



Contents lists available at ScienceDirect

Int. J. Hyg. Environ. Health

journal homepage: [www.elsevier.de/ijheh](http://www.elsevier.de/ijheh)

## Short Communication

## Feasibility of testing three salivary stress biomarkers in relation to naturalistic traffic noise exposure

Jasmin Wagner<sup>a</sup>, Michael Cik<sup>b</sup>, Egon Marth<sup>a</sup>, Brigitte I. Santner<sup>a</sup>, Eugen Gallasch<sup>c</sup>, Andreas Lackner<sup>d</sup>, Reinhard B. Raggam<sup>a,\*</sup><sup>a</sup> Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, Austria<sup>b</sup> Institute of Highway Engineering and Transport Planning, Graz University of Technology, Graz, Austria<sup>c</sup> Institute of Physiology, Medical University of Graz, Austria<sup>d</sup> Department of Neurotology, ENT University Hospital, Medical University of Graz, Austria

## ARTICLE INFO

## Article history:

Received 20 April 2009  
 Received in revised form  
 18 August 2009  
 Accepted 20 August 2009

## Keywords:

Traffic noise  
 Stress biomarkers  
 Saliva

## ABSTRACT

**Background and Objectives:** Stress dependent alterations of the salivary biomarkers alpha-amylase (sAA), salivary chromogranin A (sCgA) and salivary cortisol (sC) have been reported in numerous studies recently. The aim of this pilot study was to investigate the feasibility of testing sAA, sCgA and sC in relation to naturalistic traffic noise exposure in order to monitor a direct stress response in a laboratory setup.

**Methods:** A total of twenty study participants were exposed to binaurally recorded naturalistic traffic noise samples containing 75 dB ( $L_{A,eq}$ ) for 20 minutes via a loudspeaker system. Saliva was collected directly before and after defined exposure to naturalistic traffic noise. Determination of sAA was performed enzymatically on a Hitachi 912 laboratory analyzer, sCgA was determined by ELISA technique and sC was determined using a RIA assay.

**Results and Conclusions:** There was a significant increase of sAA and sC concentrations after traffic noise exposure ( $p = 0.045$ ;  $p = 0.01$ ), whereas for sCgA this was not observed ( $p = 0.48$ ). Measuring of sAA and sC appear to be feasible to investigate direct stress effects in relation to naturalistic traffic noise exposure in a laboratory setup. Considering the small sample size of this pilot study, these observations need to be further proved in a larger explorative study.

© 2009 Elsevier GmbH. All rights reserved.

## Introduction

Traffic noise is an increasing problem in our environment nowadays, especially the kind of “unwanted sounds” that influence our well-being and health negatively. A lot of different studies demonstrate that traffic noise as a stressor can cause discomfort, sleep disorders, and can disturb daily-live activities (Evans, 2001; Ising and Kruppa, 2007; Maschke and Hecht, 2007; Griefahn et al., 2008; Michaud et al., 2008). Moreover it may also lead to hypertension, cardiovascular disease and an increased risk of myocardial infarction (Babisch, 2006; Babisch, 2008; Jarup et al., 2008).

Several studies have demonstrated an increased secretion of salivary stress biomarkers like the salivary alpha-amylase (sAA), salivary chromogranin A (sCgA) and salivary cortisol (sC) under different stress conditions (Takai et al., 2004; Miyakawa et al., 2006; Nater et al., 2006; Hebert and Lupien, 2007; Filaire et al.,

2009; Hellhammer et al., 2009; Lewis, 2006). Investigation of stress biomarkers in saliva has achieved recognition because sample collection is standardized, non-invasive and easy to handle (Nater et al., 2007; Filaire et al., 2009). Different collection devices for standardized collection of saliva are available as described in previous studies (Poll et al., 2007; Raggam et al., 2008; Filaire et al., 2009).

The aim of this pilot study was to investigate the feasibility of testing the salivary biomarkers sAA, sCgA and sC in order to evaluate direct effects of naturalistic traffic noise, presented under strict defined laboratory conditions, on the two main physiological stress axes: the sympathetic-adreno-medullary system (SAM) and the hypothalamic-pituitary-adrenal axis (HPA).

## Methods and study design

## Study participants and selection criteria

This study included 20 healthy voluntary participants with no record of cardio-vascular disease and normal hearing function.

\* Corresponding author. Tel.: +43 316 380 4375; fax: +43 36380 9648.  
 E-mail address: [reinhard.raggam@medunigraz.at](mailto:reinhard.raggam@medunigraz.at) (R.B. Raggam).

Participants were between 20 and 44 years of age, 10 females and 10 males. The study was approved by the local Ethic Committee of the Medical University of Graz, Austria. All study participants gave written informed consent.

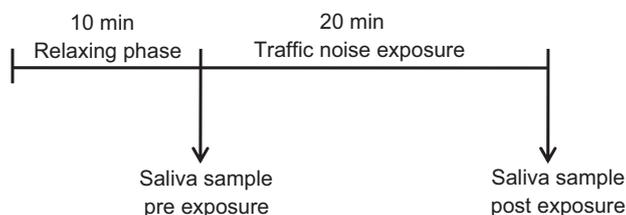
#### Naturalistic traffic noise samples and study design

For the laboratory attempt, binaurally recorded naturalistic pass-by road and rail traffic vehicle noise, taken from an existing database of the Institute of Highway Engineering and Transport Planning, Graz University of Technology, Graz, Austria, were selected and used for presentation (Raggam et al., 2007). The naturalistic road and rail traffic pass-by samples contained A-weighted sound levels with 75 dB, typical A-weighted frequency spectra meeting the national standards of traffic noise frequencies (OENORM EN 1793-3: 1998 03 01:N). All laboratory attempts were performed between 1:00 to 4:00 pm in a specially adapted listening room at the psychoacoustic laboratory of the Institute of Highway Engineering and Transport Planning, Graz University of Technology. The study participants were exposed for 20 min to binaurally recorded naturalistic road and rail traffic pass-by vehicle noise samples with a level of 75 dB ( $L_{A,eq}$ ) via a loudspeaker system after a 10 min relaxing phase lacking any acoustic content (Fig. 1).

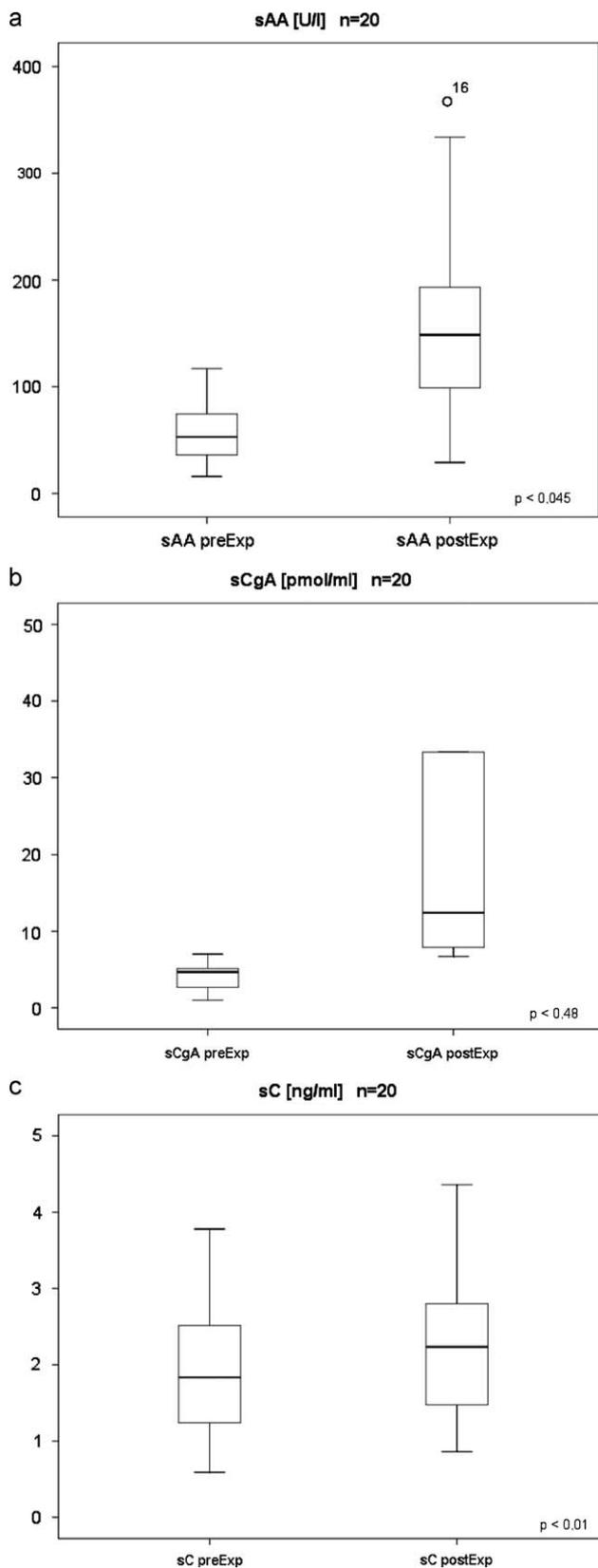
Saliva was collected using the Saliva Collection System, Greiner Bio-One GmbH, Kremsmünster, Austria, according to the manufacturer's instructions. Smoking, eating and drinking were not permitted 30 min prior. Saliva samples were obtained directly before and after noise exposure and immediately stored at  $-70^{\circ}\text{C}$ .

#### Analysis of salivary stress biomarkers

Prior to the analysis, saliva samples were thawed and centrifuged for 3 min at 3300 rpm and processed according to the manufacturer's instructions as previously described (Raggam et al., 2007). The determination of sAA was performed on a Hitachi 912 laboratory analyzer (Roche Diagnostics, Mannheim, Germany). The saliva samples were diluted 1:100 with distilled water and transferred to the analyzer using the corresponding alpha-amylase kit (Roche Diagnostics). Alpha-amylase activity was expressed as international units per liter of saliva (U/l). For the determination of sCgA, the RSCYK070R Chromogranin A (Human) EIA kit, BioVendor GmbH, Heidelberg, Germany, was used. Values of sCgA were calculated on values of total salivary protein measured in the same sample. Chromogranin A activity was expressed as pmol/ml. The determination of sC was performed using the RIA technique as described previously (Hebert and Lupien, 2007). Cortisol activity was expressed as ng/ml.



**Fig. 1.** Time course of the laboratory attempt including 10 min relaxing phase, lacking of acoustic content, followed by 20 min naturalistic traffic noise exposure. The saliva samples were collected immediately before (pre exposure) and after (post exposure) traffic noise exposure.



**Fig. 2.** (a) Box-and-Whisker Plots showing sAA concentrations pre- and post exposure to 20 minutes of naturalistic traffic noise. (b) Box-and-Whisker Plots showing sCgA concentrations pre- and post exposure to 20 minutes of naturalistic traffic noise. (c) Box-and-Whisker Plots showing sC concentrations pre- and post exposure to 20 minutes of naturalistic traffic noise.

### Statistical analysis

Statistics were calculated with SPSS 17.0 software,  $p < 0.05$  was considered as statistically significant. The correlation analysis was calculated by Pearson-test.

### Results and discussion

All 20 study participants included in the study underwent audiometry and showed normal audiograms (data not shown). The collection of saliva samples with the Saliva Collection System was uncomplicated, easy to handle and yielding a sufficient amount of saliva for laboratory testing. Immediate storage of collected saliva samples at  $-70^{\circ}\text{C}$  allowed en bloc testing without repeated freeze-thaw phases ensuring high stability of biomarkers tested.

The study participants were exposed for 20 minutes to naturalistic road and rail traffic pass-by vehicle noise samples containing noise levels ranging from 48 dB to 83 dB, (75 dB  $L_{A,eq}$ ). This specific noise levels were chosen to meet the standards of critical values according the Austrian guidelines on the influence of noise on humans (ÖAL-6/18 Richtlinie, 1991). Salivary stress biomarkers were then analyzed in order to evaluate possible differences in concentrations between pre- and post traffic noise exposure.

A significant increase of sAA and sC concentrations measured post naturalistic traffic noise exposure was observed when compared to concentrations measured directly pre exposure ( $p = 0.045$ ;  $p = 0.01$ ) (Fig. 2a and c); whereas the observed increase of the sCgA concentration post traffic noise exposure was not statistically significant ( $p = 0.48$ ) (Fig. 2b).

In several studies, the biomarkers sAA, sCgA and sC have proven to be reliable stress markers reflecting the activity of the SAM and HPA axes. (Bigert et al., 2005; Miyakawa et al., 2006; Nater et al., 2006) In this pilot study, the biomarker sAA showed a significant increase due to defined naturalistic traffic noise exposure, indicating activation of the SAM axis. Determination of sAA is less expensive compared to the determination of sCgA and sC. Performing of tests is possible under standardized, fully automated conditions and therefore making this parameter suitable for large scale testing.

When the biomarker sCgA was determined, an increase after naturalistic traffic noise exposure could be observed, however, this was not statistically significant. This seems to be caused by the test specific (ELISA) wide range of sCgA concentrations obtained from saliva samples collected post exposure. Another reason for such inhomogeneous distribution of results may be due to the poor stability of this metabolite in the sample matrix (Bender et al., 1992). Moreover, measuring sCgA with ELISA technique is strongly investigator dependent and is laborious compared to sAA determination.

The measurement of sC showed significantly higher concentrations in saliva samples collected after traffic noise exposure, indicating an activation of HPA. These results are in concordance of those previously reported (Bigert et al., 2005; Filaire et al., 2009). This also reflects a sufficient reproducibility of selected naturalistic traffic noise samples and their presentation in a laboratory setup.

In consideration of the small sample size, this pilot study showed that standardized collection of saliva samples was easy to handle and well tolerated by the study participants. The stress biomarkers sAA and sC showed a significant increase in concentrations in relation to naturalistic traffic noise exposure and they appear to be feasible biomarkers to measure direct effects of naturalistic traffic noise on the SAM/HPA axes in a laboratory setup. It therefore seems to be meaningful to better

support this associations, to conduct a further explorative study including a larger sample size and the implementation of sham-exposed controls, to exclude a possible stressful influence of the room and the equipment it-self.

### Acknowledgement

This study was financed by the Hygiene Fund of the Medical University of Graz.

The authors want to thank Michaela Winkler for her valuable, experienced technical support in performing CgA ELISA, Bettina Schmied and Ursula Schmitt for their technical support in the Clinical Chemistry laboratory.

### References

- Babisch, W., 2006. Transportation noise and cardiovascular risk: updated review and synthesis of epidemiological studies indicate that the evidence has increased. *Noise Health* 8 (30), 1–29.
- Babisch, W., 2008. Road traffic noise and cardiovascular risk. *Noise Health* 10 (38), 27–33.
- Bender, H., Maier, A., Wiedenmann, B., O'Connor, D.T., Messner, K., Schmidt-Gayk, H., 1992. Immunoluminometric assay of chromogranin A in serum with commercially available reagents. *Clin. Chem.* 38 (11), 2267–2272.
- Bigert, C., Bluhm, G., Theorell, T., 2005. Saliva cortisol – a new approach in noise research to study stress effects. *Int. J. Hyg. Environ. Health.* 208 (3), 227–230.
- Evans, G.W., 2001. Environmental stress and health. In: Baum, A., Revenson, T., Singer, J.E. (Eds.), *Handbook of Health Psychology*. Erlbaum, Mahwah, NJ, pp. 365–385.
- Filaire, E., Dreux, B., Massart, A., Nourrit, B., Rama, L.M., Teixeira, A., 2009. Salivary alpha-amylase, cortisol and chromogranin A responses to a lecture: impact of sex. *Eur. J. Appl. Physiol.* 106 (1), 71–77.
- Griefahn, B., Brode, P., Marks, A., Basner, M., 2008. Autonomic arousals related to traffic noise during sleep. *Sleep* 31 (4), 569–577.
- Hebert, S., Lupien, S.J., 2007. The sound of stress: blunted cortisol reactivity to psychosocial stress in tinnitus sufferers. *Neurosci. Lett.* 411 (2), 138–142.
- Hellhammer, D.H., Wust, S., Kudielka, B.M., 2009. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology* 34 (2), 163–171.
- Ising, H., Kruppa, B., 2007. Stress effects of noise. In: Luxon, L., Prasher, D. (Eds.), *Noise and its Effects*. John Wiley & Sons, Chichester.
- Jarup, L., Babisch, W., Houthuijs, D., Pershagen, G., Katsouyanni, K., Cadum, E., Dudley, M.L., Savigny, P., Seiffert, I., Swart, W., Breugelmans, O., Bluhm, G., Selander, J., Haralabidis, A., Dimakopoulou, K., Sourtzi, P., Velonakis, M., Vigna-Taglianti, F., 2008. Hypertension and exposure to noise near airports: the HYENA study. *Environ. Health Perspect.* 116 (3), 329–333.
- Lewis, J.G., 2006. Steroid Analysis in Saliva: An overview. *Clin. Biochem. Rev.* 27 (3), 139–146.
- Maschke, C., Hecht, K., 2007. Stress and noise – the psychological/physiological perspective and current limitations. In: Luxon, L., Prasher, D. (Eds.), *Noise and its Effects*. John Wiley & Sons, Chichester.
- Michaud, D.S., Keith, S.E., McMurphy, D., 2008. Annoyance and disturbance of daily activities from road traffic noise in Canada. *J. Acoust. Soc. Am.* 123 (2), 784–792.
- Miyakawa, M., Matsui, T., Kishikawa, H., Murayama, R., Uchiyama, I., Itoh, T., Yoshida, T., 2006. Salivary chromogranin A as a measure of stress response to noise. *Noise Health* 8 (32), 108–113.
- ÖAL-6/18 Richtlinie, 1991. Die Wirkung des Lärms auf den Menschen – Beurteilungshilfen für den Arzt. 1. ÖAL-Richtlinie 1 (11), 17–18.
- Nater, U.M., La Marca, R., Florin, L., Moses, A., Langhans, W., Koller, M.M., Ehlert, U., 2006. Stress-induced changes in human salivary alpha-amylase activity – associations with adrenergic activity. *Psychoneuroendocrinology* 31 (1), 49–58.
- Nater, U.M., Rohleder, N., Schlotz, W., Ehlert, U., Kirschbaum, C., 2007. Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology* 32 (4), 392–401.
- Poll, E.M., Kreitschmann-Andermahr, I., Langejuergen, Y., Stanzel, S., Gilsbach, J.M., Gressner, A., Yagmur, E., 2007. Saliva collection method affects predictability of serum cortisol. *Clin. Chim. Acta* 382 (1–2), 15–19.
- Raggam, R.B., Cik, M., Holdrich, R.R., Fallast, K., Gallasch, E., Fend, M., Lackner, A., Marth, E., 2007. Personal noise ranking of road traffic: subjective estimation versus physiological parameters under laboratory conditions. *Int. J. Hyg. Environ. Health* 210 (2), 97–105.
- Raggam, R.B., Santner, B.L., Kollrosner, M., Gossler, W., Schmied, B., Schmitt, U., Stelzl, E., Lackner, A., Wagner, J., Marth, E., Kessler, H.H., 2008. Evaluation of a novel standardized system for collection and quantification of oral fluid. *Clin. Chem. Lab. Med.* 46 (2), 287–291.
- Takai, N., Yamaguchi, M., Aragaki, T., Eto, K., Uchihashi, K., Nishikawa, Y., 2004. Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults. *Arch. Oral. Biol.* 49 (12), 963–968.