. It has however been demonstrated that oxytocin, a closely related peptide that is produced in a similar way as vasopressin in the same hypothalamic nuclei, is still detectable in patients with nearly complete destruction of the pituitary (Gebert et al., 2018).

To the best of our knowledge, this is the first study investigating the effects of a complete reversal of the sex-hormonal milieu on HPA-axis activity in humans. Using the dex/CRH-test, we firstly hypothesized that GAHT would increase ACTH and cortisol secretion following a pharmacological challenge in transwomen and decrease it in transmen in line with data in rodents (Viau and Meaney, 2004; Weiser and Handa, 2009). Moreover, since it has been demonstrated that T attenuates adrenal ACTH sensitivity (Kirschbaum et al., 1996) (i.e., men require higher daily ACTH secretion for comparable cortisol secretion (Roelfsema et al., 2016, 1993)), we secondly hypothesized that T treatment mitigates adrenal ACTH sensitivity. Third, we hypothesized that copeptin levels would predict HPA-axis dynamics during the test as having been reported before (Schinke et al., 2017).

2. Methods

2.1. Patient recruitment

We recruited 27 transgender patients of central European origin, eleven transwomen and sixteen transmen, aged 18–59 years who at the time received treatment at the Interdisciplinary Transgender Health Care Center at the Institute for Sex Research and Forensic Psychiatry, Hamburg, Germany. All participants were part of an observational multicenter study in Germany to assess the effects of medical interventions on psychological and metabolic outcomes of transition-related health care (TRANSIT) (Auer et al., 2017). All patients were diagnosed with gender dysphoria (DSM-5, 302.85) or Transsexualism (ICD-10, F64.0) and were treated according to the 7th version of the Standards of Care (SoC 7) published by the World Professional Association for Transgender Health, although there are some national peculiarities due to health insurance reimbursement policies. After receiving an average of one year of psychotherapy and meeting the requirements for GAHT, hormonal treatment was started in accordance with their treating endocrinologist (Table 1). Patients were included in the study and tested once before they started hormone treatment (M0) and then after three months (M3) of treatment. Eleven transwomen and sixteen transmen were investigated at baseline. One transwoman and one transman were lost during follow-up. One transman presented with extremely low morning cortisol (< 10 µg/l) levels on day one of follow-up suggestive for complete adrenal insufficiency and was therefore suspected to have taken dexamethasone the day before, despite having been instructed

Table 1
General characteristics at baseline.

Variable	Transwomen (N = 10)		Transmen (N = 15)		p-value
	N	%	N	%	
Living alone	6	60	8	53.3	0.398
History of depression	5	50	8	53.3	0.951
Family history depression					0.433
yes	1	10	4	26.7	
no	6	60	9	50	
unknown	3	30	2	13.3	
Hormonal treatment					
Testosterone undecanoate 1000 mg every 12 weeks	NA	NA	15	100	NA
Estradiol gel	8	80	NA	NA	
	Median (min-max)				
Dosage (mg)	2.2 (1.2-3.6)				
	N	**%**	**N**	**%**	
Estradiol oral	2	20	NA	NA	
	Median (min-max)				
Dosage (mg)	3 (2-4)		NA	NA	
	N	**%**	**N**	**%**	
Cyproterone acetate use	9	90	NA	NA	
	Median (min-max)				
Cyproterone acetate dosage (mg)	7.2 (2.0-25.0)		NA	NA	
	N	**%**	**N**	**%**	
Leuprorelin acetate 11,25 mg every 3 months	*0*	0	3	20.0	
Current antidepressant use	4	40	3	20.0	0.275
SSRI	2	20	1	6.7	
SSNRI	2	20	2	13.3	
Cycle phase M0					
Follicular phase	NA	NA	8	53.3	
Luteal phase	NA	NA	6	40	
Missing	NA	NA	1	6.7	
Cycle phase M3					
Amenorrhea	NA	NA	12	80	
Follicular phase	NA	NA	3	20	
Current smoking M0	8	80	9	60	**0.022**
Current smoking M3	7	70	7	46.6	0.098

Group comparison by X2 or Mann-Whitney-U-test, NA = not applicable, Transwomen (male-to-female) = transwomen, Transmen (female-to-male) = transmen. SSNRI = Selective Serotonin-Noradrenalin-Reuptake-Inhibitor, SSRI = Selective Serotonin Reuptake Inhibitor.

otherwise. Two transwomen showed no dex-suppression or even higher cortisol levels following dexamethasone intake at timepoint one and two respectively and were therefore suspected to not having taken dexamethasone the evening before testing. Same was true for one transman at timepoint two. These subjects were therefore excluded from the corresponding analyses. This resulted in a final group size of eight transwomen and thirteen transmen from whom complete data were available for both timepoints. The study was approved by the local ethics committee (Ärztekammer Hamburg). The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All participants gave written informed consent. This study is registered at clinicaltrials.gov (identifier: NCT02185274).

2.2. Dex/CRH-Test

The procedure of the combined dex/CRH tests was described in detail elsewhere (Heuser et al., 1994b) and depicted in Fig. 1. Blood was drawn on day one (d1) at 8:00 a.m. to assess dex-suppressed HPA parameters. Patients were then pretreated with 1.5 mg dexamethasone (Jenapharm, Jena, Germany) p.o. at 23:00 h the same day and blood was drawn at 8:00 the following day (d2) to assess dexamethasone-induced suppressibility of ACTH and cortisol. HPA-axis was then stimulated with 100 mg human CRH (Ferring, Kiel, Germany) intravenous application at 15:00 h in the afternoon (blood samples before CRH application are referred to as baseline values). Blood samples were drawn at 15:00 h, 15:30 h, 15:45 h, 16:00 h, and 16:15 h through an intravenous catheter and collected in EDTA tubes. CRH was injected immediately after the first blood collection. The subjects rested supine and were awake throughout the test.

2.3. Hormone measurements

All hormonal parameters were determined at d1, d2 and before CRH stimulation (= baseline). Cortisol and CBG were measured by RIA (DRG International, Inc., U.S.A.) with an intra-assay CV of 8.6% and 6.2% and an interassay CV of 10.8% and 8.7% respectively. The lower detection limit for cortisol was 0.9 µg/l. ACTH was measured by RIA (MP Biomedicals, Solon, U.S.A.) with an intra-assay CV of 6.8% and an interassay CV of 10.7%, the lower detection limit was 5.7 pg/ml. E2 was measured by RIA with coated tube technique (DRG Marburg, Germany). The intra-assay CV was below 4% and the interassay CV was below 10%. Testosterone was measured by RIA with coated tube technique (DRG Marburg, Germany) with an intra-assay CV below 7% and an interassay CV below 10%. SHBG was determined by RIA with coated tube technique (DRG Marburg, Germany), intra- and interassay CV were below 8%, respectively. Copeptin was determined using a commercially available luminescence assay device (BRAHMS Kryptor, Berlin, Germany). Intra- and interassay CVs were below 8%, respectively. Free androgen index (FAI) was calculated as follows: total T/SHBG *100.

2.4. Instruments used

2.4.1. Beck depression inventory II (BDI-II)

The Beck Depression Inventory (BDI)-II was used as an instrument for measuring symptoms of depression. It comprises 21 questions about how the patient has been feeling in the last two weeks. A total score of 0–9 indicates minimal, a score of 10–18 mild, a score of 19–29 moderate and a score of 30–63 severe depression (Hautzinger et al., 2006).

2.4.2. State-trait anxiety inventory form X (STAI-X)

The STAI-X is a psychological inventory consisting of 40 questions on a self-report basis. The STAI-X measures two types of anxiety: (i) state anxiety (X1) or anxiety about an event and (ii) trait anxiety (X2) or anxiety level as a personal characteristic. Higher scores indicate more anxiety (Spielberger and Sydeman, 1994).

2.5. Statistics

All statistical analyses were performed using SPSS for Windows, version 23 (IBM, Chicago, IL). Group comparisons with respect to sociodemographic variables were evaluated using Mann-Whitney-U- and χ2-test for dichotomous variables. Except for HPA-axis-parameters and -indices, Mann-Whitney-, respectively Wilcoxon signed-rank test, were used to test for group differences and within-subject changes, accounting for the usual non-normal distribution of hormonal values. For analysis of HPA-axis parameters, data was examined for normality using histograms and, if skewed, were normalized by log transformation or if negative and hereby preventing logarithmic transformation by transforming into Fisher-Yates normal scores prior to any further analysis. For reasons of clarity only untransformed values are reported. The total integrated ACTH and cortisol concentration following CRH-administration were assessed by the area under the curve (AUC) calculated by the trapezoidal rule (Pruessner et al., 2003) with regard to ground (gAUC), reflecting total secretion following CRH stimulation and AUC with regard to increase (iAUC) reflecting the difference to baseline secretion. Due to the unbalanced design, to evaluate the effects of 3 months of GAHT on different outcome variables, group, time, and interaction effects during the three-month study period were examined using linear mixed-effects model analysis with time and group effects as

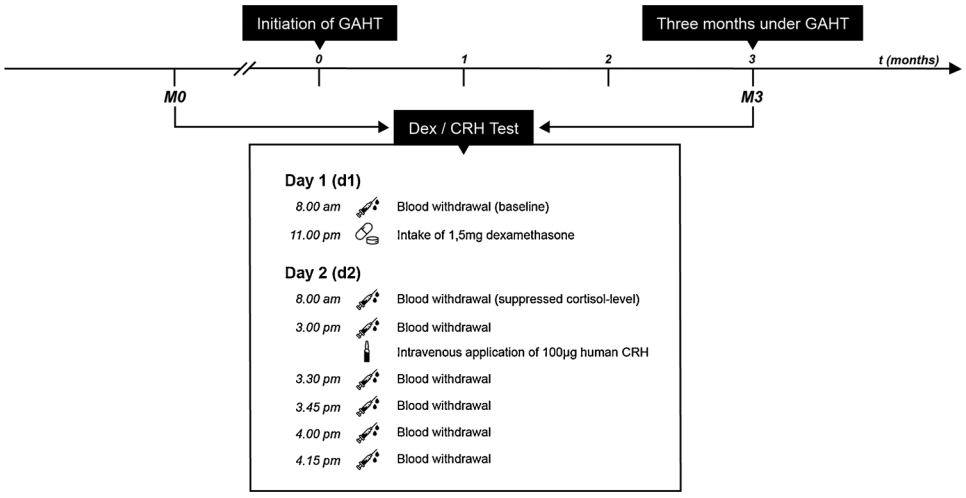

Fig. 1. Experimental timeline.
ACTH and cortisol at 8:00 a.m. before (d1) and after (d2) dexamethasone suppression before (M0) and three months following initiation of gender-affirming hormone treatment (M3).
Linear mixed-effects model analysis with time and group effects as fixed, and subject effects as random and an unstructured covariance structure, adjusted for BDI and STAIX1 sum scores, BMI and age. CBG was further added to cortisol outcomes. Data are presented as means + SD. * indicate significant time and/or groups effects ($p < 0.05$).

fixed, and subject effects as random and an unstructured covariance structure. To ascertain whether changes in HPA-outcome variables occurred independently of changes in potential confounders, BDI and STAI-X1 sum scores, BMI and age were used as covariates as well as CBG values before CRH administration for cortisol outcomes. The cortisol:ACTH-ratio for the gAUC and iAUC for both measures was used to assess adrenal responsivity. To further estimate the relative contributions of potential confounding variables on gAUC/iAUC ACTH and gAUC/iAUC cortisol, separate mixed effects regression analyses were performed with the following factors included in the predictive model: group, time, FAI, E2, CBG, ACTH respectively cortisol before CRH administration and copeptin. This procedure permits assessment of each of the factors in the presence of the effects of the other variables of interest. All statistical tests were performed with $\alpha \leq 0.05$ (2-tailed).

3. Results

General characteristics are presented in Table 1. There were no significant differences between groups regarding potential confounders such as (family-) history of depression, current antidepressant use or smoking habits. 80% of transmen achieved amenorrhea following three months of treatment defined as last menstrual bleeding > 35 days.

There was a small increase in BMI on trend-level in transmen (+0.8%, z =–1.852; p = 0.064), a significant decrease in STAIX1 as a measure of current state anxiety in transwomen (z = -2.374; p = 0.018) and a decrease in BDI-II score in transmen (z = -2.034; p = 0.042) following 3 months of GAHT (Table 2).

3.1. HPA-axis measures and indices

In both groups, GAHT led to a significant, albeit small decrease in dex-suppressed ACTH levels ($F_{1,20.868} = 29.468$; $p < 0.001$) as well as dex-suppressed cortisol levels ($F_{1,24.861} = 7.960$; $p = 0.010$) in the morning. In contrast, unsuppressed ACTH ($F125_{.646} = 11.380$; $p = 0.002$) and cortisol levels decreased in transmen (204.9 ± 46.8 ug/dl to 175.5 ± 36.7 ug/dl), while they increased in transwomen (189.8 ± 43.5 ug/l to 228.7 ± 51.9 ug/dl) after initiation of GAHT, reflected in a significant effect of group ($F_{1,26.592} = 5.979$; $p = 0.022$) and time x group interaction ($F_{1,28.436} = 14.436$; $p = 0.001$) (Fig. 2).

Three months after initiation of GAHT, overall ACTH secretion following CRH-administration was significantly increased in transwomen (2079.6 ± 893.7 ng/l*min to 2471.7 ± 921.6 ng/l*min) and decreased in transmen (2480.0 ± 721.7 ng/l*min to 2156.3 ± 727.4 ng/l*min) compared to M0, reflected in a significant time x group interaction for gAUC_ACTH ($F_{1,24.364} = 4.956$; $p = 0.036$; Fig. 3). In line, overall cortisol secretion was also increased in transwomen (2970 ± 1257.5 ug/dl*min to 3354.1 ± 2341 µg/dl*min) and decreased in transmen (2045.9 ± 1318 ug/dl*min to 1202.9 ± 363.7 µg/dl*min), reflected in a significant effect of group for gAUC_Cortisol ($F125_{.023} = 10.444$; $p = 0.004$) and a significant group x time interaction ($F125_{.662} = 13.931$; $p = 0.001$; Fig. 3). In contrast, iAUC of cortisol and ACTH secretion were not affected by GAHT in transmen and –women. Of note, variance in terms of standard deviation of cortisol response following CRH administration was significantly attenuated in transmen following treatment. Moreover, adrenal sensitivity decreased after GAHT in transmen, but no change occurred in transwomen, reflected by a significant effect of group for the gAUC_ACTH/gAUC_Cortisol-ratio ($F_{1,24.139} = 17.249$; $p < 0.001$)

Table 2
Changes in potential confounders between timepoints.

Variable	Transwomen			Transmen			Group differences	Transwomen		Transmen	
	Median	Percentiles		Median	Percentiles			Changes between timepoints		Changes between timepoints	
		25th	75th		25th	75th	p	z	p	z	p
Age	27.0	20.0	28.0	24.0	21.00	29.0	0.889	**NA**	**NA**	**NA**	**NA**
BMI M0 (kg/m²)	22.9	21.1	28.0	21.4	20.1	31.1	0.646	−1.274	0.203	−1.852	0.064
BMI M3 (kg/m²)	24.1	21.1	28.1	22.2	20.4	29.3	0.625				
BDI M0	8.0	2.0	17.0	10.0	2.75	16.5	0.893	−0.513	0.606	−2.034	**0.042**
BDI M3	9.0	4.0	13.0	5.0	1.75	13.5	0.647				
STAIX1 M0	50.0	43.0	57.0	48.0	44.5	56.0	0.936	−2.374	**0.018**	−0.725	0.468
STAIX1 M3	46.0	39.0	48.0	47.5	45.3	56.0	0.075				

Group comparisons by Mann-Whitney-U, longitudinal analysis Wilcoxon paired signed-ranks test. NA = not applicable, SD = Standard deviation, bold numbers indicate significant differences.

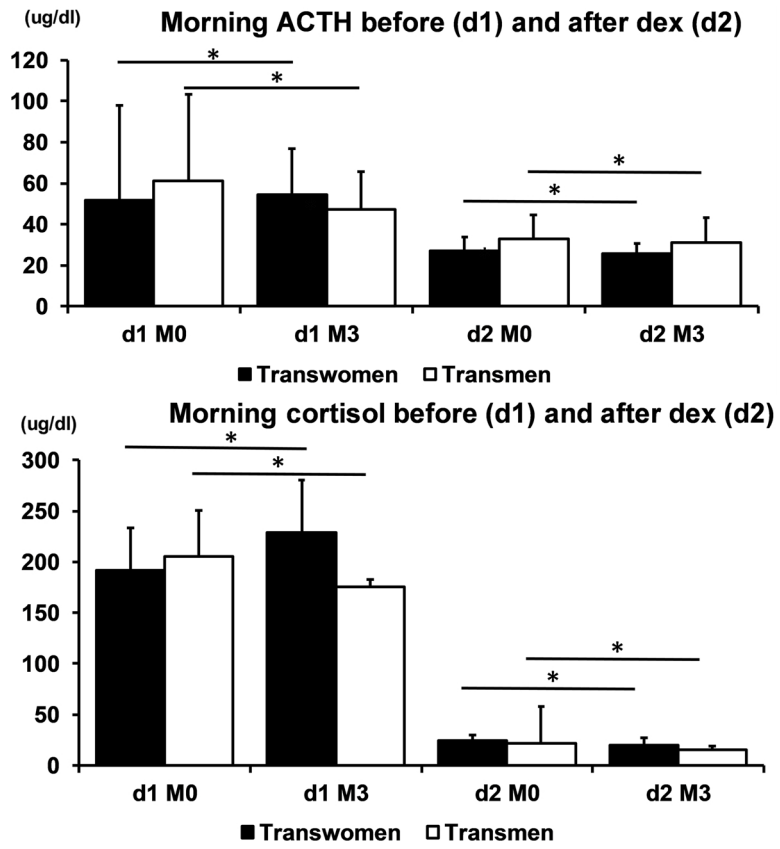

Fig. 2. ACTH and cortisol suppression following dexamethasone.
The dex/CRH test was performed before (M0) and three months after initiation of gender-affirming hormone treatment (M3).

as well as a significant time*group interaction ($F_{1,27.597}$ = 7.674; p = 0.010) (Table 3).

3.2. Diurnal variance in endocrine parameters and changes between timepoints

A diurnal variance in ACTH, copeptin and cortisol levels was observed while there was no difference in CBG and SHBG levels. E2 levels were significantly lower in the afternoon in transwomen at both timepoints while, in accordance with use of long-lasting T injections in transmen, there was only a significant decline in T in treatment-naïve subjects at baseline. For the assessment of the effects of sex steroids on HPA-axis measures in multivariate models, the corresponding sex steroid values at the given timepoint were used (Table S1). As expected, GAHT induced a reversal of the sex-typical hormonal milieu (Table S2). Further CBG and SHBG were significantly decreased by GAHT in transmen and SHBG significantly increased in transwomen. Copeptin levels were significantly lower on d1 at follow-up in transwomen (p = 0.047).

3.3. Assessing contribution of potential determinants of HPA-axis activity

Next, we were interested which factors potentially determine HPA-axis function. In univariate analyses there were no significant correlations across groups and time of T and E2 with ACTH and cortisol, neither at baseline nor following stimulation (data not shown). To account for interactions, separate mixed models were calculated to assess contributions of variables of interest on the HPA sum indices iAUC and gAUC for ACTH and cortisol, respectively. Each model included as covariates time, group, age, sex, BMI, STAIX1, BDI-II, FAI, E2 at baseline and the corresponding baseline hormone values for ACTH, cortisol, CBG and copeptin.

gAUC cortisol was positively determined by CBG (F $141_{.525}$ = 7.043; p = 0.011), STAIX1 (F$141_{.765}$ = 5.884; p = 0.020), baseline copeptin ($F_{1,33.050}$ = 31.233; p < 0.001), FAI ($F_{1,34.448}$ = 5.125, p = 0.030) and baseline cortisol ($F_{1,31.478}$; p = 0.005) and negatively by BMI ($F_{1.22,664}$ = 5.093; p = 0.034). In contrast, gAUC ACTH was only positively affected by ACTH at baseline ($F_{1,\ 34.981}$ = 70.938; p < 0.001) (Table 4). Estimates and confidence intervals are provided as supplements (Table S3-S4).

4. Discussion

In the present study, we demonstrate that a reversal of sex hormonal milieu has significant effects on HPA-axis reactivity as determined by the combined dex/CRH-test. As hypothesized, we found that GAHT leads to a significant increase in overall ACTH as well as cortisol output following CRH administration in transwomen, while the opposite was true for transmen. As indicated by the iAUC for both measures, GAHT did however not affect the dynamic response of ACTH or cortisol after CRH administration. In addition, the ACTH/Cortisol ratio following CRH challenge indicated an attenuated sensitivity for ACTH at the adrenal level in transmen after GAHT. Moreover, we found that copeptin levels predicted cortisol output while baseline ACTH levels predicted ACTH output in the dex/CRH test.

Most obviously, GAHT increased gAUC for ACTH in transwomen by 18% and decreased it by 15% in transmen which was accompanied by a 13% increase in overall cortisol secretion in transwomen and a strong decrease by 58% in transmen.

There are three other studies (Rubinow et al., 2005; Roelfsema et al., 2016; Sharma et al., 2014), which have investigated the effects of sex steroid withdrawal and replacement in men and women. In line with our results, the first study, including ten men, demonstrated that androgen withdrawal for one months by means of gonadotropin

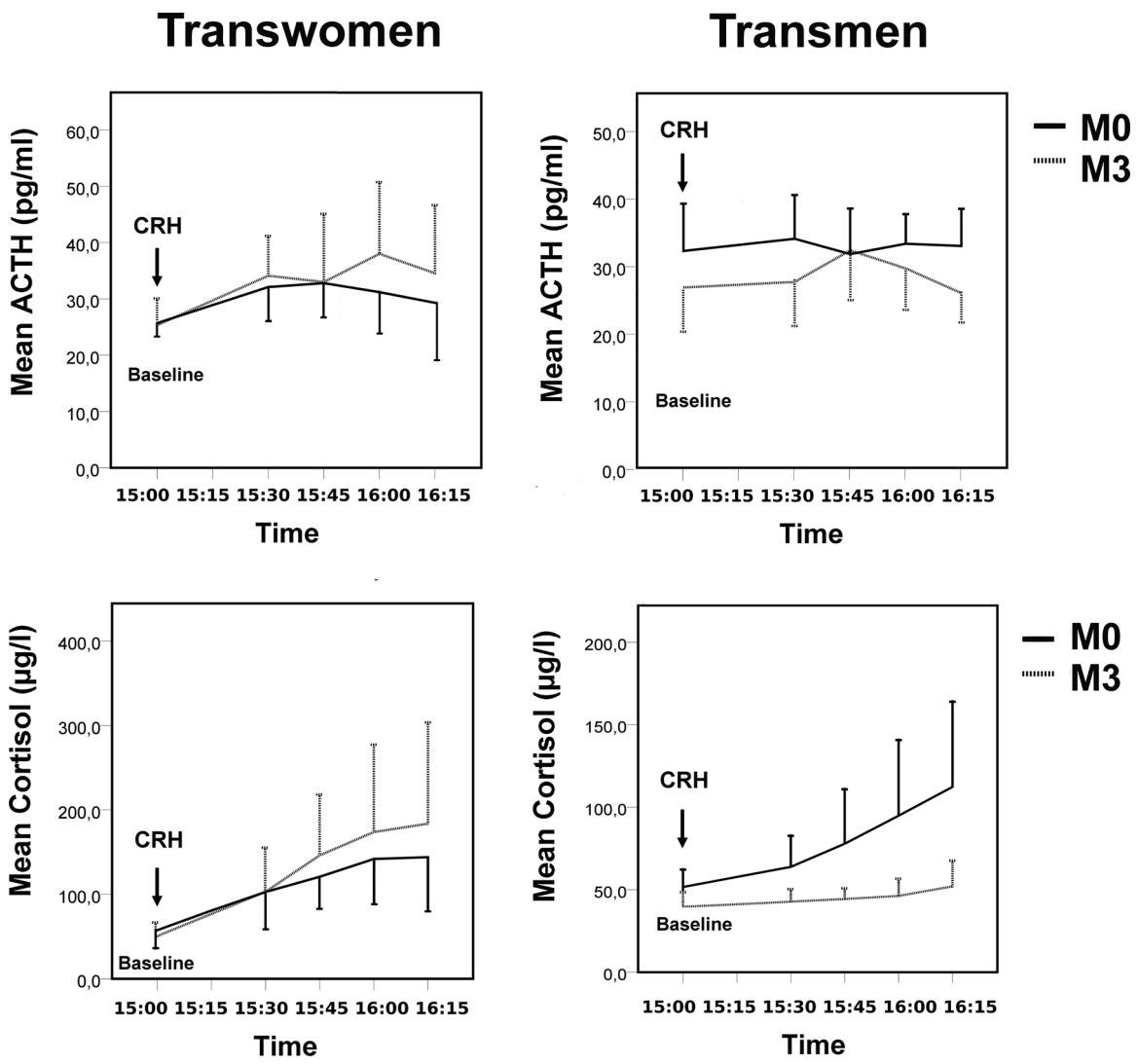

Fig. 3. ACTH and cortisol response following CRH administration.
ACTH and cortisol response following CRH-administration in the combined dex/CRH test before (M0, black line) and 3 months (M3, grey line) after initiation of gender affirming hormone therapy.

releasing hormone (GnRH)- analogue treatment results in an exaggerated CRH response for cortisol, although the opposite was true in terms of ACTH (Rubinow et al., 2005). In contrast, the study by Roelfsema et al. (2016) including 16 men and 25 women, did not observe any effect of a 4-weeks sex steroid withdrawal in men or women on overall or CRH-mediated ACTH secretion, while another comparable study (n = 24 men, n = 24 postmenopausal women) showed that spontaneous ACTH and cortisol secretion was reduced by E2 in women while there was no effect of T in men (Sharma et al., 2014).

A potential explanation for discrepant findings to our study is that in contrast to earlier studies, GAHT not only induces sex steroid withdrawal but reverses the sex steroid milieu. In addition, none of the above-mentioned studies has investigated long-term effects of a modulated sex steroid milieu for 12 weeks as in our study. Transwomen were not only T deprived, but E2 substituted, as were transmen not only lacking E2 and progesterone but treated with high dosages of T. The hypogonadal state induced by GnRH-analogue treatment in the study by Rubinow et al. e.g. did not only result in a reduction in T levels but also in E2 levels which is mainly derived from peripheral conversion of T. Although the authors did not observe a significant contribution of E2 on HPA-axis measures when including T in their model, it must be anticipated that there was a high multicollinearity between T and E2 making separation of the two effects difficult. However, at least in terms of gAUC for cortisol, free T was also a significant negative predictor in our study, while E2 was not. It must be kept in mind that due to the high fluctuations of E2 levels in transwomen in comparison to T in transmen, attributable to the different application, the effects of a single measure of sex steroids in this context has always to be interpreted with caution.

The influence of E2 on HPA reactivity in men was previously demonstrated (Kirschbaum et al., 1996) by short-term treatment of young men with transdermally applied E2 or placebo. HPA-axis activity was assessed by means of a psychosocial stressor paradigm in this study. Despite the short study period of 24–48 h, and an only marginally decrease in T levels, E2 treatment resulted in an increased ACTH and cortisol response, which was independent of the perceived stress as reported by the subjects. These findings are also in accordance with data from rodent studies indicating that treatment with estrogens in female rodents enhances HPA-axis reactivity to stressors (Burgess and Handa, 1992; Carey et al., 1995; McCormick et al., 2002).

T treatment in transmen resulted in a decrease in the ACTH/cortisol-ratio following CRH stimulation, indicating a desensitization effect for ACTH at the adrenal level. A similar observation was made before (Rubinow et al., 2005) in GnRH analogue-induced hypogonadal men.

Table 3
Mixed effects models for HPA-axis measures and indices.

Variable	Transwomen			Transmen			Group		Time		Time x Group	
	N	Mean	SD	N	Mean	SD	F	p	F	p	F	p
ACTH d1 M0 (ng/l)	10	51.8	45.9	15	61	42.3	1.054	0.314	**11.38**	**0.002**	0.099	0.755
ACTH d1 M3 (ng/l)*	9	54.4	22.3	14	47.4	18.4						
ACTH d2 M0 (ng/l)	10	26.9	7	14	32.8	11.7	0.198	0.662	**29.47**	**< 0.001**	1.077	0.309
ACTH d2 M3 (ng/l)*	9	25.8	4.8	14	31.1	12.1						
ACTH baseline M0 (ng/l)	9	25.7	4.0	15	32.3	13.5	2.987	0.097	1.263	0.274	1.907	0.180
ACTH baseline M3 (ng/l)	9	25.3	6.9	14	26.9	11.0						
gAUC ACTH M0 (ng/l*min)*	9	2079.5	893.7	14	2480	721.7	0	0.984	0.348	0.561	4.956	**0.036**
gAUC ACTH M3 (ng/l*min)*	9	2471.7	921.6	14	2156.3	727.4						
iAUC ACTH M0 (ng/l*min)	9	327.3	377.7	14	55	471.8	4.685	**0.045**	0.121	0.732	0.026	0.873
iAUC ACTH M3 (ng/l*min)	9	571.7	585.6	14	137.5	392.3						
Cortisol d1 M0 (ug/dl)	10	191.4	41.6	15	205.7	45.2	**5.979**	**0.022**	1.709	0.204	**14.436**	**0.001**
Cortisol d1 M3 (ug/dl)	10	228.7	51.9	14	175.5	36.7						
Cortisol d2 M0 (ug/dl)	9	24.4	5.2	15	21.5	7.6	3.052	0.095	**7.96**	**0.01**	0.044	0.836
Cortisol d2 M3 (ug/dl)	9	20.3	6.8	14	15.6	3.7						
Cortisol baseline M0 (ug/dl)	9	14.6	4.6	14	10.6	3.4	**12.466**	**0.002**	2.166	0.153	0.169	0.684
Cortisol baseline M3 (ug/dl)	9	12.0	4.5	14	11.0	3.7						
gAUC Cortisol M0 (ug/dl*min)*	9	2970.8	1257.5	15	2045.9	1318.3	**10.444**	**0.004**	3.487	0.076	**13.931**	**0.001**
gAUC Cortisol M3 (ug/dl*min)*	9	3354.1	2341	14	1202.9	363.7						
iAUC Cortisol M0 (ug/dl*min)*	9	1407.1	1453.5	15	641.8	1081.8	2.308	0.142	1.074	0.311	0.735	0.399
iAUC Cortisol M3 (ug/dl*min)*	9	1979.1	2404.6	14	100.4	236.8						
gAUC Cortisol/gAUC ACTH M0*	9	0.3	0.1	14	0.5	0.3	**17.249**	**< 0.001**	1.77	0.196	**7.674**	**0.01**
gAUC Cortisol/gAUC ACTH M3*	9	0.3	0.2	14	−0.5	1.8						

Linear mixed-effects model analysis with time and group effects as fixed, and subject effects as random and an unstructured covariance structure, adjusted for BDI and STAIX1 sum scores, BMI and age. CBG were further added to cortisol outcomes. Baseline refers to values determined before CRH administration at 15:00 p.m. gAUC: area under the curve with regard to ground, iAUC: area under the curve with regard to increase. Bold numbers indicate significant differences; * indicates transformation into log10 or Fisher-Yates normal scores.

Table 4
Mixed model analyses of potential determinants of HPA-axis indices.

Variable	gAUC Cortisol			gAUC ACTH		
	F	p	t	F	P	t
Group	1.143	0.295		0.060	0.808	
Time	0.095	0.761		0.187	0.669	
Age	2.517	0.126		1.520	0.229	
BMI	5.093	**0.034**	−2.257	0.214	0.648	
BDI	0.691	0.411		1.322	0.258	
STAIX1	5.884	**0.020**	2.426	0.002	0.964	
Estradiol	1.713	0.203		0.822	0.373	
FAI	5.125	**0.030**	−2.264	0.004	0.947	
Copeptin	31.233	**< 0.001**	5.589	2.131	0.153	
ACTH baseline	3.039	0.092		70.935	**< 0.001**	8.421
Cortisol baseline	8.929	**0.005**	2.988			
CBG	7.043	**0.011**	2.654			

Linear mixed-effects model analysis with time and group effects as fixed, and subject effects as random and an unstructured covariance structure. Separate mixed models were calculated to assess contributions of factors that potentially determine HPA-axis function. F-values illustrate effect sizes, while T-values are presented to illustrate direction of the significant effects.

These differences in adrenal sensitivity to ACTH have also been postulated to explain discrepancies regarding higher basal ACTH secretion but equal cortisol levels in men compared to women (Roelfsema et al., 1993) and in vitro experiments confirmed that T inhibits ACTH-stimulated but not basal corticosteroid output (Nowak et al., 1995).

Interestingly, baseline copeptin levels before CRH administration were a strong, although not the only, determinant of overall cortisol secretion as well as the iAUC but not for the ACTH response. In line, in a recent study, post-dex copeptin levels were shown to explain for an increase in dex/CRH responsiveness in obese subjects (Schinke et al., 2017). On the one hand, this would be in line with the idea, that the combined dex/CRH-test is suitable for demasking increased chronic vasopressin secretion (Rivier and Vale, 1983b), on the other hand it seems counterintuitive, as the effects of vasopressin should be mediated via an increase in ACTH secretion. However, it must be kept in mind that vasopressin is also capable of directly stimulating adrenal cells in the zona fasciculata to release cortisol (Perraudin et al., 1993) and is also produced locally (Ang and Jenkis, 1984), hereby exerting autocrine functions in terms of cortisol production. We might e.g. speculate that a high vasopressin tone, reflected by copeptin levels, increases responsiveness to ACTH (Enyeart et al., 1993) via increased intracellular calcium levels in adrenal cells (Guillon et al., 1995). CRH is also capable of directly stimulating the adrenal cells via CRHR1 and 2 (Müller et al., 2001).

As mentioned before, changes in HPA-axis activity have been reported in a variety of psychiatric disorders (Deuschle et al., 1998; Heuser, 1998; Heuser et al., 1994a; Weber et al., 2000; Yehuda et al., 1993). Importantly, however, data are partly conflicting, and a detailed discussion of the results would go beyond the scope of this paper. In particular, there is a paucity of studies examining the prognostic values of HPA-axis dysregulation on the incidence of psychiatric disorders. As the dex/CRH test is quite costly and time-consuming to perform, there are only studies examining the effects on cortisol and ACTH dynamics in already depressed subjects (von Bardeleben and Holsboer, 1989), their first-line relatives (Holsboer et al., 1995) and/or on the value for predicting relapse in terms of depressive episodes (Ising et al., 2007). Some, but not all (Carpenter et al., 2009) of these studies have shown that depression (Heuser, 1998; Heuser et al., 1994b; Ising et al., 2007; von Bardeleben and Holsboer, 1989), as well as risk for depression (Holsboer et al., 1995) and treatment response (Ising et al., 2007) is associated with a blunted dexamethasone suppression of cortisol and an exaggerated ACTH and cortisol secretion following CRH administration. Although we accounted for the effects of current mood and anxiety on HPA-axis measures by means of the used questionnaires, it remains to be seen if the increased, respectively blunted ACTH and cortisol response has prognostic implications in transgender people, particularly in predicting mood and anxiety disorders.

Another important finding of our study is that both treatments induced an attenuated suppression of ACTH and cortisol by dexamethasone. This indicates a higher sensitivity of the GR at the

pituitary level. In line with the results for transmen, women with polycystic ovary syndrome (PCOS) presenting with hyperandrogenism and disturbances in menstrual cycle, show a higher sensitivity to dexamethasone suppression (Milutinović et al., 2011). These results are also in accordance with animal studies demonstrating that E2 induces downregulation of both GR binding and transcript levels in anterior pituitary (Peiffer and Barden, 1987; Turner, 1990) and upregulation of arginine vasopressin and corticotrophin-releasing-hormone derived from the paraventricular nucleus (PVN) (Seale et al., 2004).

4.1. Strengths and limitations

To the best of our knowledge, this is the first study investigating the effects of a complete reversal of the sex-hormonal milieu on HPA-axis activity in humans.

However, some limitations must be considered. First, although it has been shown before that women show a stronger response in the dex/CRH test (Heuser et al., 1994a; Kunugi et al., 2006), our study was underpowered with regard to detect inter-individual gender differences.

In line, it must be emphasized that the sample size of our study is rather small, given the low prevalence of gender dysphoria. Accordingly, we could not account for every potential confounder, otherwise “overstretching” our models.

In addition, as dexamethasone bioavailability has been shown to affect the results of the dex/CRH test (Menke et al., 2016), we cannot exclude that this may have also affected the observed GAHT-induced changes. However, it has been repeatedly shown that dexamethasone does not bind to corticosteroid-binding globulins which would be the most obvious explanation for GAHT-induced changes in dexamethasone bioavailability. Differences in bioavailability of dexamethasone between men and women have however indeed been demonstrated (Pasquali et al., 2002). The activity of the 11β-hydroxysteroid dehydrogenase type 1 and 2, which catalyze interconversion of dexamethasone to 11-dehydrodexamethasone may explain the difference between the sexes. Previous studies have shown that obesity and sex may have different effects on 11β-hydroxysteroid dehydrogenase activity and, consequently, on cortisol concentrations (Andrew et al., 1998) and HPA-axis responsiveness to CRH and AVP (Schinke et al., 2017).

Furthermore, information about anxiety and depressive symptoms were only available at the level of self-report questionnaires, but not at the level of clinical diagnoses. Moreover, we did not study other brain systems with a putative relevance. For example, a potential explanation for our findings is a direct effect of sex hormones on brain neurotransmitter systems that influence the HPA axis such as the serotonin system or BDNF levels. For example, T in contrast to E2 activates the serotonin system in transgender people (Kranz et al., 2015) and has antidepressive and anxiolytic properties in rodents that are mediated, in part, by its aromatization in the dentate gyrus (Carrier et al., 2015). In contrast, brain-derived neurotrophic factor (BDNF) levels, which are also critical for emotional behaviors are influenced by E2 treatment but not T treatment in transgender people (Fuss et al., 2015). Interestingly, median BDI levels in transmen were descriptively lower after 3 months of GAHT but were unaffected in transwomen. Thus studying the interplay between HPA axis, emotional behaviors and the serotonin system may be fruitful for future studies in transgender people.

A further limitation in this context is that anti-androgenetic therapy by CPA itself in transwomen may affect stress response due to its ability not only to bind to the androgen receptor (AR) but also to a lesser degree to the progesterone receptor (PR), the GR and the MR which are involved in the regulatory feedback system. However, several studies have shown that CPA up to 100 mg/d in transgender patients (de Vries et al., 1986) or men undergoing androgen deprivation therapy due to prostate cancer (Schürmeyer et al., 1986) does not induce secondary adrenal insufficiency, indicating if any, only minor effects on the hypothalamic-pituitary part of the HPA-axis. The situation may be different with regard to spironolactone used as an antiandrogen outside Europe in transgender patients, due to its high affinity and antagonistic activity to the MR that is thought to be involved in determining the threshold for activation of the HPA-axis (Cornelisse et al., 2011; Heuser et al., 2000). However, a study by Roelfsema et al. (Roelfsema et al., 2016) did show that at least in terms of pulsatile negative feedback by cortisol the GR and not the MR is the major determinant.

4.2. Conclusion

Here, we demonstrate that sex hormones critically influence HPA-axis reactivity. This finding on the one hand expands the current knowledge of HPA-axis physiology and may on the other hand be particularly relevant for long-term mental health of transgender people as a relation between HPA-axis activity and mental health has been repeatedly claimed. Future studies should assess how the up- and downregulation of HPA-axis reactivity to stressors may affect mental health in this vulnerable population.

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Conflict of interest

The authors declare that there are no competing interests.

CRediT authorship contribution statement

Johannes Fuss: Conceptualization, Investigation, Supervision, Writing - original draft, Writing - review & editing. **Lena Claro:** Investigation, Writing - original draft, Writing - review & editing. **Marcus Ising:** Conceptualization, Writing - review & editing. **Sarah V. Biedermann:** Formal analysis, Writing - review & editing. **Klaus Wiedemann:** Formal analysis, Writing - review & editing. **Günter K. Stalla:** Funding acquisition, Methodology, Writing - review & editing. **Peer Briken:** Funding acquisition, Supervision, Writing - review & editing. **Matthias K. Auer:** Conceptualization, Project administration, Supervision, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.02.023.

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