

**DAYTIME-RELATED RHYTHMICITY
OF GDV PARAMETER GLOW IMAGE AREA:
TIME COURSE AND COMPARISON TO
BIOCHEMICAL PARAMETERS MEASURED IN SALIVA**

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1. Introduction and Aims of Study

Chronobiology is a relatively new field of science investigating periodic phenomena in the organism. It is well established that the flow rate and composition of saliva vary rhythmically depending on daytime (1-3). A number of parameters contained in saliva have been suggested to be reliable measures for detecting stress and related bodily answers on external noxae. In the context of stress and immune answers, alpha-amylase, immunoglobulin A (IgA), cortisol and the regulatory peptide substance P deserve special attention. Relatively fast changes of the levels of those biochemical parameters in saliva have been reported, and their significance to show response on stressors appears to be high (e.g., 4-9).

Gas discharge visualization (GDV), as developed by Professor Konstantin Korotkov (St. Petersburg University; www.korotkov.org), has been used to analyze a number of instrument-inherent physical and complementary parameters (10-30). Using GDV, evoked corona discharges from the tips of all 10 fingers can be recorded by a specifically designed apparatus essentially based on the Kirlian effect, carefully designed to meet the reproducibility and sensitivity demands of scientific research. By application of computer image analysis, digital photographs of the electro-photonic glow are transferred into a number of expedient parameters based on the sizes and distributions of the

flashes recorded. Of interest are not only complementary medical analyses made possible by integrating the meridian system of acupuncture (31-33). A powerful feature of GDV is to use arithmetic means of repeated recordings of the glow image areas of all fingers, to reliably detect stress reactions to - at least certain types of - external stressors (e.g., 12, 34). Some colleagues, however, still doubt that GDV might really be a reliable instrument meeting scientific standards. The study presented here is an attempt to provide pilot evidence potentially able to change this dissatisfactory situation towards higher general scientific acceptance, by analyzing and comparing the time courses of accepted clinical stress parameters and GDV glow image areas recorded in parallel.

As a number of biochemical parameters related to stress are known to vary during daytime, it became obvious to us that mean GDV glow image area values should also follow diurnal changes. Apparently, circadian rhythms of GDV parameters had not been reported yet in literature.

2. Test Persons and Methods

Nine persons participated at this experiment (mean age: 38,9 a; median 35 a; SD 12,11; range 24 to 53 years). Five of them were women (mean 41,2 a; range 24 to 53 a), four were men (mean 36 a; range 24 to 43 a). The results presented here were part of the control group of a larger study carried out to examine possible effects of GSM mobile phone base stations on the organism (36-37). Only non-invasive techniques were carried out. The guidelines of the enlarged Helsinki declaration were carefully followed, also including informed consent letters and taking care of data security (39-41). Persons with severe acute infections or diseases, or those taking certain kinds of medication (cortisone, psychotropics (psychotropic drugs), beta blockers etc.) were excluded. All test persons were advised to strictly avoid eating an exuberant dinner or breakfast at the evening and morning before the experiments, not to drink alcohol and only very small amounts of light coffee, black or green tea 20 hours before the experiments. During the tests, no food intake was allowed, only to drink tap water. Mobile phones had to be switched off at least one hour before the tests.

2.1. Experimental Timing

During the experiments, test persons sat relaxed in a comfortable chair. After one hour of adaptation to the laboratory conditions and anamnesis questioning, 4 experimental phases were carried out (**Fig. 1**): 10, 25 and 45 minutes after the start of each phase, saliva samples were taken. After the second saliva sampling, i.e. 30 minutes after the start of each phase (i.e., in between the 2nd and the 3rd sampling of saliva in each phase), GDV measurements were carried out. At the

50 minutes time point of each phase, breaks of 5 minutes each were included. During that period, test candidates were asked to go to the bathroom, drink tap water (thereby flushing their oral cavity), and carefully wash their hands – as a prerequisite for reliable GDV measurements. No hand cream was allowed. The GDV camera was switched on at 7:30 a.m. at each of the experimental days.

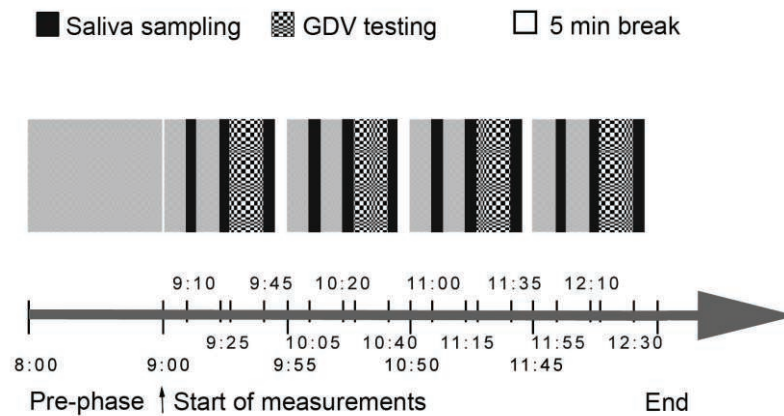


Fig. 1: Schematic representation of the time course during the experiment. After a pre-phase during which anamnestic data and a series of psychological questionnaires have been taken, the actual test period started at 9.00 a.m. for each of the test persons. Saliva samples were taken after 10 minutes, 25 minutes and 45 minutes in each of the 4 phases tested (black blocks in scheme). After 30 minutes, in each of the 4 phases GDV measurements were carried out (figured blocks in scheme). After 55 minutes of each test phase, test persons were asked to go to the bathroom to drink a glass of tap water, and then to wash their hands carefully (white blocks in scheme). Each next phase started 55 minutes after the start of the prior phase. Shortly after 12:35 a.m. of each experimental day, the program for that specific test person was completed.

2.2. Laboratory Shielding

The experimental room was carefully shielded from high frequency electromagnetic fields using “YShield” shielding paint, a grounded electro-conductive coating attached to the walls, the ceiling and the floor (type HSF53; www.yshield.com; YShield, Pocking, Germany), as well as “Swiss Shield naturell” shielding curtains (www.esag.at; Esag, Vienna, Austria).

Any electric, magnetic and electromagnetic radiation present, as well as possibly influencing acoustic or vibration-related stressors were examined and documented by an accredited company (ANBUS Co., Fürth, Bavaria; Dr. Ing. Martin Virnich). During the experiments, concomitant high frequency (HF)

measurements were carried out continuously, using a Rohde & Schwarz “FSH 3” spectrum analyzer (100 kHz to 3 GHz), and a mobile HD dosimeter (type “ESM-140”; Maschek systems, Bad Wörishofen, Germany; www.maschek.de) (42). Whilst carrying out the experiments documented here, the field strength was at 70 mV/m at mean, corresponding to a mean power density of about 13 $\mu\text{W}/\text{m}^2$ at the GSM 900 MHz band as the predominating HF field. For other types of HF electromagnetism, the field strengths detected were even below. Static magnetic influences were at 45,7 μT (mean) only, and LF electric fields were below 1 V/m. LF magnetic fields were at 42 nT (mean). None of the equipment used, including the GDV system, did change the electric, magnetic or electromagnetic field strengths significantly when powered.

During all experimental phases, the test person was comfortably sitting on a wooden chair (*Fig. 2*); there was no need to stand up for taking saliva samples or GDV measurements.

Fig. 2: Test person (face scrambled in photo) comfortably sitting on a wooden chair. Adjacent to his head, is the Maschek ESM-140 dosimeter one of the measurement devices used for continuous monitoring of mobile phone band electromagnetic fields. The GDV Compact camera is at the left side of the picture, situated at a wooden table. For the actual GDV measurements, that table was moved to the test person. Behind and partly surrounding the test person, is an opened Swiss Shield baldachin.



2.3. Saliva Analysis

2.3.1. Saliva Sample Collection

At each of the experimental time points shown in Fig. 1, during 5 minutes each, saliva samples were taken using *Salivette* saliva collection devices (Sarstedt, Nuernbrecht, Germany). Immediately thereafter, the salivettes were centrifuged for 5 min at 1000 x g, and the saliva specimen spun into 100 μl of 100 mM HEPES pH 7.0 containing 1 mg/ml bovine aprotinin to prevent proteolytic

degradation of one of the markers measured (i.e., substance P). The individual saliva samples were aliquoted and frozen at -20°C until analysis. All biochemical assays used were carried out in triplicate for each of the samples and parameter. Total protein content of saliva samples was measured by the method of Bradford (43) using bovine serum albumin as standard.

2.3.2. Biochemical Tests

Salivary alpha-Amylase (1,4 a-D-glucanohydrolase, EC 3.2.1.1) was assayed essentially according to the method of Gillard et al. (44) with 1 mM p-nitrophenyl a-maltoside as substrate with some minor modifications and adaptation to a microplate format. Enzyme activities were calculated as mU/ml.

IgA concentrations in saliva were measured by using a sandwich enzyme-linked immunosorbent assay (ELISA) with a matched pair of mouse monoclonal anti-human-IgA antibodies (G18-1 for capture, and alkaline phosphatase-labeled G20-359 for detection, both from Pharmingen (Becton-Dickinson, Vienna, Austria). IgA concentrations were calculated with respect to appropriate standard concentrations of human IgA run on each plate.

Salivary cortisol levels were examined by a competitive ELISA on microplates coated with goat-anti-rabbit-IgG with rabbit anti-cortisol-antiserum (Fitzgerald, Concord, MA, USA) and a cortisol-3-O-adipic acid dihydrazide-horseradish peroxidase (HRP) conjugate as specific competitor, synthesized essentially as described by Basu et al. (45). HRP activity was measured with 0.1 mg/ml tetramethyl benzidine and 0.01 % H_2O_2 in 0.1 M sodium acetate pH 6.0 at room temperature. Cortisol concentrations were calculated with respect to appropriate standard concentrations of cortisol (hydrocortisone) run on each plate using a 4 parameter fit equation.

Substance P levels contained in saliva were measured by a competitive luminescence immunoassay (LIA) on black microplates coated with goat-anti-rabbit-IgG with a rabbit antiserum directed against substance P and a substance P biotin conjugate, synthesized from substance P with N-hydroxy-succinimidyl-biotin, as specific competitor. Bound substance P-biotin was detected by an ExtrAvidin-HRP conjugate (Sigma). HRP activity was measured by enhanced chemiluminescence (ECL) using a commercial ECL substrate (Roche Diagnostics, Mannheim, Germany). Substance P concentrations were calculated with respect to appropriate standard concentrations of substance P run on each plate using a 4 parameter fit equation.

All chemicals and biochemicals used for saliva assays were purchased from Sigma-Aldrich (Taufkirchen, Germany) unless noted otherwise. 96-well

microplates were from Greiner BioOne (Nuertingen, Germany). For absorbance measurement, a *Sunrise* microplate reader (Tecan, Groedig, Austria) was used, and chemiluminescence was monitored with a *Lucy 2* microplate luminometer (Anthos, Wals, Austria). Washing of ELISA and LIA-plates was done with a *Wellwash 4* microplate washer (Thermo Electron, Waltham, MA, USA). Data calculation was performed using *Deltasoft* software (Biometallics, Princeton, NJ, USA).

2.3.3. Data Handling

Values obtained for the different biochemical parameters of each individual test person were corrected for dilution by the aprotinin-buffer additive and, for some of the diagrams presented here, normalized to the mean values of the first phase to compensate for the variation in individual levels of the parameters tested.

2.4. Gas Discharge Visualization

As schematically shown in Fig. 1, GDV measurements were performed after 30 minutes in each of the 4 test phases. A *GDV Camera Compact* (www.korotkov.org) has been used to capture “static GDV images” obtained during periods of 0,5 s of exposure for each image. Corona discharge images have been recorded from each of the 10 fingertips of the test person, 5 times for each finger. *GDV Capture* software was version 1.9.9., and for further calculations and analyses, the *GDV Meridian Analysis* and the *GDV Diagram* software packages (both: version no. 1.9.9.), as well as the *GDV Scientific Laboratory* software (version 1.1.5.) were applied.

During the experiments, the complete GDV system was mounted on a wooden table, making it possible to carry out measurements without moving the test person to another place, whilst sitting on the experimental chair. For each test person and test phase, 50 single static measurements were performed (each finger tip was measured 5 times for 0,5 s). The overall GDV image area values were calculated as mean values of 50 single finger GDV images in total, per test period. In addition to analyzing the arithmetic GDV mean glow image areas, the parameter *spatial fractality* was included.

A whole set of safety and reproducibility precautions also influencing stability and reproducibility were followed carefully in order to gain reliable data (10, 14, 21, 29, 34, 38).

2.5. Biomedical Statistics

All experimental data were analyzed using the software packages Sigma-Plot 9.0

(www.systat.com; Systat, San Jose, CA, USA), SPSS 14.0 (www.spss.com; SPSS, Chicago, IL, USA), and Excel 2003 (Microsoft; Redmond, WA, USA). In addition to descriptive data (mean, median, standard deviation etc.), a number of time course diagrams were calculated.

3. Results

Each of the measures tested showed distinctive time profiles (*Figs. 3 & 4*), with the exception of spatial fractality. In some of the parameters measured, markedly high inter-individual differences were detected. *Tab. 1* shows an overview on some of the key values obtained.

For **salivary alpha-amylase**, at the first test point of the first phase (sample collection in between 9:10 and 9:15 a.m. of each experimental day), the mean concentration measured was 2,29 mU/ml (ranging from 1,03 to 3,73). The amylase levels went down to a minimum mean of 1,55 mU/ml (ranging from 0,76 to 3,0), obtained from the final samples taken in between 12:30 and 12:35 p.m.

IgA showed marked inter-individual differences: The mean level measured at the first testing point (at 9:10 to 9:15 a.m.) was 146,23 µg/ml (ranging from 55,64 to 470,76). The highest value measured in one of the test phases was 539,86 µg/ml, the lowest value was 40,79 µg/ml. At the final test point of each day (at 12:30 to 12:35 p.m.), the arithmetic mean concentration was 157,94 µg/ml (at that time, values ranged from 62,40 to 451,73).

For **cortisol** too, high inter-individual variations were observed: The mean cortisol levels in saliva started at 3,75 ng/ml (ranging from 1,77 to 7,57) obtained from the first sampling time point, and the mean level at the final experimental time point have was at 2,05 ng/ml.

Salivary **substance P** levels detected started at 0,84 ng/ml in the morning at 9:15 a.m. (ranging from 0,10 to 2,16) and ended at a mean of 0,33 ng/ml (ranging from 0,10 to 0,84 ng/ml). Maximum mean levels obtained were at 0,97 ng/ml saliva.

The mean **GDV glow image area** at the first test point was at 14.325 pixels (ranging from 11.539 to 17011 pixels at about 9:30 a.m.), and the mean maximum was 20.118 pixels (ranging from 16.080 to 21.388 pixels), reached at the final time point (at about 12.30 p.m.).

GDV spatial fractality showed no recognizable daytime dependency: The mean maximum value was 1,98, the mean minimum value was 1,92. The absolute maximum was 2,04 and the absolute minimum calculated was 1,88. As no rhythmicity was observed for the spatial fractality, no time flow diagrams are

presented here for this parameter.

Tab. 1: Summary of descriptive values obtained from the very first and the very last measurements of salivary and GDV parameters. According to Fig. 1, “first measurements” for the biochemical parameters represents time point 1, i.e. the samples taken in between 9:10 and 9.15 a.m., for the GDV parameters the measurements done between 9:25 and 9:40 a.m. “Final measurements” presents data obtained from the saliva sampling in between 12:30 and 12:35 p.m., whereas for GDV, measurements done between 12:15 and 12:30 p.m. were used. Mean = arithmetic mean; SEM = standard error of the mean; Min = lowest value detected; Max = highest value detected at time point.

Factor:	<i>First Measurements:</i>				<i>Final Measurements:</i>			
	Mean	SEM	Min	Max	Mean	SEM	Min	Max
alpha-Amylase (mU/ml)	2,29	0,31	1,03	3,73	1,55	0,23	0,79	2,69
IgA (µg/ml)	146,23	45,04	55,64	470,76	157,94	40,39	62,40	451,73
cortisol (ng/ml)	3,75	0,57	1,77	7,59	2,05	0,23	1,13	3,03
substance P (ng/ml)	0,84	0,29	0,10	2,16	0,33	0,09	0,10	0,84
GDV glow image area	14325,0	798,0	11539,0	17011,0	19121,0	751,00	16081,0	21389,0
GDV spatial fractality	1,93	0,02	1,87	2,02	1,95	0,03	1,75	2,03

The following two sets of figures show normalized time course diagrams: progression in time of the different parameters are presented in two ways: The first set (*Fig. 3 a-d*) shows comparisons of the GDV glow image area time flow with the time courses of each of the biochemical parameters measured in a “smoothed” version: Arithmetic means were calculated for the biochemical results obtained during the three measurements (at 10, 25 and 45 minutes) in each of the four test phases. E.g., the first value contained in the diagram for alpha-amylase represents the arithmetic mean obtained from the three saliva sampling time points from the first test phase; the second value in the same diagram is an arithmetic mean of the three measurements of the second test phase, and so on. This calculatory setup has been applied for all four diagrams shown in Fig. 3:

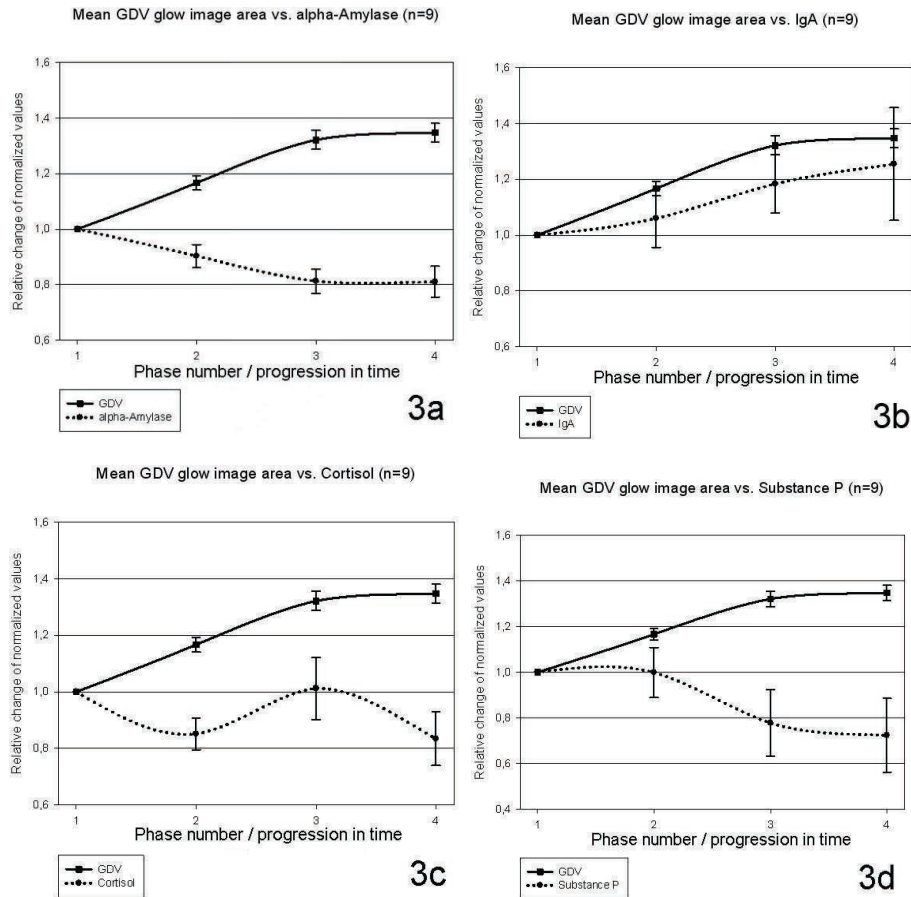


Fig. 3 a-d: Time course of GDV glow image area measurements in comparison to normalized concentrations of the salivary parameters measured biochemically. Fig. 3 a shows the comparison between GDV and alpha-amylase, Fig. 3 b compares GDV and IgA, Fig. 3 c is on GDV and the cortisol levels, and Fig. 3 d on GDV and substance P. In each diagram, GDV glow image areas are presented as a continuous time course line in which the mean values of the 4 phases tested are shown as black quadrates. For the associated parameter measured in saliva, dotted time course lines are used, and slightly bigger black dots each show the time centers of the 4 test phases sampled. The bars show the associated standard errors of the mean (SEM). The y-axis represents the relative change of each parameter, normalized for the starting point (phase 1). The x-axis in each diagram represents a relative time line in which the 4 experimental phases are labeled as 1 to 4. Each experiment started at 9:00 a.m. with phase 1 (see Fig. 1) and ended at approximately 12:35 pm. after phase 4.

Different Research Lines

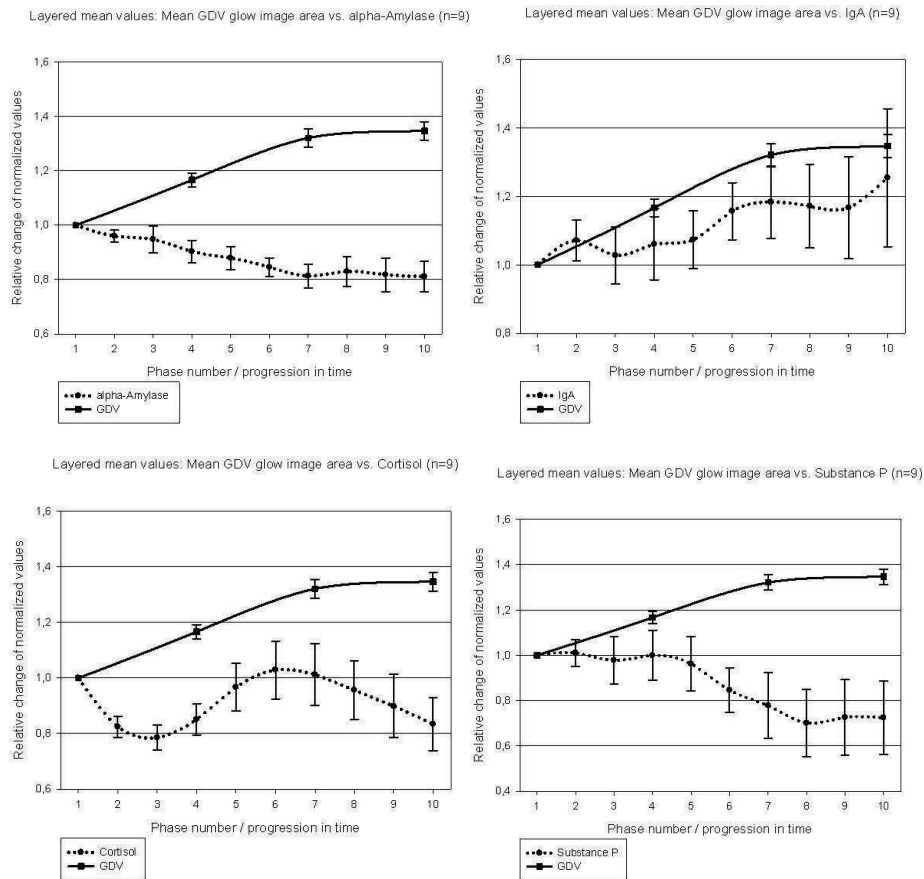


Fig. 4 a-d: Diagrams resulting from an alternative approach, showing the progressions in time of GDV glow image area in comparison to those of the markers detected biochemically in saliva in a finer resolution than in Fig. 3. Apparently, each of those salivary parameters shows a (characteristic?) time course, some of which appear to change rhythmically during the daytimes measured.

The following “layered diagrams” (*Fig. 4 a-c*) are the results of an alternative approach of presenting, yielding a finer resolution of the time courses of the salivary parameters detected. They were calculated as follows: Whilst the GDV glow image area curves were done as in Fig. 3, each marking point of the associated biochemical parameters (shown as 10 black spots) was calculated as the mean value of 3 consecutive experimental concentration measurements.

Spot 1 represents the mean of the level values obtained at time points 1, 2 and 3 in the first phase; spot 2 is the mean level of the values obtained at time points 2 and 3 of the first phase and of time point 1 of the next phase; spot 4 is the mean level of the values obtained at time points 3 of the first phase plus the following two time points, i.e. of point 1 and 2 of phase 2; and so on. This special way of calculation allows observing a finer resolution of the rhythmicity than seen in the diagrams of Fig. 3.

4. Discussion and Conclusions

Some scientists now and then are skeptical that GDV can be used as a reliable biomedical instrument. However, during the years, a great number of manuscripts have been published, evidencing that GDV can be used in pure scientific manner, as a reproducible and highly sensitive instrument with a variety of different applications (10-30, 35, 38). One of the scientific fields in which GDV appears to be a highly powerful, non-invasive apparatus for experimental research, is its use for the sensitive detection of certain stress response reactions (e.g., 12, 34, 38). Using GDV, we were able to reproducibly detect effects of certain geologic phenomena (so-called geopathic zones) on the human organism, and also to present statistically highly significant evidence for the compensatory and harmonizing potential of the “Geowave” device (Geowave-Research, Hallein bei Salzburg, Austria; www.geowave.at) (34, 38). Regarding the scientific validity of the GDV glow image area parameter, the pilot study described in the present book chapter, although done with a comparatively low number of test persons (n=9), can be regarded as kind of a bridge between “school medicine” and complementary medicine. For the first time, the possible existence of a circadian rhythmicity of the mean GDV glow image area was detected. Using standardized clinico-biochemical technology, we could show that there appears to be a characteristic relationship between the diurnal progressions of the mean GDV glow image area and a number of key parameters of stress detected in human saliva. A closer look comparing the time progressions of the parameters tested shows that each of them appears to follow the expected directions in a remarkably foreseeable way, thus reinforcing that GDV indeed is a scientifically valuable tool to detect stress.

Circadian clocks evolved as an adaptation to the planet’s 24 h rotation and its attendant rhythms of light and temperature on the Earth’s surface (46). The circadian system allows organisms to make use of those regular cycles, timing biological processes to a part of the cycle (a phase) that benefits from external light and warmth, or the absence of conflicting internal processes (47). In addition to day/night rhythms, biologic clocks reflect other temporal cycles

prevailing life on Earth: tides, month, and year also regulate their temporal programs (48).

Circadian rhythms can be found in most organisms and enable them to anticipate, rather than passively adjust to the changes imposed by alternation between day and night. The changes relate to positive functions such as vision and negative mechanisms such as UV light damage, and also include indirectly generated alternations such as temperature, availability of food and prevalence of predators. At least part of the rhythmicity is generated endogenously and approximates the length of a day and a night (e.g., 49). The mechanisms including circadian rhythms are regulated at cellular and molecular levels (48).

GDV has been used to detect stress in a number of studies; lower GDV glow image area values have been described to indicate stress, whereas higher values would be a sign of relaxation (e.g., 12, 34, 38). As stress is reflected in a number of physiological markers that show diurnal rhythmicity, it became necessary to experimentally check whether there would be a relation in between the levels of biochemical key parameters of stress reactions and the corresponding mean GDV glow image area values. As to our hypothesis, GDV glow image area would have to reflect bodily levels and rhythmicities of such physiological parameters (see also: 10-31, 50-53, 55-56) in certain “characteristic” ways.

Alpha amylase, an enzyme contained in saliva with manifold functions, can be regarded as an acknowledged enzyme chemical parameter showing stress reactions in saliva (e.g., 6-7, 9, 52, 54): In case of stress, the values of alpha-amylase should increase. If our hypothesis is right, in times of progressing relaxation, GDV glow image areas should go up, whereas in parallel, alpha amylase levels should decrease. The results of our present study show that such an inverse relationship indeed appears to be present: Not only that GDV glow image area shows a distinct progression during the daytimes checked; the two curves, as seen in *Fig. 3a*, apparently follow a mirror-like progression in between 9 a.m. and 12.30 p.m. If one looks at a finer resolution of the biochemical levels, it can be seen that this is still the case in a markedly picturesque way.

When looking at salivary IgA (2-9, 51, 55), the concentrations of this biochemical marker in our hypothesis should increase when GDV glow image area do too. *Fig. 3b* gives an indication that this appears to be true, i.e., the two curves progress in parallel-like manner. Looking at a finer resolution as shown in *Fig. 4b*, the same trend can still be observed, although for IgA, an additional “sub-rhythmicity” might to be present - a finding which remains to be

confirmed in follow-up studies to be carried out, testing the progression of the markers presented here a higher number of people and in different experimental setups.

Looking at the stress hormone cortisol, the situation is far more complicated: Cortisol appears to follow smaller “sub-rhythms” that change inter-individually and heavily depending on type and stressing potential of external noxae (e.g., 53, 56-57). Nevertheless, both diagrams, low (*Fig. 3c*) and higher resolution type (*Fig. 4c*) apparently show a well comparable outcome when looking at progression during the times tested. Chronobiological concentration variations in time detected here are in agreement with those reported in literature. A distinct relationship to the GDV time flow was not identifiable in the present pilot study.

The neuropeptide substance P, also contained in saliva, is involved in both, pain reception and immunological response and shows circadian rhythms too (58-63). Apparently chronobiological variations of substance P appear not to be detectable in all individuals (62). When looking at *Figs. 3d* and *4d*, it appears that the curve relationship between GDV and substance P in certain aspects might resemble the figures presented for alpha-amylase (*Figs. 3a* and *4a*). Consecutive experiments with a much higher number of test persons should contribute to bring more light into the relationship indicated here.

Although it seems likely that the progressions of the levels / values of the parameters tested may reflect real diurnal curves, it is very likely that superimposed effects of the specific experimental setup used are present, too. The participating test persons, at the beginning of the experiments, might have been stressed, e.g. by fear of the unexpected, but later on, got used to the new situation, leading to relaxation. The GDV curve progression seen therefore might be understood as kind of an adaptation to the specific experimental situation. Some of the “sub-rhythms” (those might be called second order “(beta-)rhythms” superimposed upon spontaneous “alpha-rhythms”; 62) seen in the “finer resolution” diagrams, such as for IgA, might also have been caused by the hourly breaks for drinking water, using the bathroom and washing hands: After such breaks, additional bits of relaxation could lead to distortions of the “real diurnal curves”. For cortisol, however, curves comparable to those of our study had been described earlier (e.g., 57).

In line with the biochemical findings, probable connections between GDV glow image area levels and standardized psychological questionnaires concerning strain and stress should be mentioned. During the mobile phone base station

study of whose control group the “side-results” presented here were taken, a number of psychological questionnaires had been used, including a symptom check list (SCL-90-R, measuring various kinds of psychological strain; 64), the STAI test (state-anxiety; 65), and a B-L questionnaire (somatic and psychosomatic disturbances and symptoms; 66). In those tests, high scores indicate high strain. All SCL-subcales and sum scores, as well as sum scores of STAI and B-L, showed negative (although mostly not significant) correlations with GDV. Chance seems to be an unsatisfying explanation (the binomial test shows a p value $< 0,001$), and further research should therefore include this matter too.

The data presented here undoubtedly justify follow-up studies aimed to test a higher number of persons during longer periods of the whole day. Designing such studies will not be easy, as no person would agree to sit still or participate at such experiments lasting longer than a few hours. Also, external and intrinsic parameters (hunger, need for sleep etc.) would heavily influence the results. Therefore it might have to be a compromise, in which overlapping groups during several time windows of the day need to be tested, e.g. one group as done here on forenoons, another group at afternoons, but also groups over “lunchtime” and in the evening hours. Such setups would probably also contribute to elucidate possible influences of the experimental setup on the results. Taking salivary samples during the night would be likely to be impossible in the way done during the pilot experiments presented here. Although methods of nighttime sampling of saliva have been described (67), using automatic blood samples might be another way of approaching. However, taking representative GDV glow image areas in between sleep breaks would cause stress likely to be seen in changes of the GDV glow image area. For such “during night” experiments, hypnosis might be successfully used for allowing GDV tests to be performed during sleep periods.

As a conclusion of the pilot study presented in this manuscript, closer evidence has been presented for the validity of using the mean GDV glow image area as a reliable measure for (at least, certain types of) stress. Comparative analyses of the curve progressions of the parameters tested here in parallel showed the expected connections: Higher GDV glow image area values went in line with increased IgA production in saliva, whilst at the same time, the alpha amylase curve progressed in the opposite way. The meaning of the connections between GDV and cortisol and substance P detected needs to be clarified in subsequent experiments. Overall, such studies will have to include a higher number of test persons, as well as specifically designed experimental setups also needed to

elucidate influences of water drinking breaks, of fear and other factors likely to be present.

5. Acknowledgements:

The pilot study presented here is an ancillary result of a major grant given by the Land Salzburg for studying possible influences of GSM mobile phone base stations on the human organism. Dr. Schwammerger (Institute of Biochemistry at University of Salzburg, Faculty of Natural Sciences) had been appointed on order to perform the biochemical tests reported. We cordially thank Dr. Gerd Oberfeld (Health department of the Salzburg federal government) and Dr. Ing. Martin Virnich (ANBUS Analytic Co., Fürth, Bavaria) for their most professional support and advice concerning NF and HF electromagnetic fields and their shielding in the laboratory used for the tests reported.

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**DAYTIME-RELATED RHYTHMICITY
OF GDV PARAMETER GLOW IMAGE AREA:
TIME COURSE AND COMPARISON TO
BIOCHEMICAL PARAMETERS MEASURED IN SALIVA**

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1. Introduction and Aims of Study

Chronobiology is a relatively new field of science investigating periodic phenomena in the organism. It is well established that the flow rate and composition of saliva vary rhythmically depending on daytime (1-3). A number of parameters contained in saliva have been suggested to be reliable measures for detecting stress and related bodily answers on external noxae. In the context of stress and immune answers, alpha-amylase, immunoglobulin A (IgA), cortisol and the regulatory peptide substance P deserve special attention. Relatively fast changes of the levels of those biochemical parameters in saliva have been reported, and their significance to show response on stressors appears to be high (e.g., 4-9).

Gas discharge visualization (GDV), as developed by Professor Konstantin Korotkov (St. Petersburg University; www.korotkov.org), has been used to analyze a number of instrument-inherent physical and complementary parameters (10-30). Using GDV, evoked corona discharges from the tips of all 10 fingers can be recorded by a specifically designed apparatus essentially based on the Kirlian effect, carefully designed to meet the reproducibility and sensitivity demands of scientific research. By application of computer image analysis, digital photographs of the electro-photonic glow are transferred into a number of expedient parameters based on the sizes and distributions of the

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**ENERGY FIELDS
ELECTROPHOTONIC
ANALYSIS
IN HUMANS
AND NATURE**

*Be governed by your knowledge, and proceed
I the sway of your own will*

*William Shakespeare
King Lear IV, 7*