Universiteit Leiden
The Netherlands

Neurobiological correlates of antisociality across adolescence and young adulthood: a multi-sample, multi-method study

Blankenstein, N.E.; Rooij, M. de; Ginkel, J. van; Wilderjans, T.F.; Ruigh, E.L. de; Oldenhof, H.C.; … ; Jansen, L.M.C.

Original Article

CAMBRIDGE
UNIVERSITY PRESS

Neurobiological correlates of antisociality across adolescence and young adulthood: a multi-sample, multi-method study

Neeltje E. Blankenstein[1] , Mark de Rooij[2] , Joost van Ginkel[2] ,
Tom F. Wilderjans[2,3] , Esther L. de Ruigh[1] , Helena C. Oldenhof[1] ,
Josjan Zijlmans[1] , Tijs Jambroes[1], Evelien Platje[4] , Marjan de Vries-Bouw[5],
Susan Branje[6] , Wim H. J. Meeus[6] , Robert R. J. M. Vermeiren[7] ,
Arne Popma[1] and Lucres M. C. Jansen[1]

[1]Child and Adolescent Psychiatry and Psychosocial Care, Amsterdam UMC, Vrije Universiteit Amsterdam, de Boelelaan 1117, Amsterdam, the Netherlands; [2]Unit of Methodology and Statistics, Institute of Psychology, Faculty of Social and Behavioral Sciences, Leiden University, Leiden, the Netherlands; [3]Research Group of Quantitative Psychology and Individual Differences, Faculty of Psychology and Educational Sciences, KU Leuven, Leuven, Belgium; [4]Applied University Utrecht, Utrecht, the Netherlands; [5]GGNet, Apeldoorn, the Netherlands; [6]Department of Youth and Family, Utrecht University, Utrecht, the Netherlands and [7]Department of Child and Adolescent Psychiatry, Curium-Leiden University Medical Center, Leiden, the Netherlands

Abstract

Background. Antisociality across adolescence and young adulthood puts individuals at high risk of developing a variety of problems. Prior research has linked antisociality to autonomic nervous system and endocrinological functioning. However, there is large heterogeneity in antisocial behaviors, and these neurobiological measures are rarely studied conjointly, limited to small specific studies with narrow age ranges, and yield mixed findings due to the type of behavior examined.

Methods. We harmonized data from 1489 participants (9–27 years, 67% male), from six heterogeneous samples. In the resulting dataset, we tested relations between distinct dimensions of antisociality and heart rate, pre-ejection period (PEP), respiratory sinus arrhythmia, respiration rate, skin conductance levels, testosterone, basal cortisol, and the cortisol awakening response (CAR), and test the role of age throughout adolescence and young adulthood.

Results. Three dimensions of antisociality were uncovered: 'callous-unemotional (CU)/manipulative traits', 'intentional aggression/conduct', and 'reactivity/impulsivity/irritability'. Shorter PEPs and higher testosterone were related to CU/manipulative traits, and a higher CAR is related to both CU/manipulative traits and intentional aggression/conduct. These effects were stable across age.

Conclusions. Across a heterogeneous sample and consistent across development, the CAR may be a valuable measure to link to CU/manipulative traits and intentional aggression, while sympathetic arousal and testosterone are additionally valuable to understand CU/manipulative traits. Together, these findings deepen our understanding of the fundamental mechanisms underlying different components of antisociality. Finally, we illustrate the potential of using current statistical techniques for combining multiple datasets to draw robust conclusions about biobehavioral associations.

Introduction

Antisociality puts adolescents and young adults at high risk of developing a wide range of problems (Brazil, van Dongen, Maes, Mars, & Baskin-Sommers, 2018). However, there is large heterogeneity in the severity and type of antisociality across adolescence and young adulthood (Moffitt, 2018). To better understand these individual differences, researchers related neurobiological measures such as autonomic nervous system (ANS) and neuroendocrinological functioning to antisociality (e.g. Alink et al., 2008; Dekkers et al., 2019; Portnoy & Farrington, 2015). However, diverse findings and limitations in study designs hinder firm conclusions on these biobehavioral associations across age. Therefore, we examined the continuous relation between multiple neurobiological measures and distinct dimensions of antisociality across the entire adolescent and young adult age range (9–27 years) by harmonizing six heterogeneous study samples (*N* = 1489).

A heightened tendency for antisociality has been associated with low arousal (sensation seeking hypothesis; Zuckerman, 1990) and a lack of fear (fearlessness hypothesis; Raine & Liu, 1998). Lower resting heart rate (HR), a marker of low ANS activity, is often found to

be associated with higher levels of aggression, delinquency, (violent) offending, and psychopathic traits, irrespective of age or sex (Portnoy & Farrington, 2015). In contrast, a higher respiration rate (RR) – a general measure of ANS functioning – was related to antisociality characterized by heightened emotionality and was more prominent in girls than in boys (and was higher in girls than in boys; Oldenhof et al., 2019). This fits well with models stating that callous-unemotional (CU) aggressive behavior is related to low arousal, while anxiety- or frustration-based aggression is related to high arousal (Blair, 2013; Fanti, 2018).

In addition, studies on the *parasympathetic* branch of the ANS (promoting resting conditions) show that respiratory sinus arrhythmia (RSA) an index of PNS activity and a marker of self-regulation is *lowered* in antisocial youths (Beauchaine & Thayer, 2015). This seems related to dysfunctions in emotion-regulation abilities. However, findings diverge between sexes, or studies included only boys, and non-significant findings have also been reported (Beauchaine, Hong, & Marsh, 2008; Bimmel, Van IJzendoorn, Bakermans-Kranenburg, Juffer, & De Geus, 2008; De Vries-Bouw et al., 2011; De Wied, Boxtel, Posthumus, Goudena, & Matthys, 2009; de Wied, van Boxtel, Matthys, & Meeus, 2012; Marsh, Beauchaine, & Williams, 2008; Oldenhof et al., 2019).

Findings on the *sympathetic* branch of the ANS (promoting fight or flight reactions) are also mixed depending on the type of antisociality studied. Lowered SNS (longer pre-ejection period, PEP) has been associated with conduct disorder, while heightened SNS (higher skin conductance levels, SCLs) with more grandiose-manipulative traits, with effects primarily found in adolescence, and less in childhood (Beauchaine, Gatzke-Kopp, & Mead, 2007; Beauchaine, Katkin, Strassberg, & Snarr, 2001; MacDougall, Salekin, & Gillen, 2019; Marsh et al., 2008). However, non-significant findings have also been reported (van Zonneveld, Platje, de Sonneville, van Goozen, & Swaab, 2017). Thus, findings on ANS functioning suggest specific associations with different aspects of antisociality, yet importantly, findings are inconclusive, mostly limited to males, and the role of age remains unclear.

Findings on neuroendocrinological measures and antisociality in youth also depend on the specific behavior examined. First, testosterone has been associated with aggression (although modestly), social dominance, impulsivity, and approach-related behaviors (Archer, 2006; Carré & Archer, 2018; Geniole et al., 2020; Peper, Braams, Blankenstein, Bos, & Crone, 2018; Rowe, Maughan, Worthman, Costello, & Angold, 2004), but there is limited evidence for associations with conduct disorder- or CU-like traits (e.g. Loney, Butler, Lima, Counts, & Eckel, 2006; but see Pajer et al., 2006). Second, lower levels of basal cortisol or a lower cortisol awakening response (CAR) have been related to higher levels of externalizing behavior in children (but not adolescents, Alink et al., 2008). In adolescents, associations were found with psychopathic/CU-traits (Jambroes et al., 2019; Loney et al., 2006; but see Feilhauer, Cima, Korebrits, & Nicolson, 2013), persistent aggression (McBurnett, Lahey, Rathouz, & Loeber, 2000; Platje *et al.* 2013a; Yi-Zhen & Jun-Xia, 2009), and impulsivity (Feilhauer et al., 2013). However, these testosterone and cortisol studies too included mostly males, and findings diverge across age ranges. Finally, it has been suggested that high testosterone combined with low cortisol levels relate to status-seeking behaviors such as dominance (Mehta & Josephs, 2010). A recent meta-analysis that focused on these behaviors, and in addition

on risk taking, aggression, and psychopathy, only moderately supports this 'dual-hormone hypothesis' (Dekkers et al., 2019). It is currently unclear if this dual-hormone hypothesis applies to specific dimensions of antisociality across adolescence and young adulthood. This warrants a robust study on neuroendocrinological factors and antisociality across the entire adolescent age range in the context of multiple neurobiological measures.

In sum, although prior studies have laid the groundwork for biological underpinnings of antisociality in youth, many used case-control designs, narrow age ranges, and modest sample sizes. As a result, little is known about the relation between the *severity* of distinct *dimensions* of antisociality and neurobiological functioning *throughout adolescence and young adulthood*, ranging from typically developing individuals to individuals with severe antisociality. Indeed, recent frameworks call for a more dimensional approach to understand mental dysfunctions (Insel et al., 2010), which may eventually aid in the assessment of specific forms of antisocial behavior in practice (Glenn, 2019). Studying a large heterogeneous sample may therefore provide a deeper, fundamental insight into these dynamics.

The current study

We studied (1) effects of multiple neurobiological measures in unison (i.e. conjointly), (2) in a large heterogeneous sample, (3) across a broad age range, and (4) on multiple dimensions of antisociality. We combined six heterogeneous samples (1489 participants, combined age range = 9–27 years, 67% male) to examine associations between ANS and neuroendocrinological measures and antisociality across the entire adolescent and young adult age range. Because we set out to examine heterogeneity in antisociality throughout adolescence and young adulthood, we included individuals from populations with varying backgrounds in antisociality severity: adolescents from the general population, adolescents referred to a diversion program (i.e. due to minor delinquent acts), adolescents with a conduct disorder diagnosis, adolescents and young adults currently in closed youth care or detained in juvenile detention center due to severe antisocial behavior, and young adults characterized by a multitude of problems. The inclusion of these varied samples thus enables to study this heterogeneity. In this study, a prominent focus was thus to harmonize and find the optimal statistical approach for analyzing multiple datasets in order to robustly answer our research questions.

Our main goal was to uncover specific dimensions of antisociality and subsequently aimed to relate these to ANS measures [HR, RR, PEP (SNS), RSA (PNS), SCL (SNS)], testosterone, cortisol, and the CAR. We focused on resting measures, which give an index of the body's neurophysiological and neuroendocrinological attunement of the stress system. We included testosterone for its role in aggression and social-dominance-related behaviors. We expected that ANS measures would differentially relate to specific dimensions of antisociality (Portnoy & Farrington, 2015), whereas testosterone would relate to dimensions such as aggression and social dominance (Archer, 2006; Dekkers et al., 2019). Finally, we expected that heightened cortisol functioning related to reduced psychopathic/CU-like traits (Loney et al., 2006) and heightened aggression (McBurnett et al., 2000; Yi-Zhen & Jun-Xia, 2009). For each dimension of antisociality we tested associations with all neurobiological measures in unison, and tested the role of age and sex.

Methods and materials

Participants

Participants came from six samples of cross-sectional and longitudinal studies, collected in the Netherlands between 2002 and 2016. All studies were approved by their respective ethical committees and all participants and caregivers gave written informed consent. The complete sample included 1489 participants with a total of 2443 observations, due to the longitudinal nature of two of the samples (see below). Participants were between 9.00 and 27.18 years old (M_{age} = 16.54, S.D.$_{age}$ = 2.39), and 67% were male (note that the age range of girls was limited to 18 years, warranting a cautious interpretation of related effects). Table 1 provides an overview of each sample's participants' characteristics and descriptive statistics of the behavioral outcome variables and the neurobiological independent variables. Below we give a brief description of each sample (see also online Supplementary Appendix A and Table S1). Papers that include data that were used in the current study are indicated with an asterisk in the reference list. All samples included at least five neurobiological measures, and at least one self-report measure on antisociality.

Sample descriptions

Sample 1 included three annual waves of data of the longitudinal population sample RADAR-Y (Branje & Meeus, 2018). Sample 2 included two waves of data of male adolescents who were referred to a delinquency diversion program after having committed a minor offense, and non-delinquent controls (Popma et al., 2006, 2007). Sample 3 consisted of adolescent girls and boys with conduct disorder and controls from the Dutch portion of a European multi-center study (Freitag, 2014; Oldenhof et al., 2019). Sample 4 consisted of adolescents (girls and boys) in a closed treatment facility for compulsory treatment due to severe antisocial behavior (Jambroes et al., 2019). Sample 5 included adolescent boys in juvenile justice institutions, referred because of severe behavioral problems or criminal offenses (de Ruigh, Jansen, Vermeiren, & Popma, 2019). Finally, sample 6 included multi-problem adolescent and young adult males who struggle with a variety of psychosocial problems and have a history of juvenile justice problems (Zijlmans et al., 2018, 2019).

Self-report measures

To derive dimensions of antisociality we used the following self-report measures: the Reactive and Proactive Aggression questionnaire (RPQ; Raine et al., 2006), the Youth Self-Report (YSR; Achenbach & Rescorla, 2001), the Adult Self-Report (ASR; Rescorla & Achenbach, 2004), and the Youth Psychopathic Index-short version (YPI-sv; van Baardewijk et al., 2010). The RPQ assesses 23 proactive and reactive aggressive actions on a three-point Likert scale (0: *never*, 1: *sometimes*, 2: *often*; for example: 'Got angry when I did not get my way'). For the YSR and ASR we included 31 and 29 items of aggressive and rule-breaking behaviors, respectively, which are scored on a three-point Likert scale (0: *never*, 1: *sometimes*, 2: *often*; for example: 'I don't keep by the rules at school/work or somewhere else'). Finally, the YPI-sv assessed 18 psychopathic traits, on a four-point Likert scale (ranging from 0: *Does not apply at all*, to 3: *Applies very well*; for example: 'It's easy for me to manipulate people').

Importantly, these questionnaires were not administered in each sample, but there was overlap in used items from the questionnaires across samples (see online Supplementary Table S1), and our goal was to study associations between broad dimensions of antisociality and neurobiology. Therefore, we entered *all raw item scores* – of all samples together – in a principal component analyses (PCA)-like method [coupled matrix factorization (CMF)] that took the overlap in questionnaires (items) between samples into account. As such, broad dimensions of antisociality were derived in a data-driven way rather than including pre-defined subscales of these questionnaires in only a portion of the samples. Importantly, this technique derived antisociality measures representing the same broad dimensions across the different samples (see section 'Data analyses').

Neurobiological measures

Neurobiological measures included resting HR, PEP, RSA, RR, SCLs, testosterone levels, cortisol, and the CAR. Specific data collection procedures and analysis methods for each sample can be found in the original respective papers (indicated with an asterisk in the reference list). In the online Supplementary materials (Appendix B), we briefly describe assessment protocols across samples. Online Supplementary Table S1 depicts data availability of the samples, and Fig. 1(*d–l*) depicts raw observed data of the neurobiological measures across age, showing considerable between-subjects heterogeneity.

Data analyses

We used three sets of analyses, which we briefly summarize here and are further described in Appendix C of the online Supplementary materials. Code is available via https://osf.io/qn95r/. It is important to note that in these analyses – in which we combined and analyzed the datasets – we did not control for sample. This was done to not remove any interesting variance between the samples, i.e. by controlling for sample, one removes heterogeneity. First, to derive broad general dimensions of antisociality from the – partially overlapping – questionnaires assessed across the samples, we used coupled matrix factorization (CMF) (Sorber, Van Barel, & De Lathauwer, 2015; Van Deun, Smilde, Van Der Werf, Kiers, & Van Mechelen, 2009). CMF reduces the items across all questionnaires from all samples – even when not all questionnaires (items) are administered in each sample – to their underlying dimensions. We tested for a one-, two-, three-, and four-component solution and retained the solution that optimally balances model fit (i.e. sum of squared residuals) and model complexity (i.e. the number of components). The items' content and the component loadings of the chosen solution were used to label each component and to link each component to an underlying dimension of antisociality. We used a varimax rotation to ensure a clear structure of the loadings of each item per component (i.e. simple structure), thus facilitating interpretation of the components. Components were allowed to correlate, as they all reflect dimensions of antisociality.

Next, we applied multiple imputations to deal with missing neurobiological data (Rubin, 2004; Van Buuren & Groothuis-Oudshoorn, 2010). The data structure and availability of the variables across the samples are depicted in online Supplementary Table S1, and details of the multiple imputation procedure are outlined in online Supplementary Appendix C. The data were imputed 100 times. Each of the 100 imputed datasets has a rather

Table 1. Descriptive statistics of the total sample and of each subsample

Sample	Total	1: Population sample[a]	2: Diversion program sample[a]	3: Dutch FemNAT-CD sample	4: Closed youth care sample	5: Juvenile justice institution sample	6: Multi-problem young adult sample
Sample characteristics							
Sample size N	**1489**	450	185	195	119	413	127
% boys	**67**	57	100	16.0	39	100	100
% typically-developing	**61**	100	33	46	0	0	0
Age M (s.d.)	**16.54 (2.39)**	15.99 (0.92)	15.23 (2.50)	14.39 (2.26)	15.79 (1.34)	18.58 (1.71)	22.42 (2.47)
Age range	**9.00–27.18**	13.67–19.56	11.26–20.66	9.00–21.95	13.17–20.28	13.92–24.45	16.68–27.18
Behavioral outcomes M (s.d.)							
CU/manipulative traits	**0.02 (0.05)**	0.01 (0.03)	0.03 (0.05)	0.03 (0.07)	0.03 (0.09)	0.02 (0.04)	0.05 (0.08)
Intentional aggression/ conduct	**0.02 (0.05)**	0.01 (0.03)	0.03 (0.05)	0.02 (0.07)	0.06 (0.07)	0.02 (0.05)	0.03 (0.08)
Reactivity/irritability/ impulsivity	**0.03 (0.04)**	0.02 (0.02)	0.03 (0.05)	0.05 (0.05)	0.05 (0.07)	0.03 (0.04)	0.06 (0.06)
Neurobiological independent variables M (s.d.)							
HR (beats/min)	**71.77 (10.63)**	70.36 (10.15)	76.35 (10.91)	75.50 (10.13)	77.25 (10.52)	71.66 (10.27)	65.61 (9.49)
PEP (ms)	**107.98 (20.44)**	113.88 (18.84)	99.05 (21.60)	100.90 (19.19)	100.19 (18.45)	99.84 (19.73)	111.49 (17.17)
RSA (ms)	**84.31 (46.76)**	85.71 (48.18)	76.05 (42.95)	90.98 (46.59)	91.04 (49.44)	79.91 (43.64)	85.83 (42.92)
RR (breaths/min)	**17.51 (2.57)**	17.31 (2.52)	17.83 (2.61)	17.76 (2.59)	18.01 (2.61)	17.89 (2.65)	16.88 (2.37)
SCL (μS)	**5.11 (3.17)**	5.48 (3.33)	3.71 (2.22)	5.30 (3.19)	4.81 (2.72)	4.89 (2.92)	5.11 (3.28)
Testos (pmol/l)	**182.17 (120.14)**	156.46 (105.03)	167.77 (112.54)	77.47 (78.17)	125.00 (99.87)	289.71 (78.32)	343.46 (79.86)
Cortisol (nmol/l)	**6.13 (5.38)**	4.77 (3.21)	4.98 (6.74)	4.36 (3.44)	6.53 (3.78)	9.59 (3.37)	13.99 (11.93)
CAR AUCg (nmol/l)	**1044.06 (463.28)**	1086.03 (438.14)	700.95 (447.41)	1005.30 (426.83)	1071.59 (323.33)	1054.30 (427.41)	1317.40 (575.93)
CAR AUCi (nmol/l)	**47.87 (401.73)**	−42.74 (405.20)	111.73 (297.70)	96.12 (406.44)	288.22 (297.13)	126.30 (382.21)	285.54 (411.19)

CU, callous-unemotional; HR, heart rate; PEP, pre-ejection period; RSA, respiratory sinus arrhythmia; RR, respiration rate; SCL, skin conductance level; Testos, testosterone; CAR AUCg, cortisol awakening response: area under the curve with respect to the ground; CAR AUCi, cortisol awakening response: area under the curve with respect to the increase.
[a]Sample includes two or more measurement waves. Descriptive statistics depicted here are collapsed across all measurement waves for parsimony.
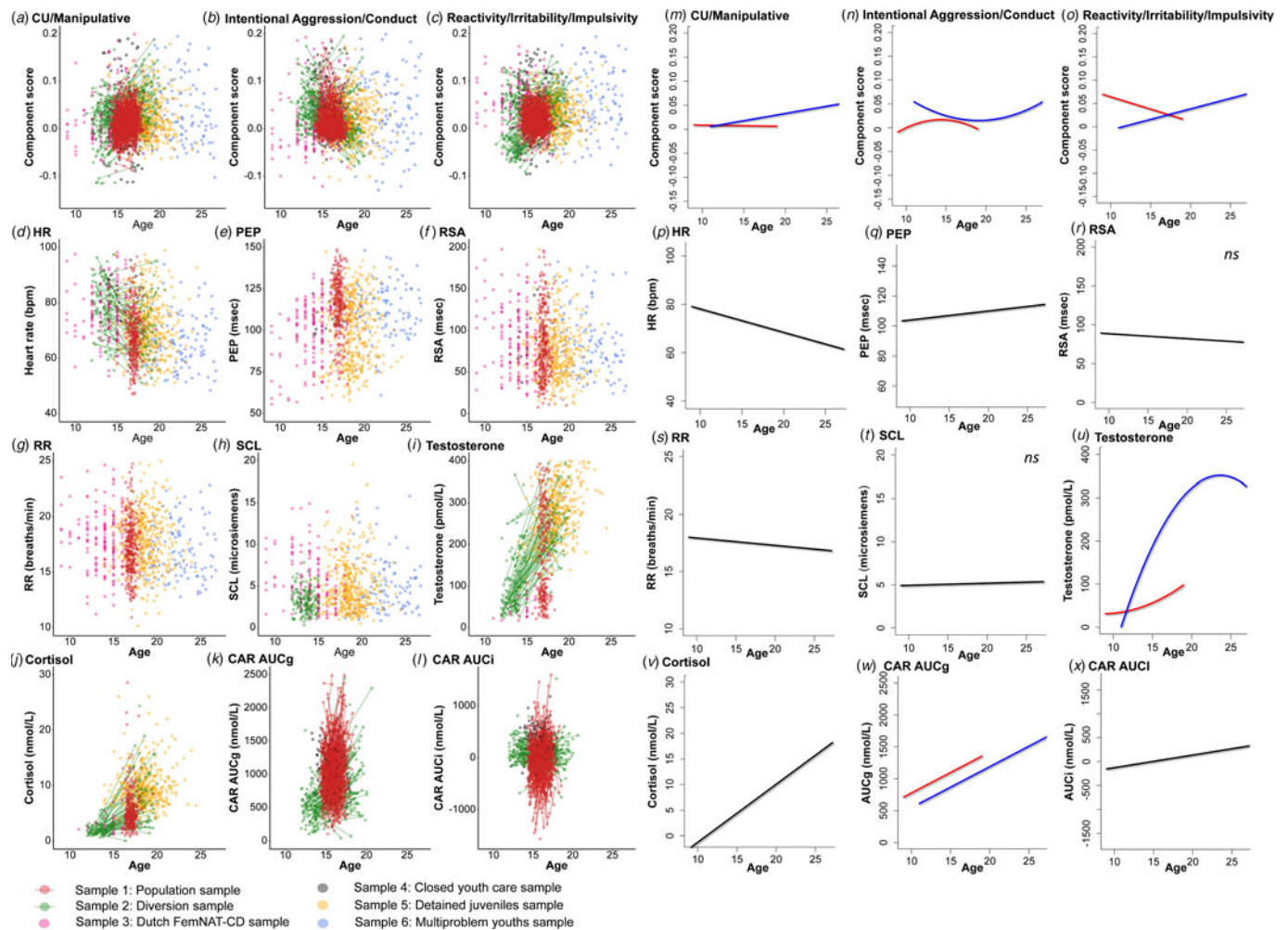
Fig. 1. (a)–(l) Observed data of the behavioral dimensions of antisociality across age, before multiple imputations, for CU/manipulative traits, intentional aggression/conduct, and reactivity/irritability/impulsivity, and the neurobiological measures. The different colors indicate the different samples. The data show considerable heterogeneity. (m)–(x) Results of the general linear models with clustered bootstraps for the models testing age (linear and quadratic) and sex effects across 100 imputed datasets, for CU-traits/manipulative aggression, intentional aggression/conduct, and reactivity/irritability/impulsivity and the neurobiological measures. The blue line represents males and the red line represents females. Note that the age overlap for girls and boys is limited to 13–18 years, therefore the developmental patterns can only be compared with caution. HR, heart rate; PEP, pre-ejection period; RSA, log-transformed respiratory sinus arrhythmia RR, respiration rate; SCL, skin conductance level; CAR AUCg, cortisol awakening response area under the curve with respect to the ground; CAR AUCi, cortisol awakening response, area under the curve with respect to the increase.

complicated structure where some parts have a nested structure (i.e. multiple waves within persons; samples 1 and 2) and other parts do not have such a nested structure (samples 3–6). To deal with this complexity, we applied a general linear model with clustered bootstrap that takes into account dependency in parts of the data (GLMCB; Deen & de Rooij, 2019). This technique is better able to deal with this partial dependency than multilevel/linear mixed effects models, a common technique applied to nested data. An additional advantage of the clustered bootstrap approach is that almost no distributional assumptions need to be satisfied for the data (and the model residuals).

Model specification

We calculated quasi-likelihood information criteria (QIC) values (Pan, 2001) for different models for each of the 100 imputed datasets. QIC values indicate model fit and lower values indicate a better fitting model. We compared the resulting 100 QIC values between the models via box plots. The model with the lowest

QIC values across the 100 imputed datasets was retained as the best-fitting model. In the case of equal model fits, the simplest model was chosen. The best-fitting model was subsequently entered in a GLMCB, using 100 bootstrap samples for each imputed dataset. To obtain the estimated parameter value, we took the average of the 100 estimates from the 100 imputed datasets (Rubin, 2004). To obtain a confidence interval we used the percentile approach on the 100×100 estimated values so that both the variance within the imputed datasets as well as the variance between the imputed datasets was taken into account (van Ginkel & Kiers, 2011). If zero was *not* included in the confidence interval, the effect was considered significant (at $\alpha = 0.05$).

The models that were compared to describe age and sex patterns of all measures are specified in online Supplementary Table S2 (upper half). Using QIC values we compared models with age as a linear effect, age as a quadratic polynomial, and models including an interaction between sex and these age effects. Our main hypotheses concerned the associations of the neurobiological measures with the dimensions of antisociality, and the role

of age and sex. Using QIC values, for each dimension we compared a model with the main effects of neurobiological measures only, with models additionally including age as a linear effect, age as a quadratic polynomial, and models including an interaction between sex and these age effects. These model specifications are depicted in online Supplementary Table S2 (lower half). When there was an age effect, we additionally compared different models in which age interacted with the neurobiological measures.

Results

Disclosing dimensions of antisociality through CMF

The CMF analysis showed that the three-component solution was most appropriate as it optimally balanced model fit and model complexity. In particular, although being more complex, the three-component solution (loss = 31 920, explained variance of 56.9%) fitted the data clearly better than the one- (loss = 36 459, 50.8%) and two-component (loss = 33 965, 54.2%) solutions. The four-component solution fitted the data better (loss = 30 394, 58.9%) but not so much better to warrant the added complexity. Moreover, the three-component solution was preferred over the four-component solution as the items loaded relatively unambiguously on the three components, whereas none of the items had a highest loading on the fourth component, which explained only 5.74% of the variance. Online Supplementary Fig. S1A shows, for each pair of the three components, the loadings (after varimax rotation) of each item on their respective components and illustrates the separation of the items based on the component loadings. The three-component solution explained 56.9% of the variance in the data, with all three components being more or less of equal importance (i.e. each component explains more or less the same amount of variance in the data). Online Supplementary Table S2 gives an overview of explained variances per solution, Table 2 provides a complete list of the items loading substantially on each component for the chosen three-component solution, and depicts the items' original subscales, and online Supplementary Table S4 additionally shows the item loadings for the one-, two-, and four-component solutions.

After inspection of the loadings of each item on the components and the item content, we labeled the components:

Callous-unemotional/Manipulative traits: included items such as 'To be nervous or worried is a sign of weakness', 'I have talents that go far beyond other people's', 'I don't let my feelings affect me as much as other people's feelings seem to affect them'.

Intentional aggression/conduct: included items such as 'Had fights with others to show who was on top', 'I physically attack people', 'I bully others a lot'.

Reactivity/irritability/impulsivity: included items such as 'Gotten angry when frustrated', It often happens that I do something without thinking about it', 'I am stubborn, sullen, or irritable'.

The varimax-rotated participant scores on these components were used in the subsequent analyses. The components were only moderately and negatively interrelated. To give some indication on how the components relate to subscales the raw items were originally derived from, online Supplementary Fig. S1B shows a Pearson correlation plot. However, note that these correlations are biased, because items of these subscales partly make up the components, and correlations are limited to participants who completed these questionnaires in the original studies. Finally, Fig. 1(*a–*

c) shows the raw data of the component scores across age and samples, showing considerable heterogeneity in these behavioral measures. This set the stage for our main analyses.

Development of behavioral outcome measures

Here, we describe the developmental patterns of our behavioral outcome measures, i.e. the components derived from the CMF. Test statistics are summarized in Table 3 and effects are visualized in Fig. 1 (m–o). Recall that we evaluated model fits by comparing the QIC values per multiply imputed dataset via box plots (online Supplementary Fig. S2). The model with the lowest QIC values across the 100 imputed datasets was retained as the best-fitting model. It should be noted that the age overlap for girls and boys is limited, therefore these developmental patterns should be compared with caution.

First, CU/manipulative traits was best described by a model including the interaction between (linear) age and sex (i.e. CU/manipulative = Age × Sex). For girls, this dimension appeared stable (but note the limited age range until 18 years), while for boys an increase from until 27 years was observed. Intentional aggression/conduct was best described by a quadratic age by sex interaction effect (i.e. intentional aggression/conduct = Age² × Sex). Here, reactive aggression peaked around 14 years for girls, while for boys, a quadratic dip was observed around 18–19 years. Finally, reactivity/irritability/impulsivity showed a linear age by sex interaction effect (i.e. reactivity/irritability/impulsivity = Age × Sex). Across age this measure decreased for girls while it increased for boys.

Development of neurobiological measures

Observed data of neurobiological measures across age, before multiple imputations, are shown in Fig. 1*d–l*. Results of the GLMCBs on the imputed datasets are visualized in Fig. 1*p–x* and summarized in Table 3. ANS measures were best described by models including age linear only (see online Supplementary Fig. S2 for QIC values across multiply imputed datasets, lower values indicate a better fit). While HR and RR decreased linearly with age, PEP increased with age, and RSA and SCL remained stable across age. Testosterone was best described as a model including a quadratic age by sex interaction effect, but note the limited age range in girls. Testosterone levels increased steeply for boys and leveled off into young adulthood. For girls, testosterone levels increased moderately during puberty. Basal cortisol and cortisol awakening response, area under the curve with respect to the increase (CAR AUCi) were best described by models including a linear age term only and increased across age, while cortisol awakening response, area under the curve with respect to the ground (CAR AUCg) showed an additional main effect of sex (girls > boys). These analyses across the imputed dataset show that the development of these neurobiological measures were in the expected direction and support the use of these imputed measures for our main analyses.

Relation between neurobiological measures and dimensions of antisociality

Next, we tested associations between neurobiological measures and the antisociality dimensions. Online Supplementary Fig. S3 shows a correlation plot of all measures across imputed datasets. These include zero-order correlations and partial correlations controlling

Table 2. Raw items per component and original subscales

Original subscale	Item content
Component 1: Callous-unemotional/manipulative traits	
RPQ Proactive aggression	20. Gotten others to gang up on someone else
YSR Rule-breaking	105. I use drugs
YPI Affective	3. I think that crying is a sign of weakness even if no one sees you
YPI Interpersonal	4. I have the ability to con people by using my charm and smile
YPI Interpersonal	5. I am good at getting people to believe me when I make something up
YPI Affective	6. When other people have problems it is often their own fault. Therefore one should not help them
YPI Interpersonal	8. I have talents that go far beyond other people's
YPI Interpersonal	9. It's easy for me to manipulate people
YPI Affective	10. To be nervous and worried is a sign of weakness
YPI Interpersonal	14. When I need to, I use my smile and my charm to use others
YPI Affective	15. I don't understand how people can be touched enough to cry by watching things on TV or movie
YPI Interpersonal	16. I am destined to become a well-known, important and influential person
YPI Affective	17. To feel guilty and remorseful about things you have done that have hurt other people is a sign of weakness
YPI Affective	18. I don't let my feelings affect me as much as other people's feelings seem to affect them
Component 2: Intentional Aggression/Conduct	
RPQ Proactive aggression	2. Had fights with others to show who was on top
RPQ Proactive aggression	4. Taken things from other students
RPQ Proactive aggression	6. Vandalized something for fun
RPQ Reactive aggression	7. Had temper tantrums
RPQ Reactive aggression	8. Damaged things because you felt mad
RPQ Proactive aggression	9. Had a gang fight to be cool
RPQ Proactive aggression	10. Hurt others to win a game
RPQ Proactive aggression	12: Used physical force to get others to do what you want
RPQ Reactive aggression	13. Gotten angry or mad when you lost a game
RPQ Proactive aggression	15. Used force to obtain money or things from others
RPQ Reactive aggression	16. Felt better after hitting or yelling at someone
RPQ Proactive aggression	17. Threatened or bullied someone
RPQ Proactive aggression	18. Made obscene phone calls for fun
RPQ Proactive aggression	21. Carried a weapon to use in a fight
RPQ Reactive aggression	22. Gotten angry or mad or hit others when teased
RPQ Proactive aggression	23: Yelled at others so they would do things for you
YSR Rule-breaking	2. I drink alcohol without my parents' permission.
YSR Aggression	3. I argue a lot
YSR Aggression	16. I am mean to others
YSR Aggression	20. I damage my own things
YSR Aggression	21. I damage other people's things
YSR Aggression	22. I don't obey my parents
YSR Aggression	23. I am disobedient at school
YSR Rule-breaking	26. I don't feel guilty after have done something I shouldn't have
YSR Rule-breaking	28. I don't keep by the rules at home, at school, or somewhere else
YSR Aggression	28. I fight a lot

(Continued)

Table 2. (*Continued.*)

Original subscale	Item content
YSR Rule-breaking	39. I deal with boys and girls who get into trouble
YSR Rule-breaking	42. I lie and cheat
YSR Aggression	57. I physically attack people
YSR Rule-breaking	67. I run away from home
YSR Rule-breaking	72. I set fires
YSR Rule-breaking	81. I steal from home
YSR Rule-breaking	82. I steal outdoors
YSR Aggression	94. I bully others a lot
YSR Rule-breaking	96. I thing about sex too much
YSR Aggression	97. I threaten people to hurt them
ASR Aggression	3. I argue a lot
ASR Aggression	37. I get in fights a lot
ASR Aggression	57. I physically attack people
ASR Aggression	68. I scream or yell a lot
Component 3: Reactivity/Irritability/Impulsivity	
RPQ Reactive aggression	1. Yelled at others when they have annoyed you
RPQ Reactive aggression	3. Reacted angrily when provoked by others
RPQ Reactive aggression	5. Gotten angry when frustrated
RPQ Reactive aggression	11. Become angry or mad when you do not get your way
RPQ Reactive aggression	14. Gotten angry when others threatened you
RPQ Reactive aggression	19. Hit others to defend yourself
YSR Aggression	19. I try to get a lot of attention
YSR Rule-breaking	63. I rather hang out with older boys and girls than same-aged peers
YSR Aggression	68. I yell a lot
YSR Aggression	86. I am stubborn
YSR Aggression	87. My mood or feelings change suddenly
YSR Aggression	89. I am suspicious
YSR Rule-breaking	80. I curse or use dirty words
YSR Aggression	95. I am hot tempered
YSR Rule-breaking	99. I smoke tobacco
YSR Rule-breaking	101. I skip classes or skip school
YSR Rule-breaking	104. I am louder than other boys or girls
YPI Behavioral	1. I have probably skipped school or work more than most other people
YPI Behavioral	2. I consider myself as a pretty impulsive person
YPI Behavioral	18. It often happens that I talk first and think later
YPI Behavioral	11. I get bored quickly by doing the same thing over and over
YPI Behavioral	12. It often happens that I do things without thinking ahead
YPI Behavioral	13. It has happened several times that I've borrowed something and then lost it
ASR Aggression	5. I blame others for my problems
ASR Rule-breaking	6. I use drugs
ASR Aggression	16. I am mean to others
ASR Rule-breaking	20. I damage my own things

(*Continued*)

Table 2. (*Continued.*)

Original subscale	Item content
ASR Rule-breaking	23. I don't keep by the rules at work or somewhere else
ASR Rule-breaking	26. I don't feel guilty when I've done something I shouldn't have
ASR Aggression	28. I get along badly with my family
ASR Rule-breaking	39. I deal with people who get into trouble
ASR Rule-breaking	41. I am impulsive or do things without thinking
ASR Rule-breaking	43. I lie or cheat
ASR Aggression	55. My moods change between elation and depression
ASR Rule-breaking	76. My behavior is irresponsible
ASR Aggression	81. My behavior is very changeable
ASR Rule-breaking	82. I steal
ASR Aggression	85. I am stubborn, sullen, or irritable
ASR Aggression	87. My mood or feelings change suddenly
ASR Rule-breaking	90. I drink too much alcohol or get drunk
ASR Rule-breaking	92. I do things that can get me in trouble with the law
ASR Aggression	95. I am hot tempered
ASR Aggression	97. I threaten people to hurt them
ASR Rule-breaking	114. I fail to pay debts or fulfill other financial obligations
ASR Aggression	116. I am easily upset
ASR Rule-breaking	117. I have trouble managing money or payment cards
ASR Aggression	118. I am too impatient
ASR Rule-breaking	122. I have trouble keeping jobs

RPQ, Reactive Proactive Aggression Questionnaire; YPI, Youth Psychopathic Trait Index (short version); YSR, Youth Self Report; ASR, Adult Self Report.

for age and sex. These exploratory correlations indeed suggest meaningful associations between neurobiological measures and our dimensions of antisociality, which we formally tested using GLMCB. Results of the GLMCB models are summarized in Table 4 and significant effects are visualized in Fig. 2. Recall that we inspect QIC values across multiple imputed datasets to assess which model best fitted the data. QIC values are visualized in online Supplementary Fig. S4, and lower values indicate a better fit.

First, QIC values showed that CU/manipulative traits were best described by a model with neurobiological main effects only (i.e. the model: CU/manipulative = HR + PEP + log_RSA0 + RR + SCL + Testosterone + Cortisol + AUCg + AUCi; see online Supplementary Fig. S4A, left panel). After bootstrapping this model, we observed significant effects of PEP, testosterone, and CAR AUCi. Here, shorter PEP, more testosterone, and greater cortisol awakening reactivity response were related to higher levels of CU/manipulative traits (see Fig. 2a–c).

Second, intentional aggression/conduct was also best described by a model with neurobiological main effects only (i.e. intentional aggression/conduct = HR + PEP + log_RSA0 + RR + SCL + Testosterone + Cortisol + AUCg + AUCi, online Supplementary Fig. S4B, left panel). The bootstrapped model showed that a higher AUCi (cortisol awakening reactivity) was significantly related to higher levels of reactive/frustration-based aggression (see Fig. 2d). QIC values in online Supplementary Fig. S4B suggest that the model including an $Age^2 \times Sex$ interaction may marginally outperform the model with neurobiological main effects only. Bootstrapping this model (HR + PEP +

log_RSA0 + RR + SCL + Testosterone + Cortisol + AUCg + AUCi + $Age^2 \times Sex$) showed that the effect of CAR AUCi remained significant (with $\beta = 0.141$). Thus, the effect of CAR AUCi on intentional aggression/conduct remained robust when controlling for the $Age^2 \times Sex$ interaction effect.

Third, reactivity/irritability/impulsivity was best described by a model including the main effects of the neurobiological measures, as well as a (linear) age by sex interaction effect (i.e. reactivity/irritability/impulsivity = HR + PEP + log_RSA0 + RR + SCL + Testosterone + Cortisol + AUCg + AUCi + $Age^1 \times Sex$). Models including interactions between neurobiological measures and age and sex did not indicate a better fit. After bootstrapping the best model, we observed that only effects of sex, age, and their interaction were significant. Here, reactivity/irritability/impulsivity values increased for boys and decreased for girls.

Finally, to add to existing literature on the dual-hormone hypothesis, we also explicitly examined whether models including testosterone-by-cortisol interactions improved model fits. This was not the case: for none of the antisociality components did QIC values indicate a better fit when including any of the possible testosterone-by-cortisol interactions (i.e. testosterone × cortisol, testosterone × CAR AUCg, testosterone × CAR AUCi; see online Supplementary Fig. S4, right panels for QIC box plots).

Discussion

This multi-sample study is unique in that we were able to examine associations between multiple neurobiological measures conjointly

Table 3. Results of the best age models of each variable

Variable	b	CI lower	CI upper	β
CU/manipulative traits				
Intercept	0.0112	−0.0208	0.0453	–
Sex	**−0.0388**	**−0.0786**	**−0.0017**	**−0.008**
Age linear	−0.0003	−0.0024	0.0018	−0.013
Sex × Age linear	**0.0033**	**0.0010**	**0.0058**	**0.002**
Intentional Aggression/Conduct				
Intercept	−0.1700	−0.3480	0.0322	–
Sex	0.4047	0.1793	0.6145	**0.084**
Age linear	0.0261	−0.0017	0.0508	1.366
Age quadratic	−0.0009	−0.0018	0.0000	−0.114
Age linear × Sex	**−0.0492**	**−0.0769**	**−0.0192**	**−0.024**
Age quadratic × Sex	0.0015	0.0005	0.0025	**0.002**
Reactivity/Irritability/Impulsivity				
Intercept	0.1172	0.0856	0.1486	–
Sex	**−0.1693**	**−0.2054**	**−0.1339**	**−0.039**
Age linear	**−0.0053**	**−0.0073**	**−0.0033**	**−0.310**
Age linear × Sex	**0.0098**	**0.0076**	**0.0121**	**0.005**
Heart rate				
Intercept	87.7960	84.0890	91.4401	–
Age linear	**−0.9691**	**−1.1767**	**−0.7594**	**−0.218**
Pre-ejection period				
Intercept	98.0285	88.8593	106.2537	–
Age linear	**0.6013**	**0.1454**	**1.0978**	**0.070**
Respiratory sinus arrhythmia				
Intercept	94.8839	76.4982	114.2942	–
Age linear	−0.6395	−1.6954	0.3845	−0.033
Respiration rate				
Intercept	18.5822	17.4705	19.7596	–
Age linear	**−0.0646**	**−0.1291**	**−0.0036**	**−0.060**
Skin conductance level				
Intercept	4.7035	3.1665	6.1534	–
Age linear	0.0246	−0.0444	0.0951	0.018
Testosterone				
Intercept	77.1134	−61.6840	245.3216	–
Age linear	−10.7027	−35.0738	9.6264	−0.213

(*Continued*)

Table 3. (*Continued.*)

Variable	*b*	CI lower	CI upper	*β*
Age quadratic	0.6223	−0.1109	1.4964	−0.431
Sex	**−953.2643**	**−1210.6165**	**−716.8602**	**−0.075**
Age linear × Sex	**114.6445**	**83.9609**	**149.3242**	**0.022**
Age quadratic × Sex	**−2.8224**	**−3.9678**	**−1.8233**	**−0.001**
Cortisol				
Intercept	−12.5764	−18.3970	−8.4867	–
Age linear	**1.1316**	**0.8811**	**1.4968**	**0.500**
CAR AUCg				
Intercept	136.1294	−264.4061	484.2551	–
Sex	**−230.2575**	**−285.2945**	**−171.5003**	**−0.005**
Age	**64.2381**	**42.0723**	**89.1338**	**0.331**
CAR AUCi				
Intercept	−388.1281	−653.7942	−114.1861	–
Age linear	**26.3613**	**9.3043**	**42.7556**	**0.157**

CI, confidence interval; CAR AUCg, cortisol awakening response, area under the curve with respect to the ground; CAR AUCi, cortisol awakening response, area under the curve with respect to the increase.
Variables in the first column indicate the included independent variables based on the model selection via QICs. Significant effects ($\alpha = 0.05$) are in bold.

in 1489 participants ranging from none to severe antisocial behavior problems across the entire adolescent age range (9–27 years, 67% male). Three dimensions of antisociality emerged: CU/manipulative traits, intentional aggression/conduct, and reactivity/irritability/impulsivity, which showed considerable heterogeneity. Our main analyses revealed that (1) more CU/manipulative traits related to shorter PEPs, higher levels of testosterone, and higher cortisol awakening reactivity, independent of age and sex; (2) higher intentional aggression/conduct related to higher cortisol awakening reactivity, independent of age and sex; and (3) reactivity/irritability/impulsivity was explained by age and sex only, in which a decrease across age was found for girls and an increase across age for boys.

To derive broad general dimensions of antisociality that are consistent across the different samples, we used CMF. This is a PCA-like method that can deal with multiple samples by assuming overlap in items between samples. A solution with three dimensions was retained which reflected CU and manipulative, intentional aggressive, and reactive and irritable behaviors. These dimensions resonate with existing models on (adolescent) antisocial behavior, which also consider similarly differentiated aspects of antisociality (e.g. Blair, 2013) and prior factor analyses (although on aggression specifically) in adolescents (Smeets et al., 2017), and adults (Van Donkelaar et al., 2020). This indicates that CMF results in meaningful components which could be used in subsequent analyses.

Our developmental analyses showed that CU/manipulative traits increased moderately with age for boys, and was stable for girls. An important caveat is the limited overlap in age ranges between boys (10–27) and girls (9–18 years), therefore these comparisons have to be interpreted with caution. Prior research indicates that CU-traits are relatively stable from early childhood on, but primarily for

those individuals with elevated CU-traits (Frick, Ray, Thornton, & Kahn, 2014). Our sample was more heterogeneous as it included a wider range of individuals, ranging from typically-developing participants to participants with moderate to severe problem behavior. The developmental pattern for intentional aggression/conduct fits well with prior study on the development of physical aggression and violence (Tremblay, 2010), although overrepresentation of youths with problem behavior in late adolescence/young adulthood may in part explain the increase for males in late adolescence. Finally, reactivity/irritability/impulsivity decreased for girls and increased for boys. Prior research in typical development (including both sexes) have shown both decreases (Harden & Tucker-Drob, 2011), increases, and adolescent peaks (Peper et al., 2018) in impulsivity measures, while irritability remains stable across adolescence (Brotman, Kircanski, & Leibenluft, 2017; Caprara, Paciello, Gerbino, & Cugini, 2007). Our dimension covers both impulsivity as well as irritability. Therefore, future research should confirm the developmental pattern of this construct.

Most neurobiological measures showed expected developmental patterns. HR, PEP, and RR all decreased with age, confirming prior research. Although RSA also decreased linearly with age, in line with prior research, this effect did not attain significance. SCL was stable throughout adolescence and young adulthood, which extends prior research in childhood and early-middle adolescence (El-Sheikh, 2007). Furthermore, basal cortisol levels and the CAR also increased with age (Gunnar, DePasquale, Reid, & Donzella, 2019; Kiess et al., 1995; Oskis, Loveday, Hucklebridge, Thorn, & Clow, 2009; Platje et al., 2013b). Finally, testosterone showed the expected increase for boys, and to a lesser extent for girls (Hiort, 2002; Peper et al., 2018). In sum, these findings confirm existing knowledge on neurobiological development, and extend these findings by robustly documenting the developmental

Table 4. Results of the bootstrapped models relating the antisociality dimensions to the neurobiological measures

Variable	b	CI lower	CI upper	β
CU/manipulative traits				
Intercept	0.04680	−0.01271	0.12175	–
HR	−0.00019	−0.00051	0.00010	−0.043
PEP	**−0.00016**	**−0.00030**	**−0.00002**	**−0.069**
log_RSA0	−0.00279	−0.01177	0.00347	−0.033
RR	0.00025	−0.00096	0.00136	0.014
SCL	−0.00015	−0.00216	0.00252	−0.010
Testos	**0.00005**	**0.00003**	**0.00008**	**0.137**
Cortisol	0.00081	−0.00009	0.00181	0.095
CAR AUCg	0.00000	−0.00002	0.00001	−0.049
CAR AUCi	**0.00001**	**0.00000**	**0.00003**	**0.109**
Intentional Aggression/Conduct				
Intercept	0.01394	−0.04174	0.07063	–
HR	0.00011	−0.00017	0.00038	0.025
PEP	−0.00011	−0.00026	0.00003	−0.051
log_RSA0	0.00161	−0.00501	0.00781	0.019
RR	0.00056	−0.00058	0.00180	0.032
SCL	−0.00120	−0.00331	0.00099	−0.083
Testos	0.00002	−0.000002	0.00005	0.062
Cortisol	−0.00057	−0.00167	0.00038	−0.068
CAR AUCg	0.00000	−0.00002	0.00001	−0.024
CAR AUCi	**0.00002**	**0.00000**	**0.00003**	**0.150**
Reactivity/Irritability/Impulsivity				
Intercept	**0.28219**	**0.19062**	**0.37471**	**–**
Sex	**−0.15680**	**−0.19379**	**−0.11859**	**−0.037**
Age linear	**−0.01408**	**−0.01830**	**−0.00968**	**−0.825**
Sex × Age linear	**0.00879**	**0.00625**	**0.01122**	**0.005**
HR	0.00017	−0.00012	0.00045	0.044
PEP	−0.00002	−0.00016	0.00010	−0.012
log_RSA0	−0.00195	−0.00947	0.00479	−0.026
RR	−0.00063	−0.00164	0.00043	−0.040
SCL	0.00111	−0.00047	0.00286	0.086
Testos	0.00001	−0.00002	0.00005	0.040
Cortisol	0.00084	−0.00004	0.00185	0.112
CAR AUCg	−0.00001	−0.00002	0.00000	−0.095
CAR AUCi	0.00001	−0.000001	0.00002	0.092

CI, confidence interval; HR, heart rate; PEP, pre-ejection period; log_RSA0, log-transformed respiratory sinus arrhythmia; RR, respiration rate; SCL, skin conductance level; CAR AUCg, cortisol awakening response, area under the curve with respect to the ground; CAR AUCi, cortisol awakening response, area under the curve with respect to the increase.
Variables in the first column indicate the included independent variables based on the model selection via QICs. Significant effects ($\alpha = 0.05$) are in bold.

pathways across the entire adolescent and young adult age range in a heterogeneous sample.

Our main focus was on associations between neurobiological measures and dimensions of antisociality. First, we found *increased* SNS activity (specifically, shorter PEP) and hypothalamus–pituitary–adrenal (HPA)-axis functioning (specifically, a higher CAR) to be related to increased CU/manipulative traits. Other research reported blunted – rather than heightened – SNS activity with
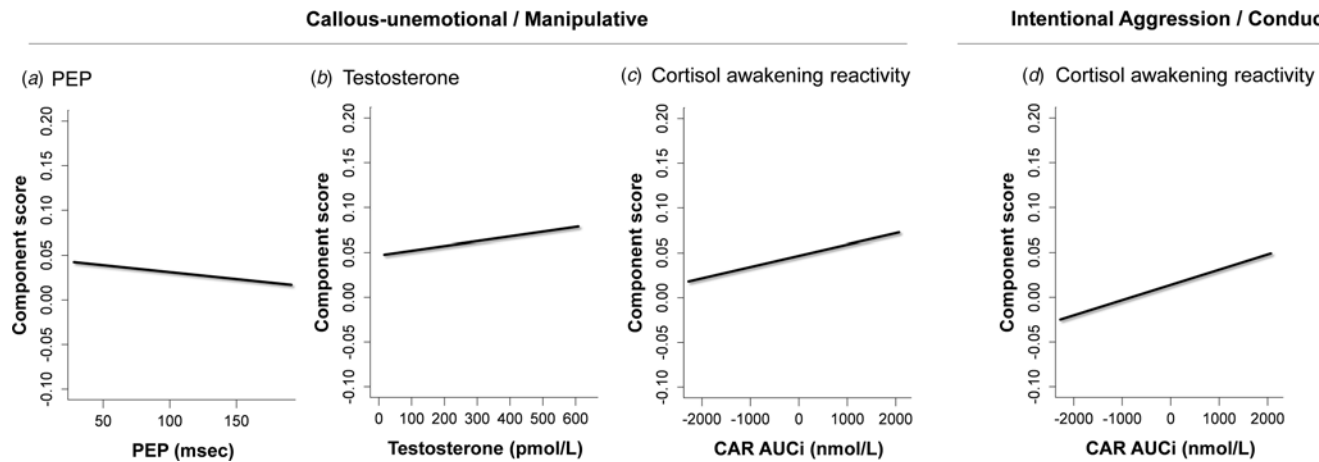
Fig. 2. Results of the general linear models with clustered bootstraps for the biobehavioral models. Displayed are significant associations between dimensions of antisociality and neurobiological measures. PEP, pre-ejection period; RR, respiration rate; CAR AUCi, cortisol awakening response, area under the curve with respect to the increase (i.e. cortisol awakening reactivity).

psychopathic traits, although these studies focused on youths with problem behaviors (Fanti, 2018; Fanti et al., 2019). Yet, other research does report heightened SNS functioning (although reflected in higher SCL) in adolescent males with – specifically – more grandiose-manipulative traits (MacDougall et al., 2019). Our dimension reflected both CU as well as (grandiose-)manipulative aspects. Moreover, our study included a wide range of individuals, ranging from typically developing adolescents to adolescents with more moderate to severe problems. Findings may differ when examining a wide range of individuals rather than focus on specific subgroups. Alternatively, the low arousal theory (e.g. Raine & Liu, 1998; Zuckerman, 1990), suggesting blunted SNS and HPA-axis activation, may only be pronounced in subgroups of individuals characterized by severe antisociality. In our exploratory analyses limited to the samples that could be characterized by more severe antisociality we did find negative associations between cortisol and Intentional aggression/conduct and between RR and reactivity/irritability/impulsivity (see online Supplementary materials p. 19), but not with CU/manipulative traits. Because examining biobehavioral associations in such subgroups in particular was not the goal of this study, future studies better equipped to define and select individuals with 'severe' antisociality, may formally address this research question. Instead, the current findings add to the literature by yielding a deeper understanding of these fundamental biobehavioral dynamics across a large heterogeneous sample.

Furthermore, a pronounced finding was that testosterone related positively CU/manipulative traits. This is in line with prior research on behaviors related to social dominance (Archer, 2006; Carré & Archer, 2018). However, no evidence was found for the dual-hormone hypothesis (Mehta & Josephs, 2010) on any of the dimensions of antisociality. The dual-hormone hypotheses suggest that a combination of low levels of cortisol combined with high levels of testosterone is related to higher levels of status-seeking behaviors, such as dominance. A recent meta-analysis (Dekkers et al., 2019) found modest evidence for this hypothesis for status, dominance, risk taking, aggression, and psychopathy. We add to existing research in showing that the dual-hormone effect is absent across adolescent and young adult heterogeneous development for these dimensions of antisociality, in the context of multiple neurobiological measures.

Furthermore, a higher CAR was also related to more intentional aggression/conduct, and this effect was slightly stronger than for CU/manipulative traits. This fits well with models suggesting that increased threat sensitivity gives rise to this dimension of antisociality (Blair, 2013) through hyperactivity of the ANS and HPA-axis (Fanti, 2018; Oldenhof et al., 2019). Finally, reactivity/irritability/impulsivity was not related to any of the neurobiological measures, although prior research did find associations between impulsivity and testosterone in typically developing adolescents (Peper et al., 2018) and with cortisol in detained adolescents (Feilhauer et al., 2013). The current study adds to this literature by showing absent associations across a heterogeneous sample, and extends prior research by covering aspects of both irritability and impulsivity.

One of our key aims was to test these associations across the entire adolescent age range. We found that these neurobiological associations were stable throughout adolescence and young adulthood and across both sexes. Indeed, prior research shows developmentally stable and sex-independent associations (Portnoy & Farrington, 2015). We show that this holds for multiple SNS and PNS measures. Regarding the CAR, our findings confirm a meta-analysis showing sex-independent associations, but diverge from this meta-analysis showing an absent association in adolescence (Alink et al., 2008). However, other, longitudinal, research did find associations between the CAR and CU-traits in adolescents (Jambroes et al., 2019; Loney et al., 2006). Future research should confirm these findings, preferably within a longitudinal study from childhood to early adulthood, which allows us to capture within-person developmental changes.

In contrast to prior research, we found no associations with HR, RSA, SCL, basal cortisol, and CAR AUCg (i.e. total cortisol during wakening). Possibly, the inclusion of multiple other ANS and hormonal measures resulted in these particular measures accounting for little additional variance. Importantly, our findings underscore that multiple neurobiological measures should be included, which yields more specific information about the contribution of indices of ANS and HPA-axis functioning (Alink et al., 2008; Oldenhof et al., 2019), and that specifically PEP, testosterone, and cortisol awakening reactivity play a pivotal role in explaining distinct dimensions of antisociality.

Strengths, limitations, and future directions

This study has a number of strengths, such as a robust, heterogeneous, and large combined sample size spanning a broad age range, and multiple neurobiological measures. Despite sample differences in the items used and data availability, we could combine and analyze these samples using a sophisticated set of analyses: CMF, multiple imputations, and GLMCB. This combination may be promising for future studies wishing to combine different datasets to increase sample size and robust findings. With the transition toward open science, data-harmonization techniques become increasingly important. Moreover, the current data-driven approach yields more insights into underlying components and mechanisms of antisociality. Insight into fundamental concepts and mechanisms underlying antisociality may subsequently inform clinicians in understanding the origins of antisocial behavior. Future studies may focus on relating these mechanisms to (preventive) interventions (e.g. see de Ruigh et al., 2021).

Despite these strengths, this study also had some limitations. First, sex was not equally distributed across age because of the sampling of the original studies. Conclusions about developmental trajectories for girls are thus limited to a less broad age range. Relatedly, in late adolescence/young adulthood, typically developing controls were underrepresented. Future research should strive for an equal distribution of males and females, and participants from various backgrounds, across all developmental phases. Furthermore, although factor loadings differentiated the three dimensions of antisociality, it should be noted that the dimensions were small-to-moderately negatively correlated. This may have had consequences for the interpretation of the three separate regression models (one for each dimension), which we interpreted independently. A solution would be to run a multivariate model, but this may lead to a less conservative analysis and a more complex – and thus less interpretable – model. Finally, for our behavioral measures we included self-report measures only. An opportunity for future research is to include multiple informants (e.g. self-, teacher-, and parent reports), as well as juvenile-justice registrations, psychosocial stress and emotion regulation tasks, and psychosocial factors such as socio-economic status, substance use, the influence of (delinquent) peers, and trauma (Moffitt, 2018). Such a comprehensive biopsychosocial perspective of multifaceted antisociality may give rise to person-based predictions of an individual's sensitivity to intervention.

Conclusions

This study is the first to examine associations between multiple dimensions of antisociality and multiple neurobiological measures conjointly in such a large, heterogenous sample across the full adolescent and young adult age range. We found that CU/manipulative traits were related to higher arousal (reflected in higher SNS), and higher levels of testosterone, whereas cortisol awakening reactivity was consistently related to both CU/manipulative traits and intentional aggression/conduct. These findings deepen our understanding of – developmentally stable – neurobiological correlates of antisociality components. Finally, this study also highlights the potential of using the current techniques to harmonize existing datasets, to optimize data use of unique populations, and for robust analyses. Together, this study yields fundamental insights into underlying components and mechanisms of antisociality across adolescence and young adulthood.

Author contributions. NEB: data curation, formal analysis (multiple imputations, CMF and GLMCB), visualization, writing – original draft, writing – review and editing; MdR: formal analysis (GLMCB), writing – original draft (methods and results); writing – review and editing (methods and results); JvG: formal analysis (multiple imputations), writing – original draft (methods and results); writing – review and editing (methods and results); TW: formal analysis (CMF), writing – original draft (methods and results); writing – review and editing (methods and results); EdR, HO, JZ, TJ, EP, and MdVB: investigation; SB, WM, RV, and AP: conceptualization (of original studies), investigation, writing – review and editing; LJ: conceptualization, investigation, writing – review and editing, supervision, funding acquisition. All authors approved the final version of the manuscript.

References

Note. References marked with an asterisk (*) are studies of which data are included in the current study.

Achenbach, T. M., & Rescorla, L. (2001). *Manual for the ASEBA school-age forms & profiles: An integrated system of multi-informant assessment.* New York, NY: Springer.

Alink, L. R., van IJzendoorn, M. H., Bakermans-Kranenburg, M. J., Mesman, J., Juffer, F., & Koot, H. M. (2008). Cortisol and externalizing behavior in children and adolescents: Mixed meta-analytic evidence for the inverse relation of basal cortisol and cortisol reactivity with externalizing behavior. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*, 50(5), 427–450.

Archer, J. (2006). Testosterone and human aggression: An evaluation of the challenge hypothesis. *Neuroscience & Biobehavioral Reviews*, 30(3), 319–345.

Beauchaine, T. P., Gatzke-Kopp, L., & Mead, H. K. (2007). Polyvagal theory and developmental psychopathology: Emotion dysregulation and conduct problems from preschool to adolescence. *Biological Psychology*, 74(2), 174–184.

Beauchaine, T. P., Hong, J., & Marsh, P. (2008). Sex differences in autonomic correlates of conduct problems and aggression. *Journal of the American Academy of Child & Adolescent Psychiatry*, 47(7), 788–796.

Beauchaine, T. P., Katkin, E. S., Strassberg, Z., & Snarr, J. (2001). Disinhibitory psychopathology in male adolescents: Discriminating conduct disorder from attention-deficit/hyperactivity disorder through concurrent assessment of multiple autonomic states. *Journal of Abnormal Psychology*, 110 (4), 610.

Beauchaine, T. P., & Thayer, J. F. (2015). Heart rate variability as a transdiagnostic biomarker of psychopathology. *International Journal of Psychophysiology*, 98 (2), 338–350.

Bimmel, N., Van IJzendoorn, M. H., Bakermans-Kranenburg, M. J., Juffer, F., & De Geus, E. J. (2008). Problem behavior and heart rate reactivity in adopted adolescents: Longitudinal and concurrent relations. *Journal of Research on Adolescence*, 18(2), 201–214.

Blair, R. J. R. (2013). The neurobiology of psychopathic traits in youths. *Nature Reviews Neuroscience*, 14(11), 786–799.

*Branje, S., & Meeus, W. H. J. (2018). Research on adolescent development and relationships (young cohort). DANS. doi: 10.17026/dans-zrb-v5wp

Brazil, I. A., van Dongen, J. D., Maes, J. H., Mars, R., & Baskin-Sommers, A. R. (2018). Classification and treatment of antisocial individuals: From behavior to biocognition. *Neuroscience & Biobehavioral Reviews*, 91, 259–277.

Brotman, M. A., Kircanski, K., & Leibenluft, E. (2017). Irritability in children and adolescents. *Annual Review of Clinical Psychology*, 13, 317–341.

Caprara, G. V., Paciello, M., Gerbino, M., & Cugini, C. (2007). Individual differences conducive to aggression and violence: Trajectories and correlates of irritability and hostile rumination through adolescence. *Aggressive Behavior*, 33(4), 359–374.

Carré, J. M., & Archer, J. (2018). Testosterone and human behavior: The role of individual and contextual variables. *Current Opinion in Psychology*, 19, 149–153.

Deen, M., & de Rooij, M. (2019). ClusterBootstrap: An R package for the analysis of hierarchical data using generalized linear models with the cluster bootstrap. *Behavior Research Methods*, 52(2), 572–590.

Dekkers, T. J., van Rentergem, J. A. A., Meijer, B., Popma, A., Wagemaker, E., & Huizenga, H. M. (2019). A meta-analytical evaluation of the dual-hormone hypothesis: Does cortisol moderate the relationship between testosterone and status, dominance, risk taking, aggression, and psychopathy? *Neuroscience & Biobehavioral Reviews*, 96, 250–271.

*de Ruigh, E. L., Bouwmeester, S., Popma, A., Vermeiren, R. R. J. M., van Domburgh, L., & Jansen, L. M. (2021). Using the biopsychosocial model for identifying subgroups of detained juveniles at different risk of re-offending in practice: A latent class regression analysis approach. *Child and Adolescent Psychiatry and Mental Health*, 15(1), 1–15.

*de Ruigh, E. L., Jansen, L. M., Vermeiren, R., & Popma, A. (2019). Psychopathic traits and empathic functioning in detained juveniles: Withdrawal response to empathic sadness. *International Journal of Forensic Mental Health*, 18(4), 1–14.

De Vries-Bouw, M., Popma, A., Vermeiren, R., Doreleijers, T. A., Van De Ven, P. M., & Jansen, L. M. (2011). The predictive value of low heart rate and heart rate variability during stress for reoffending in delinquent male adolescents. *Psychophysiology*, 48(11), 1597–1604.

De Wied, M., Boxtel, A. V., Posthumus, J. A., Goudena, P. P., & Matthys, W. (2009). Facial EMG and heart rate responses to emotion-inducing film clips in boys with disruptive behavior disorders. *Psychophysiology*, 46(5), 996–1004.

de Wied, M., van Boxtel, A., Matthys, W., & Meeus, W. (2012). Verbal, facial and autonomic responses to empathy-eliciting film clips by disruptive male adolescents with high versus low callous-unemotional traits. *Journal of Abnormal Child Psychology*, 40(2), 211–223.

El-Sheikh, M. (2007). Children's skin conductance level and reactivity: Are these measures stable over time and across tasks? *Developmental Psychobiology*, 49(2), 180–186.

Fanti, K. A. (2018). Understanding heterogeneity in conduct disorder: A review of psychophysiological studies. *Neuroscience & Biobehavioral Reviews*, 91, 4–20.

Fanti, K. A., Eisenbarth, H., Goble, P., Demetriou, C., Kyranides, M. N., Goodwin, D., … Cortese, S. (2019). Psychophysiological activity and reactivity in children and adolescents with conduct problems: A systematic review and meta-analysis. *Neuroscience & Biobehavioral Reviews*, 100, 98–107.

Feilhauer, J., Cima, M., Korebrits, A., & Nicolson, N. A. (2013). Salivary cortisol and psychopathy dimensions in detained antisocial adolescents. *Psychoneuroendocrinology*, 38(9), 1586–1595.

Freitag, C. (2014). Neurobiology and treatment of adolescent female conduct disorder: FemNAT-CD consortium: A new European cooperation. *European Child & Adolescent Psychiatry*, 23(8), 723.

Frick, P., Ray, J., Thornton, L., & Kahn, R. (2014). A developmental psychopathology approach to understanding callous-unemotional traits in children and adolescents with serious conduct problems. *Journal of Child Psychology and Psychiatry*, 55, 532–548.

Geniole, S. N., Bird, B. M., McVittie, J. S., Purcell, R. B., Archer, J., & Carré, J. M. (2020). Is testosterone linked to human aggression? A meta-analytic examination of the relationship between baseline, dynamic, and manipulated testosterone on human aggression. *Hormones and Behavior*, 123, 104644.

Glenn, A. L. (2019). Using biological factors to individualize interventions for youth with conduct problems: Current state and ethical issues. *International Journal of Law and Psychiatry*, 65, 101348.

Gunnar, M. R., DePasquale, C. E., Reid, B. M., & Donzella, B. (2019). Pubertal stress recalibration reverses the effects of early life stress in postinstitutionalized children. *Proceedings of the National Academy of Sciences*, 116(48), 23984–23988.

Harden, K. P., & Tucker-Drob, E. M. (2011). Individual differences in the development of sensation seeking and impulsivity during adolescence: Further evidence for a dual systems model. *Developmental Psychology*, 47(3), 739.

Hiort, O. (2002). Androgens and puberty. *Best Practice & Research Clinical Endocrinology & Metabolism*, 16(1), 31–41.

Insel, T., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D. S., Quinn, K., … Wang, P. (2010). Research domain criteria (RDoC): Toward a new classification framework for research on mental disorders. *The American Journal of Psychiatry*, 167(7), 748–751. doi:10.1176/appi.ajp.2010.09091379

*Jambroes, T., Jansen, L. M. C., Oostermeijer, S., Ven, P. M. v. d., Doreleijers, T. A. H., Vermeiren, R. R. J. M., & Popma, A. (2019). CU-traits and HPA-axis reactivity conjointly relate to treatment effect in adolescents with severe antisocial behavior. *Journal of Criminal Justice*, 65, 8.

Kiess, W., Meidert, A., Dressendörfer, R., Schriever, K., Kessler, U., Köunig, A., … Strasburger, C. (1995). Salivary cortisol levels throughout childhood and adolescence: Relation with age, pubertal stage, and weight. *Pediatric Research*, 37(4), 502–506.

Loney, B. R., Butler, M. A., Lima, E. N., Counts, C. A., & Eckel, L. A. (2006). The relation between salivary cortisol, callous-unemotional traits, and conduct problems in an adolescent non-referred sample. *Journal of Child Psychology and Psychiatry*, 47(1), 30–36.

MacDougall, E. A., Salekin, R. T., & Gillen, C. T. (2019). Adolescent psychopathy, heart rate, and skin conductance. *Psychophysiology*, 56(6), e13344.

Marsh, P., Beauchaine, T. P., & Williams, B. (2008). Dissociation of sad facial expressions and autonomic nervous system responding in boys with disruptive behavior disorders. *Psychophysiology*, 45(1), 100–110.

McBurnett, K., Lahey, B. B., Rathouz, P. J., & Loeber, R. (2000). Low salivary cortisol and persistent aggression in boys referred for disruptive behavior. *Archives of General Psychiatry*, 57(1), 38–43.

Mehta, P. H., & Josephs, R. A. (2010). Testosterone and cortisol jointly regulate dominance: Evidence for a dual-hormone hypothesis. *Hormones and Behavior*, 58(5), 898–906.

Moffitt, T. E. (2018). Male antisocial behaviour in adolescence and beyond. *Nature Human Behaviour*, 2(3), 177.

*Oldenhof, H., Prätzlich, M., Ackermann, K., Baker, R., Batchelor, M., Baumann, S., … Popma, A. (2019). Baseline autonomic nervous system activity in female children and adolescents with conduct disorder: Psychophysiological findings from the FemNAT-CD study. *Journal of Criminal Justice*, 65, 101564.

Oskis, A., Loveday, C., Hucklebridge, F., Thorn, L., & Clow, A. (2009). Diurnal patterns of salivary cortisol across the adolescent period in healthy females. *Psychoneuroendocrinology*, 34(3), 307–316.

Pajer, K., Tabbah, R., Gardner, W., Rubin, R. T., Czambel, R. K., & Wang, Y. (2006). Adrenal androgen and gonadal hormone levels in adolescent girls with conduct disorder. *Psychoneuroendocrinology*, 31(10), 1245–1256.

Pan, W. (2001). Akaike's information criterion in generalized estimating equations. *Biometrics*, 57(1), 120–125.

Peper, J. S., Braams, B. R., Blankenstein, N. E., Bos, M. G., & Crone, E. A. (2018). Development of multifaceted risk taking and the relations to sex steroid hormones: A longitudinal study. *Child Development*, 89(5), 1887–1907.

*Platje, E., Jansen, L. M., Raine, A., Branje, S. J., Doreleijers, T. A., de Vries-Bouw, M., … Meeus, W. H. (2013a). Longitudinal associations in adolescence between cortisol and persistent aggressive or rule-breaking behavior. *Biological Psychology*, 93(1), 132–137.

*Platje, E., Vermeiren, R. R., Branje, S. J., Doreleijers, T. A., Meeus, W. H., Koot, H. M., … Jansen, L. M. (2013b). Long-term stability of the cortisol awakening response over adolescence. *Psychoneuroendocrinology*, 38(2), 271–280.

*Popma, A., Jansen, L. M., Vermeiren, R., Steiner, H., Raine, A., Van Goozen, S. H., … Doreleijers, T. A. (2006). Hypothalamus pituitary adrenal axis and autonomic activity during stress in delinquent male adolescents and controls. *Psychoneuroendocrinology*, 31(8), 948–957.

*Popma, A., Vermeiren, R., Geluk, C. A., Rinne, T., van den Brink, W., Knol, D. L., … Doreleijers, T. A. (2007). Cortisol moderates the relationship between testosterone and aggression in delinquent male adolescents. *Biological Psychiatry*, 61(3), 405–411.

Portnoy, J., & Farrington, D. P. (2015). Resting heart rate and antisocial behavior: An updated systematic review and meta-analysis. *Aggression and Violent Behavior*, 22, 33–45.

Raine, A., Dodge, K., Loeber, R., Gatzke-Kopp, L., Lynam, D., Reynolds, C., … Liu, J. (2006). The reactive–proactive aggression questionnaire: Differential correlates of reactive and proactive aggression in adolescent boys. *Aggressive Behavior*, 32(2), 159–171.

Raine, A., & Liu, J. H. (1998). Biological predispositions to violence and their implications for biosocial treatment and prevention. *Psychology, Crime and Law*, 4(2), 107–125.

Rescorla, L. A., & Achenbach, T. M. (2004). The Achenbach System of Empirically Based Assessment (ASEBA) for Ages 18 to 90 Years.

Rowe, R., Maughan, B., Worthman, C. M., Costello, E. J., & Angold, A. (2004). Testosterone, antisocial behavior, and social dominance in boys: Pubertal development and biosocial interaction. *Biological Psychiatry*, 55(5), 546–552.

Rubin, D. B. (2004). *Multiple imputation for nonresponse in surveys* (Vol. 81). Hoboken, NJ: John Wiley & Sons.

Smeets, K. C., Oostermeijer, S., Lappenschaar, M., Cohn, M., Van der Meer, J., Popma, A., … Buitelaar, J. K. (2017). Are proactive and reactive aggression meaningful distinctions in adolescents? A variable-and person-based approach. *Journal of Abnormal Child Psychology*, 45(1), 1–14.

Sorber, L., Van Barel, M., & De Lathauwer, L. (2015). Structured data fusion. *IEEE Journal of Selected Topics in Signal Processing*, 9(4), 586–600.

Tremblay, R. E. (2010). Developmental origins of disruptive behaviour problems: The 'original sin' hypothesis, epigenetics and their consequences for prevention. *Journal of Child Psychology and Psychiatry*, 51(4), 341–367.

van Baardewijk, Y., Andershed, H., Stegge, H., Nilsson, K. W., Scholte, E., & Vermeiren, R. (2010). Development and tests of short versions of the youth psychopathic traits inventory and the youth psychopathic traits inventory-child version. *European Journal of Psychological Assessment*, 26(2), 122–128.

Van Buuren, S. V., & Groothuis-Oudshoorn, K. (2010). mice: Multivariate imputation by chained equations in R. *Journal of statistical software*, 45(1), 1–68.

Van Deun, K., Smilde, A. K., Van Der Werf, M. J., Kiers, H. A., & Van Mechelen, I. (2009). A structured overview of simultaneous component based data integration. *BMC Bioinformatics*, 10(1), 246.

Van Donkelaar, M. M., Hoogman, M., Shumskaya, E., Buitelaar, J. K., Bralten, J., & Franke, B. (2020). Monoamine and neuroendocrine gene-sets associate with frustration-based aggression in a gender-specific manner. *European Neuropsychopharmacology*, 30, 75–86.

van Ginkel, J. R., & Kiers, H. A. (2011). Constructing bootstrap confidence intervals for principal component loadings in the presence of missing data: A multiple-imputation approach. *British Journal of Mathematical and Statistical Psychology*, 64(3), 498–515.

van Zonneveld, L., Platje, E., de Sonneville, L., van Goozen, S., & Swaab, H. (2017). Affective empathy, cognitive empathy and social attention in children at high risk of criminal behaviour. *Journal of Child Psychology and Psychiatry*, 58(8), 913–921.

Yi-Zhen, Y., & Jun-Xia, S. (2009). Relationship between levels of testosterone and cortisol in saliva and aggressive behaviors of adolescents. *Biomedical and Environmental Sciences*, 22(1), 44–49.

*Zijlmans, J., Bevaart, F., van Duin, L., Luijks, M., Popma, A., & Marhe, R. (2019). Error-related brain activity in relation to psychopathic traits in multi-problem young adults: An ERP study. *Biological Psychology*, 144, 46–53.

*Zijlmans, J., Marhe, R., Bevaart, F., Luijks, M.-J. A., van Duin, L., Tiemeier, H., & Popma, A. (2018). Neural correlates of moral evaluation and psychopathic traits in male multi-problem young adults. *Frontiers in Psychiatry*, 9, 248.

Zuckerman, M. (1990). The psychophysiology of sensation seeking. *Journal of Personality*, 58(1), 313–345.