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Dynamics of the human stress system in depression

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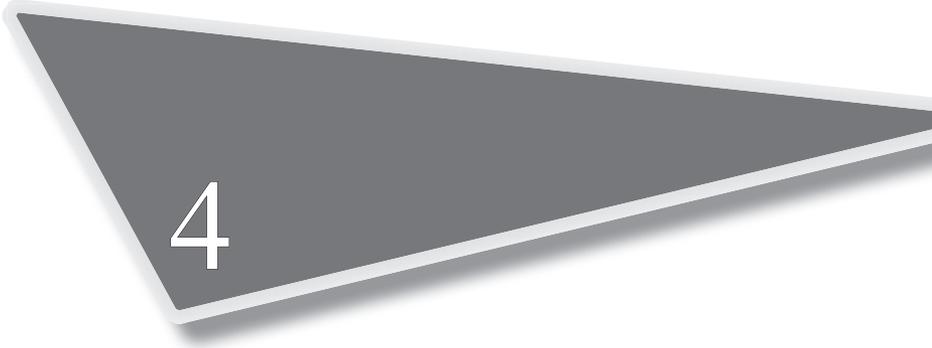
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How to assess stress biomarkers for idiographic research?

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ABSTRACT



Associations between stress-related biomarkers, like cortisol or catecholamines, and somatic or psychological symptoms have often been examined at the group level. Studies using this nomothetic approach reported equivocal findings, which may be due to high levels of intra-individual variance of stress biomarkers. More importantly, analyses at the group level provide information about the average patient, but do not necessarily have meaning for individual patients. An alternative approach is to examine data at the level of individual patients in so-called idiographic research. This method allows identifying individuals in whom symptoms are explained by preceding alterations in specific stress biomarkers, based on time series of symptoms and stress biomarkers. To create time series of sufficient length for statistical analysis, many subsequent stress biomarker measurements are needed for each participant. In the current paper different matrices (i.e., saliva, urine, nail and hair) are discussed in light of their applicability for idiographic research. This innovative approach might lead to promising new insights in the association between stress biomarkers and psychological or somatic symptoms. New collection tools for stress biomarkers, like the use of sweat pads, automated microdialysis systems, dried blood spots, or smartphone applications, might contribute to the feasibility and implementation of idiographic research in the future.

EXAMINING PSYCHONEUROENDOCRINOLOGY AT THE LEVEL OF INDIVIDUALS

Since the last half of the previous century, stress-related peripheral biomarkers, like cortisol and catecholamines, have been examined in patients suffering from psychological and somatic disorders (Tak et al., 2009b; Vogelzangs et al., 2010; Vrshek-Schallhorn et al., 2013). In these studies, it is generally assumed that alterations found at the group level are present in all individual patients. However, in order to generalize findings at the group level to the level of the individual, two assumptions have to be met: 1) the study population has to be homogeneous, and 2) the processes under study should have a stable mean and (co)variance function over time (Molenaar, 2004). Regarding the first assumption, studies provide evidence for significant intra-individual heterogeneity with regard to the importance of stress biomarkers in disease (Kudielka et al., 2009; Tak et al., 2009a, 2009b). Differences between groups of patients and controls are often smaller than the differences within these groups. The second assumption, stability over time most likely does not hold for most stress biomarkers. Studies looking at within-individual stability of cortisol levels over time show large day-to-day fluctuations, shifts in a person's mean level over the course of days, and cyclical trends (Platje et al., 2013; Schubert et al., 2012). Moreover, the psychological processes to which these stress biomarkers are often linked are inherently unstable over time (Molenaar and Campbell, 2009). When the process under study violates the homogeneity and/or stability assumption, findings at the population level cannot be generalized to the individual level. A new approach in the field of psychoneuroendocrinology, adopted from fields such as econometrics and engineering, might aid overcoming these problems. This time-series method, which is an idiographic approach, aims at identifying relationships within individuals. The method can for example be used to link multiple repeated measurements (time series) of a suspected stress biomarker to somatic or psychological symptoms within a single patient. Such an approach provides information about a single patient and thus allows determining whether a biomarker is related to the symptoms in that particular patient. These analyses do not need a priori decisions about which variable is the determinant and which variable the outcome, implying that the effect of physiological alterations on symptom fluctuations, and the effect of fluctuations in symptoms on physiology can be modeled simultaneously. Therefore, idiographic research is ideally suited for the field of psychoneuroendocrinology, in which it is often unclear whether physiological alterations precede or follow the fluctuation in symptoms in patients.

DIFFERENCES BETWEEN IDIOGRAPHIC RESEARCH AND NOMOTHEIC APPROACHES

The main difference between an idiographic and a nomothetic approach is that idiographic research aims to answer questions at an individual level (e.g. 'Does an increase in cortisol level predict deterioration of mood in this participant?'), whereas



nomothetic research aims to answer questions at a group level (e.g. ‘Do participants with low mood have higher cortisol levels?’). The statistical power of idiographic analysis is determined by the number of observations obtained for each individual, whereas the power in nomothetic research is determined by both the number of participants as the number of observations for each individual. While nomothetic research is possible with one observation for each individual, idiographic research requires as many observations as possible, as outlined in the next section (‘Assumptions for performing idiographic analyses’). Further, it is good to note that some potential confounders in nomothetic research do not apply to idiographic research. Factors that are stable within persons during the measurement period (such as age and sex) do not need to be taken into account in idiographic research, since analyses are performed within individuals. Differences between nomothetic and idiographic research with regard to research questions, analytical differences, and practical issues are summarized in Table 1.

A discussion of the plethora of statistical techniques for nomothetic and idiographic research is beyond the scope of the current paper. We will only briefly address the differences between the idiographic and nomothetic approach. With nomothetic techniques, such as structural equation modeling, longitudinal data are processed at the group level to examine (bidirectional) relationships between variables. Nested subgroup analysis can be performed, by applying different models on subgroups of patients, but analyses are, in contrast to idiographic research, not performed at the individual level. Multilevel structural equation models allow differentiating between within-subject and between-subject variances. Although the within-subject variance allows the level and strength of the association (i.e. the random intercept and the slope) to differ between individuals, individual estimates are generated posthoc, relative to group estimates, and no significance tests are provided for the individual estimates (Rovine & Lo, 2012). Moreover, assumptions are only tested at the group level and not at the individual level. Therefore, even multilevel structural equation modeling cannot define whether changes in neuroendocrinological factors predict symptom increases or vice versa for individual participants, which is possible with idiographic analyses, such as time-series analysis.

For further reading, we refer to standard introductory texts on time-series analyses (Chatfield, 2013; Durbin and Koopman, 2012), structural equation modeling (Bentler, 1980) and multilevel modeling (Hruschka et al., 2005), and to papers on techniques such as vector autoregressive modeling (Rosmalen et al., 2012), unified structural equation modelling (Gates et al., 2010; Kim et al., 2007), and convergent cross-mapping (Sugihara et al., 2012).

Table 1. Differences between idiographic and nomothetic research with regard to psychoneuroendocrinologic research

	Idiographic	Nomothetic
Analysis level	Individual level	Group level
Research questions	E.g. Does a rise in cortisol level predict deterioration of mood in this participant?	E.g. Do participants with low mood have higher cortisol levels?
Power	Determined by number of observations for each individual	Determined by number of participants and number of observations for each individual
Number of data points for each participant	As high as possible, at least 30 for vector autoregressive analyses	Possible with one observation for each participant
Confounders	Factors that fluctuate within the measurement period (e.g. weather, stressful events, number of cigarettes smoked)	Factors that differ between participants (e.g. age, gender, glucocorticoid sensitivity, biological challenge)
Practical consequences	Data preferably equidistant and stationary. Highly intensive collection period, thus concessions have to be made to make collection feasible (e.g. no food intake 30 minutes before saliva sample)	Data do not need to be stationary or equidistant. Less intensive collection for individuals, thus less concessions (e.g. no food intake 2 hours before saliva sample)
Statistical techniques	E.g. Vector autoregressive models, unified structural equation models, dynamic models	E.g. Regression models, multilevel models, structural equation models

ASSUMPTIONS FOR PERFORMING IDIOGRAPHIC ANALYSES

Certain assumptions have to be met when performing time-series analyses. Most importantly, sufficient data points need to be available for each individual, since in time-series analyses the number of observations gathered for each participant determines the statistical power to reveal an association. However, it is difficult to determine how many data points are exactly needed for time-series analyses. This is

because the direction of the associations and the timing of lagged influences in the system under investigation are usually unknown and bidirectional and feedback effects can be present as well. For example, a decrease in cortisol level might lead to an increase in pain, while at the same time an increase in pain might lead to an increase in cortisol level. These influences can be modeled simultaneously in time-series models. Further, estimation of more parameters or non-linear associations decreases power and increases the number of data points needed (Brandt and Williams, 2006). Time-series analyses described in the context of the current how-to paper are based on vector autoregressive models. These are linear models that can relate fluctuations in symptoms, such as depression, fatigue, and pain, to preceding or subsequent fluctuations in stress biomarkers. The required number of observations to yield enough statistical power depends, like in nomothetic research, on measurement error and the strength of the relationship between the studied variables. However, in contrast to nomothetic research, the measurement error and strength of association ‘within’ and not ‘between’ participants is important, and therefore the number of observations needed might differ between individuals. Simulation studies have shown that linear vector autoregressive models provide valid results with 30 time points, although larger numbers of observations yield more reliable results (Lütkepohl, 2007). Especially for stress biomarkers, which are normally influenced by many factors, larger numbers than 30 are recommended, such as the 63 samples used in the study of Schubert et al. (2012) or the 90 samples in the study of Bouwmans et al. (2014) and Booij et al. (2015). Regarding the sample frequency, psychological and somatic symptoms fluctuate in a time frame of hours to days and stress biomarkers in a matter of milliseconds to months. Sampling frequency depends on the frequency at which the studied biomarker fluctuates. High-frequency biomarkers such as heart rate variability need to be sampled at a higher frequency (several times per second) than for instance salivary cortisol (hourly) to discern meaningful patterns in their time series. The duration of the sampling period is determined by the biomarker that fluctuates at the lowest frequency.

Two other assumptions are not absolutely necessary for time-series analysis, but ease statistical analysis, and diminish the number of data points needed. The first is that data points need to be equidistant, meaning that the time period between observations is equal (Lütkepohl, 2007). Second, some statistical models require time series to be stationary, that is, have a stable mean and (co) variance function over time (Lütkepohl, 2007).

SCOPE OF THIS HOW-TO PAPER

The assumptions of idiographic statistical models lead to specific requirements for collection of stress biomarkers that do not apply to nomothetic studies. In this paper, an overview is given of practical issues encountered while performing idiographic studies and their potential solutions. Most importantly, the total measurement period

is usually one to several weeks, during which data have to be collected one to several times a day. This intensive data collection period usually asks for measures that can be obtained ambulatory during the normal daily routines of participants.

Many sources for ambulatory assessment of assumed stress-related biomarkers exist: blood (Monk et al., 2013), saliva (Kudielka et al., 2012), sweat (Cizza et al., 2008; Russel et al., 2013), urine (Schubert et al., 2003), hair (D'Anna-Hernandez et al., 2011), nails (Warnock et al., 2010), breast milk (Patacchioli et al., 1992), feces (Ebensperger et al., 2013), and tears (Monk et al., 2013) can be used. Of these, saliva and urine are most widely used in ambulatory studies in humans. Also hair and nails are easy to collect and increasingly used. The focus of this methodological how-to paper will therefore be on stress biomarkers collected in saliva, urine, hair and nails. We will particularly focus on the stress biomarker cortisol, since it can easily be collected in different matrices, is quite stable at room temperature, and shows large within-person fluctuation. All these issues will be discussed in depth. To the best of our knowledge, only five idiographic time-series studies have been published in the field of psychoneuroendocrinology so far (i.e. Blackburn et al., 1987; Booij et al., 2015; Bouwmans et al., 2015; Schubert et al., 2003; Schubert et al., 2012). We will only discuss methodological issues related to these articles and not mention their results in this how-to-paper.

GENERAL PRACTICAL ISSUES

In light of the earlier recommendations, data is best collected at fixed and equidistant intervals. This is most challenging for saliva, which should, given the high fluctuation rate of salivary stress biomarkers, be collected on fixed intervals during the day. For example, if samples are to be collected three times a day with eight-hour intervals, this could be at 8 a.m., 4 p.m., and midnight. However, this might interfere with the natural sleep-wake rhythm of some participants, and thereby reduce compliance and ecological validity. An alternative for the eight-hour interval would be to sample every six hours, for example, at 10 a.m., 4 p.m. and 10 p.m., without taking a fourth night sample at 4 a.m.. To indicate the relatively long interval between the evening and the morning sample a dummy variable can be included in the time-series analysis that marks the 10 a.m. samples. An alternative is to impute the 4 a.m. samples, based on previous cortisol levels, but these imputations might be imprecise, since cortisol night and day curves systematically differ. In our experience both methods can lead to different results. Unfortunately, no research has currently been performed to sort out the best method for systematically correcting for missing values in time series in psychoneuroendocrinology. In the field of econometrics where stock market data for the weekend are always missing, including a dummy variable to indicate the Monday morning is standard practice (e.g. Gao & Wang 1999). If one is interested in fluctuations in the cortisol awakening response, a daily collection scheme can be provided to participants to obtain equidistant intervals. Equidistant intervals for pooled urine, hair

and nail samples are more feasible, since daily, weekly or monthly collection frequencies can be used. Another assumption to bear in mind is the assumption of stationarity. This implies that the association between the psychological parameter and stress biomarker of interest is stable during the measurement period. However, if stationarity during the measurement period cannot be assumed, statistical methods exist which can take this lack of stationarity into account, such as non-linear or dynamical models (Sugihara et al., 2012). Alternatively, data can be processed to become stationary by for example applying filter techniques to remove linear or cyclical trends during raw data analyses.

Further, biomarkers in stress research, such as cortisol and α -amylase, are influenced by several daily life factors that might be highly variable within individuals in the short term, for instance lifestyle factors, such as smoking. This implies that information about such measures should be repeatedly collected as well. If registered properly, idiographic analyses can deal with and further scrutinize interindividual differences in the effects of these lifestyle factors by including them as covariates.

One needs to bear in mind that fluctuation of a biomarker over time constitutes both true within-individual change as well as measurement error (e.g. laboratory measurement error). In order to detect meaningful relations between variables, “noise” (i.e. variation in the time series caused by analytical variation) needs to be kept to a minimum, and needs to be proportionally small compared to true change. Measurement error in the form of laboratory error (e.g. batch effects) or errors due to faulty storage applies to idiographic research as it does to nomothetic research. The difference is that in idiographic research it is much more important to prevent measurement error within the time series of a single individual than to prevent measurement error between individuals, as statistical analysis is carried out at the within-individual level. We thus recommend to store samples of each individual separately, and to analyze all samples of one individual in one run in order to prevent batch effects within an individual time series. Moreover, it is thus particularly important for idiographic research that the intra-assay coefficient of variation of an analytical method is low. The intra-assay coefficient of variation (CV) is the standard deviation divided by the mean value after repeated measurement of the same samples within one run. It therefore reflects the precision of an analytical technique. In Table 2, we have provided a referenced overview of reported intra- and inter-assay CVs for the analysis of urinary, salivary, and hair cortisol with Enzyme-Linked Immuno Sorbent Assay (ELISA) and liquid chromatography tandem mass spectrometry (LC-MS/MS) in relation to the test-retest correlation of the biomarker. It shows that analytical variation is within the range of 5-10%. This variation is proportionally small compared to the test-retest correlation of the biomarkers in question. Internal standards can be used to create a separate time series, which can then be regressed on the time series of the stress biomarker in a multivariate time series model to account for measurement error in the data.

Table 2. Intra- and inter-assay coefficients of variation in cortisol concentration

	Test-retest reliability	Intra-run CV Elisa	Intra-run CV LC-MS-MS	Inter-run CV Elisa	Inter-run CV LC-MS-MS
Salivary cortisol	0.16 & 0.54 (Kirschbaum et al., 1990)	≤ 7% (Bio-vendor Research and Diagnostic products)	≤ 5% (Fus-tinoni et al., 2013)	≤ 10% (Bio-vendor Research and Diagnostic Products)	≤ 5% (Fus-tinoni et al., 2013)
Urinary free cortisol	0.69 - 0.72 (Rosmalen et al., 2014)	≤ 7% (Alp-co)	≤ 5% (Wood et al., 2008)	≤ 9% (Alp-co)	≤ 5% (Wood et al., 2008)
Hair cortisol	0.68 - 0.79 (Stalder et al., 2012)	≤ 8% (St-alder et al., 2012)	≤ 8% (Chen et al., 2013)	≤ 8% (St-alder et al., 2012)	≤ 7% (Chen et al., 2013)

Further, although for instance cortisol is stable up to at least three freeze-thaw cycles (Barrett et al., 2005), not all analytes have good freeze-thaw stability. If several analytes are to be determined on different occasions, they can best be aliquoted into different tubes for separate storage. It should be realized that, when studying several biomarkers, this results in hundreds of samples and thus a large amount of freezer space for each individual, preferably at -80 °C.

Finally, the matrix used to collect stress biomarkers, is partly determined by the idiographic research question. This is because the optimal measurement frequency differs per matrix, depending on the time frame of the stress biomarker level that is assessed. A summary of the time frames of cortisol reflected in different matrices is provided in Table 3. Salivary cortisol has been found to reflect cortisol levels of the last couple of minutes to hours, and can therefore best be linked to psychological or somatic symptoms which fluctuate within a timeframe of minutes or hours. Urinary cortisol (especially 12 or 24 h) reflects cortisol levels during the past day and could therefore be related to daily psychological or somatic symptom fluctuations. Cortisol assessed in hair reflects cortisol levels during the past weeks or months and can therefore be associated with weekly to monthly fluctuations in somatic or psychological syndromes. Examples of idiographic research questions related to each matrix are provided in Table 4.

Table 3. Time frames related to different stress biomarker matrices

Time frame	Level and type	Authors	Year	Marker	Results	Analysis method
Minutes	Group Observational	McLean et al.	2005	Salivary cortisol	Pain and cortisol levels in the morning were concurrently associated in patients with FM	Pearson's correlation
Minutes	Group EMA	Sonnenschein et al.	2007	Cortisol awakening response	Smaller CAR increase was associated with more exhaustion symptoms after waking up	Mixed model
Minutes - hours	Group EMA	Van Eck et al.	1996	Salivary cortisol	Stressful events predicted subsequent cortisol levels; ongoing stressors predicted current cortisol levels	Mixed model
Hours	Group Experimental	Henckens et al.	2010	Cortisol supplementation	Cortisol supplementation desensitized amygdala responsivity rapidly (+/- 75 min), and resensitizes amygdala responsivity slowly (+/- 285 min).	ANOVA and PPI
Days	Individual Time series	Schubert et al.	2003	Urine cortisol 12-hours	12-hour urine cortisol levels predicted anticipated stressful events one day later. Unanticipated stressful events predicted 12-hour urine cortisol levels one day later	ARIMA
Weeks	Group Observational	Wei et al.	2015	Cortisol in hair	Cortisol in hair was significantly higher in individuals with a first episode, compared to healthy individuals and those with a recurrent episode. These differences were not present at baseline, before disease onset.	ANOVA
Weeks - months	Group Observational	Gao et al.	2015	Cortisol in hair	After the earthquake, cortisol levels increased 6 and 22 weeks after. Decreased again 43 weeks after outburst.	Repeated measures ANOVA

Note: CAR = Cortisol awakening response, ANOVA = analysis of variance, ARIMA = Autoregressive integrated moving average, PPI = Psychophysiological interaction, FM = Fibromyalgia

Table 4. Comparison of different ways of collecting stress biomarkers for idiographic research

	Fluctuations	Research questions
Saliva	Minutes to hours	• Does an increase in salivary cortisol predict decrease in affect several hours later in this participant?
Urine (overnight, 24-h)	Daily	• Does overnight urine level predict energy level during the day in this participant?
Hair or nail	Weeks to months	• Is hair cortisol related to mood in this bipolar patient?

SALIVA COLLECTION

Saliva can be used to examine associations between stress biomarkers and fluctuations in somatic or psychological symptoms within a time frame of minutes to hours (see Kudielka et al. 2012 and Rohleder and Nater 2009 for extensive reviews about salivary cortisol and α -amylase, respectively). Many biomarkers in saliva are influenced by timing, e.g. salivary cortisol shows a circadian, ultradian, and infradian rhythm (Lightman and Conway-Campbell, 2010). If sampled frequently enough, these natural rhythms can be modeled. The most appropriate time frame of saliva collection depends on the fluctuations a researcher aims to study and might differ from several times a day to once a day. Examples of biomarker fluctuation rates in relation to suitable research questions are provided in Table 4.

Practical issues

Saliva is obtained by chewing on a roll-shaped plain synthetic or cotton swab (which one is most suitable depends on the analyte measured), i.e. by use of Salivettes®. Whereas cortisol enters the saliva through passive diffusion, other analytes including alpha-amylase are actively transported. The latter process is influenced by chewing, implying that participants should not chew if one aims to collect actively transported molecules (Gröschl, 2008). When collecting saliva several times a day, compliance issues might arise. Participants are not allowed to eat, drink, smoke or brush their teeth prior to sample collection, because food particles can contaminate saliva, which may subsequently lead to unreliable assay results (e.g. Gröschl et al. 2001). Beyond that, food intake itself may increase cortisol levels (e.g. Gibson et al. 1999). While some studies argue that these effects dissipate after 30 minutes (e.g. Smyth et al. 2013), other studies found that dissipation takes up to two hours (e.g. Hansen et al. 2008). Thus, ideally food intake should be restricted two hours before the sampling. However, adding life style restriction to the sampling protocol interferes with normal daily life and in this way decreases ecological validity of the study. As a compromise, in line with Bouwmans et al. (2014) and Booij et al. (2015), food intake can be restricted

to 30 minutes in advance of the sample collection. In addition, information on food intake before the sample collection could be gathered and this information can be incorporated in the time-series models. A sampling schedule that suits the participant is essential to increase compliance, particularly if the schedule requests participants to get up at the same time every morning in order to obtain equidistant data. Participants could be designated personalized daily sampling schedules (again with predefined time points) depending on their sleep-wake rhythm, since cortisol levels depend on awakening time (Federenko et al., 2004). A benefit of personalized sampling schedules is that participants' daily lives are less likely to be obtruded by the sampling, thereby increasing compliance of the participant and ecological validity of the study. Idiographic research is associated with specific problems with storage of samples. Although cortisol remains relatively stable at room temperature for a period of at least 2 days (Clements and Parker, 1998), cortisol is preferably stored in participants' fridges or in a cool bag for transport during daily activities. Cortisol decreases at a more rapid rate when stored on a salivette compared to centrifuged samples both at room temperature and at 4 °C (Garde and Hansen, 2005; Gröschl et al., 2001), and a significant decrease starts to occur after 5 days (Garde and Hansen, 2005). Since centrifuging of the samples directly after collection is not feasible in this type of study, samples should be brought to the lab frequently to be centrifuged and frozen at -80 °C.

Compliance

In one of our own studies (Bouwman et al., 2014; Booij et al. 2015), we asked participants (n=30) to (anonymously) evaluate feasibility of their saliva collection. They performed these saliva collections three times a day for 30 days. Two statements were rated by means of a visual analog scale (0 mm = fully disagree, 100 mm = fully agree). The first statement 'Saliva sampling always took place at the prescribed moment' was given an average rating of 82 mm. The second statement 'I found it difficult to refrain from eating or drinking anything (except water) for half an hour prior to saliva sampling', was given an average rating of 31 mm. The average percentage of missing saliva samples in this study was 4.0%. Thus, compliance with this protocol seems high and the proportion of missing data was low. Compliance might have been enhanced by two strategies in this study. First, participants who completed the 30-day protocol received a personal feedback report on the psychological data they provided in the diary. Second, the fee that they received depended on the number of measurements that they completed, with more completed measurements resulting in a higher fee. These factors may have contributed to the seemingly high compliance rates. Compliance might be further enhanced by use of Medication Event Monitoring System (MEMS) Track caps, which provides information about sampling times (Kudielka et al., 2003). This device can store up to 3800 events, has a 36 months battery life, and can transport data on compliance wirelessly to the researcher. Incorporating these sampling times in statistical models will improve the estimates derived from these models.

Urine collection

24-Hour urine collection provides an integrative measure of stress biomarkers, like cortisol output, that is not disturbed by circadian or ultradian rhythms. 24-Hour urine samples can be used to examine the association between stress biomarkers and daily fluctuations in psychological or somatic symptoms. Table 4 provides an example of a research question.

Practical issues

Collecting 24-hour urine samples for idiographic research requires a huge effort of participants, since they have to collect all their urine during a period of several weeks. Women might not appreciate collecting their urine during their menstrual period, but this period is typically included in the measurement period of idiographic research, which usually takes at least one month in case urine samples are collected. In our experience, it is helpful to mention and discuss the practicalities of urine collection before the start of the study.

Urinary cortisol can be collected in urine containers that can be stored outside the fridge for a period of at least 24 hours (Gouarne et al., 2004). The containers should therefore be collected on a regular basis. To reduce the burden of urine collection, some nomothetic studies assessed cortisol in overnight urine samples instead of in 24-hours samples (e.g. Castro-Diehl et al., 2014, Mitchel et al., 2010). Although this method is less burdensome, it might be not as informative as cortisol assessment in 24-hour collection, since effects of daily hassles, alternating affect, and uplifts on cortisol are especially expected during the day (e.g. Luecken et al. 1997).

The measurement of urinary catecholamines in 24-hour urine in idiographic studies is more problematic than that of cortisol for two reasons. First, it is difficult to control for the influence of diet on catecholamine levels. A diet rich in fruits and nuts for instance can lead to a 1.5 fold increase in urinary free dopamine and norepinephrine levels (Ausman et al, 2008). Adjusting for this would require detailed diet diaries for the entire assessment period, which poses an extra burden on participants. Second, catecholamines are unstable molecules which are easily oxidized. At room temperature, a drop in epinephrine and norepinephrine levels of 28% and 39% respectively is to be expected in the course of 24 hours (Gouarne et al., 2004). The speed of degradation varies widely between individuals (Gouarne et al., 2004; Willemsen et al., 2007) and was shown in one study to depend on the urinary pH (Gouarne et al., 2004). The urinary pH itself depends heavily on diet (Ausman et al., 2008) and can thus also fluctuate within the day and from day-to-day at a within-individual level. Although it is true that the degradation of catecholamines can be prevented by acidifying the urine samples to a pH <4 with hydrochloric acid, this is difficult to accomplish outside a laboratory setting. Pre-adding acid to the collection container would expose participants to the potential harm of coming into contact with highly acidic fluid when the container is still relatively empty. Cooling the sample to 4 °C is also a good way of preserving catecholamine levels for 24 hours, but this is generally not feasible.

Non-compliance with the cooling protocol is detrimental for the reliability of the catecholamine measures and compliance cannot be verified afterwards. Therefore, the only practically feasible way of measuring urinary catecholamines in an idiographic study might be to collect overnight urine samples (i.e. from the time of going to bed until the first morning void).

Compliance

Compliance to urinary collection is often checked by measuring creatinine excretion (e.g. Luecken et al., 1997). However, previous studies found that this method is not sufficient to exclude incomplete urine samples (De Keyzer et al., 2012). Moreover, there is considerable within-individual variability in day-to-day creatinine excretion. A review article mentions a within-subject CV ranging from 3-20% with an average of 10% (Boeniger et al., 1993). This is also the case for studies checking completeness with para-aminobenzoic acid administration that found an average within-person CV of 10% (Bingham et al., 1988). This variation is quite understandable as creatinine excretion depends not only on turnover from the lean muscle mass, but also on dietary (meat) intake, exercise, hydration status and protein loading as the latter two change the glomerular filtration rate (Perrone et al., 1992). In one of our own idiographic studies, 10 (3 males, 7 females) healthy participants collected their 24-hour urine for 63 consecutive days. Samples were collected at participants homes every two days, after which they were stored at -80 °C. Urinary creatinine was measured with the creatinine plus enzymatic assay on the Roche Modular. The intra-run coefficient of variation was 0.9% and the inter-run coefficient of variation was 2.4 %. All samples of one participant were analyzed in one run. Each participant showed considerable day-to-day fluctuations in total creatinine output (Figure 1). As this variation was either normally distributed around the mean or slightly positively skewed, we assume, in accordance with other studies (Bingham et al., 1988; Boeniger et al., 1993; Perrone et al., 1992), that this concerns mostly natural physiological variation. In line with the idiographic nature of the study, we decided to exclude samples from statistical analysis if the 24-hour creatinine output was lower than 2 standard deviations from the person's own mean, instead of looking at between-person variations as an estimate for 24-hour urine completeness. To increase compliance, we informed our participants of the fact that we use urinary creatinine to check the completeness of each 24-hour urine sample, and provide a financial incentive for every compliant day.

How to assess stress biomarkers for idiographic research?

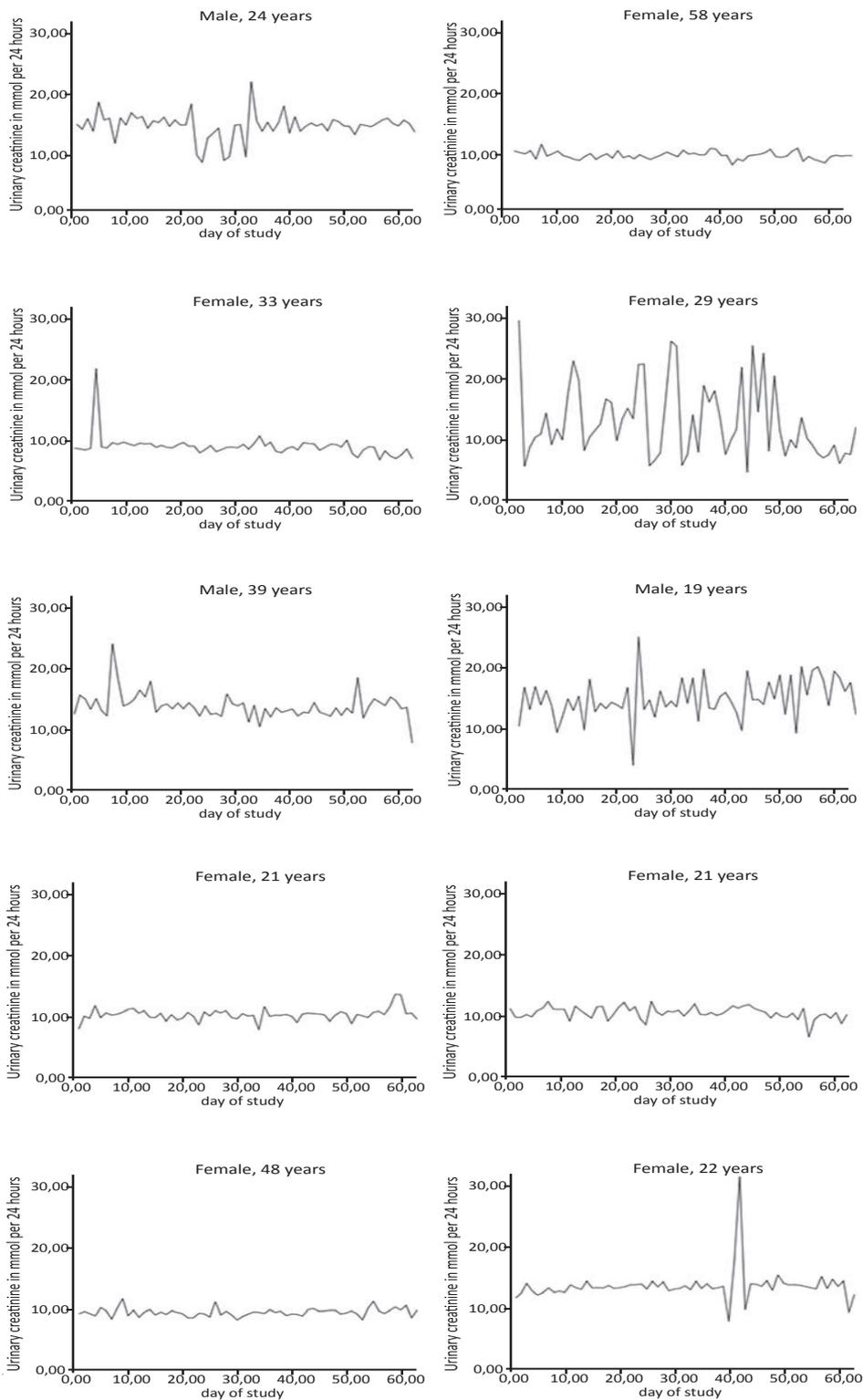


Figure 1. Urinary creatinine of 10 healthy individuals

COLLECTION OF HAIR OR NAILS

Stress hormones can be measured in keratinized matrices such as hair and nails. The state of the art with regard to measuring cortisol in hair and its relationship to psychosocial stress and mental illness has recently been reviewed elsewhere (Stadler and Kirschbaum, 2012; Staufenbiel et al., 2013). Likewise, nail biology has extensively been reviewed elsewhere (de Berker et al., 2007; Palmeri et al., 2000) albeit not with respect to psychoneuroendocrine research. To our knowledge, only one psychoneuroendocrinological study has currently reported on the use of fingernails, indicating higher cortisol levels in nails during a stressful period (Warnock et al., 2010). Since samples taken from hair and nails represent biomarker levels in the past weeks or months, they can be linked to weekly or monthly fluctuations in psychological or somatic factors. Hair and nail samples are suitable methods for idiographic research if one is interested in long-term effects and willing to follow-up an individual during a period of months to years. For example, hair or nail samples can be used to examine whether fluctuations in cortisol level predict manic or depressed episodes in bipolar patients, see Table 4.

Measuring stress biomarkers in hair

Hair samples can be obtained both by cutting or shaving the hair as closely to the scalp as possible. By shaving or cutting exactly on the same spot on the scalp at predefined time intervals equidistant time intervals can be obtained. For idiographic research, a sample frequency of once a month is feasible, since hair grows on average about 1 cm each month (Wennig, 2002). Collecting a bundle of hair repeatedly instead of retrospectively will increase the precision of the estimated time period represented by each hair segment, since individual differences in growth rate can be accounted for (LeBeau et al., 2011). A more accurate estimation of this represented time period is helpful if it is relevant to take into account seasonal effects (Randall and Ebling, 1991) and hormonal influences (Randall, 2008), which are known to influence cortisol concentrations in hair. Moreover, obtaining ‘fresh’ hair samples repeatedly avoids the so-called wash-out effect in which cortisol concentrations progressively decrease in older more distal hair segments, possibly due to repeated washing (Hamel et al., 2011), as has been noted in several studies (D’Anna-Hernandez et al., 2011; Gao et al., 2010; Kirschbaum et al., 2009). Finally, assessment of hair by shaving will result in more reliable results than by cutting, since differences between investigators in cutting accuracy are large. Cutting closely to the scalp proves to be difficult even for experienced investigators, leaving an average length of 0.8 cm of hair behind on the scalp (LeBeau et al., 2011).

Measuring stress biomarkers in nails

Growth rate of fingernails is approximately 0.1 mm per day or 3 mm per month (de Berker et al., 2007), but varies widely between individuals from 2 to 4.4 mm per month (Gupta et al., 2005). A within-individual study showed that the nail growth rate increased with temperature and declined with age (Bean, 1980). Based on this study,

seasonal effects should thus be taken into account in idiographic studies. The hand and fingers from which the nails are taken need to be standardized within the study, as growth rate differs both between hands and between different fingers (Dawber, 1970). At least 1 mg of fingernails is required for measuring cortisol in fingernails (Ben Khelil et al., 2011). The use of nail clippings of only one thumb yielded sufficient material for LC-MS/MS analyses, on average 5.7 mg (Ben Khelil et al., 2011). Participants should clip their fingernails as short as they can on the first day, and again at predefined time intervals throughout the duration of the study. In our experience, weekly sampling yields unacceptably small amounts of nail samples in most participants, whereas two-weekly intervals were suitable. Participants can be provided with a nail clipper surrounded by a plastic enclosure capturing the nail inside the clipper, or instructed to clip their nails securely in a Ziploc plastic bag. Nails can be stored at room temperature (Daniel et al., 2004) into micro tubes.

It is currently unclear whether the use of cosmetic nail products influences hormone levels in nails. Although there is some evidence that the hormone levels in nails are not influenced by nail polish (Ben Khelil et al., 2011), the use of nail polish during idiographic studies is not recommended until more solid research has been performed.

LIMITATIONS OF IDIOGRAPHIC RESEARCH

One of the limitations of idiographic research is that it is not possible to examine the relationship between alterations in affect and biomarkers if someone's affect is quite stable during the research period. However, affect and mood normally show daily and weekly fluctuations, even in depressed individuals (Bylsma et al., 2011, Peeters et al., 2003, Gordijn et al., 1994), so lack of fluctuation is probably only a problem in a small subset of study participants. One might wonder whether these daily and weekly fluctuations in mood are clinically relevant, since psychiatric diagnoses such as major depressive disorder are assumed to be relatively stable over time. Nevertheless, previous studies showed that these short-time fluctuations can predict the progression of psychiatric diseases like depression, and thus can provide clinically relevant information (see for example Kuppens et al., 2012 and Van der Leemput et al., 2014).

Further, since many biological samples are needed for each participant, idiographic research might not only be burdensome for the participant, but is also quite expensive. However, these high costs do also apply to other methods in clinical practice, such as MRI research. To reduce the costs of statistical analyses, a software package called 'AutoVAR' has been developed by our research group (Emerencia et al, in press), which performs automated autoregressive analyses.

THE RELEVANCE OF IDIOGRAPHIC RESEARCH IN THE FIELD OF PSYCHONEUROENDOCRINOLOGY

Idiographic research provides very rich datasets that can provide crucial information

for clinical practice. Idiographic research can for instance be used to detect whether a change in salivary cortisol level predicts worsening of fatigue in a specific patient, and might thus be used to get more insight into mechanisms contributing to specific symptoms. This ultimately might preclude the use of standard reference values, based on average biomarker levels in the population, and lead to reference change values for individuals instead. For generalizable knowledge development we believe that the idiographic and nomothetic approach can best be combined. For example, it might be interesting to perform idiographic research in a longitudinal multiwave cohort study, combining widely spaced measurements with occasional intensive collection periods (i.e. measurement bursts), see Sliwinski (2008). Estimates (coefficients) of physiological stress responsiveness obtained from idiographic analyses of salivary or urinary cortisol can also be used as between-individual predictors of hair cortisol slopes in for instance multilevel models. Such an approach enables testing whether persons with higher physiological stress responsiveness have greater ‘wear and tear’ of the stress responsive systems than persons with lower physiological responsiveness, as hypothesized by the allostatic load theory (McEwen and Seeman, 1999).

FUTURE DIRECTIONS

Since ecological assessment becomes increasingly popular, collection methods for stress biomarkers more suitable for daily life are expected to become available. Recently developed cutaneous sweat patches (Marques et al., 2010) might be an example of a new collection method. Sweat patches can be attached to the skin and collect data about stress biomarkers for 24 hours. Therefore, this method might, just like 24-hour urine collection, overcome problems with circadian rhythm. Compliance of proper self-application of the sweat patch is high (Marques et al., 2010). Participants just have to replace the sweat patch every day and store them in their fridge (Marques et al., 2010). After collection, stress biomarkers can be detected by recycling immunoaffinity chromatography (RIC) coupled with laser-induced fluorescence. Although this method seems promising, the patches are not yet available for commercial use and the method awaits replication. Another recently developed method is a portable automated sampling system which combined with microdialysis, is capable of sampling subcutaneous tissue free cortisol for a period of 24 hours (Bhake et al., 2013). Also dried blood spots, which are drops of capillary whole blood that are collected from finger sticks, can be used for minimally invasive collection of blood samples in daily life (e.g. McDade, 2014). Finally, smartphone applications might ease ecological assessment of stress biomarkers in the future. For example, a urinary dipstick has been developed, that changes color based on hormone levels in urine. This color change can be quantified by a smartphone application. Validation of such method is required before incorporating such methods in research. These less invasive collection methods are essential for successful idiographic research, since idiographic research is generally more burdensome for participants than nomothetic research, given the large number of repeated samples needed for each participant.

CONCLUSION

Almost all studies investigating psychological phenomena and their relation to stress biomarkers examine *between-individual variation*, implicitly assuming that inferences made at a population level reflect *within-individual change*. This assumption is untenable when a group of people is not homogeneous or when a process is not stable over time. Such processes can be studied at the within-individual level by multiple repeated measurements, creating time series of the variables of interest. Idiographic analyses, adopted from fields such as engineering and econometrics, are useful to quantitatively examine data of individual patients. These techniques require many repeated subsequent stress biomarker and symptom measures in individual patients, which has several practical consequences. Urinary and saliva samples can be used to examine associations with stress biomarkers and short-term fluctuations in psychological measures, whereas hair and nail samples are more suitable for psychological measures characterized by long-term fluctuations. In the current paper, we have discussed the practicalities of collecting stress biomarkers using these various matrices for idiographic research. Collecting such data requires significant effort from both the participant and the researcher, but can lead to promising new insights in the association between stress biomarkers and psychological or somatic symptoms. The main focus of the article was on the stress biomarker cortisol. We did not discuss immune stress biomarkers in the current article, because we believe it is beyond the scope of the current paper. Nonetheless, it should be noted that immune biomarkers are very suitable for idiographic analyses. Immune biomarkers do not only show large fluctuations over time (Haberhorn et al., 2013; Schubert et al., 2012), but have also shown to be good predictors of physical and sometimes even mental illness (e.g., Dahl et al., 2014). To test the clinical relevance of idiographic research, intervention studies should be performed. For example, it can be examined whether hydrocortisone administration is especially effective in fatigued patients for whom idiographic analyses show that decreases in cortisol levels predict higher fatigue levels. So, although idiographic research in psychoneuroendocrinology seems promising, further research is needed to test its clinical relevance.

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