Short-term effects of nocturnal transportation noise on cardio-metabolic outcomes and its association to sleep

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TABLE OF CONTENT

ABSTRACT

Transportation noise is one of the most important environmental risk factors for cardio-metabolic diseases. It is assumed that short-term noise exposure activates a physiological stress response, which in a chronic state can cascade to long-term critical health problems. Sleep disturbances are regarded as the main mediator of transportation noise induced cardio-metabolic diseases. However, no study so far investigated short-term effects of nocturnal transportation noise exposure on glucose regulation and stress markers in association with alterations in sleep macro- and microstructure. This thesis aimed at investigating the following main questions: does short-term nocturnal transportation noise exposure impair glucose regulation and activate stress responses, and are they both related to sleep changes? Do individual covariates such as age and sex modulate the observed noise effects?

Twenty-six (12 women) young (19-33 years) and 16 (8 women) older (52-70 years) volunteers participated in a six day controlled laboratory study. The experiment started and ended respectively with a noise-free baseline and a noise-free recovery night; in-between four different transportation noise scenarios (low/medium/high intermittent road or rail scenarios with an identical equivalent continuous sound level of 45dB) were presented in a randomized order to the volunteers. Sleep was recorded polysomnographically, glucose tolerance and insulin sensitivity were assessed during an oral glucose tolerance test (OGTT) in the morning of the baseline, the last noise night and the recovery night, nocturnal catecholamine was assessed in the urine, daytime cortisol in the saliva and morning inflammatory markers in the fasting blood samples of the OGTT. Blood pressure as well as heart rate variability (HRV) and autonomic arousals were also recorded during the six experimental nights.

Sleep macrostructure and the number of cortical arousals were not significantly altered by nocturnal transportation noise exposure. However, cumulative autonomic arousals duration increased during the highly intermittent road noise scenario night for the young group and during the highly intermittent railway noise scenario night for the entire experimental group compared to baseline night. Four nights of nocturnal transportation noise exposure reduced glucose tolerance and insulin sensitivity in both age groups. Moreover, the reduction in glucose tolerance was associated with the increase in cumulative autonomic arousals duration. Additionally, highly intermittent noise had a stronger deleterious effect on glucose regulation than lower intermittent noise. Highly intermittent road noise increased next evening cortisol levels only in the young subgroup. This increase was associated with the increase in cumulative autonomic arousal duration during the previous sleep

episode. Nocturnal blood pressure, HRV spectral analysis and catecholamine as well as morning inflammatory markers were not significantly changed by the noise exposure.

Taken together, even if the effect observed on glucose regulation was not clinically significant, these results underline the harmful effect of nocturnal transportation noise on subcortical activation during sleep and its repercussions on the stress and metabolic system. In a chronic state these effects may lead on long-term to cardio-metabolic diseases.

LIST OF ABBREVIATIONS

AA:	Autonomic arousal
AASM:	American academy of sleep medicine
ACTH:	Adrenocorticotrophic hormone
ANS:	Autonomic nervous system
ARAS:	Ascending reticular activating system
AUC:	Area under the curve
BL:	Baseline night
BP:	Blood pressure
CA:	Cortical arousal
CAR:	Cortisol awakening response
CHD:	Coronary heart disease
CRH:	Corticotropin-releasing hormone
CRP:	C-reactive protein
CVD:	Cardiovascular diseases
ECG:	Electrocardiogram
EEG:	Electroencephalogram
EMG:	Electromyogram
EOG:	Electrooculogram
ESS:	Epworth Sleepiness Scale
FFT:	Fast Fourier transform
G_0:	Fasting plasma glucose concentration
G_{120}:	Glucose concentration 2 hours after glucose intake
$Glucose_{AUC}$:	Area under the curve of the OGTT glucose profile
HPA:	Hypothalamic-pituitary-adrenal
HR:	Heart rate
HRV:	Heart rate variability
I_0:	Fasting serum insulin concentration
Il-1:	**I**nterleukin 1
Il-6:	Interleukin 6
$Insulin_{AUC}$:	Area area under the curve of the OGTT insulin profile
IR:	Intermittency ratio
ISI:	Insulin sensitivity index
KSS:	Karolinska sleepiness scale
LA50:	Median sound level
LAeq:	A-weighted equivalent continuous sound level
LAmax:	A-weighted maximum sound level
LE:	Less eventful
LEF-K:	Lärmempfindlichkeitsfragebogen
Leq:	Equivalent continuous sound level
MCTQ:	Munich chronotype questionnaire
ME:	More eventful

NoiseQ:	Noise Sensitivity Questionnaire
OFS:	Office fédéral de la statistique
OGTT:	Oral glucose tolerance test
PANAS:	Positive and negative affect schedule
PSG:	Polysomnography
PSQI:	Pittsburgh Sleep Quality Index
PVN:	Paraventricular nucleus
PVT:	Psychomotor vigilance task
RC:	Recovery night
REM:	Rapid eye movement sleep
SAM:	Sympathetic-adrenal-medullary
SE:	Sleep efficiency
SNS:	Sympathetic nervous system
SPL:	Sound pressure level
SSC:	Sleep stage change
SWA:	Slow-wave activity
SWS:	Slow-wave sleep
T2D:	Type 2 diabetes
TNFα:	Tumor necrosis factor alpha
TST:	Total sleep time
VAS:	Visual analog scale
VNTR:	Variable number tandem repeat
WASO:	Wake after sleep onset
WHO:	World Health Organization

Chapter I

INTRODUCTION

Cardiovascular diseases (CVD) comprise all disorders impacting the heart and blood vessels. With 17.7 million deaths in 2015, CVD represent 31% of all deaths and are the number one killer globally (WHO, 2017a). Also in Switzerland, CVD are the first cause of death with 34% of the total deaths for women and 30% for men in 2015 (OFS, 2018). Diabetes which is one of the most powerful risk factor for CVD, but also a disease *per se,* is rising with a global prevalence of 9% in 2014 compared to 5% in 1980 (WHO, 2017b) and is expected to be the seventh leading cause of death in 2030 (Mathers & Loncar, 2006).

Physiological risk factors for CVD include hypertension, thrombosis, high blood glucose or diabetes, high blood lipids, overweight or obesity and inflammation, which favor atherosclerosis and CVD on the long-term. These risk factors are often the consequence of behavioral risk factors comprising unhealthy diet, physical inactivity, tobacco or alcohol abuse (WHO, 2017a, 2017b). Socioeconomic status, education level, and environmental factors, such as air and noise pollution or lack of green space, can also affect cardio-metabolic health (Tzoulaki et al., 2016). It has been stated that more than 75% of the burden of diseases coming from environmental factors is attributable to particulate matter air pollution and transportation noise (Hänninen et al., 2014). With globalization and urbanization, transportation noise can become a critical risk factor. Indeed, already more than 30% of the EU population is exposed to nocturnal levels exceeding 55 dB(A), the recommended interim level fixed by the World Health Organization (WHO) (Fritschi et al., 2011; Münzel, Sorensen, et al., 2017b). Above this level the risk of cardio-metabolic disorders is considered to increase.

A growing number of epidemiological studies associated transportation noise with cardio-metabolic diseases (Münzel, Sorensen, et al., 2017b). Recent studies emphasize the importance of the nocturnal hours for transportation noise induced diabetes (Eze, Foraster, et al., 2017; Eze, Imboden, et al., 2017) and CVD and mortality (Héritier et al., 2018; Jarup et al., 2008). Indeed, sleep is vital for restoring the cardio-metabolic system and sleep impairment is known to increase cardio-metabolic disorders (Cappuccio & Miller, 2017). However, the pathway linking transportation noise exposure and cardio-metabolic disturbances is not completely elucidated yet, and so is its association to sleep.

Moreover, covariates, such as noise characteristics, and individual factors can significantly modify the effect of nocturnal transportation noise on health. Most of the studies modeling health effects of noise used average noise metrics over longer time periods. This kind of metrics may lose information in averaging complex time patterns of exposure in a single value. Thus, in the framework of our national SiRENE (Short and Long Term Effects of Transportation Noise Exposure) project, a new acoustical metric focusing on single events, the intermittency ratio (IR) (Wunderli et al., 2015), has been elaborated and will be used in this thesis as a potential covariate of the effect of nocturnal transportation noise exposure on sleep and cardio-metabolic regulation. Furthermore, the

implication of individual factors such as age, sex, and genetic predisposition, as other potential covariates will be investigated.

In sum, this thesis aims at determining whether already short-term nocturnal transportation noise exposure disturbs cardio-metabolic regulation, and whether it is associated with sleep disturbances. Moreover, we were interested to determine if covariates such as IR, sex, age or genetic predisposition may influence the observed noise effects.

Chapter II

BACKGROUND

1. Sleep assessment and physiology

We spend more than one third of our life asleep, thus "if sleep does not serve an absolutely vital function, then it is the biggest mistake the evolutionary process has ever made" (Rechtschaffen, 1971). Sleep is a complex state composed of different sleep stages presenting distinct biological functions. A physiological night sleep is organized in 4 to 6 sleep cycles, each of it composed of four different sleep stages. A sleep cycle starts with light sleep (sleep stages N1 and N2) followed by deep slow-wave sleep (SWS) and rapid eye movement (REM) sleep. Sleep stages are defined by visual scoring of 30 sec polysomnography (PSG) epochs according to standard criteria (Berry et al., 2016). PSG is the gold standard method for assessing sleep and comprises an electroencephalogram (EEG), an electrooculogram (EOG), an electromyogram (EMG) and an electrocardiogram (ECG) (Berry et al., 2016).

1.1. Sleep stages characteristics

In a calm situation with closed eyes, **wakefulness** is in 80% of the population characterized by alpha activity (8-12 Hz) in the occipital region (Santamaria & Chiappa, 1987) (*Figure II.1*).

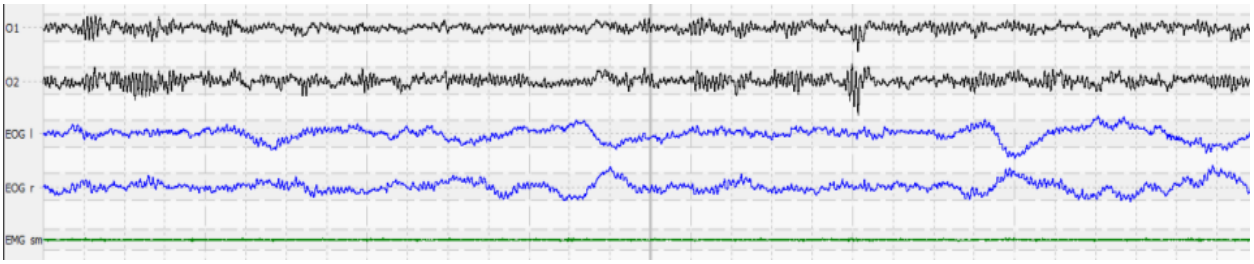

Figure II.1: Thirty seconds of cerebral activity at occipital electrodes level (black), eye movements (blue) and muscle activity (green) during wakefulness, eyes closed. *Personal data.*

With relaxation, the amount of alpha activity slowly decreases at the expense of theta activity (4-8 Hz); the eyes roll slowly and muscle activity decreases compared to wakefulness. This sleep stage is called **N1**, a transition state between wake and sleep (*Figure II.2*).

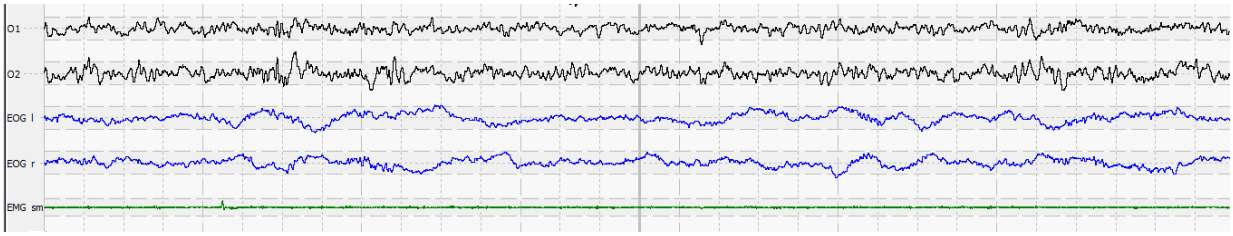

Figure II.2: Thirty seconds of cerebral activity at occipital electrodes level (black), eye movements (blue) and muscle activity (green) during N1. *Personal data.*

Sleep normally continues with **N2**, the predominant sleep stage present during approximately 50% of the night sleep. N2 is characterized by the presence of sleep spindles (burst of activity of 11-16Hz) and K-complexes (low-frequency high-amplitude EEG waves (0.5-1Hz)) in the central region (*Figure II.3*).

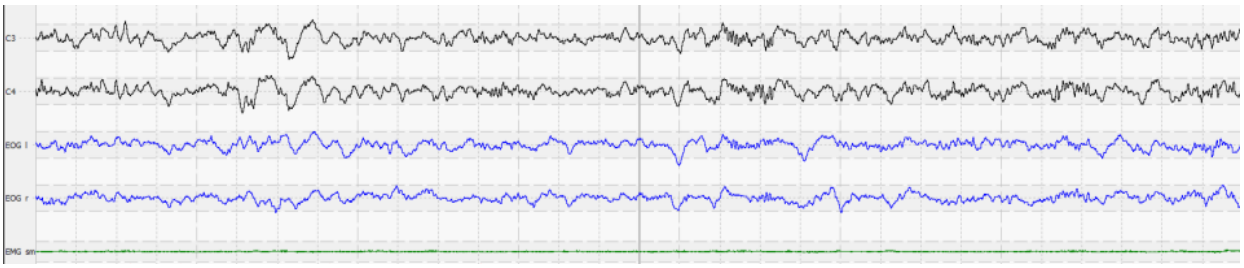

Figure II.3: Thirty seconds of cerebral activity at central electrodes level (black), eye movements (blue) and muscle activity (green) during N2. *Personal data.*

The deepest and most restorative sleep stage is **N3**, also called slow-wave sleep (SWS) because of its easily distinguishable low-frequency high-amplitude waves in the frontal brain area (*Figure II.4*). A healthy adult spends approximately 20% of the night in SWS, the percentage decreases with age.

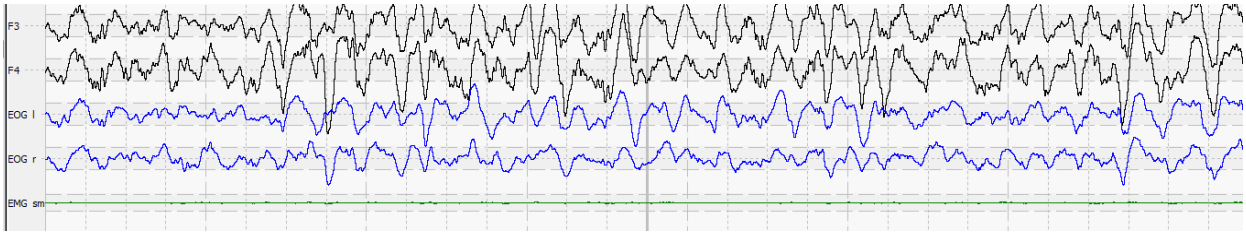

Figure II.4: Thirty seconds of cerebral activity at frontal electrodes level (black), eye movements (blue) and muscle activity (green) during N3. *Personal data.*

The remaining sleep stage, **REM** sleep, is the one during which we dream the most. Michel Jouvet called it paradoxical sleep as it is a state with "an active brain in a quiet body" (Naquet, 2004). Indeed, cerebral activity is similar to the wake state, but the body is motionless with a complete muscle atonia. Only the eyes continue to move rapidly thus the name of REM (rapid eye movement) sleep (*Figure II.5*). A healthy adult spends approximately 20% of the night in REM sleep.

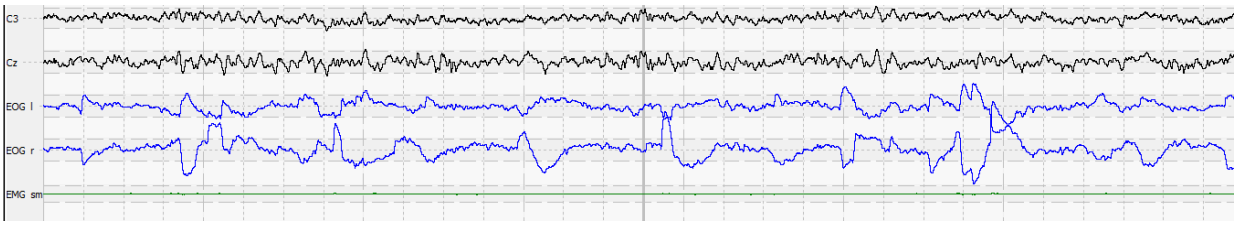

Figure II.5: Thirty seconds of cerebral activity at central electrodes level (black), eye movements (blue) and muscle activity (green) during REM sleep. *Personal data.*

1.2. Sleep microstructure

During sleep, subtle activations, of vegetative and cortical origin as well as awakenings, can occur at each moment.

Vegetative activation, also called **autonomic arousals (AA)**, can be identified by an increase in heart rate (HR) (*Figure II.6*). AA occur at the subcortical brain stem level (Guilleminault et al., 2006) and correspond to transient elevations in the sympathetic tone. They are frequently accompanied by cortical arousals (CA) but they may also occur separately (Griefahn et al., 2008).

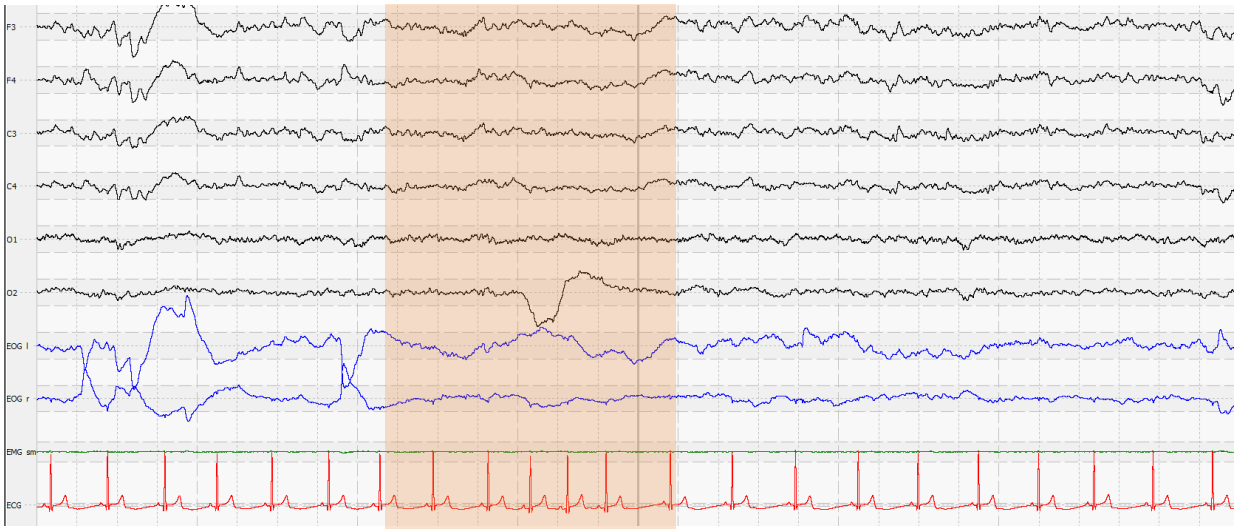

Figure II.6: Thirty seconds of cerebral activity at frontal, central and occipital electrodes level (black), eye movements (blue), muscle activity (green) and cardiac activity (red) during an autonomic arousal (marked in orange). *Personal data.*

Cortical arousals (CA) are defined as abrupt shift in EEG frequency, which may include theta, alpha and/or frequencies greater than 16Hz but not spindles (Berry et al., 2016) (*Figure II.7*).

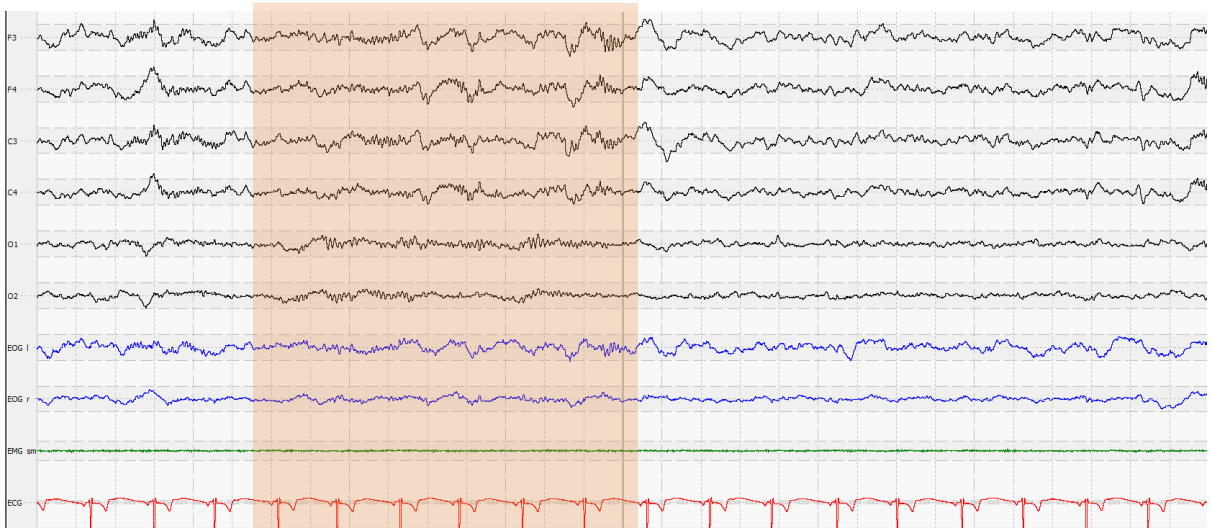

Figure II.7: Thirty seconds of cerebral activity at frontal, central and occipital electrodes level (black), eye movements (blue), muscle activity (green) and cardiac activity (red) during a cortical arousal (marked in orange). *Personal data.*

1.3. Spectral analysis

While sleep scoring is a qualitative measure of sleep architecture, it may be insensitive to smaller but clinically relevant variations in sleep structure. For example, slow-wave activity (SWA), which is important for glucose regulation (Tasali et al., 2008), is mainly present during SWS but it is also visible, to a smaller amount, during light sleep. Power spectral analysis allows a more quantitative analysis of the EEG signal with the fast Fourier transform (FFT) dividing the signal into its different frequency components. SWA corresponds to the so-called delta frequency band between 0.75 and 4.5 Hz.

1.4. Sleep regulation

As described by the two process model, two mechanisms control timing, duration and quality of sleep and wakefulness (Borbely, 1982; Daan et al., 1984). According to this model, sleep is regulated by a homeostatic process, mirrored in an accumulation of sleep pressure during wakefulness and its dissipation during sleep. SWA and the amount SWS are the principal markers of this sleep homeostatic process; the longer an individual stays awake the higher SWA during the following sleep. SWA is at its maximum at the beginning of the sleep period and dissipates towards the end of the sleep period (Borbely, 1982). Sleep is also regulated by a 24h periodical rhythm, relatively independent of sleep pressure. This regulatory mechanism is called circadian process (Borbely, 1982). REM sleep, for example, shows a circadian regulation and is predominantly present in the early morning mostly independent of prior time spent asleep.

2. Sleep and cardio-metabolic regulation

The sleep-wake homeostasis and the circadian process are crucial for the cardio-metabolic regulation. *Figure II.8* shows mean profiles of blood glucose, insulin and cortisol secretion during 53h in healthy young men (Van Cauter et al., 1991). After an 8h nocturnal sleep period participants were kept awake during 28h followed by an 8h recovery daytime sleep. The increase in glucose and insulin, observed during nocturnal sleep, was also present during daytime sleep and to a lesser extent during nocturnal wakefulness, underlining the importance of sleep *per se* but also of circadian-dependent mechanisms. In contrast, cortisol is mainly driven by the circadian rhythm. The typical quiescent period of the cortisol profile, habitually observed during late evening/beginning of nocturnal sleep, was still present during nocturnal wake episode but not during daytime sleep.

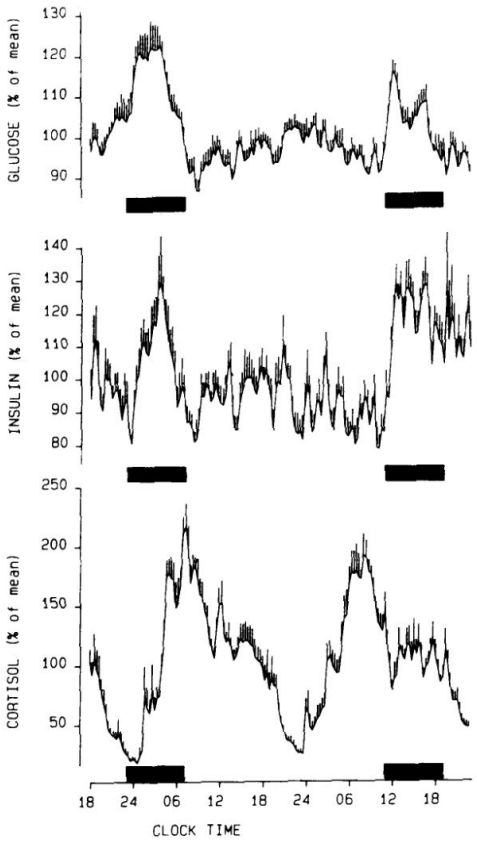

Figure II.8: Glucose, insulin, and cortisol mean profile of eight young men first sleeping 8h during the night (indicated by 1st black bar) followed by 28h wakefulness and 8h of daytime recovery sleep (2nd black bar). *Blood samples were assessed in 20 min intervals. A constant perfusion of glucose served as caloric intake. Adapted from Van Cauter et al. (1991).*

Moreover, SWS, which is predominant in the first half of the night, is associated with transient metabolic, hormonal, and neurophysiologic changes that affect glucose homeostasis including decreased cerebral glucose utilization, stimulation of growth hormone release and inhibition of the hypothalamic-pituitary-adrenal (HPA) axis. Measure of heart rate variability (HRV), a non-invasive and reliable tool to evaluate cardiovascular autonomic control, varies with the sleep stages. NREM sleep presents a predominance of the parasympathetic activity on the sympathetic tonus, while REM sleep is characterized by a sympathetic predominance (Jurysta et al., 2003; Spiegel et al., 2009). The decrease of the sympatho-vagal activity during NREM sleep results in lower blood pressure (BP) and HR. SWS plays therefore a major role in the cardio-metabolic protective function of sleep (Tobaldini et al., 2017).

2.1. Sleep disruption and T2D

A number of epidemiological studies associate sleep disturbances with the rise of type 2 diabetes (T2D) and CVD (Cappuccio & Miller, 2017). T2D, the commonest form of diabetes in the world, is characterized by two components: a) the inability of the body to use circulating glucose properly (glucose intolerance) and b) the development of an insulin resistance leading to chronic hyperglycemia. The most important risk factors for the development of T2D are excess body weight and physical inactivity. Recently, it has been observed that the risk of developing T2D in chronically

sleep disturbed individuals is comparable to that attributable to traditional cardio-metabolic risk factors (Anothaisintawee et al., 2016). Reducing sleep duration by four hours, decreases glucose tolerance and insulin sensitivity in young healthy men as first demonstrated experimentally by Spiegel et al. (1999). Sleep quality and sleep continuity are also important for glucose regulation; suppression of SWS (Herzog et al., 2013; Tasali et al., 2008) as well as the increase of CA (Stamatakis & Punjabi, 2010) lead to physiological changes, such as an increase of sympathetic activity and cortisol, that are deleterious for glucose and insulin regulation, and could on long-term predispose to T2D.

2.2. Sleep disruption and CVD

In large population studies, impaired sleep quantity and quality has been associated with increased risk of developing coronary heart diseases (CHD) (Cappuccio et al., 2011; Chandola et al., 2010), fatal and non-fatal strokes (Cappuccio et al., 2011; Leng et al., 2015), and all-cause mortality (Cappuccio et al., 2010). The incidence of hypertension, which already affects 30-40% of the adult population worldwide (Münzel, Sorensen, et al., 2017b), is even higher in short sleepers (≤ 6h per night), who have a 21% higher risk of developing this pathology (Meng et al., 2013). Under physiological conditions, the BP decrease during sleep is due to a supine body position, muscle relaxation and reduced sympathetic tone (Cappuccio & Miller, 2017). However, an increasing number of individuals do not show this nocturnal dip anymore, even if daytime BP is in the physiological range (Huang et al., 2018). The so-called non-dippers have a higher risk of developing CVD and mortality (Cappuccio & Miller, 2017; Ohkubo et al., 1997).

Figure II.9 illustrates the possible pathways through which sleep disturbances increase the risk of cardio-metabolic disorders. Sleep disturbances impair cortisol secretion which in turn impairs glucose homeostasis and increases the risk of diabetes and obesity (Leproult et al., 1997; Spiegel et al., 1999). Disturbed sleep is associated with higher sympathetic activity, disrupting the sympatho-vagal balance and HRV (Castro-Diehl et al., 2016), increasing blood pressure (BP) and impairing glucose homeostasis. Sleep deprivation leads also to inflammation and cytokines release (e.g., IL1, IL6, TNFα, CRP) (Mullington et al., 2009).

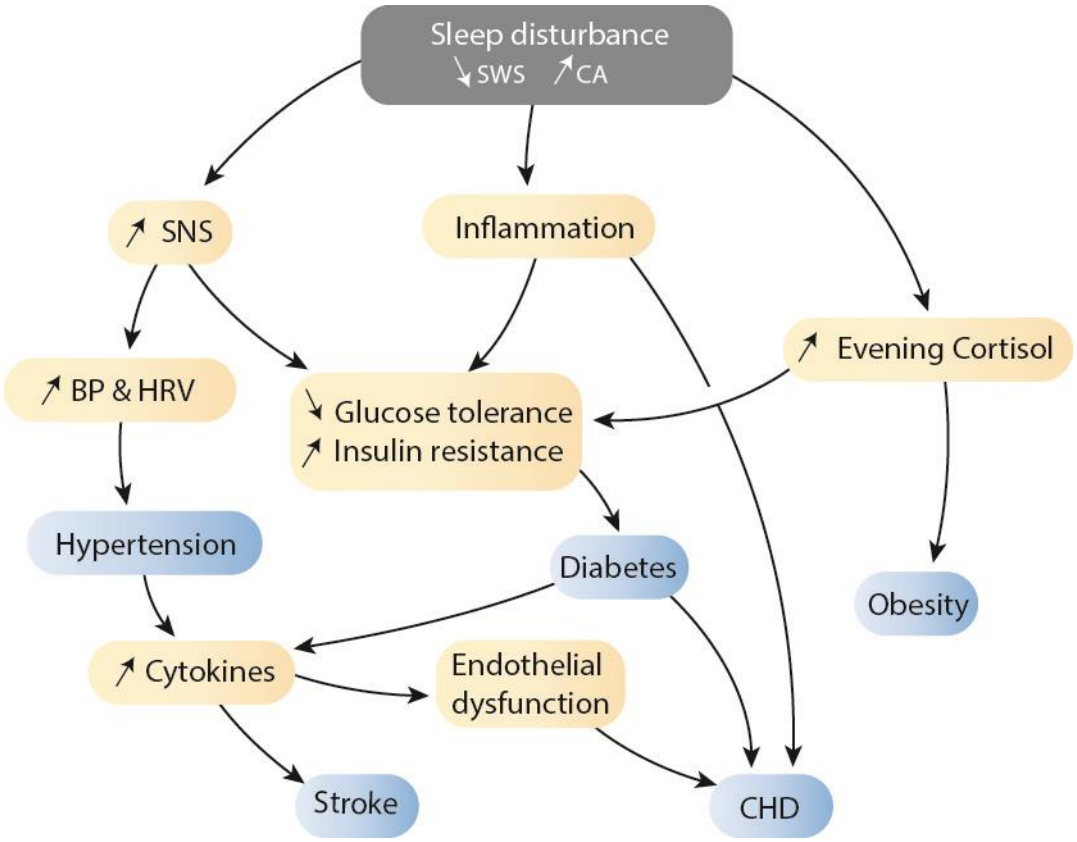

Figure II.9: Consequences of disturbed sleep on cardio-metabolic health. Adapted from Cappuccio and Miller (2017). *SWS: slow wave sleep; CA: cortical arousal; SNS: sympathetic nervous system; BP: blood pressure; HRV: heart rate variability; CHD: coronary heart disease.*

3. Noise processing during sleep

Sleep can be disrupted by endogenous or environmental stimuli, such as noise. Indeed, in contrast to anesthesia or coma, sensory stimuli are still processed during sleep, and sleep is rapidly reversible, allowing reacting to a potential danger. Noise is defined as an unwanted and unpleasant sound. Its perception during sleep involves the ascending reticular activating system (ARAS) in the midbrain, which integrates most of the wake-regulating stimuli (Saper et al., 2005). The auditory pathway takes root in the inner ear, at the organ of Corti. Through the cochlea-vestibular nerve the auditory information reaches the reticular formation of the brainstem where the ARAS originates (see *Figure II.10*). The ARAS is connected with the thalamus, the relay region to the cortex (Hurtley, 2009; Krone et al., 2017). Thus, external noise can provoke CA or awakenings from sleep and lead to sleep disruption as well as to a delayed sleep onset (Atienza et al., 2001). However, the thalamo-cortical gating may also prevent cortical activation. Whether an external input leads to cortical activation or whether sleep remains undisturbed at the cortical level depends on the sensory information and the current state of the central nervous system (Cote et al., 2000; Dang-Vu et al., 2010). The ARAS also connects to the autonomic nervous system (ANS) and the neuroendocrine system. Via the

hypothalamus, noise stimuli can directly activate the Sympathetic-Adrenal-Medullary (SAM) and the neuroendocrine Hypothalamic–Pituitary–Adrenal (HPA) axis, the two main stress pathways (detailed information in the section II.6.1.) (Westman & Walters, 1981). The reaction to noise is therefore fine-grained, ranging from an isolated AA to an associated CA or to awakening. Stronger cortical activations are associated with longer and more severe AA (Basner et al., 2007; Griefahn et al., 2008). In the following I will refer thus to a direct stress pathway where noise activates the autonomic system and an indirect stress pathway where cortical activation amplifies the autonomic response.

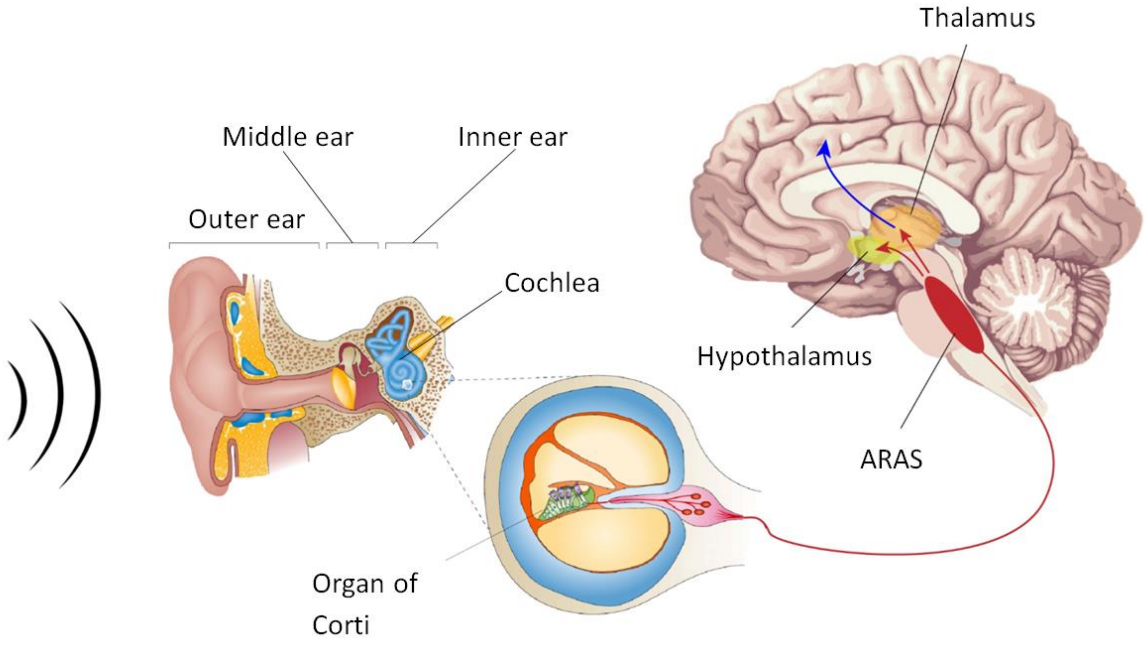

Figure II.10: Noise pathway in a sleeping individual. Adapted from Ng et al. (2013)*. Noise reaches the organ of Corti situated in the cochlea in the inner ear. The auditory information is relayed via the cochlea-vestibular nerve to the reticular formation of the brainstem where the ascending reticular activating system (ARAS) originates. From the ARAS, auditory information is sent to the hypothalamus and the thalamus, the relay region to the cortex.*

4. Noise assessment

Several acoustical exposure metrics are used to quantify noise effects for environmental noise regulation. The most commonly used measures are:

- The sound pressure level (SPL) represents the ratio of the sound pressure to a reference value, which depends on the medium of propagation (20 µPa for air).
- The equivalent continuous SPL (Leq) is an average of the total sound energy measured over a defined time period.
- The A-weighted Leq (LAeq) filter covers the range processed by the human ear (20 Hz-20 kHz).
- The maximum Leq (LAmax).

- The LA50, corresponding to the level exceed for 50% of the time. It represents the median of the fluctuating noise levels.
- The duration of events.
- The slope of rise of the SPL.

Most epidemiological studies model health effects of noise by using noise metrics based on average energetic doses over longer time periods. However, these metrics summarize complex time patterns of exposure into a single value and lead to information loss: noise scenarios, which differ in number, acoustical properties, and placement of noise events, may have the same average energetic dose, but differ substantially in their effects on sleep. Therefore, acoustical metrics of single events might be more relevant to describe noise effects during sleep. Wunderli et al. (2015) proposed the IR, an integral measure of the energy contribution of distinct noise events on the total sound exposure. At the same LAeq, two noise situations can have a low IR in the case of continuous sound exposure and small differences between LAmax and LAeq such as a highway or in the case of railway line with single events present a high IR (*Figure II.11*). This metric has been recently associated with cardiovascular mortality (Héritier et al., 2017), and highly intermittent nocturnal noise seems to increase arterial stiffness (Foraster et al., 2017).

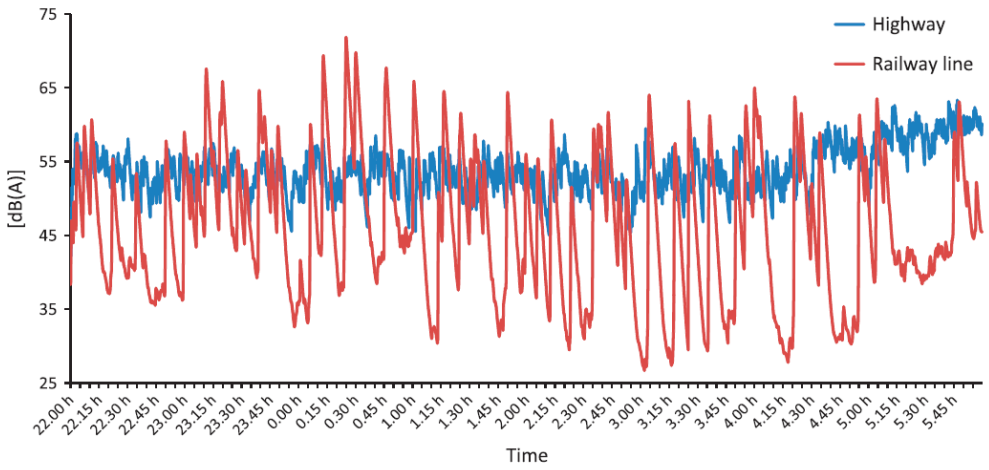

Figure II.11: Illustration of intermittency ratio with the time course of the sound pressure level produced by road traffic on a highway, at 7.5m distance, and along a railway line with predominantly freight traffic events, at 250m distance, for a time period of 8h*. From Wunderli et al. (2015).*

5. Effects of nocturnal transportation noise on sleep structure

Nocturnal road, rail and air traffic noise has been shown to negatively impact sleep macrostructure but results are not straightforward as summarized in *Table II.1*. Sleep efficiency (SE) was reported to decrease in Griefahn et al. (2006) and Saremi et al. (2008), while it remained unchanged in Basner et al. (2011) and Smith et al. (2017). Wakefulness after sleep onset (WASO) increased in Griefahn et al.

(2006) but did not change for others (see *Table II.1*). Moreover, the recent review from Basner and McGuire (2018) reports only moderate evidences of transportation noise induced cortical awakenings. Time spent in SWS and REM decreased linearly with increasing LAeq (Griefahn et al., 2006). Reduced SWS was also observed in another laboratory study with road and railway noise exposure at LAeq =40dB (Basner et al., 2011). REM duration and REM latency were affected by railway (LAeq =50dB) (Griefahn et al., 2006) and road traffic (LAeq ≥ 44dB) exposure (Griefahn, 1986). However, these results could not always be replicated. Saremi et al. (2008) with an LAeq between 40 and 50 dB and Smith et al. (2017) with an LAeq between 42 and 46 dB did not observe changes in SWS and REM duration during railway noise exposure.

At the level of the sleep microstructure, nocturnal transportation noise exposure lead to more sleep stage changes (Basner et al., 2011; Smith et al., 2017), CA (Basner et al., 2011; Saremi et al., 2008; Smith et al., 2017), and HR acceleration, i.e. AA (Basner et al., 2011; Griefahn et al., 2008; Smith et al., 2017). Several studies point out that CA, but not subcortical arousals, habituate to noise exposure over time (Basner et al., 2011; Griefahn et al., 2008; Hofman et al., 1995; Muzet, 2007).

Taken together, effects of nocturnal transportation noise on sleep macro- and microstructure are ambiguous. Nevertheless, sleep microstructure, and especially AA, seems more severely impacted than sleep macrostructure and should be a critical aspect to investigate in future studies (Basner et al., 2011; Saremi et al., 2008).

Study	Nb of participants (Female)	Age range or mean	Traffic noise source	LAeq (LAmax)	Nb of events	Sleep macrostructure				Sleep microstructure		
						SE	WASO	REM	SWS	SSC	CA	AA
Griefahn, 1986	36 (18)	21-30	road	37-64		NA	NA	↘	NA	NA	NA	NA
Griefahn, 2006	24 (12)	19-28	road+rail+air	39-50 (45-77)	172/195/261	↘	↗	↘	↘	NA	NA	NA
Griefahn, 2008	24 (12)	19-28	road+rail+air	39-50 (45-77)	162/195/261	NA	NA	NA	NA	NA	NA	↗
Saremi, 2008	young: 20 (16) middle-aged: 18 (15)	25.8 ± 2.6 52.2 ± 2.5	rail	40/50 (51-66)	48	↘	=	=	=	=	↗	NA
Basner, 2011	72 (40)	18-71	road+rail+air	40 (45-65)	40/80/120	=	=	=	↘	↗	↗	↗
Smith, 2017	23 (13)	19-30	rail	42-46 (47-50)	36/52	=	=	=	=	↗	↗	↗

***Table II.1**: Studies investigating the effect of nocturnal transportation noise exposure on sleep macro- and microstructure.*

6. Effects of nocturnal transportation noise on cardio-metabolic markers

6.1. The stress response pathway

It is already well documented that long-term exposure to transportation noise leads to cardio-metabolic disorders. The primary pathway is assumed to be a physiological activation of a stress response, which in a chronic state can become harmful for the organism (Babisch, 2002). The two main stress pathways are the SAM and the HPA axes (see *Figure II.12*). In response to a stressor, the

sympathetic nervous system (SNS) is immediately activated in the hypothalamus and stimulates the release from the adrenal medulla of the catecholamines epinephrine and norepinephrine. These hormones stimulate glycogenolysis (breakdown of glycogen to glucose), increase BP, breathing and metabolic rate to increase cellular oxygenation. The SNS has also cardio-stimulatory effects (increased HR, decreased HRV and increased arrhythmia) and immunological effects, such as stimulation of Il-6 and TNFα release (DeRijk et al., 1994; van Gool et al., 1990). The endocrine HPA axis is activated in a second step and sustains the stress response. The paraventricular nucleus (PVN) neurons from the hypothalamus releases corticotropin-releasing hormone (CRH) activating the adrenocorticotrophic hormone (ACTH) release from the pituitary gland which in turn stimulates the secretion of glucocorticoids from the adrenal cortex. Cortisol, the main glucocorticoid in humans, induces gluconeogenesis (the production of glucose from non-carbohydrate carbon substrates such as proteins or lipids), and increases BP and central adiposity. Cortisol also affects inflammatory processes.

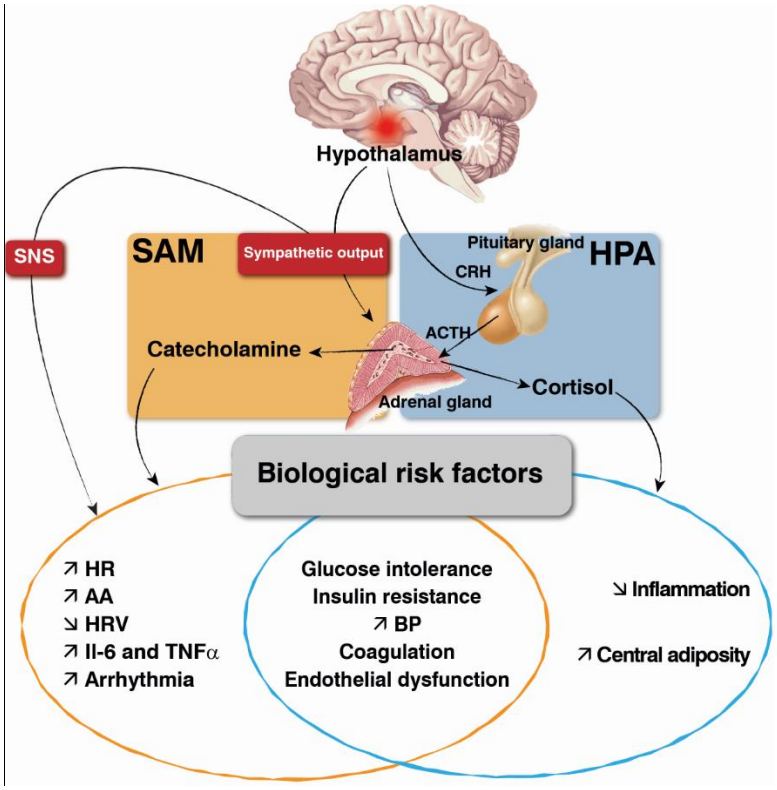

***Figure II.12*: Stress response pathway.** *SAM: Sympathetic-Adrenal-Medullary, HPA: Hypothalamic–Pituitary–Adrenal, SNS: sympathetic nervous system, CRH: corticotropin-releasing hormone, ACTH: adrenocorticotrophic hormone, HR: heart rate, AA: autonomic arousals, HRV: heart rate variability, BP: blood pressure.*

Night-time has been reported to be a critical time window for cardio-metabolic noise-induced impairments (Héritier et al., 2018; Jarup et al., 2008). The pathophysiological pathway is considered to be the same as during wakefulness with the activation of a stress response in the sleeping

individual. Studies investigating the possible link between nocturnal transportation noise exposure and one or several of these stress outcomes are listed in the following section.

6.2. Effects of nocturnal transportation noise on stress indicators

Stress hormones act early in the cause-effect chain of noise-induced physiological disorders and can be measured easily. Therefore, they have been widely analyzed in studies investigating the effect of noise on CVD (Babisch, 2003). However, the number of studies focusing on the effect of nocturnal exposure on these hormones is sparse and results are diverging.

Catecholamines are acutely released in response to a stressor and have a short half-life. They should ideally be assessed during the night in temporal proximity to actual noise exposure. Laboratory studies which investigated nocturnal urinary catecholamine levels show ambiguous results. Maschke et al. (1993) found an increase in nocturnal epinephrine levels during aircraft noise exposure in eight adults living near airports, a result which could not be replicated by Carter et al. (1994) in cardiac arrhythmia patients exposed to aircraft or road noise. Furthermore Maaß and Basner (2006) did not find any changes in catecholamine levels of healthy individuals exposed to aircraft noise with similar noise characteristics (see *Table II.2*). The authors highlighted the possible lack of sensitivity of the total overnight urinary value, which might be not precise enough to detect acute subtle catecholamine changes. On the other side, single blood samples during the night could be a supplementary stressor impacting the interpretation of the results and is therefore not an adequate solution.

Cortisol has a longer half-life compared to catecholamines (Babisch, 2003) and can therefore be assessed during the following day. Cortisol undergoes a circadian baseline secretion profile with a peak level in the morning, followed by a decline over the day to reach minimum levels in the late evening (Horrocks et al., 1990). Cortisol awakening response (CAR) is considered as a reliable indicator of the reactivity of the HPA axis (Schmidt-Reinwald et al., 1999) and has been studied in the context of nocturnal transportation noise exposure. In laboratory conditions, two studies, which investigated the effect of nocturnal transportation noise on next day CAR in young healthy volunteers, did not observe significant effects (Griefahn & Robens, 2010; Waye et al., 2003). Up to now, the largest field study which investigated the effect of aircraft noise exposure on morning saliva cortisol levels, is the HYENA study, including 439 older participants living near airports and found an increase of morning cortisol only for the women (Selander, Bluhm, et al., 2009). Lefèvre et al. (2017) could not replicate this result but found a decrease in cortisol variation over the day and increased evening cortisol levels for people living with higher nocturnal aircraft noise exposure.

Study	Nb of participants (female)	Age range or mean	Traffic noise source	LAeq (LAmax)	Nb of events	Morning cortisol	Evening cortisol	Nocturnal epinephrine	Nocturnal norepinephrine
Experimental studies									
Maschke, 1993	8	18-40	air	36-56 (>55)	16/32/64	NA	NA	↗	=
Carter, 1994	9	61	air or trucks	32 (65-72)	50	NA	NA	=	=
Maaß, 2004	128 (75)	18-65	air	30-54 (50-80)	4-128	NA	NA	=	=
Waye, 2003	12	25	road	35 (50)	75+ 2-3 lorry/h	=	NA	NA	NA
Griefahn, 2010	12 (6)	18-26	rail	44-58 (65-74)	20/40/80	=	NA	NA	NA
Griefahn, 2010	46 (24)	19-30	road+rail	42-56	rail=20/400/57 road=1300/4300/8600	=	NA	NA	NA
Field studies									
Selander, 2009	439 (230)	45-70	air			↗*	NA	NA	NA
Lefèvre, 2017	954 (554)	>18	air			=	↗	NA	NA

Table II.2: Studies investigating the effect of nocturnal transportation noise exposure on daytime cortisol level or nocturnal catecholamines*. CAR: cortisol awakening response. * in women only.*

6.3. Effects of nocturnal transportation noise on biological risk factors

Beside stress indicators, it is of interest to investigate the effect of nocturnal transportation noise on biological risk factors for cardio-metabolic diseases.

Sympatho-vagal balance: Nocturnal transportation noise exposure has been shown to increase the sympatho-vagal balance with decreased parasympathetic activity in the second part of the night after six day road and railway noise exposure (Graham et al., 2009). As described before in the previous section II.5, nocturnal transportation noise induces AA in humans during sleep without inducing awakenings.

Heart rate: In most of the studies, nocturnal transportation noise exposure did not impact mean HR (Basner et al., 2011; Haralabidis et al., 2008; Schmidt et al., 2013) except for Hofman et al. (1995) who observed an increase in HR when exposed to LAeq=47 dB transportation noise compared to the quiet situation (LAeq=38 dB).

Blood pressure: The effect of nocturnal transportation noise on BP has been assessed in field studies only and results are mixed. In healthy subjects, Schmidt et al. (2013) did not observe significant changes in BP while Haralabidis et al. (2008) reported a minor increase in systolic BP (+0.81 mmHg per 5dB increase in LAmax,indoor road noise).

Inflammatory markers: Inflammatory markers in humans have been assessed once so far. Schmidt et al. (2013) did not observe any changes in morning CRP and Il-6 after exposure to nocturnal transportation noise.

Glucose tolerance and insulin sensitivity in association to nocturnal transportation noise exposure has never been assessed in humans so far.

Most of these studies did not assess sleep with PSG and were therefore not able to associate potential changes with sleep disturbances. Only studies investigating the autonomic nervous activity, and more precisely cardiac activity, recorded EEG in parallel; they observed that cardiac activations

increased without substantial changes in sleep macrostructure or awakenings (Basner et al., 2011; Griefahn et al., 2008; Smith et al., 2017). These AA do not seem to habituate across nights (Basner et al., 2011; Griefahn et al., 2008; Smith et al., 2017). AA are of particular interest, given that repeatedly induced AA have been proposed as a risk factor for developing CVD (Griefahn et al., 2008). Consequently, it is still unclear how sleep disturbances potentiate the effect of transportation noise exposure on cardio-metabolic health.

7. Possible individual moderators

Finally, the effect of nocturnal transportation noise exposure on sleep and cardio-metabolic health may differ between individuals and some groups of individuals may be more vulnerable to nocturnal transportation noise exposure than others.

7.1. Age

Most epidemiological studies which showed deleterious effects of transportation noise on cardio-metabolic health investigated large age ranges including young, middle-aged, and older populations. However, age could be an important covariate as it is well known that both the cardio-metabolic system and sleep change with age. Moreover, it has been shown that some associations between road traffic noise and health outcomes, such as stroke for instance, are age-dependent (Sorensen et al., 2012). Aging is the largest risk factor for cardio-metabolic disorders; with aging, the contractility of the left ventricle, the ejection fraction as well as the sympathetic modulation of HR and beta-adrenergic receptor activation decrease leading to decreased HRV (Antelmi et al., 2004). Aging also increases arterial thickening and stiffness leading to increased systolic BP (North & Sinclair, 2012). Moreover, glucose regulation is impaired with age, increasing the risk of developing T2D (Brewer et al., 2016). With advancing age, sleep duration, and the amount of SWS decrease while sleep latency increases (Mander et al., 2017); older individuals also report to be more noise sensitive than younger individuals (Matsumura & Rylander, 1991; Schreckenberg et al., 2010).

7.2. Sex

Sex could also influence the physiological response to nocturnal transportation noise exposure as the incidence of CVD is also sex-dependent. Before menopause, women are relatively protected by their endogenous sex hormones; women have higher insulin sensitivity and lipid storage (Varlamov et al., 2014) and men have a preponderance of the sympathetic over vagal control of cardiac function compared to women (Salerni et al., 2015). Some epidemiological studies noticed sex differences in the response to transportation noise exposure with increased cortisol levels (Selander, Bluhm, et al., 2009) and higher risk of developing T2D only in women (Eriksson et al., 2014) and higher risk of

hypertension (Eriksson et al., 2010), and myocardial infarction (Babisch et al., 2005) for men. However, sex differences in the reactivity to nocturnal transportation noise have not been found consistently (Evrard et al., 2017; Griefahn et al., 2008; Jarup et al., 2008; Lefèvre et al., 2017; Selander, Nilsson, et al., 2009).

7.3. Genetic predisposition

It may be of interest to identify possible genetic markers of nocturnal transportation noise sensitivity. Specific polymorphism in so-called clock genes are implicated in human sleep-wake regulation (Franken & Dijk, 2009). The coding region of the clock gene PERIOD3 (PER3) contains a variable number tandem repeat polymorphism (VNTR) where a motif encoding 18 amino acids is repeated either four ($PER3^{4}$) or five times ($PER3^{5}$) (Ebisawa et al., 2001). The $PER3^{5/5}$ carriers have been reported to be more vulnerable and less resilient to the detrimental effects of sleep loss than $PER3^{4/4}$ individuals (Groeger et al., 2008; Viola et al., 2007). This polymorphism could also influence parameters of the ANS such as BP, HR and HRV as it has been shown that $PER3^{5/5}$ carriers have higher sympathetic and lower parasympathetic tone compared to $PER3^{4/4}$ (Viola et al., 2008).

Chapter III

MAIN RESEARCH QUESTIONS, HYPOTHESES & DESIGN

The main goal of this thesis was to determine whether short-term nocturnal transportation noise, at a LAeq=45dB, activates a stress response and impairs glucose regulation in healthy sleeping adults. We hypothesized that noise may disrupt sleep and lead to cortical activation (CA or awakening), which in turn activates subcortical areas to initiate a stress response (indirect pathway illustrated in *Figure III.1).* As an alternative hypothesis, noise may also directly activate subcortical areas without cortical activation (direct pathway). Moreover, we were interested in determining if the source and intermittency of the noise as well as individual moderators such as sex, age or genetic predisposition may influence the observed noise effects.

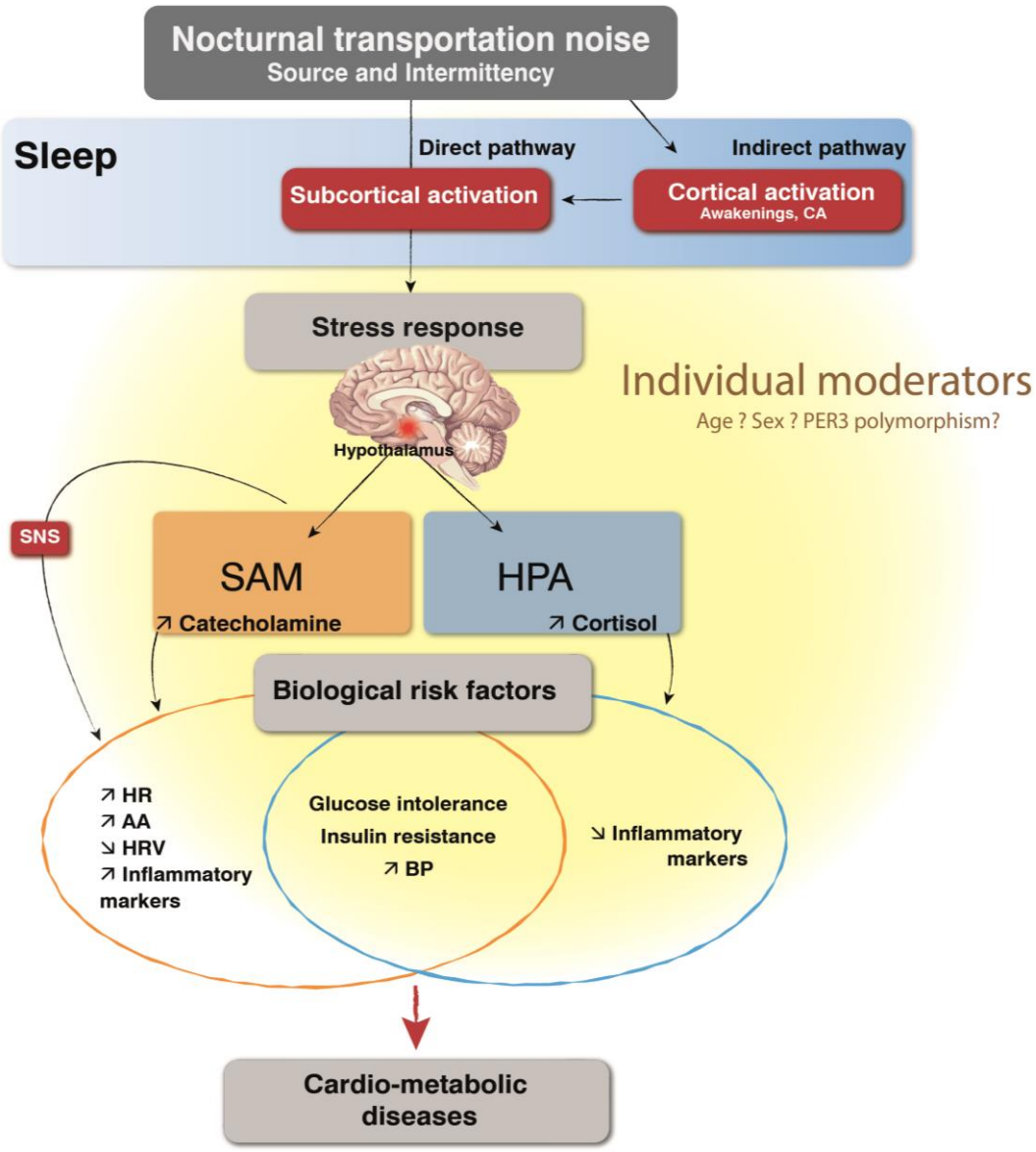

Figure III.1: Possible mechanistic pathways linking nocturnal transportation noise exposure in sleeping individuals and cardio-metabolic diseases*. CA: cortical arousal, SAM: Sympathetic-Adrenal-Medullary, HPA: Hypothalamic–Pituitary–Adrenal, SNS: sympathetic nervous system, HR: heart rate, AA: autonomic arousals, HRV: heart rate variability, BP: blood pressure.*

To answer these questions, we asked 26 (12 women) young (19-33 years) and 16 (8 women) older (52-70 years) volunteers to participate in a five 24h-days, including the following morning, laboratory

study. Half of the younger volunteers were PER3$^{4/4}$ carriers and the other half were PER3$^{5/5}$ carriers. Older participants were not stratified according to their PER3 polymorphism, but the information has been collected. *Figure III.2* illustrates the study protocol with the scheduling of the main outcome variables of this thesis. The experiment started and ended with a baseline (BL) and a recovery (RC) night during which individuals were exposed to an ambient noise scenario (LAeq=30dB); during NN2-NN5, four different transportation noise scenarios, with LAeq=45dB measured at participants ear, were presented in a randomized order to the volunteers starting at lights-OFF and ending with lights-ON during habitual bedtime (noise characteristics are summarized in *Table III.1*). Young volunteers were balanced according to sex and to PER3 polymorphism, while the older group was only balanced according to sex. Subjective sleep quality was assessed each morning with the LEEDS questionnaire. Objective sleep was continuously recorded by PSG during the night. Cognitive performance was tested throughout the day (at 2h30, 6h30, 10h and 13h after scheduled wake-up time) using different tests (memory, working memory (N-back), and sustained attention (PVT)). Subjective sleepiness (KSS), well-being (VAS), positive and negative mood (PANAS), hunger and appetite as well as saliva samples for cortisol and melatonin profile were assessed every 30 min during the first 3h after wake-up, 4h30 before lights-OFF and every 2h in-between. Noise annoyance of the different nights was retrospectively evaluated during the last morning of the study. Results concerning cognitive performance as well as well-being, mood, hunger and appetite and melatonin profile will not be reported in this thesis. Specific methods for each main research question are detailed below.

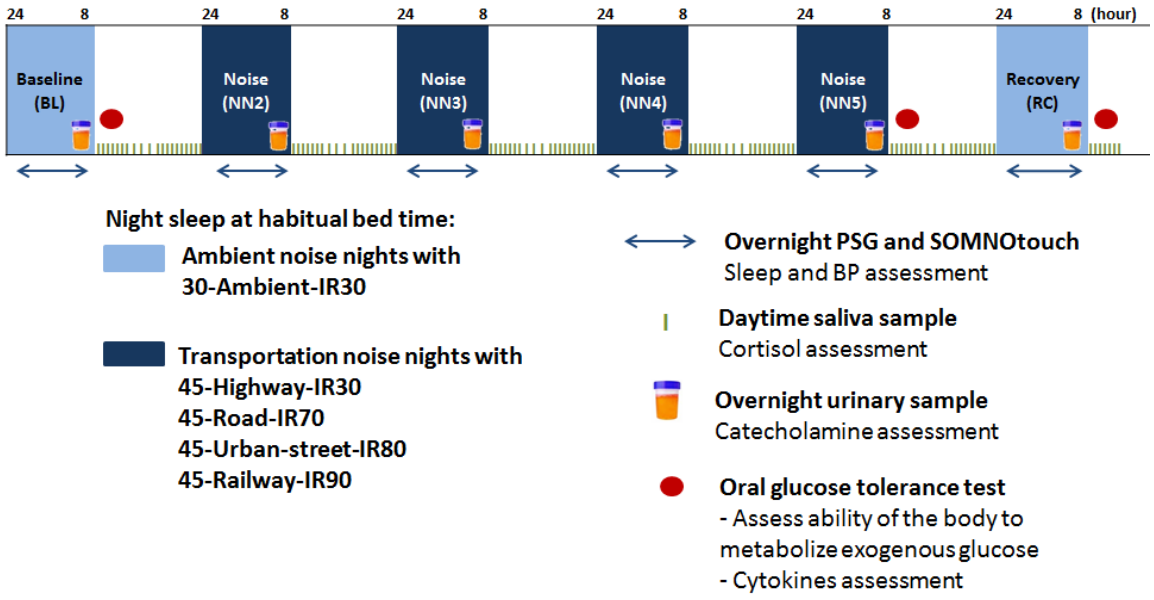

Figure III.2: Illustration of the laboratory study protocol. *PSG: polysomnography, BP: blood pressure, noise scenario: «LAeq,1h-Noise source-IR (intermittency ratio)».*

Scenario	Chapter IV terminology	Source	Type of noise	Posted speed limit (km/h)	Distance (m)	Pass-bys (n/h)	Laeq (dB)	IR	Noise eventfulness
30-Ambient-IR30	0	ambient	Noise-free				30	0.3	
45-Highway-IR30	A	road	4-lane highway	120	400	1000	45	0.3	Less eventful
45-Road-IR70	B	road	2-lane country road	80	50	250	45	0.7	Less eventful
45-Urban-street-IR80	C	road	1-lane urban road	50	15	100	45	0.8	More eventful
45-Railway-IR90	D	rail	freight and regional trains			10	45	0.9	More eventful

Table III.1: Noise characteristics. *IR: intermittency ratio.*

The present thesis aimed at answering the following three main research questions:

1. **Does short-term exposure to different nocturnal transportation noise scenarios impair glucose regulation, and is this related to concomitant sleep changes? (Chapter IV)**

Based on the above mentioned literature, we hypothesized that four nights of nocturnal transportation noise exposure deteriorates sleep macro- and/or microstructure, in particular the amount SWS and CA, impairing glucose tolerance and insulin sensitivity. We also hypothesized a return to baseline levels after one noise-free recovery night. The exposure to higher IR noise scenarios on NN5 was expected to elicit stronger deterioration than lower IR noise scenarios.

To investigate this question, we implemented a two hour 75gr oral glucose tolerance test (OGTT) one hour after awakening from BL, NN5 and RC (see *Figure III.2)*. This test comprised eight blood samples scheduled at precise time intervals (*Figure III.3*). The test started with two blood samples taken in a fasting state; participants were then instructed to drink a bottle of 75gr diluted glucose within five min and blood was collected first each ten min during half an hour and then each 30 min during one and a half hour. Using this test, it was possible to assess fasting glucose and insulin as well as glucose tolerance and insulin sensitivity.

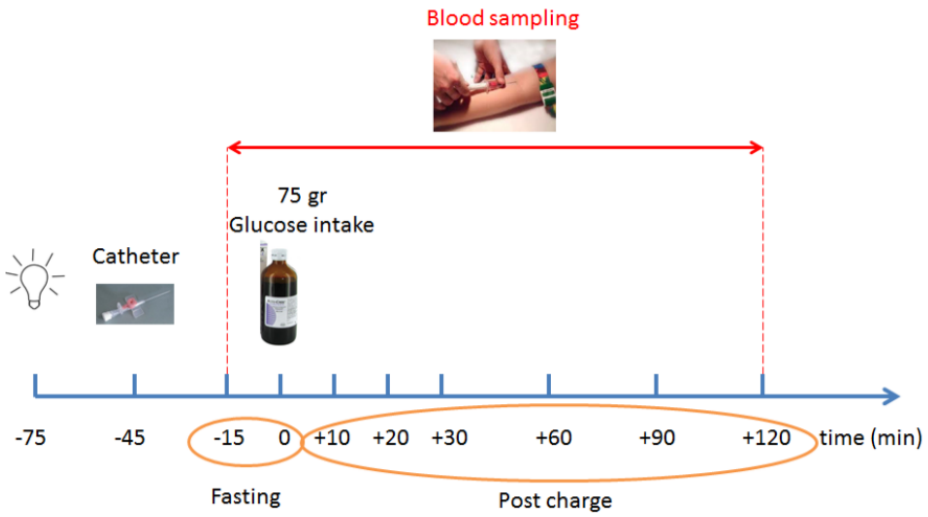

Figure III.3: Protocol of the oral glucose tolerance test (OGTT). *The OGTT started one hour after waking-up. A catheter was inserted 30 min before the start of the OGTT. The test started with two fasting blood samples (t-15 and t0), at t0 the participants was instructed to drink a bottle of 75gr diluted glucose within five min and blood was collected first each 10 min during 30min (t10,t20, t30) and then each 30 min during 1h30 (t60, t90, t120).*

2. Does short-term exposure to different nocturnal transportation noise scenarios impair the stress axes, and is this related to concomitant sleep changes? (Chapter V)

We hypothesized that nocturnal transportation noise exposure deteriorates sleep, in particular increases CA (indirect pathway) and/or AA (direct pathway), which in turn, impact on stress markers. We expected to observe impaired hemodynamic variables (HR and BP), increased sympathetic or decreased parasympathetic nervous activity and increased nocturnal catecholamine levels. Additionally, daytime cortisol levels and inflammatory markers were expected to increase after nocturnal noise exposure compared to noise-free nights. Finally, we hypothesized that higher IR would have a stronger impact on the above mentioned outcomes than scenarios with lower IRs.

In order to answer this question, we collected nocturnal urine to assess catecholamine levels, daytime saliva to assess cortisol profile and we used the fasting blood samples of the OGTT to measure inflammatory markers. We also assessed nocturnal BP using a cuffless continuous BP monitor (SOMNOtouch^{TM} NIBP), and HR and AA were obtained from the ECG recording (*see Figure 14*). HRV was analyzed with the time-domain analysis and the spectral analysis of the signal.

3. Do age, sex and a PER3 polymorphism influence the impact of nocturnal transportation noise exposure on cardio-metabolic outcomes? (Chapter VI)

We finally investigated if age, sex and a PER3 polymorphism could modulate the response of nocturnal transportation noise on the observed significant outcomes reported in the previous two chapters (Chapter IV and V; i.e. increased AA duration, increased evening cortisol level and increased glucose and insulin response to the OGTT). Furthermore, we were also interested to determine if the potential increase in AA duration or evening cortisol level could be a mediator of the observed impairment of glucose tolerance and insulin sensitivity.

Based on the cited literature in section II.7, we predicted stronger effects of nocturnal transportation noise exposure in the older subgroup. We also expected stronger noise-related effects on glucose and cortisol regulation in women compared to men. PER3$^{5/5}$ carriers were expected to be more sensitive to noise induced sleep and cardio-metabolic deterioration. Moreover, based on the results of the two previous chapters, we hypothesized that high evening cortisol level or increased autonomic activation are both related to next-morning impaired glucose regulation.

The next three chapters consist of three research papers, to which I contributed as a first author, including the design of the experimental protocol, recruitment of volunteers, study conduction, data acquisition and processing, statistical analyses, and manuscript writing.

Chapter IV

Adverse impact of nocturnal transportation noise on glucose regulation in healthy young adults: effect of different noise scenarios

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Keywords: Traffic noise, intermittent noise, glucose tolerance, insulin sensitivity, sleep, type 2 diabetes

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Conflict of interest: The authors do not report any conflicts of interest in the present study.

ABSTRACT

Background: Epidemiological evidence indicates an association between transportation noise exposure and a higher risk of developing type 2 diabetes. Sleep disturbances are thought to be one of the mechanisms as it is well established that a few nights of short or poor sleep impair glucose tolerance and insulin sensitivity in healthy good sleepers.

Objectives: The present study aimed to determine the extent to which exposure to nocturnal transportation noise affects glucose metabolism, and whether it is related to noise-induced sleep alterations.

Methods: Twenty-one young healthy volunteers (nine women) participated in a six-day laboratory study starting with a noise-free baseline night, then four nights sleeping with randomly-presented transportation noise scenarios (three road and one railway noise scenario) with identical average sound level of 45dB but differing in eventfulness and ending with a noise-free recovery night. Sleep was measured by polysomnography. Glucose tolerance and insulin sensitivity were measured after the baseline, the last noise night and the recovery nights with an oral glucose tolerance test using Matsuda and Stumvoll insulin sensitivity indexes. Eleven participants were assigned a less eventful noise scenario during the last noise night (LE-group), while the other ten had a more eventful noise scenario (ME-group). Baseline metabolic and sleep variables between the two intervention groups were compared using a non-parametric Mann-Whitney U-test while mixed models were used for repeated measure analysis.

Results: All participants had increased $glucose_{AUC}$ (Mean ± SE, 14 ± 2%, p<0.0001) and $insulin_{AUC}$ (55 ± 10%, p<0.0001) after the last noise night compared to the baseline night. 2h-glucose level tended to increase only in the ME-group between baseline (5.1 ± 0.22 $mmol.L^{-1}$) and the last noise night (6.1 ± 0.39 $mmol.L^{-1}$, condition: p=0.001, interaction: p=0.08). Insulin sensitivity assessed with Matsuda and Stumvoll indexes respectively decreased by 7 ± 8% (p=0.001) and 9 ± 2% (p<0.0001) after four nights with transportation noise. Only participants in the LE-group showed beneficial effects of the noise-free recovery night on glucose regulation (relative change to baseline: $glucose_{AUC}$: 1 ± 2%, p=1.0 for LE-group and 18 ± 4%, p<0.0001 for ME-group; Stumvoll index: 3.2 ± 2.6%, p=1.0 for LE-group and 11 ± 2.5%, p=0.002 for ME-group). Sleep was mildly impaired with increased sleep latency of 8 ± 2 min (<0.0001) and more cortical arousals per hour of sleep (1.8 ± 0.6 arousal/h, p=0.01) during the last noise night compared to baseline. No significant associations between sleep measures and glucose tolerance and insulin sensitivity were found.

Conclusion: In line with epidemiological findings, sleeping four nights with transportation noise impaired glucose tolerance and insulin sensitivity. Based on the presented sound exposure, the eventfulness of the noise scenarios seems to play an important role for noise-induced alterations in glucose regulation. However, we could not confirm our hypothesis that transportation noise impairs glucose regulation via deterioration in sleep quality and quantity. Therefore, other factors, such as stress-related pathways, may need to be considered as potential triggers for noise-evoked glucose intolerance in future research.

1. INTRODUCTION

Exposure to transportation noise is a major public health issue ranking among the top environmental risk factors for health in Europe (Hänninen et al., 2014; Vienneau, Perez, et al., 2015). Long-term exposure to transportation noise has been associated with increased risk for cardiovascular diseases (Foraster et al., 2017; Héritier et al., 2017; Selander, Nilsson, et al., 2009; Van Kempen & Babisch, 2012; Vienneau, Schindler, et al., 2015) and type 2 diabetes (T2D) (Clark et al., 2017; Eze, Foraster, et al., 2017; Eze, Imboden, et al., 2017; Kempen et al., 2018; Sorensen et al., 2013). However, the underlying mechanism linking noise exposure and development of T2D remains unclear (Cui et al., 2016; Liu et al., 2016), and the dose-response is poorly understood with adverse effects observed below the WHO recommended threshold (Héritier et al., 2017; Hurtley, 2009).

Both epidemiological and field studies attributed a key role to sleep in the regulation of glucose homeostasis and incident T2D. Short sleep duration and poor sleep quality were found to impair glucose regulation (Anothaisintawee et al., 2016). Several experimental studies confirmed the importance of sleep duration on glucose regulation (Reutrakul & Van Cauter, 2014; Spiegel et al., 1999). Donga et al. (Donga et al., 2010), for example found that one night with a 4-h sleep restriction resulted in a marked decrease in insulin sensitivity and glucose tolerance. Sleep quality, and more precisely the amount of deep sleep and the severity of sleep fragmentation, also seems crucial for glucose regulation (Reutrakul & Van Cauter, 2014). Sleep fragmentation as a consequence of selective (Herzog et al., 2013; Tasali et al., 2008) and nonselective (Stamatakis & Punjabi, 2010) auditory slow wave sleep (SWS) suppression, without reducing total sleep duration, was found to initiate glucose intolerance and insulin resistance. The underlying mechanisms include increased brain energy metabolism (Maquet, 1995) and increased sympathetic activity during slow wave sleep (Brandenberger et al., 2001; Tasali et al., 2008). As several studies reported impaired sleep quality due to nocturnal transportation noise exposure (Basner & McGuire, 2018), we hypothesized that transportation noise impairs glucose regulation by its deleterious effects on sleep.

To date, environmental noise effects on health are typically evaluated using the average energetic dose over longer time periods expressed, for example, as the LAeq (i.e, A-weighted equivalent continuous sound pressure level) (Fritschi et al., 2011). However, such measures have limited explanatory power for predicting specific noise effects such as annoyance or sleep disturbances (Griefahn et al., 2006). Acoustical characteristics of noise events, such as the distribution of maximum sound pressure level and the slope of rise of the level, explain some physiological reactions including awakenings and increased heart rate better than the LAeq (Basner et al., 2011; Brink et al., 2008; Griefahn et al., 2006; Marks et al., 2008). Thus, Wunderli et al. proposed, the intermittency ratio (IR), an integral measure of the energy contribution of distinct noise events on the total sound exposure, which reflects the "eventfulness" of a noise situation. For example, passing trains yield a

higher IR than a highway, which produces rather continuous noise (Wunderli et al., 2015). A recent study from Héritier and colleagues indicated that a moderate IR at night (2^{nd}-4^{th} quintile) was more relevant than continuous noise (quintile 1) or highly variable noise (quintile 5) for increased risk of all cardiovascular and ischemic heart diseases (Héritier et al., 2017).

The goal of the present laboratory study was to determine if short-term (a few nights) nocturnal transportation noise exposure affects glucose regulation in healthy adults. Furthermore, we tested if the eventfulness of transportation noise is related to effects on glucose metabolism as well as sleep alterations, and whether the latter confers changes in glucose regulation. Additionally, we tested for laboratory stay effects on glucose regulation by applying the same protocol to a control group sleeping only under noise-free conditions.

2. MATERIAL AND METHODS

2.1. Study participants

Participants were recruited between July 2014 and August 2016 through advertisements on university websites, in newspapers and in public buildings in Switzerland, Germany and France. They were screened for medical, psychological and sleep disorders through questionnaires, a medical examination and a full polysomnography during an adaptation night in the laboratory. Exclusion criteria were > 15 periodic leg movements per hour and an apnea-hypopnea index > 10. Participants underwent blood tests to ensure that haematology and fasting glucose levels were within normal range, as well as a hearing test to guarantee normal hearing threshold according to age and gender. Participants habitually slept 7 to 9 hours per night and indicated good subjective sleep quality (Pittsburgh Sleep Quality Index, (Buysse et al., 1989) PSQI ≤ 5) and no daytime sleepiness (Epworth Sleepiness Scale, (Johns, 1991) ESS ≤ 10). To control for potential circadian phase misalignment, we excluded extreme chronotypes (Munich Chronotype Questionnaire, (Roenneberg et al., 2003) MCTQ < 2 or MCTQ ≥ 7), shift workers, and did not permit trans-meridian flights within the month preceding study participation. Noise sensitivity was assessed using the short version of the German *Lärmempfindlichkeitsfragebogen* (LEF-K) (Zimmer & Ellermeier, 1998) and the *Noise Sensitivity Questionnaire* (NoiSeQ) (Schutte et al., 2007). Although it was not part of the selection criteria, noise sensitivity did not differ between study volunteers and only 3 out of the 21 participants, and none of the control group, reported living in a rather noisy environment. All participants were non-smokers and medication-free (including drugs and hormonal contraceptives). Women were tested for pregnancy prior to study admission; none were excluded on this basis. For all but two women, the entire lab protocol was conducted between day 0 and 11 after menses onset, i.e. during the follicular phase. The two remaining women started the study during the late luteal phase and were

subsequently excluded from the analysis. One additional woman could not be included in this analysis because of difficulties with blood collection during the oral glucose tolerance test (OGTT). Thus, 21 participants (nine women) out of 286 (184 women), were included in the present analyses. A control group of six young men, matched in age and BMI to the intervention group, was also studied. All participants gave written informed consent. The study protocol, screening questionnaires and consent forms were approved by the local ethics committee (EKNZ/Ethikkommission Nordwest- und Zentralschweiz, Switzerland), conformed to the Declaration of Helsinki and were performed in accordance with international ethical standards.

2.2. Methods

2.2.1. Pre-study condition

To maintain a regular sleep-wake rhythm, one week prior to study begin in the laboratory participants were asked to maintain their habitual bedtimes within ± 30 minutes, spend 8 hours in bed and not take naps. Compliance was assessed via actigraphy (Actiwatch L, Cambridge Neurotechnologies, Cambridge, UK). Participants were asked to avoid stimulating nutriments (coffee, tea, chocolate) and alcohol, to eat as usual without extreme fatty meals and to avoid extreme physical activities in order to match the laboratory conditions as best as possible.

2.2.2. Laboratory study conditions

Participants spent six consecutive days and nights (*Figure IV.1*) in the laboratory in individual windowless and sound proof bedrooms (12.5m^2, http://www.chronobiology.ch/wp-content/uploads/2013/05/room_single_5_web.jpg). The reverberation time of the bedrooms was 0.6 s and the background noise level was below 20 dB(A). Light intensity during the day was kept constant at 150 lux and room temperature was set at 22°C. Participants had an 8-h sleep opportunity per night scheduled at their habitual bedtimes.

2.2.3. Noise exposure characteristics

The sound stimuli were created by sampling portions of real-world field sound recordings. Thereby, outdoor sound recordings of single vehicle pass-by events were mixed and played back on a loudspeaker installed in the bedrooms. The spectral effect of sound transmission through a tilted window was simulated using a digital filter. *Figure IV.1* illustrates the study protocol. All participants started and ended the study week respectively with a noise-free baseline (BL) and recovery (RC) night during which a very low volume ambient sound scenario (Scenario 0) was applied to reproduce a tilted window situation (*Figure IV.1*). It consisted of cricket chirps and distant traffic, with a LAeq of

30 dB at the ear of the sleeper. In between, participants were exposed from lights OFF to lights ON to four different noise scenarios (A, B, C and D), which were incompletely counterbalanced between noise nights NN2 to NN5: less eventful (LE) noise scenarios (A and B) alternated with more eventful (ME) noise scenarios (C and D). All noise scenarios had an identical hourly LAeq of 45 dB at the ear of the sleeper, which corresponds to an outdoor level of approximately 60 dB for a tilted window (Locher et al., 2018). Scenario A corresponded to a 4-lane highway, scenario B to a 2-lane country road, scenario C to a 1-lane urban road, and scenario D to a railway noise situation with five (four freight and one regional trains) different train pass-bys (see *Table IV.A* in appendix for more information). The noise scenarios differed with respect to median sound level (LA50) and IR, two noise characteristics that negatively correlate with each other and that describe the eventfulness of the noise scenario (*Table IV.1*). LA50 is the noise level exceeded during 50% of the time. The noise scenarios with a low difference between LAeq and LA50 (i.e. scenarios 0 and A) had a low IR because of the steady sound level. During the last noise night (NN5), eleven participants slept with a LE noise scenario (LE-group, *Figure IV.1a*), while ten slept with a ME noise scenario (ME-group, *Figure IV.1b*). The control group underwent exactly the same laboratory condition and was instructed the same way. While they thought they would be exposed to transportation noise, they actually slept all six nights with Scenario 0 (*Figure IV.1c*). During the last morning of the study, participants were asked to retrospectively evaluate noise annoyance of each night on a scale of 0 to 100 with the question "How annoyed were you during the respective night (BL-NN5-RC) by the noise"? Retrospective recall had the benefit to allow comparison between the noise scenarios and avoid directing participant's attention too much to the noise exposure during the study. Further details of the noise characteristics are described in Rudzik et al. (Rudzik et al., 2018).

Scenario	Source	LAeq [dB]	LA50 [dB]	LAeq-LA50 [dB]	IR	Noise eventfulness
0	ambient	30	29	1	0.3	
A	road	45	44	1	0.3	Less eventful
B	road	45	39	6	0.7	Less eventful
C	road	45	33	12	0.8	More eventful
D	rail	45	31	14	0.9	More eventful

Table IV.1: Acoustical characteristics of the noise scenarios*. "0" represents the noise scenario used during the noise-free baseline and recovery nights in the experimental group and during all 6 nights in the control group. A, B, C and D are the four different noise scenarios randomly introduced during the four noise nights in the noise group. LAeq: average level, LA50: median level, IR: Intermittency ratio (Wunderli et al., 2015).*

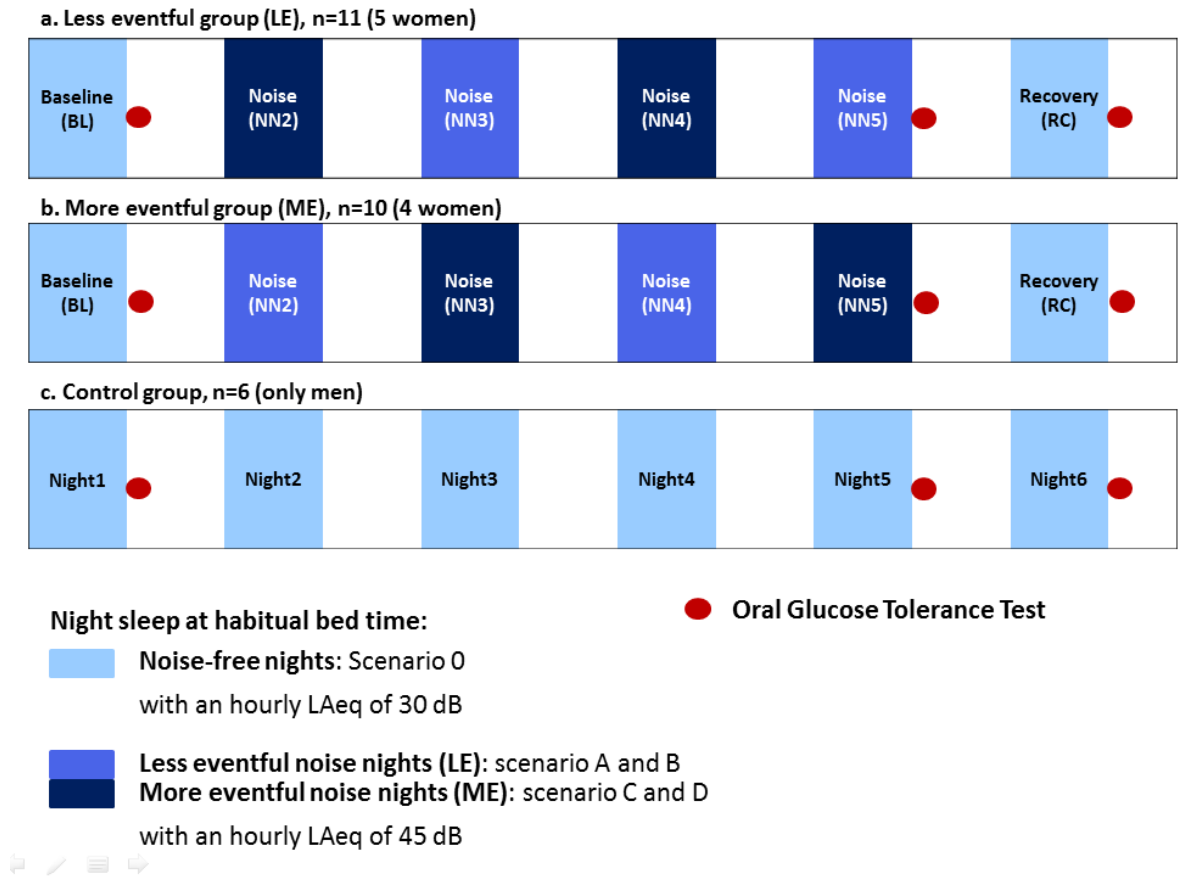

Figure IV.1: Schematic illustration of the study protocol. *Sleep episodes were scheduled according to habitual bedtimes. Glucose tolerance and insulin sensitivity were assessed via three oral glucose tolerance tests (OGTTs) (red circles). The two intervention groups (a., LE-group n=11 and b., ME-group n=10) started and ended the laboratory stay with the noise-free Scenario 0. LE-group slept with a less eventful noise scenario during the night preceding the second OGTT (NN5) whereas the ME-group slept with a more eventful noise scenario. The order of less (A or B) vs. more (C or D) eventful noise scenarios was balanced across the night NN2 to NN5. The control group (c.) slept each night with the noise-free Scenario 0.*

2.2.4. Control of diet and physical activity

Daily caloric intake during the laboratory was individually estimated using the Mifflin equation (resting energy expenditure = 9.99 x weight + 6.25 x height – 4.92 x age + 166 x sex – 161 x 1.3 for the low activity factor) (Frankenfield et al., 2003; Mifflin et al., 1990) and was kept constant. Each meal included 35% lipids, 50% carbohydrates and 15% protein. Meal timing was adjusted to the participant's wake up time and no snacks were allowed. Participants were encouraged to walk through the windowless corridor to ensure some light physical activity. In the morning of BL, NN5 and RC, weight was measured upon awakening after the participants used the toilet.

2.2.5. Glucose metabolism

Glucose metabolism was assessed via a two hour 75-g OGTT administered one hour after awakening following BL, NN5 and RC, 30 min after inserting the venous catheter (*Figure IV.1*). Two fasting (t-15 and t0) and six post-load (time points: t10, t20, t30, t60, t90 and t120) blood samples were collected.

Assays Blood was distributed in Na-fluorid tubes and immediately centrifuged for plasma glucose measurement. Serum insulin measurement was obtained from another tube after 30 min clotting at room temperature. Tubes were centrifuged at 4°C for 10 min at 3500rpm. All samples were then stored at -80°C until assay. Plasma glucose was assayed via the hexokinase method (Glucose GOD-PAP test, Roche) with a limit of sensitivity of 0.11 mmol.L^{-1} and an intra-assay variation coefficient of 0.9%. Serum insulin was measured with an ELISA test (80-INSHU-E01.1; ALPCO) with a limit of sensitivity of 2.78 pmol.L^{-1} and an intra-assay variation coefficient of 6%.

Measures Fasting glucose (G_0) and insulin (I_0) levels were calculated by averaging the values from blood samples at t-15 and t0. Fasting insulin resistance was assessed using the HOMA-IR ((G_0 x I_0)/22.5, (Matthews et al., 1985)). The area under the curve (AUC) for glucose and insulin was calculated using the trapezoidal rule. Glucose tolerance was assessed by calculating $glucose_{AUC}$ and via glucose concentration at t120 (G_{120}). Insulin sensitivity was estimated using the Matsuda index ($10{,}000/\sqrt{G_0 \text{ x } I_0 \text{ x mean G x mean I}}$ (Matsuda & DeFronzo, 1999)) and the Stumvoll ISI index (0.226 - 0.0032 x BMI - 0.0000645 x I_{120} - 0.00375 x G_{90}, where I_{120} and G_{90} represent insulin concentration at t120 and glucose concentration at t90 respectively (Stumvoll et al., 2000)). Beta-cell function was assessed by calculating the Stumvoll first-phase (1.283 + 1.829 x I_{30} - 138.7 x G_{30} + 3.772 x I_0) and second-phase insulin release (287 + 0.4164 x I_{30} - 26.07 x G_{30} + 0.9226 x I_0 ; where I_{30} and G_{30} represent insulin and glucose concentrations at t30 respectively (Stumvoll et al., 2000)).

2.2.6. Sleep measurement

Subjective sleep quality

Subjective sleep quality was assessed 5-10 min upon awakening with the Leeds Sleep Evaluation Questionnaire (Parrott & Hindmarch, 1978) with ten visual analog scale questions assessing four parameters of sleep quality: getting to sleep (more difficult - easier than usual; slower - more quickly than usual; less sleepy - more sleepy than usual), quality of sleep (more restless - calmer than usual; with more wakeful periods - with less wakeful periods than usual), awake following sleep (more difficult - easier than usual; requires a period of time longer - shorter than usual), and behavior following wakening (tired - alert).

Polysomnographic sleep recordings

Sleep and wakefulness were continuously recorded via polysomnography (PSG) including 12 electroencephalographic (EEG; F3, FZ, F4, C3, CZ, C4, P3, PZ, P4, O1, OZ and O2), two electro-oculographic, two electromyographic and two electrocardiographic derivations (Vitaport-3 digital recorder; TEMEC Instruments BV, Kerkrade, The Netherlands). Each 30-s epoch during scheduled sleep was scored according to the AASM standard criteria (Berry et al., 2016) by four experienced

scorers in our laboratory blind to the respective noise scenario (scorer agreement >85%). All nights of a single participant were scored by the same scorer. The following sleep variables were analyzed: total sleep time (TST, time spent asleep between lights OFF and lights ON), sleep efficiency (percentage of time spent in rapid eye movement (REM) and non REM sleep between lights OFF and lights ON), time spent in light NREM (NREM1+NREM2), in SWS and in REM sleep, sleep latency (time between lights OFF and NREM2 onset) and wake after sleep onset (WASO, time awake between sleep onset and the final morning awakening). EEG slow-wave activity (SWA, EEG power density between 0.75-4.5 Hz) was computed, after removal of artifacts by visual inspection, over frontal EEG derivations (F3, Fz, F4) in 4-s bins using fast Fourier transforms (Hamming window, frequency resolution of 0.25 Hz, overlap of 50%) collapsed in to 30-s epochs in order to match the time resolution of the sleep stage scoring. Arousals were scored as an abrupt shift of EEG frequency that lasted at least 3 seconds and with at least 10 seconds of stable sleep preceding the change (Berry et al., 2016). Sleep stage changes (SSC) to deeper stages were defined as the sum of the number of wake-NREM, NREM-REM and wake-REM stage changes per hour while SSC to lighter stages were defined as the sum of the number of NREM-wake, REM-NREM and REM-wake stage changes per hour.

2.3. Statistical analysis

For all analyses, the SAS statistical software package was used (SAS Institute Inc., Cary, NC; version 9.4). Comparisons between the intervention (LE-group and ME-group) versus the control group were only explorative because the number of controls was too small to allow for formal statistical testing. We compared baseline metabolic and sleep variables between the two noise groups (LE-group vs. ME-group) with a non-parametric Mann-Whitney U-test. Mixed model analysis of variance (PROC MIXED) were carried out for each variable (glucose, insulin and sleep variables) separately and included the fix factor “group” (LE-group vs. ME-group), the repeated within-subject factor “condition” (BL, NN5 and RC) and the random factor “subject” with a variance component covariance structure. Contrasts were assessed with the LSMEAN statement and p-values were based on Kenward-Roger’s corrected degrees of freedom (Kenward & Roger, 1997). In presence of an interaction between group and condition the statistical significance of each group was separately reported. Residual outliers were removed from analysis. Multiple post-hoc comparisons were corrected using the Tukey-Kramer method. Correlations were calculated with the Pearson correlation coefficient when data were normally distributed; otherwise the Spearman correlation coefficient was used. . Normality of the distribution was evaluated by using the Shapiro-Wilk W test ($p>0.05$ for all comparisons). Statistical significance was set at $p<0.05$; $p<0.10$ was reported as marginally significant.

3. RESULTS

Twenty-seven healthy participants were included in the analysis; during the last intervention night (NN5), 11 and 10 participants respectively were exposed to a less eventful (LE-group) and more eventful noise scenario (ME-group) (see *Figure IV.1*). The six remaining participants belonged to the control group. *Table IV.2* summarizes the demographic and metabolic variables at baseline for these three groups. No significant differences were observed for any of these variables between the two intervention groups. Sleep variables did not differ between the two intervention groups (TST (min), p=0.97; SE (%), p=0.97; light NREM (min), p=0.57; SWS (min), p=0.67; REM (min), p=0.83; arousal (n/h), p=0.11; SWA (μV^2/Hz), p=0.70, see *Table IV.3* for values). Study participants lost on average 493 ±20 g ($F_{2,40}$=3.89, p=0.03) during the laboratory stay without a significant difference between the LE and ME group.

	Control group	Less eventful (LE) group	More eventful (ME) group	U-test between LE and ME groups
	n=6	n=11	n=10	(p-value)
DEMOGRAPHICS				
Sex				
Women (n)	0	5	4	
Men (n)	6	6	6	
Age (years)	26.7 ± 1.3	24.7 ± 1.0	25.1 ± 1.2	0.89
Baseline BMI (kg/m^2)	21.7 ± 0.6	21.6 ± 0.70	23.4 ± 0.54	0.06
Noise sensitivity				
LEF-K	12.0 ±2.5	10.3 ± 1.4	11.5 ± 0.97	0.62
NoiSeQ Global	1.0 ± 0.29	1.2 ± 0.16	1.3 ± 0.11	0.65
NoiSeQ Sleep	0.88 ± 0.17	1.0 ± 0.19	1.2 ± 0.23	0.67
BASELINE METABOLIC VARIABLES				
Fasting glucose ($mmol.L^{-1}$)	5.0 (4.8-5.2)	4.9 (4.8 -5.3)	5.1 (4.9 -5.5)	0.18
Fasting insulin ($pmol.L^{-1}$)	34 (16-103)	29 (20-41)	34 (24-42)	0.60
HOMA-IR	1.1 (0.50-3.4)	0.97 (0.62-1.4)	1.1 (0.79-1.4)	0.65
$Glucose_{AUC}$ ($mmol.L^{-1}.min.10^1$)	79 ± 2.8	70 ± 3.8	71 ± 3.1	0.92
$Insulin_{AUC}$ ($pmol.L^{-1}.min.10^3$)	36 ± 7.3	33 ± 4.1	34 ± 4.0	0.97
Matsuda index	9.1 ± 2.9	9.2 ± 1.4	7.4 ± 0.64	0.64
Stumvoll ISI index ($\mu mol.kg^{-1}.min^{-1}.pM^{-1}$)	0.12 ± 0.003	0.12 ± 0.004	0.12 ± 0.004	0.39

***Table IV.2: Participants' characteristics for the control group, the less eventful (LE) and the more eventful (ME) groups**. Data are expressed as mean ± SE when normally distributed and as median (25^{th}-75^{th} percentile) when not normally distributed. P values were calculated between the LE-group and ME-group using the Mann-Whitney U-test.*

3.1. Sleep

Sleep characteristics are summarized in *Table IV.3*. Subjectively, the intervention groups, the ME-group in particular, had more difficulties getting to sleep and scored their quality of sleep worse after NN5 compared to BL (and RC for quality of sleep). For both groups combined, awake following sleep and behavior following wakening scores decreased after NN5 and RC compared to BL. All participants from the intervention group scored the noise scenario during NN5 as more annoying than during BL and RC. Objectively measured TST, sleep efficiency, WASO, the amount SWS, SWA and SSC to deeper and lighter stages did not significantly differ between the noise conditions. However, sleep latency significantly increased by 8 ± 2 min during NN5 and by 11 ± 2 min during RC compared to BL. Participants spent 15 ± 5 min more in REM sleep during the RC compared to BL night. This increase in REM sleep came at the cost of a decrease of time spent in light NREM sleep (18 ± 7 min between BL and RC). The number of arousals per hour TST, as well as per hour REM and NREM sleep increased throughout the study. On average, participants had 1.8 ± 0.6 more arousals per hour TST during NN5 compared to BL. Compared to the intervention groups, the control group showed rather an improvement of all the subjective sleep quality parameters during NN5 compared to BL and RC. Noise annoyance and the objectively measured sleep parameters (TST, sleep efficiency, WASO, amount SWS, SWA, SSC and number of arousals) did not differ throughout the week. However, the increase in sleep latency and REM sleep throughout the week was also present in the control group.

3.2. Glucose metabolism

Glucose values are presented in *Table IV.4*.

3.2.1. Fasting state

For both the LE and ME groups combined, G_0 decreased from BL to NN5 and RC (*Figure IV.2a*). The I_0 (*Figure IV.2b*) and HOMA-IR (*Figure IV.2c)* did not change throughout the study.

3.2.2. Response to an oral glucose load

$Glucose_{AUC}$ increased in both groups after NN5 compared to BL (*Figure IV.2d*). However, only the LE-group returned to baseline levels after RC (p=1.0 for LE-group and p<0.0001 for ME-group, *Figure IV.2d*). Together, both groups had increased $insulin_{AUC}$ after NN5 and RC compared to BL (*Figure IV.2e*). Concerning $G_{120,}$ only the ME-group showed an increase from BL to NN5 and RC (*Figure IV.2f*). Glucose and insulin OGTT profiles are presented in the appendix (*Figure IV.A*).

Matsuda index decreased after NN5 and RC for both groups combined compared to BL (*Figure IV.2g*). Stumvoll ISI index decreased after NN5 compared to BL for both groups (*Figure IV.2h*) and tended to return to baseline levels after RC only for the LE-group (p=1.0 for LE-group and p=0.002 for ME-

group. Although overall $insulin_{AUC}$ was found to be increased after NN5 and RC compared to BL, for both groups combined the first-phase and second-phase Stumvoll insulin secretion indexes showed no significant changes throughout the week. None of these changes in the glucose metabolism variables significantly correlated with any of the sleep variables or with the noise annoyance rating ($glucose_{AUC}$-SWS and $glucose_{AUC}$-arousal correlations are shown in appendix *Figure IV.B*).

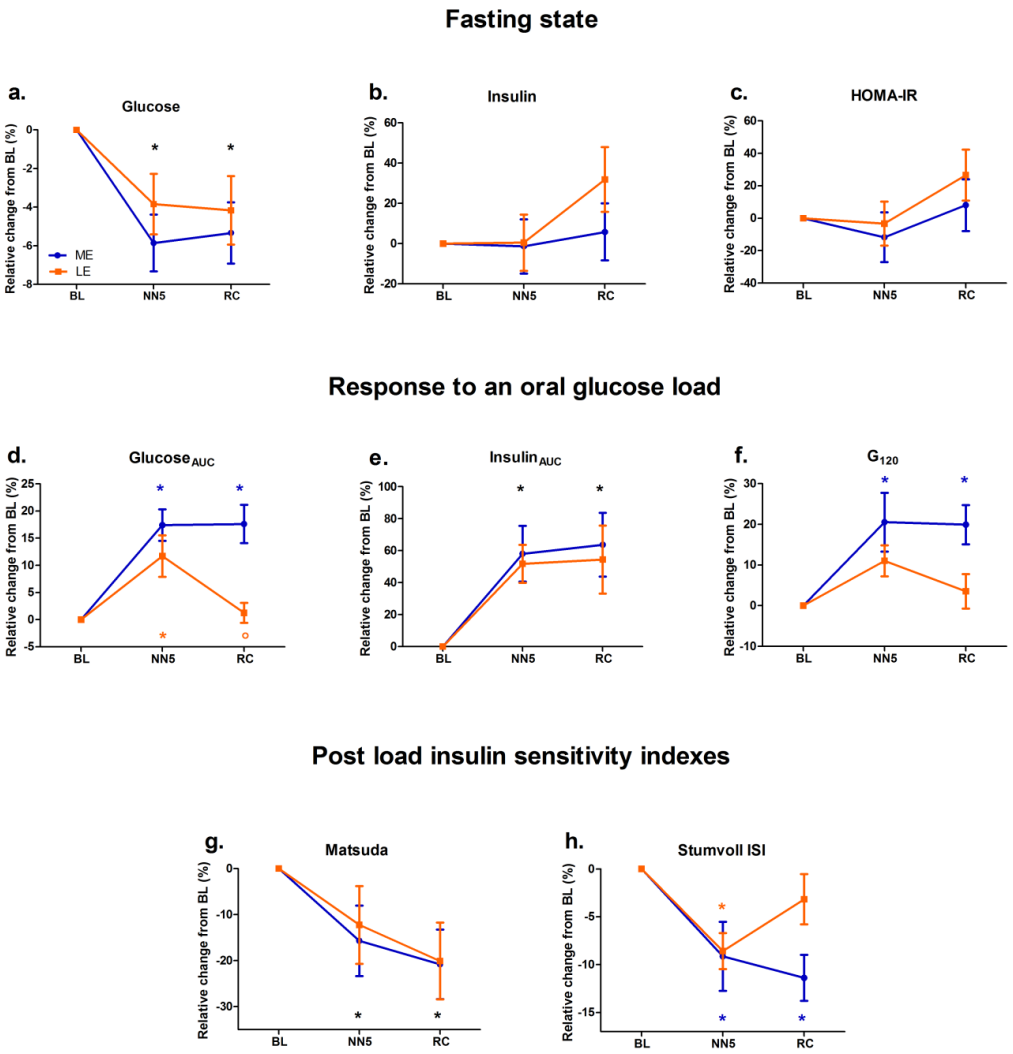

Figure IV.2: Relative changes in glucose metabolism variables between baseline (BL) and the last noise night (NN5) and recovery night (RC) (LE-group in orange and ME-group in blue). *Mean ± SE. Fasting state measures (first row): fasting glucose (condition: p<0.0001, interaction: p=0.31, a), fasting insulin (condition: p=0.23, interaction: p=0.54, b) and fasting insulin resistance index HOMA-IR (condition: p=0.15, interaction: p=0.78, c). Response to the oral glucose load (second row): $glucose_{AUC}$ (condition: p<0.0001, interaction: p=0.004, d), $insulin_{AUC}$ (condition: p<0.0001, interaction: p=0.71, e) and glucose level at t120 (G_{120}, condition: p=0.0006, interaction: p=0.08, f). Post load insulin sensitivity index (third row): Matsuda (condition: p=0.0002, interaction: p=0.74, g) and Stumvoll (condition: p<0.0001, interaction: p=0.07, h) insulin sensitivity indexes. AUC: area under the curve.* in comparison to BL, ° in comparison to NN5, p<0.05.*

3.3. Effect of the laboratory setting on glucose variables

The control group experienced the same laboratory conditions without being exposed to transportation noise during the night, which gave us the possibility to assess the effect of the

laboratory stay *per se* on the glucose regulation. *Figure IV.3* represents the percentage change in glucose variables between the OGTT in the morning of the BL and NN5 or RC for the three groups ME-group, LE-group and the control group. The control group did not show any changes in G_0. Similar to the intervention groups, the control group showed a small increase (5 (-0.8-40) %) in $glucose_{AUC}$ after night 5, but the effect was weak compared to the intervention groups. After the RC, the $glucose_{AUC}$ of the ME-group remained clearly high whereas the LE-group returned to baseline levels, similar to control group levels. $Insulin_{AUC}$ increased by comparable levels between the control group and the intervention groups. In line with these results, Stumvoll ISI index decreased for the control group however the median stayed higher than the ME-group. Refer to the appendix for details on the time course of glucose and insulin OGTT profiles in the control group (*Figure IV.C*).

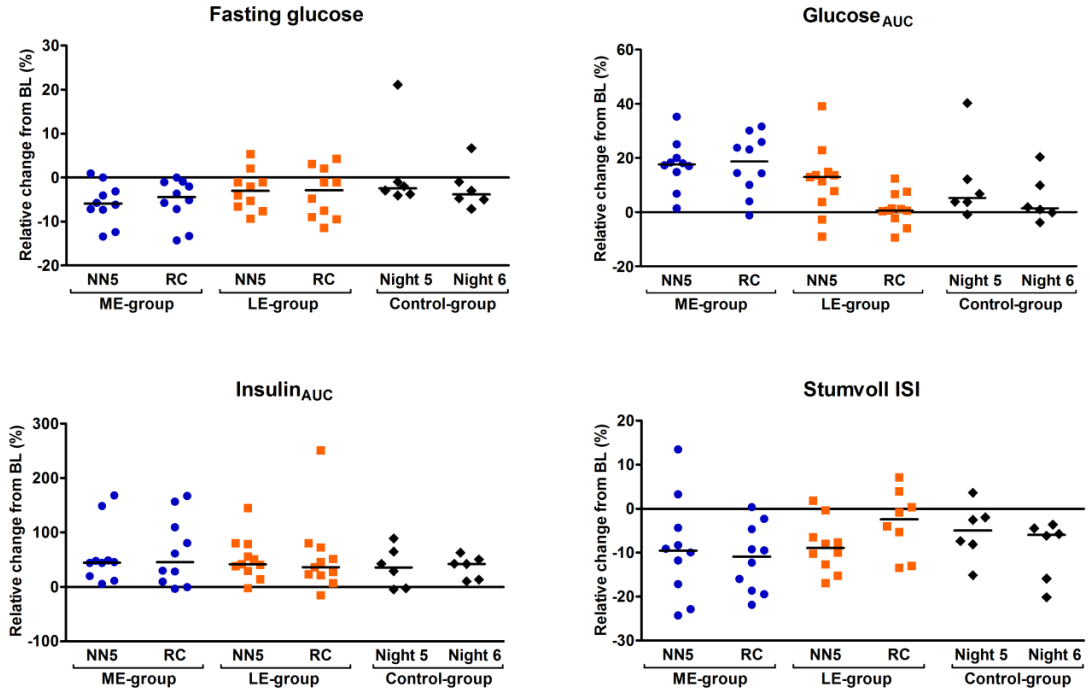

Figure IV.3: Relative change in fasting glucose, $glucose_{AUC}$, $insulin_{AUC}$ and Stumvoll ISI between BL and NN5 or RC for the ME (in blue), LE (in orange) and the control group (in black). *AUC: area under the curve. Black bars correspond to the median.*

4. DISCUSSION

Previous studies have suggested that exposure to transportation noise impairs glucose regulation leading to long term increased risk for developing T2D (Eze, Foraster, et al., 2017; Sorensen et al., 2013). To our knowledge, this is the first experimental study evaluating the effects of nocturnal transportation noise exposure for different scenarios over several nights on glucose regulation, accounting for the potential role of sleep. Despite the mild effect of transportation noise on sleep variables, we found that glucose tolerance and insulin sensitivity were significantly decreased after the noise intervention. Additionally, we tested whether the eventfulness of the noise scenarios plays

a role in reflecting these effects. We found that only participants sleeping with less eventful noise during the last noise night were able to recover to baseline glucose levels after one noise-free recovery night.

Effect of the noise intervention and laboratory conditions on glucose regulation

Compared to the quiet baseline night, we found that sleeping four nights with transportation noise exposure increased morning glucose response and decreased insulin sensitivity. Changes in overall glucose response and G_{120} indicated a significant decrease in glucose tolerance, without reaching prediabetes levels (G_{120}>7.8mmol.L^{-1}) (American diabetes association, 2018). Global insulin sensitivity, as quantified with the Matsuda and Stumvoll indexes, decreased after sleeping with transportation noise exposure. Compared to the sleep fragmentation study conducted by Herzog and colleagues, in which the arousal index increased by 172% and Matsuda index decreased by 15% (Herzog et al., 2013), our changes were more modest: 20% and 7%, respectively. The Matsuda index, considered as the gold standard to evaluate insulin sensitivity from an OGTT, integrates fasting and post-load glucose and insulin levels. The Stumvoll ISI, on the other hand, utilizes demographic data of interest, such as the BMI in our case, and does not integrate G_0 and I_0 in the formula. Given the fact that our participants lost on average 493 ± 20 g, and G_0 decreased throughout the week, the Stumvoll index may be more appropriate for interpreting our results than the Matsuda index. Although the overall insulin response was increased in response to oral glucose, no changes were apparent in the Stumvoll first and second phase of insulin secretion. These results indicate that the β-cells were unable to secrete enough insulin to compensate for the reduced insulin sensitivity, therefore leading to decreased glucose tolerance. As impaired glucose tolerance and insulin resistance are the first steps in the development of T2D, our results support the hypothesis that exposure to nocturnal transportation noise may contribute to incident T2D.

G_0 decreased after sleeping four nights with transportation noise. The changes in REM sleep duration may have contributed to these results. Indeed, during REM sleep, cerebral glucose utilization is as high as when awake, while it is reduced by more than 40% in SWS (Maquet, 1995). Accordingly, we found a significant correlation between the changes in REM sleep duration and G_0 (see *Figure IV.D* in appendix). HOMA-IR, which reflects fasting insulin resistance, was unaffected by transportation noise exposure.

Effect of noise eventfulness on the recovery of noise-induced glucose intolerance and insulin resistance

Unlike participants exposed to less eventful noise during the last noise night, those exposed to more eventful noise at the same LAeq did not return to normal baseline glucose levels after one noise-free

night. In contrast to the more eventful group, Stumvoll ISI (but not the Matsuda index) in the less eventful group tended to recover after one noise-free night. Our results indicate that more eventful transportation noise during sleep is more deleterious for glucose regulation compared to less eventful noise with the same hourly average sound pressure level.

Effect on sleep variables

Both intervention groups felt annoyed from exposure to transportation noise compared to the noise-free baseline and recovery night. In contrast, the control group did not report changes in noise annoyance throughout the week, although they thought to be exposed to transportation noise. Also only the intervention group showed impaired subjective sleep quality after the last intervention night, in line with field studies indicating that noise annoyance is a mediator for subjective but not objective sleep quality (Frei et al., 2014; Miedema & Vos, 2007). This statement is in accordance with the objective sleep measures of this study that only revealed small effects. TST, sleep efficiency, and the amount of SWS or SWA were not significantly different from the noise-free baseline and recovery nights. However, sleep latency increased after the noise exposure nights as did the number of arousals (i.e. by ca. 2 arousals per hour TST). These observed effects of transportation noise during sleep (LAeq= 45 dB) on objective sleep variables are in line with previous results (Basner et al., 2014; Basner et al., 2011), and are mild compared to clinically relevant sleep disturbances. Similar to Basner and colleagues (Basner et al., 2011), who conducted an eleven-night laboratory study with nocturnal transportation noise exposure, the amount of REM sleep increased during the recovery night compared to baseline. This may reflect the improved sleep hygiene by the imposed regular sleep-wake cycle and the 8 hours in bed.

To date, only experimental changes in sleep duration and sleep fragmentation, in particular during SWS, have been shown to play a key role in glucose regulation (Spiegel et al., 1999; Stamatakis & Punjabi, 2010; Tasali et al., 2008). As such, these were our first candidate outcomes to relate to changes in glucose metabolism. The observed traffic noise effects on sleep variables in our study were rather small compared to the above mentioned studies, and thus did not show significant associations with glucose metabolism changes. Another possible pathway through which transportation noise could have impaired glucose regulation may have been via the stress axes as observed in noise-exposed rodents (Cui et al., 2016; Liu et al., 2016). High plasma cortisol levels (Fichna & Fichna, 2017; Mazziotti et al., 2011; Plat et al., 1996), or increased sympathetic nervous activity and catecholamine levels (Lembo et al., 1994; Thorp & Schlaich, 2015), are known to lead to insulin resistance. The transportation noise scenarios that we presented to our participants could have triggered the activation of these stress axes, without profound sleep alterations, as evoked

autonomic arousals during sleep are less likely to habituate than cortical arousals (Basner et al., 2011; Muzet, 2007).

Strengths and limitations

To our knowledge, this is the first controlled laboratory study investigating the impact of transportation noise on sleep and glucose regulation. The strength of a full time laboratory study is the ability to control many parameters such as food intake, physical activity, ambient light intensity, room temperature and noise exposure during the entire stay. All these factors can potentially influence sleep and glucose regulation and their interaction. Although food intake was quantified with the Mifflin et al. equation (Mifflin et al., 1990) to estimate daily caloric need, an average weight loss of 493 ± 20 g compared to basal weight was observed; but there was no statistical difference between the LE and ME groups. Thus, the study was carried out in a mild negative energy balance state, suggesting that the harmful effect of transportation noise during sleep on glucose regulation would have been even stronger without this weight loss corroborating the study conclusions of St-Onge et al. (St-Onge et al., 2012).

A limitation of this study is the rather small sample size of the intervention group and the control group of only six young men restricted us to explorative comparisons. Nevertheless, the control group highlighted the impact of the sedentary condition of the laboratory stay on glucose regulation even under low caloric intake. This observation is in line with previous investigations on the harmful effect of physical inactivity on glucose regulation (Bergouignan et al., 2011).

One further limitation could be the retrospective recall of noise annoyance. However, the noise scenario during the baseline and recovery nights was the same and accordingly, the annoyance during these two nights did not significantly differ, supporting the idea that the retrospective recall did not impact the noise annoyance rating.

We assessed individual global noise sensitivity using the LEF-K and the NoiSeQ questionnaires which did not differ between participants. However, individual long-term exposure to transportation noise and lifestyle at home was not known, and these factors could influence susceptibility to noise and metabolic response to acute noise exposure.

5. CONCLUSION

Our laboratory findings are in line with those from epidemiological studies reporting detrimental effects of nocturnal transportation noise on glucose regulation. The novelty of this study is that it considers a shorter time scale in experimental controlled condition with objective measurements; four nights sleeping with transportation noise were enough to elicit impaired glucose tolerance and

insulin sensitivity. The effects may not be clinically significant but could become relevant over a longer time span and in combination with other risk factors for cardio-metabolic diseases. One could argue that the observed effect could be related to the sedentary laboratory conditions since the control group also showed mild glucose tolerance impairments; however the extent of the effect was evidently stronger for the intervention groups. Moreover, the efficiency of the recovery night for glucose regulation depended on the eventfulness of the last noise night, with a better recovery for more continuous than intermittent noise exposure. Even without eliciting major sleep disturbances at 45 dB, transportation noise may affect glucose regulation via other mechanisms such as the stress axis. To validate these results, a field study in a more natural and longer term setting is necessary.

Acknowledgments

We thank our research volunteers for their participation and their compliance to our study protocol, our staff team, students, civil servants, nurses, and interns Salome Bitter, Louis Germond, and Noelie Gerber for their help in conducting the study, Corrado Garbazza and Helen Slawik for the medical screening and Ruta Lasauskaite for proof reading the manuscript. We also thank the Clinical Trial Unit of the University of Basel, for statistical support.

Funding

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	Control group (n=6)			LE-group (n=11, 5 women)			ME-group (n=10, 4 women)			LE vs ME (p-values)			Tukey post-hoc (p-values)		
	Night1	Night5	Night6	BL	NN5	RC	BL	NN5	RC	Condition	Group effect	Inter-action	BL-NN5	BL-RC	NN5-RC
SUBJECTIVE ASSESSMENT															
Subjective sleep quality															
Getting to sleep	42 ± 5.8	47 ± 8.3	36 ± 6.6	48 ± 5.3	39 ± 4.5	50 ± 3.8	53 ± 6.3	34 ± 5.0	36 ± 4.1	**0.01**	0.38	**0.09**	**0.01**	0.18	0.36
Quality of sleep	33 ± 8.2	37 ± 3.8	34 ± 10	34 ± 4.5	31 ± 4.6	46 ± 5.6	40 ± 4.0	19 ± 5.0	30 ± 6.1	**0.02**	0.1	**0.07**	**0.04**	0.98	**0.03**
Awake following sleep	57 ± 9.8	66 ± 5.9	64 ± 4.1	64 ± 5.2	54 ± 6.3	47 ± 4.4	57 ± 4.0	39 ± 5.6	47 ± 4.1	**0.01**	0.1	0.34	**0.02**	**0.02**	0.99
Behavior following wakening	40 ± 9.6	45 ± 9.4	45 ± 6.1	49 ± 7.8	35 ± 6.8	38 ± 7.8	52 ± 6.4	24 ± 5.1	35 ± 6.8	**0.004**	0.6	0.46	**0.003**	**0.06**	0.47
Noise annoyance	44 ± 13	53 ± 9.9	35 ± 8.4	33 ± 8.0	53 ± 8.7	25 ± 7.2	16 ± 5.2	60 ± 8.7	20 ± 6.9	**<0.0001**	0.56	0.31	**0.0003**	0.97	**0.0002**
OBJECTIVE ASSESSMENT															
TST (min)	437 ± 15	429 ± 17	439 ± 8.3	448 ± 8.0	447 ± 6.8	459 ± 1.9	456 ± 5.0	452 ± 4.3	449 ± 6.4	0.74	0.87	0.18			
Sleep efficency (%)	91 ± 3.1	89 ± 3.4	91 ± 1.7	93 ± 1.7	93 ± 1.4	96 ± 0.4	95 ± 1.0	94 ± 0.89	94 ± 1.3	0.74	0.87	0.17			
Sleep latency (min)	19 ± 12	28 ± 6.6	24 ± 5.8	12.7 ± 1.9	18.2 ± 2.3	20.7 ± 2.0	12.5 ± 2.9	24.2 ± 4.0	27.0 ± 4.2	**<0.0001**	0.31	0.19	**0.0001**	**<.0001**	0.26
Sleep architecture															
Light NREM (min)	272 ± 20	246 ± 18	267 ± 11	280 ± 15	276 ± 12	281 ± 9.3	283 ± 7.1	266 ± 12	254 ± 10	**0.02**	0.55	0.16	0.2	**0.02**	0.49
SWS (min)	70 ± 12	72 ± 13	69 ± 14	74 ± 9.3	76 ± 10	69 ± 8.3	80 ± 8.8	81 ± 9.2	87 ± 11	0.64	0.52	0.4			
SWA ($\mu V^2/Hz$)	1.45 ± 0.05	1.47 ± 0.05	1.46 ± 0.03	1.52 ± 0.07	1.53 ± 0.06	1.56 ± 0.07	1.49 ± 0.07	1.53 ± 0.08	1.54 ± 0.08	**0.06**	0.84	0.82	0.47	**0.05**	0.41
REM (min)	94 ± 12	111 ± 9.3	102 ± 13	95 ± 6.2	96 ± 7.6	109 ± 4.7	93 ± 5.6	105 ± 5.9	108 ± 6.3	**0.01**	0.8	0.41	0.36	**0.01**	0.18
WASO (min)	37 ± 14	34 ± 16	23 ± 5.2	24.8 ± 7.6	21.2 ± 5.8	10.3 ± 1.9	16.2 ± 4.9	12.4 ± 3.3	16.7 ± 6.3	0.38	0.84	0.31			
SSC_deeper (n/h)	5.4 ± 0.68	5.0 ± 0.84	5.3 ± 1.3	3.6 ± 0.31	3.9 ± 0.36	3.8 ± 0.43	3.6 ± 0.38	4.1 ± 0.52	3.8 ± 0.40	0.34	0.95	0.89			
SSC_lighter (n/h)	4.8 ± 0.59	4.1 ± 0.64	4.3 ± 0.98	3.3 ± 0.27	3.6 ± 0.31	3.3 ± 0.41	3.0 ± 0.35	3.5 ± 0.43	3.4 ± 0.36	0.42	0.76	0.83			
Arousal (n/h)	13 ± 2.3	10 ± 1.5	12 ± 2.0	11 ± 0.82	12 ± 1.3	11 ± 0.97	8.4 ± 0.98	11 ± 0.85	9.8 ± 0.86	**0.01**	0.33	0.3	**0.01**	0.45	0.13
Arousal (n/h REM)	16 ± 3.8	13 ± 2.8	15 ± 3.6	11 ± 1.4	13 ± 1.5	12 ± 1.8	9.1 ± 1.4	13 ± 1.7	13 ± 0.8	**0.01**	0.83	0.34	**0.02**	**0.02**	0.99
Arousal (n/h NREM)	13 ± 2.1	8.7 ± 1.0	10 ± 1.9	10 ± 1.0	12 ± 1.6	10 ± 1.2	8.2 ± 1.2	11 ± 0.9	8.8 ± 1.1	**0.02**	0.31	0.6	**0.03**	0.97	**0.05**

Table IV.3: Sleep characteristics between the noise conditions (BL=baseline night, NN5=last noise night, RC=recovery night) for the control group, the less eventful (LE) and the more eventful (ME) groups. *Mean ± SE; TST: total sleep time; SWA: slow wave activity; WASO: wake after sleep onset; SSC_deeper: sleep stage changes to deeper stages per hour; SSC_lighter: sleep stage changes to lighter stages per hour (n/h: number per hour).*

	LE-group (n=11, 5 women)			ME-group (n=10, 4 women)			LE vs ME (p-values)			Tukey post-hoc (p-values)		
	BL	NN5	RC	BL	NN5	RC	Condition	Group effect	Inter-action	BL-NN5	BL-RC	NN5-RC
FASTING STATE												
Fasting glucose ($mmol.L^{-1}$)	5.0 ± 0.11	4.8 ± 0.07	4.8 ± 0.09	5.2 ± 0.11	4.8 ± 0.08	4.9 ± 0.08	**<0.0001**	0.15	0.31	0.0003	0.0003	1
Fasting insulin ($pmol.L^{-1}$)	31 ± 5.3	28 ± 3.8	36 ± 3.3	35 ± 4.8	33 ± 4.9	35 ± 4.7	0.23	0.7	0.54			
HOMA-IR	1.1 ± 0.20	0.87 ± 0.13	1.1 ± 0.11	1.2 ± 0.19	0.98 ± 0.18	1.1 ± 0.15	0.15	0.75	0.78			
RESPONSE TO AN ORAL GLUCOSE LOAD												
$Glucose_{AUC}$ ($mmol.L^{-1}.min.10^{1}$)	70 ± 3.8	78 ± 3.9	71 ± 3.8	71 ± 3.1	83 ± 2.7	83 ± 2.9	**<0.0001**	0.22	**0.004**	<0.0001	0.001	0.09
$Insulin_{AUC}$ ($pmol.L^{-1}.min.10^{3}$)	33 ± 4.1	47 ± 3.9	45 ± 3.8	34 ± 4.0	49 ± 4.2	51 ± 5.2	**<0.0001**	0.58	0.71	<0.0001	<0.0001	1
G_{120} ($mmol.L^{-1}$)	5.3 ± 0.32	5.8 ± 0.27	5.4 ± 0.44	5.1 ± 0.22	6.1 ± 0.39	6.0 ± 0.26	**0.001**	0.62	**0.08**	0.001	0.02	0.48
POST LOAD INDEXES												
Matsuda index	9.2 ± 1.4	7.2 ± 0.62	6.5 ± 0.61	7.4 ± 0.64	6.8 ± 0.92	6.6 ± 1.0	**0.0002**	0.22	0.74	0.005	0.0002	0.53
Stumvoll ISI index ($\mu mol.kg^{-1}.min^{-1}.pM^{-1}$)	0.12 ± 0.004	0.11 ± 0.003	0.11 ± 0.01	0.12 ± 0.004	0.11 ± 0.004	0.10 ± 0.003	**<0.0001**	**0.04**	**0.07**	<0.0001	0.002	0.56
Stumvoll first-phase ($pmol.L^{-1}$)	1064 ± 152	1272 ± 145	1405 ± 229	1257 ± 119	1275 ± 123	1244 ± 142	0.2	0.95	0.16			
Stumvoll second-phase ($pmol.L^{-1}$)	279 ± 35	327 ± 34	357 ± 52	320 ± 26	333 ± 27	325 ± 31	0.12	0.9	0.21			

Table IV.4: Comparison of metabolic values between the noise conditions (BL=baseline night, NN5=last noise night, RC=recovery night) for the less eventful (LE) and the more eventful (ME) groups. *Mean ± SE.*

6. APPENDICES

TABLE

Scenario	Source	Type of noise	Posted speed limit (km/h)	Distance (m)	Pass-bys (n/h)	LAFmax (dB)
A	road	4-lane highway	120	400	1000	53
B	road	2-lane country road	80	50	250	60
C	road	1-lane urban road	50	15	100	62
D	train	freight and regional trains			10	62

Table IV.A: Noise scenarios specifications. LAFmax: maximum level.

FIGURES

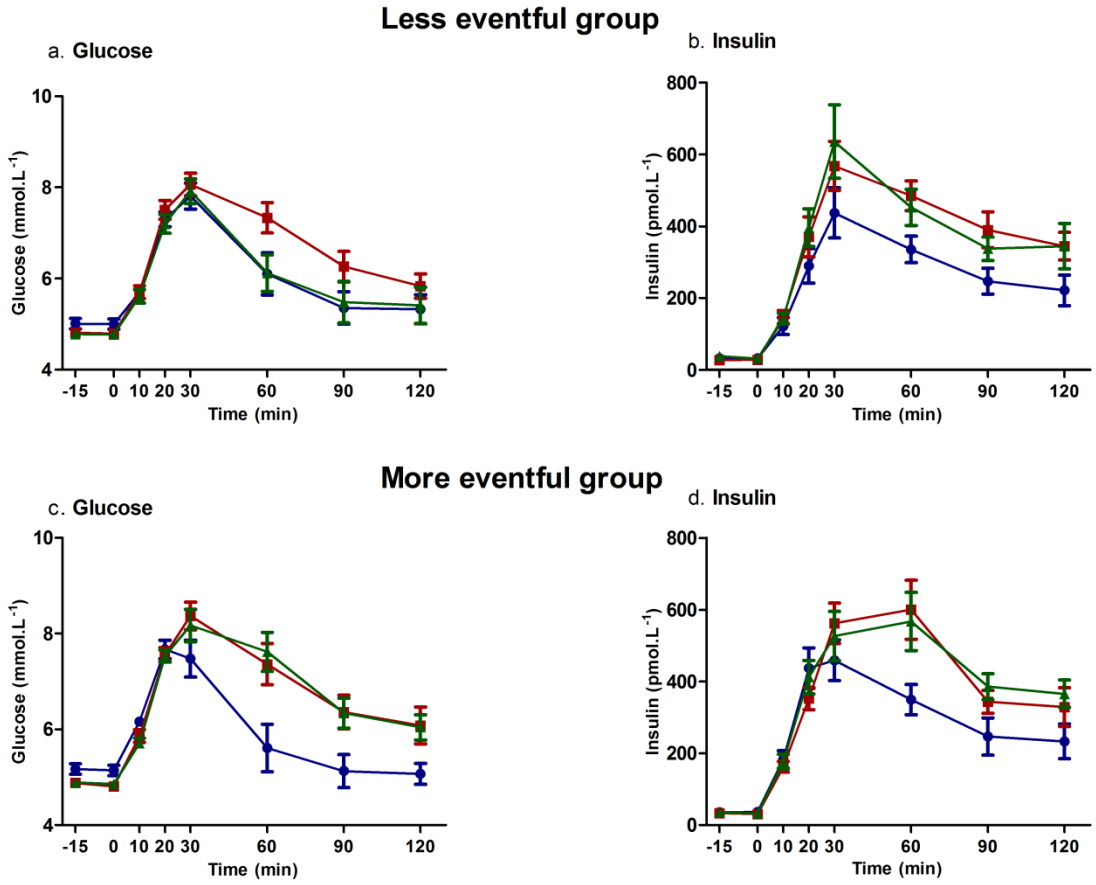

Figure IV.A: Glucose and insulin profiles during an OGTT in the less eventful group (LE) (a. and b.) and the more eventful (ME) group (c. and d.). *Mean ± SE. After baseline (blue), after NN5 (red) and after one recovery night (green).*

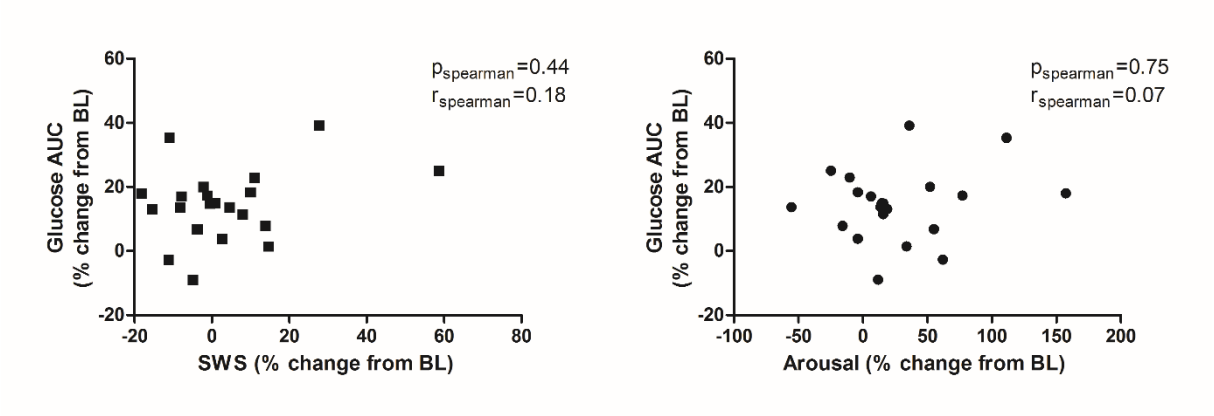

Figure IV.B: Correlations between the relative change in SWS duration or the amount arousals and Glucose$_{AUC}$ during NN5 compared to BL.

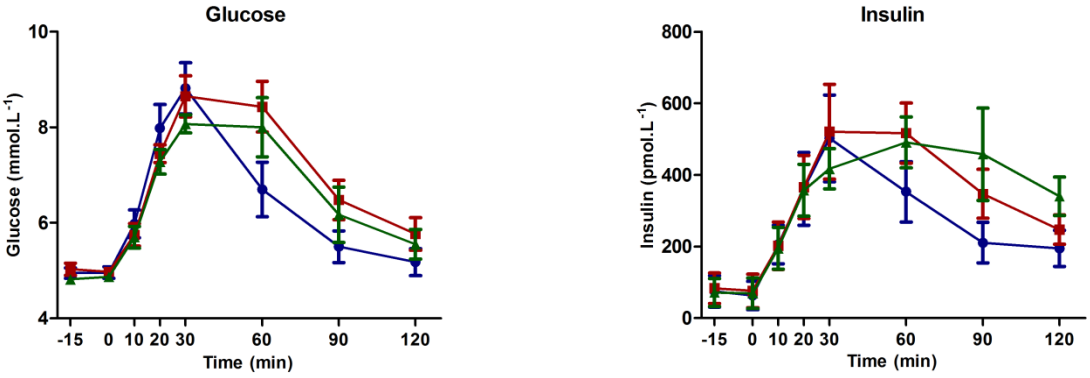

Figure IV.C: Glucose and insulin profiles during an OGTT in the control group. *Mean ± SE. After sleeping 1 night (blue), 5 nights (red) and 6 nights (green) in the laboratory.*

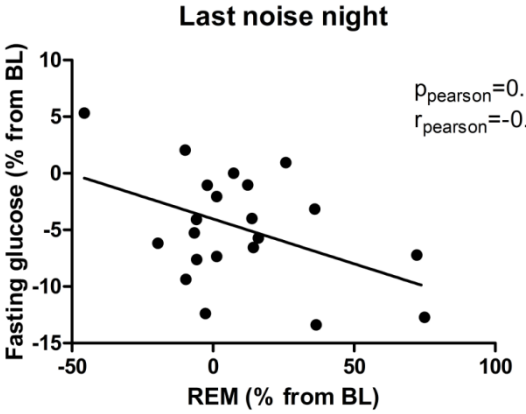

Figure IV.D: Correlation between the relative change in REM sleep duration and fasting glucose during NN5 compared to BL.

Chapter V

Transportation noise impairs cardiovascular function without altering sleep: the importance of autonomic arousals

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Keywords: Traffic noise, Intermittency, Blood pressure, Heart rate variability, Stress hormones, Inflammation

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ABSTRACT

Aims

Chronic exposure to nocturnal transportation noise has been linked to cardiovascular disorders with sleep impairment as the main mediator. Here we examined whether nocturnal transportation noise affects the main stress pathways, and whether it relates to changes in the macro and micro structure of sleep.

Methods and results

Twenty-six young healthy participants (12 women, 24.6 ± 0.7 years, mean ± SE) spent five 24-h days and one last morning in the laboratory. The first (baseline, BL) and last (recovery, RC) days comprised a noise-free night. In-between, four different noise scenarios (low/medium/high intermittent road or rail scenarios with an identical equivalent continuous sound level of 45dB) were randomly presented during the 8-h nights. Participants felt more annoyed from the transportation noise scenarios compared to the noise-free BL and RC nights ($F_{5,117}$=10.2, p<0.001). Nocturnal transportation noise did not significantly impact polysomnographically assessed sleep macrostructure, blood pressure, nocturnal catecholamine levels and morning cytokine levels. Evening cortisol levels increased after sleeping with highly intermittent road noise compared to baseline (p=0.002, noise effect: $F_{4,83}$=4.0, p=0.005), a result related to increased cumulative duration of autonomic arousals during the noise nights ($F_{5,106}$=3.4, p<0.001; correlation: $r_{pearson}$=0.64, p=0.006).

Conclusion

Under controlled laboratory conditions, highly intermittent nocturnal road noise exposure at 45dB increased the cumulative duration of autonomic arousals during sleep and next-day evening cortisol levels. Our results indicate that, without impairing sleep macrostructure, nocturnal transportation noise of 45dB is a physiological stressor that affects the hypothalamic-pituitary-adrenal axis during the following day in healthy young good sleepers.

1. INTRODUCTION

Epidemiological evidence for harmful effects of transportation noise on the cardiovascular system is rapidly growing (Kempen et al., 2018). Populations living in proximity to roads, railways or airports are at higher risk for arterial hypertension and cardiovascular diseases (CVD) (Kempen et al., 2018), also when adjusting for air pollution (Stansfeld, 2015). It is assumed that acute exposure to noise activates the two main stress pathways, the sympatho-adrenal-medullary (SAM) pathway and the hypothalamic–pituitary–adrenal (HPA) axis (Münzel, Sorensen, et al., 2017a). In response to an acute stressor, SAM is activated and releases catecholamines while HPA sustains the response via the secretion of glucocorticoids (e.g. cortisol) (Aich et al., 2009). The repetitive activation of these two stress pathways may lead to higher blood pressure (BP), dyslipidemia, impaired glucose regulation, altered heart rate variability (HRV) or even endothelial dysfunction through induction of oxidative stress and vascular inflammation (Münzel et al., 2018); all of these conditions may contribute to the development of CVD.

Nocturnal hours are particularly relevant for transportation noise induced CVD and cardiovascular mortality (Héritier et al., 2018; Jarup et al., 2008). Noise may disturb sleep and impair its restorative function on the stress system (Cappuccio & Miller, 2017). Therefore, several field and laboratory studies investigated the effect of nocturnal transportation noise on sleep or cardiovascular outcomes (Basner et al., 2011; Carter et al., 1994; Griefahn & Robens, 2010; Haralabidis et al., 2008; Schmidt et al., 2013). To our best knowledge, the potential role of polysomnographically assessed sleep variables as a proxy of the effect of nocturnal transportation noise on both stress pathways has not yet been investigated together in a single setting. Thus, our combined analysis aimed at obtaining an overall picture of the nocturnal noise-cardiovascular impairment pathway.

Here, we explored the impact of short-term effects of nocturnal transportation noise exposure on sleep and cardiovascular outcomes. The main goal was to determine the role of sleep, and more precisely of cortical and autonomic arousals (CA and AA, respectively), in the complex noise-cardiovascular system pathway. We hypothesized that nocturnal transportation noise exposure deteriorates sleep by increasing CA and AA, impairing hemodynamic variables (HR and BP), increasing sympathetic or decreasing parasympathetic nervous activity and increasing nocturnal catecholamine levels. Additionally, daytime cortisol levels and inflammatory markers were expected to increase after nocturnal noise exposure compared to noise-free nights. Since our noise scenarios differed according to their intermittency ratio (IR), a new acoustical metric that has been recently associated with arterial stiffness, glucose tolerance and cardiovascular mortality (Foraster et al., 2017; Héritier et al., 2017; Thiesse, Rudzik, Spiegel, et al., 2018), we hypothesized that higher IR would have a stronger impact on the above mentioned outcomes than scenarios with lower IR.

2. METHODS

2.1. Study participants

Participants were healthy non-smokers free of any medication as assessed by the clinical history, physical examination, routine blood and toxicological urine screening. Participants habitually slept 7 to 9 hours per night and reported good sleep quality (Pittsburgh Sleep Quality Index, (Buysse et al., 1989) PSQI ≤ 5) and no daytime sleepiness (Epworth Sleepiness Scale, (Johns, 1991) ESS ≤ 10). Volunteers with sleep disorders (diagnosed during an adaptation night), shift work, extreme chronotypes (Munich Chronotype Questionnaire, [34] MCTQ < 2 or MCTQ ≥ 7) or hearing loss were excluded. Noise sensitivity was assessed using the short version of the German *Lärmempfindlichkeitsfragebogen* (LEF-K) (Zimmer & Ellermeier, 1998) and the *Noise Sensitivity Questionnaire* (NoiSeQ) (Schutte et al., 2007) but was not part of the selection criteria.

All participants gave written informed consent. The protocol was approved by the local ethics committee (EKNZ/Ethikkommission Nordwest- und Zentralschweiz, Switzerland), and conformed to the Declaration of Helsinki.

2.2. Procedure

One week prior to the study, participants were asked to follow their habitual bedtimes within ± 30 minutes, spending 8 hours in bed and not taking naps. Compliance was assessed via actigraphy (Actiwatch L, Cambridge Neurotechnologies, Cambridge, UK). Additionally, participants were asked to avoid stimulating substances (coffee, tea, chocolate) and alcohol.

Participants spent five 24-h days and one last morning in the laboratory in individual bedrooms. Light intensity during the daytime was kept constant (between 50 and 150 lux at participant's eye) and room temperature was set at 22°C. Participants had an 8-h sleep opportunity per night scheduled at their habitual bedtimes. The study protocol is illustrated in *Figure V.1*.

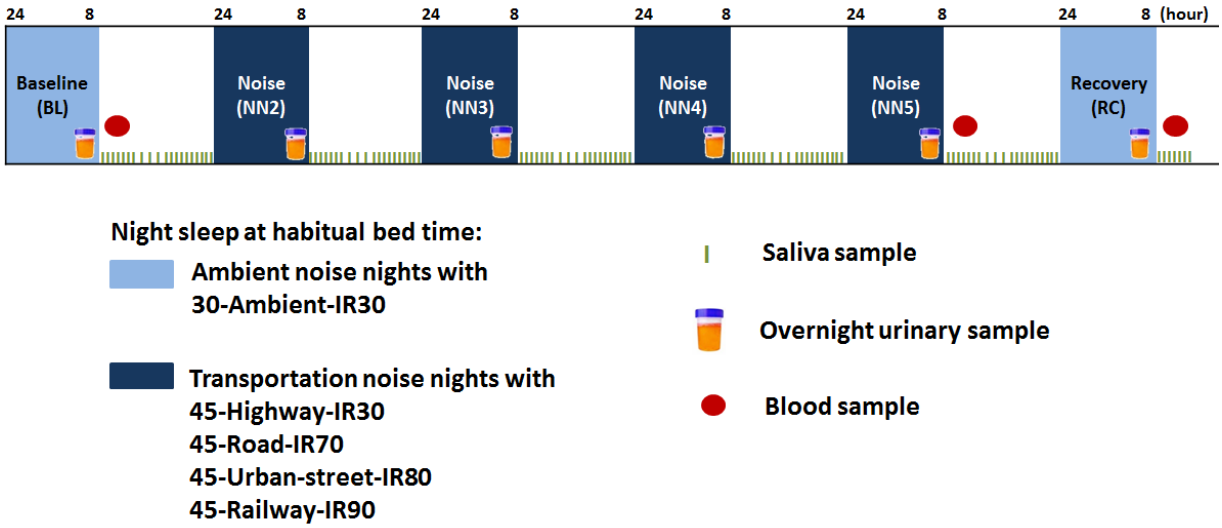

Figure V.1: Schematic illustration of the study protocol. *Time in bed was scheduled according to each participant's habits (e.g. 24-8h). The order of exposure to the four different transportation noise scenarios was randomized between participants. Noise scenario: «LAeq,1h-Noise source-IR (intermittency ratio)».*

2.3. Noise exposure

Noise exposure characteristics were extensively described in previous publications with this dataset (Rudzik et al., 2018; Thiesse, Rudzik, Spiegel, et al., 2018). In short, all participants started and ended the study respectively with a noise-free baseline (BL) and recovery (RC) night during which a quiet ambient scenario (30-Ambient-IR30) was applied (*Figure V.1*). In between, participants were randomly exposed to four different noise scenarios which had eight identical hourly equivalent continuous sound pressure levels (*L*Aeq,1h=45dB at the ear of the sleeper) during lights-OFF to lights-ON. The noise scenarios differed with respect to the noise source (road or railway) and IR, a metric which describes the share of individual noise events compared to the overall sound exposure (Wunderli et al., 2015) - for details see *Table V.1*. During the last morning of the study, participants were asked to retrospectively evaluate noise annoyance during each night on a scale of 0 to 100 with the question "How annoyed were you during the night by the noise"?

Scenario	Source	LAeq,1h [dB]	LAFmax [dB]	IR
30-Ambient-IR30	ambient	30	39	0.3
45-Highway-IR30	road	45	53	0.3
45-Road-IR70	road	45	60	0.7
45-Urban-street-IR80	road	45	62	0.8
45-Railway-IR90	rail	45	62	0.9

Table V.1: Acoustical characteristics of the noise scenarios. *30-Ambient-IR30 represents the noise-free scenario of BL and RC nights. The four transportation noise scenarios were randomly introduced during the noise nights. LAeq,1h: equivalent continuous sound level, LAFmax: maximum sound level, IR: intermittency ratio (Wunderli et al., 2015).*

2.4. Sleep measurement

Subjective sleep quality was assessed 5-10 min upon awakening with the LEEDS Sleep Evaluation Questionnaire (Parrott & Hindmarch, 1978).

Polysomnographic sleep recordings (PSG) included 12 electroencephalographic, 2 electro-oculographic and 2 electromyographic derivations (Vitaport-3 digital recorder). Rapid eye movement (REM) and Non-REM (NREM1, NREM2 and Slow-Wave Sleep, SWS)) sleep, sleep efficiency (percentage of total sleep time (TST: time spent in REM and Non-REM sleep) between lights-OFF and lights-ON), sleep latency (time between lights-OFF and NREM2 onset) and CA were scored according to standard criteria (Berry et al., 2016). Further details on sleep measurements are described in (Rudzik et al., 2018).

2.5. Salivary cortisol

Saliva samples were obtained every 30 min during the first 3h after wake-up, 4h30 before lights-OFF and every 2h in-between. Samples were kept at -20°C until assay. Cortisol was measured using a direct salivary enzyme-linked immunosorbent assay (ELISA) with a limit of sensitivity of 1.0 ng/mL and a mean intra-assay coefficient of variation (CV) <10% (ALPCO Diagnostics, Salem, NH, USA). Cortisol awakening response (CAR) was calculated as the absolute change between the first and the second morning sample (Pruessner et al., 1997). The daytime trend in salivary cortisol was quantified using the best-fit curve based on periodogram calculations (Van Cauter, 1979). The rate of decrease of the cortisol profile was calculated as the absolute change “acrophase–nadir” divided by the time interval. The area under the curve (AUC) during the last 2h before lights-OFF was calculated to assess evening cortisol levels.

2.6. Urinary catecholamines

Before bedtime, participants were instructed to use the toilet. Overnight urine was then collected until wake-up time in an acidified urine container (HCL 5M, pH<4.0). The light-protected samples were stored at -80°C until analysis. Catecholamines were measured by high-performance liquid chromatography (Recipe, Munich, Germany). Catecholamine concentrations were normalized to creatinine, assessed in the same samples (Roche / Hitachi cobas c systems) with a limit of sensitivity of 100 µmol/L.

2.7. Serum inflammatory markers

A fasting blood sample was obtained one hour after scheduled wake-up time following BL, NN5 and RC nights. Serum was obtained after 30 min clotting at room temperature and centrifugation at 4°C for 10 min at 3500rpm. IL-6, TNFα, adiponectin and CRP were measured by a commercially available

multiplex beads immunoassay with intra-assay CV <8% (Luminex Performance assay, Human Obesity Panel, R&D Systems, Minneapolis, USA). Leptin was measured with an ELISA test (11-LEPHU-E01) with a limit of detection of 0.50 ng/mL and an intra-assay CV <5.5%.

2.8. Heart rate variability

An electrocardiogram was recorded continuously throughout the laboratory study. Data acquisition and post-acquisition analyses were carried out in accordance with established standards (HRV, 1996). Accordingly, analyses were carried out on the shortest time window, i.e. 5 min, over the 8h sleep opportunity. Segments occurring during more than 2.5 min of wakefulness were excluded. Heart rate was derived from the mean normal beat-to-beat interval (N-N, HR=60/N-N). Global HRV was obtained from the standard deviation over the whole time series (SDNN) and vagal activity was indirectly measured with the root mean square of successive N-N intervals (RMSSD) and the percentage of N-N interval differences greater than 50 ms (pNN50). Spectral analysis was based on the Fast Fourier transform with low-frequency (LF, 0.04-0.15 Hz) and high-frequency (HF, 0.15-0.50 Hz) bands. The frequency bands were normalized (LFn=LF/(LF+HF) and HFn=HF/(HF+LF)). HF reflects modulation of vagal activity by respiration while LF represents vagal and sympathetic activity and LF/HF is an index of sympatho-vagal balance (Malliani et al., 1998).

2.9. Blood pressure

Nocturnal BP was obtained and analyzed every second from lights-OFF to lights-ON with the validated cuffless continuous BP monitor SOMNOtouch^{TM} NIBP (SOMNOmedics, Randersacker, Germany) which derives systolic and diastolic BP levels from pulse transit time (Bilo et al., 2015).

2.10. Autonomic arousals

AA, defined as sudden increase in HR followed by a return to initial values, were detected using Somno-Art methodology, based on an algorithm by Muzet et al. (Muzet et al., 2016). The algorithm used 1Hz HR and 1Hz wrist actimetry obtained from the SOMNOtouch^{TM} device. AA were detected and quantified as changes in the steady state of average HR to higher or lower values and were often co-occurring with body movements. AA occurring during wakefulness were excluded.

2.11. Statistical analysis

Statistical analyses were performed using the SAS statistical software package (SAS Institute Inc., Cary, NC; version 9.4). Mixed model analyses of variance were carried out for each outcome variable separately and included the repeated within-subject factor “scenario” (BL, 45-Highway-IR30, 45-Road-IR70, 45-Urban-street-IR80, 45-Railway-IR90, RC) and the random factor “subject” with a

variance component covariance structure. Contrasts were assessed with the LSMEAN statement and p-values were based on Kenward-Roger's corrected degrees of freedom (Kenward & Roger, 1997). Multiple post-hoc comparisons were corrected using the Tukey-Kramer method. Data were log-transformed when residuals were not normally distributed. Correlations were calculated with the Pearson or Spearman correlation coefficient, as appropriate. Correlation outliers were detected and removed according to the absolute deviation around the median (Leys et al., 2013). Data are presented as mean ± standard error and statistical significance was set at $p<0.05$. Although two men quit the study on the fifth day for personal reasons, their data were included in the analysis. For all women, except two, the entire lab protocol was conducted during the follicular phase; the two remaining women did not impact the statistical significances. Missing values, due to technical reasons are summarized in a supplementary table as well as the exact number of individuals and samples for each variable (*Table V.A*).

3. RESULTS

Twenty-six young lean volunteers (12 women, 24.6 ± 0.69 yrs, BMI: 22.2 ± 0.41 kg/m^2) participated in the study. Participants were good sleepers (baseline sleep efficiency= 93.9 ± 0.86 %) and not noise sensitive (LEF-K=11.0 ± 0.79, NoiseQ=1.29 ± 0.08).

3.1. Sleep and CVD markers during nocturnal noise exposure

Noise annoyance and sleep variables upon the different noise scenarios are illustrated in *Figure V.2*. Participants were more annoyed by the transportation noise than by the noise-free BL and RC scenarios. The quality of sleep was perceived as better during RC compared to 45-Highway-IR30 and 45-Road-IR70. No significant changes in objective sleep efficiency and the amount SWS could be observed between the different noise scenarios. Participants took longer to fall asleep during RC compared to the other nights. The number of CA and AA were not different between the noise scenarios. However, the mean duration of AA was higher during 45-Railway-IR90 and RC compared to BL and cumulative duration of AA was higher during 45-Highway-IR30, 45-Urban-street-IR80 and RC compared to BL.

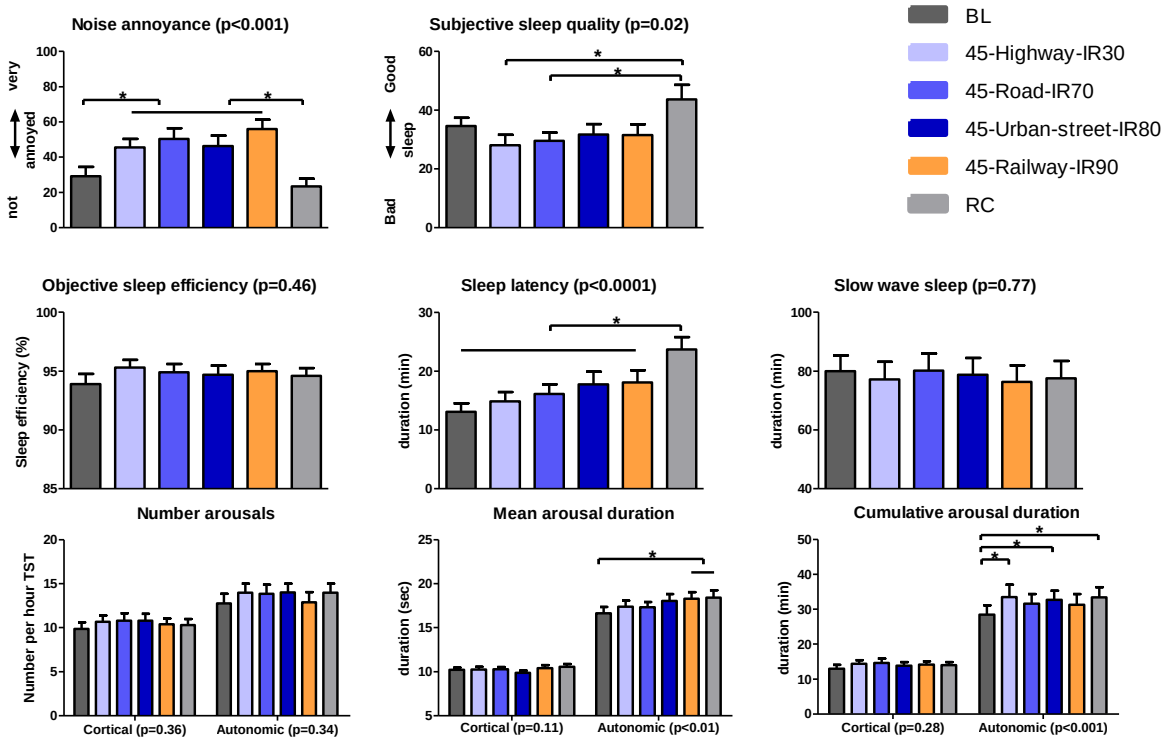

Figure V.2: Noise annoyance, subjective and objective sleep variables for the different noise scenarios. *Baseline (BL) and recovery (RC) nights were noise-free, while night 45-Highway-IR30, 45-Road-IR70, 45-Urban-street-IR80 and 45-Railway-IR90 were transportation noise exposed nights. TST: total sleep time. P-values indicate the noise effect, and asterisks significances at p<0.05.*

Nocturnal mean HR and systolic BP were not affected by the exposure to transportation noise scenarios (*Table V.3*). Nevertheless, mean overnight diastolic BP was higher during BL than during 45-Highway-IR30 (p=0.01) and 45-Urban-street-IR80 (p=0.0004). SDNN increased significantly during 45-Railway-IR90 compared to BL (p=0.02) but pNN50 and RMSSD did not differ between the nights. Spectral analysis of the full-night HRV yielded no differences in LFn and HFn powers nor in LF/HF ratio. No correlation between diastolic BP or mean SDNN and the above mentioned sleep variables were observed except for the relative change between BL and 45-Railway-IR90 in mean SDNN and the number AA per hour TST ($r_{pearson}$=0.77, p<0.01). Creatinine normalized nocturnal urinary epinephrine ($F_{5,74}$=0.52, p=0.76) and norepinephrine ($F_{5,77}$=1.7, p=0.14) concentrations were not affected by the noise exposure.

	BL	45-Highway-IR30	45-Road-IR70	45-Urban-street-IR80	45-Railway-IR90	RC	P value
Hemodynamic variables							
HR (bpm)	56.24 ± 1.34	55.00 ± 1.19	54.79 ± 1.24	54.77 ± 1.37	55.87 ± 1.41	55.61 ± 1.39	0.24
Systolic BP (mmHg)	115 ± 2.54	110 ± 2.39	111 ±2.23	109 ± 2.37	109 ± 2.43	107 ± 3.79	0.16
Diastolic BP (mmHg)	72 ± 1.41	66 ± 1.76 *	69 ± 1.54	65 ± 1.69 *	69 ±1.28	68 ± 1.61	0.001
Time domain analysis							
pNN50 (%)	47 ± 3	47 ± 3	46 ± 3	48 ± 3	48 ± 3	46 ± 3	0.43
SDNN (ms)	97.4 ± 4.20	99.8 ± 3.99	98.6 ± 4.43	103 ± 4.88	104 ± 5.35 *	97.4 ± 4.49	0.01
RMSSD (ms)	79.7 ± 5.00	79.5 ± 5.03	78.1 ± 4.85	82.0 ± 5.34	86.0 ± 6.55	77.8 ± 5.71	0.12
Spectral analysis							
LFn (ms^2)	0.55 ± 0.020	0.57 ± 0.023	0.57 ± 0.023	0.57 ± 0.022	0.57 ± 0.023	0.56 ± 0.026	0.61
HFn (ms^2)	0.45 ± 0.020	0.43 ± 0.023	0.43 ± 0.023	0.43 ± 0.022	0.43 ± 0.02	0.44 ± 0.026	0.55
LF/HF (ms^2)	1.79 ± 0.19	2.22 ± 0.35	2.08 ± 0.26	2.07 ± 0.30	2.11 ± 0.32	2.02 ± 0.28	0.35

Table V.2: Overnight hemodynamic and HRV variables*. Mean ± SE. *, p<0.05 in comparison to BL.*

3.2. CVD markers following nocturnal noise exposure

Morning cytokines IL-6 ($F_{2,44}$=2.24, p=0.12), TNFα ($F_{2,42}$=1.33, p=0.28), CRP ($F_{2,44}$=0.86, p=0.43) and the adipokines adiponectin ($F_{2,44}$=1.77, p=0.18) and leptin ($F_{2,42}$=1.64. p=0.21) were not affected by nocturnal noise exposure.

Diurnal cortisol profiles are illustrated in *Figure V.3.* The rate of decrease of the cortisol profile was not significantly altered by the preceding noise exposure ($F_{4,95}$=0.36, p=0.84). CAR tended to be marginally higher after sleeping with 45-Railway-IR90 compared to BL (p=0.08) and 45-Road-IR70 (p=0.09, *Figure V.3*). Evening cortisol levels were higher after sleeping with 45-Urban-street-IR80 compared to BL (p=0.002) (*Figure V.3*). The percentage increase in evening cortisol between BL and 45-Urban-street-IR80 was correlated with the percentage increase in cumulative duration of AA (*Figure V.4).*

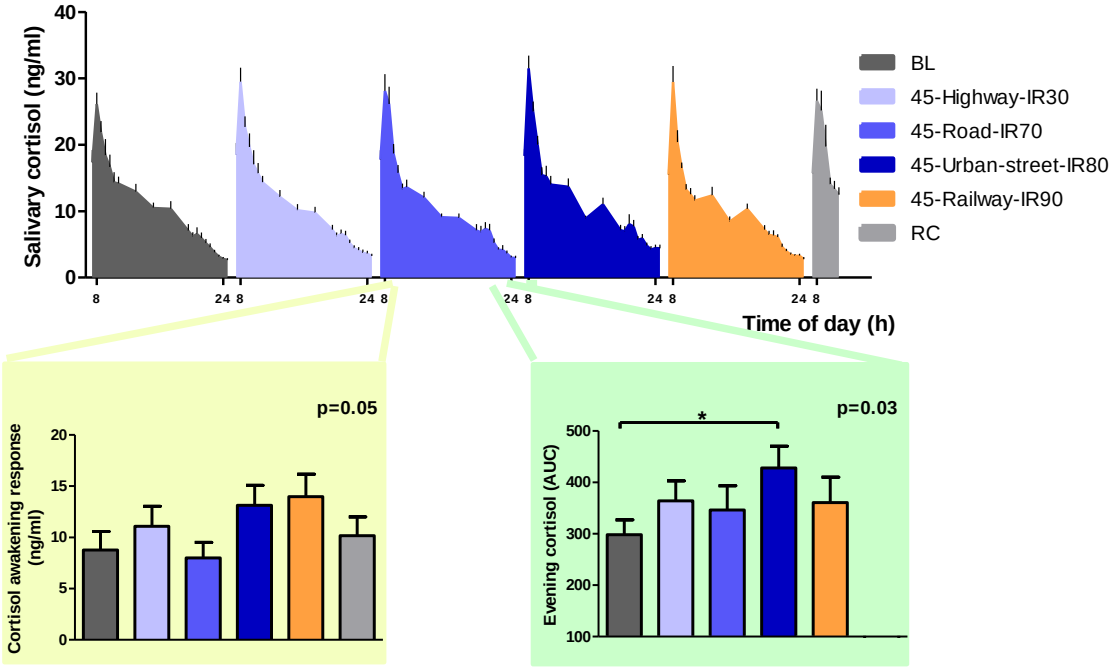

Figure V.3: Upper panel: Diurnal salivary cortisol profiles. Lower panel: Cortisol awakening responses and evening cortisol levels after sleeping with different noise scenarios. *BL and RC after sleeping with the noise-free scenario; 45-Highway-IR30, 45-Road-IR70, 45-Urban-street-IR80 and 45-Railway-IR90 after sleeping with the corresponding noise scenario. AUC: Area under the curve. Mean ± SE.*

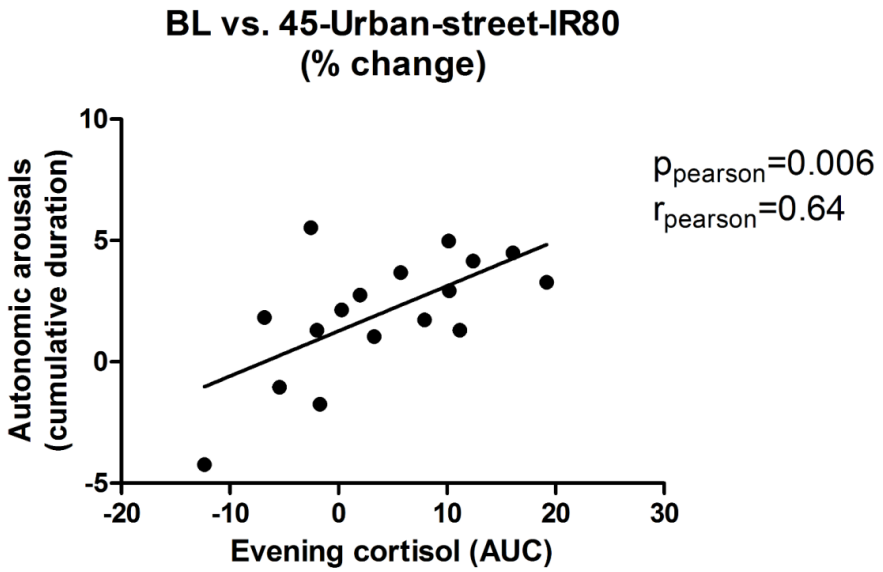

Figure V. 4: Relative change in cumulative duration of AA and next-day evening cortisol between BL and 45-Urban-street-IR80 noise scenario.

4. DISCUSSION

We aimed at determining the importance of sleep alterations as a potential mediating factor of nocturnal transportation noise related cardiovascular impairments. Additionally, we examined whether the IR is an acoustical marker for transportation noise effects on cardiovascular functions.

Effect of nocturnal transportation noise on sleep and nocturnal cardiovascular markers

In contrast to the subjective feeling of a better recovery night, sleep macrostructure and the amount of CA were not affected by noise exposure. CA are usually accompanied by AA but the reverse is not true (Sforza et al., 2000). AA are good candidates for promoting nocturnal transportation noise induced CVD (Basner et al., 2011; Griefahn et al., 2008). While we did not find more AA compared to BL, the mean duration was higher for 45-Railway-IR90 and total duration was higher during the noise nights 45-Highway-IR30, 45-Urban-street-IR80 and RC. Surprisingly, full-night spectral analysis of HRV reflected no changes in autonomic nervous activity, probably this analysis is less sensitive to detect subtle short-term changes. Catecholamines are also important markers of sympathetic activity. The effect of nocturnal transportation noise on these hormones are mixed: one laboratory study found higher overnight urinary levels (Maschke et al., 1993), while others did not (Carter et al., 1994; Maaß & Basner, 2006). Again, full-night values are possibly too rough to detect subtle acute variations. Global HRV obtained from SDNN increased during the exposure to 45-Railway-IR90 compared to the BL. Low HRV has been associated with increased risk of a first cardiovascular event (Hillebrand et al., 2013). However, increased variability also mirrors the increase in AA as the relative change from BL to 45-Railway-IR90 of SDNN was positively correlated with the number of AA. Nocturnal mean HR and BP were not affected by the presence of transportation noise, a result consistent with Schmidt et al. (Schmidt et al., 2013). Haralabidis (Haralabidis et al., 2008) reported an increase in BP but it was minor with only +0.81 mmHg per 5dB increase in LAmax,indoor road noise for systolic BP.

Effect of nocturnal transportation noise exposure on diurnal cortisol levels

Using 20 saliva samples per day, we were able to quantify the circadian baseline secretion profile with a peak level in the morning, followed by a decline over the day to reach minimum levels in the late evening (Horrocks et al., 1990). The rate of decrease of the cortisol profile was not changed after sleeping with transportation noise. In accordance with previous studies (Griefahn & Robens, 2010; Waye et al., 2003), CAR was not affected by nocturnal transportation noise exposure. Based on sleep deprivation studies, evening hours may be a more critical time window for noise induced HPA dysregulation (Leproult et al., 1997; Spiegel et al., 1999). Lefèvre et al. (2017) reported an increase in the field, and our laboratory study confirms this finding with increased evening cortisol after sleeping with 45-Urban-street-IR80 compared to BL. Moreover, this increase correlated with the increase in the cumulative duration of AA revealing the role of subcortical activation in the noise-stress response pathway of sleeping individuals.

Limitations

The laboratory study strictly controlled possible confounders such as sleep duration, caloric intake, light exposure and physical activity. Even though participants had an adaptation night in the laboratory prior to the study, the unusual environment while wearing various devices could have led to the so-called “first-“ or “last night effect”. This could explain the lack of changes in sleep variables, longer sleep latency during RC and higher diastolic BP during BL compared to the other nights. We studied a low-risk population (non-noise sensitive healthy good sleepers) which is not representative of the overall population. Finally, the level of LAeq,indoor=45dB corresponding to LAeq,outdoor=60dB for a tilted window (Locher et al., 2018) may not have been high enough to elicit strong acute health effects.

Summary and conclusion

To the best of our knowledge, this is the first study integrating polysomnographically assessed sleep variables, AA, stress hormones, hemodynamic, HRV and inflammatory markers under controlled laboratory conditions. The exposure to nocturnal transportation noise, which was perceived as more annoying than the noise-free baseline and recovery nights, had no effect on sleep macrostructure, nocturnal mean HR, BP and catecholamine levels and did not lead to inflammation in young healthy adults. Nevertheless, the cumulative duration of AA increased during the highly intermittent road noise scenario and was correlated with the increase in next evening cortisol level. This result confirms previous studies that indicated that a low LAeq (≤45dB) nocturnal noise might affect the cardiovascular function by a subcortical activation (Basner et al., 2011; Griefahn et al., 2008). Thus, cardiovascular outcomes are sensitive markers for the very early negative impact of nocturnal transportation noise before cortical sleep alterations may occur.

Acknowledgments

We thank our volunteers for their participation, our staff team, students, civil servants, nurses, and interns for their help in conducting the study, Dr. Martin Meyer and Dr. Helen Slawik for the medical screening. We also thank Dr. Antoine Viola for his advises in the study design.

5. APPENDIX

Variable	N subjects	N samples per subjects	N total samples
EEG	26	6	150
ECG	26	6	150
Autonomic arousals	25	6	140
Blood pressure	23	6	123
Catecholamine	19	6	114
Cortisol awakening response	26	6	150
Evening cortisol	26	5	124
Cytokines	23	3	69

Table V.A: Number of subjects and samples for each variable analyzed. *EEG: electroencephalogram, ECG: electrocardiogram.*

Chapter VI

Autonomic arousals during sleep and next-day glucose and cortisol regulation in response to nocturnal transportation noise exposure: age and sex differences

Keywords: Traffic noise, Intermittency, vegetative activation, glucose tolerance, insulin sensitivity, evening cortisol

About to be submitted to Environmental Research (IF: 4.7)

ABSTRACT

Strong associations between long-term transportation noise exposure and cardio-metabolic disorders have been reported in epidemiological studies, particularly for older individuals. We have recently shown that short-term exposure to nocturnal transportation noise is linked to longer duration of autonomic arousals during sleep and increased next-day glucose and cortisol levels in healthy young adults. Here we test, whether these findings depend on individual factors such as age and sex, which are known to considerably influence cardio-metabolic health.

Twenty-six young (12 women, 24.6 ± 0.7 y, mean ± SE) and 16 older healthy volunteers (8 women, 60.8 ± 1.5 y) participated in a six day laboratory study. The first and last night comprised a noise-free baseline (BL) and a noise-free recovery night (RC). In-between, four different noise scenarios with an identical equivalent continuous sound level (45dB) but different noise sources (road or rail) and intermittencies (low, medium and high), were randomly presented during four 8-h noise nights. Sleep was polysomnographically recorded and daytime cortisol levels were assessed 20 times per day. Glucose tolerance ($glucose_{AUC}$: area under the curve of the glucose profile and G_{120}: glucose level 2h after oral glucose intake) and the Stumvoll insulin sensitivity index (ISI) were calculated during an oral glucose tolerance test (OGTT) in the morning after BL, after the last noise night and after RC.

Older participants slept less efficient ($p<0.0001$), had a shorter autonomic arousal duration ($p=0.03$), and a higher glucose response in the OGTT ($glucose_{AUC}$; $p=0.04$) than the younger individuals. Age and sex did not significantly influence noise-induced increase in autonomic arousal duration and decrease in glucose tolerance and insulin sensitivity. In contrast, the next-day increase in evening cortisol levels was only observed in the younger participants after sleeping with highly intermittent road noise ("age x noise" interaction: $p=0.02$). Irrespective of age and sex, the increase in cumulative AA duration ($p=0.01$) was significantly associated with the increase in next-day glucose tolerance assessed with G_{120} ($p<0.0001$, correlation: $r_{pearson}=0.42$, $p=0.02$).

We do not have strong evidence for age and sex differences in the short-term effects of nocturnal transportation noise on autonomic arousal duration, glucose tolerance and insulin sensitivity in healthy individuals. Only younger participants had increased evening cortisol levels after sleeping with highly intermittent road noise. We conclude that autonomic activation in response to acute transportation noise during sleep is a key mediator of next-day impaired glucose tolerance mostly independent of age and sex in healthy individuals.

1. INTRODUCTION

There is epidemiological evidence for associations between long-term nocturnal transportation noise exposure and cardiovascular diseases (Héritier et al., 2018; Jarup et al., 2008) and diabetes (Eze, Foraster, et al., 2017; Sorensen et al., 2013). Field and laboratory studies reported increased cardio-metabolic risk factors such as increased blood pressure (BP) (Haralabidis et al., 2008), increased stress hormones (Lefèvre et al., 2017; Selander, Bluhm, et al., 2009) or disturbed sleep in response to nocturnal transportation noise exposure (Basner et al., 2011; Griefahn et al., 2006). We have recently reported that already four nights with transportation noise exposure of only LAeq,1h = 45 dB at the ear of the sleeper in the sleep laboratory impaired glucose tolerance and insulin sensitivity without impairing sleep macrostructure in healthy young adults (Thiesse, Rudzik, Spiegel, et al., 2018). Moreover, nocturnal highly intermittent road noise exposure increased next day evening cortisol levels, paralleled with an increase in nocturnal cumulative duration of autonomic arousals (AA) (Thiesse, Rudzik, Krämer, et al., 2018). Thus, the clinical manifestations of long-term effects of nocturnal transportation noise on cardiovascular diseases and diabetes observed in epidemiological studies, may already start after a few nights of noise exposure as indexed by subtle but significant short-term detrimental effects. Most of these long-term studies included a broader age range with young and older individuals, and some associations between transportation noise and health outcomes, such as stroke, are age dependent (Sorensen et al., 2012). As physiological aging impacts cardio-metabolic health (Brewer et al., 2016; North & Sinclair, 2012), we reanalyzed the main outcomes of our previous studies for their age dependency. Some epidemiological studies also noticed sex differences in the response to transportation noise exposure with increased cortisol levels (Selander, Bluhm, et al., 2009) and higher risk of type 2 diabetes incidence in women (Eriksson et al., 2014) and higher risk of hypertension (Eriksson et al., 2010), ischemic heart disease (Vienneau, Schindler, et al., 2015) and myocardial infarction (Babisch et al., 2005) in men. However, the results are ambiguous as some studies showed no sex differences (Evrard et al., 2017; Jarup et al., 2008; Lefèvre et al., 2017; Selander, Nilsson, et al., 2009).

Thus, here we aimed at determining the impact of age and sex on the previously observed detrimental effects of nocturnal transportation noise on AA during sleep and next-evening cortisol (Thiesse, Rudzik, Krämer, et al., 2018) and glucose regulation (Thiesse, Rudzik, Spiegel, et al., 2018). We hypothesized stronger detrimental noise-effects for older individuals who are already more vulnerable for cardio-metabolic diseases than the young and also for women who seem more sensitive to noise-induced cortisol and glucose impairment than men.

2. METHODS

2.1. Study participants

Participants were healthy non-smokers free of any medication (including hormonal substitutes) as assessed by the clinical history, physical examination, routine blood and toxicological urine screening. Participants habitually slept 7 to 9 hours per night and reported good sleep quality (Pittsburgh Sleep Quality Index, (Buysse et al., 1989) PSQI ≤ 5) and no daytime sleepiness (Epworth Sleepiness Scale, (Johns, 1991) ESS ≤ 10). Volunteers with sleep disorders (diagnosed during an adaptation night), shift work, extreme chronotypes (Munich Chronotype Questionnaire, [34] MCTQ < 2 or MCTQ ≥ 7) or hearing loss were excluded. Noise sensitivity was assessed using the short version of the German *Lärmempfindlichkeitsfragebogen* (LEF-K) (Zimmer & Ellermeier, 1998) and the *Noise Sensitivity Questionnaire* (NoiSeQ) (Schutte et al., 2007). Both noise sensitivity questionnaires were not part of the selection criteria.

All participants gave written informed consent. The protocol was approved by the local ethics committee (EKNZ/Ethikkommission Nordwest- und Zentralschweiz, Switzerland), and conformed to the Declaration of Helsinki.

2.2. Procedure

One week prior to the study, participants were asked to follow their habitual bedtimes within ± 30 minutes, spending 8 hours in bed and not taking naps. Compliance was assessed via actigraphy (Actiwatch L, Cambridge Neurotechnologies, Cambridge, UK). Additionally, participants were asked to avoid stimulating substances (coffee, tea, chocolate) and alcohol, to eat as usual without extreme fatty meals and to avoid extreme physical activities in order to match the laboratory conditions as best as possible.

Participants spent five 24-h days and one last morning in the laboratory in individual bedrooms. Light intensity during the daytime was kept constant (between 50 and 150 lux at participant's eye) and room temperature was set at 22°C. Participants had an 8-h sleep opportunity per night scheduled at their habitual bedtimes. Meal timing was adjusted to the participants wake up time and consisted of three calorie-adjusted, using Mifflin et al. (1990) formula, main meals (breakfast, lunch and dinner). The study protocol is illustrated in *Figure VI.1*.

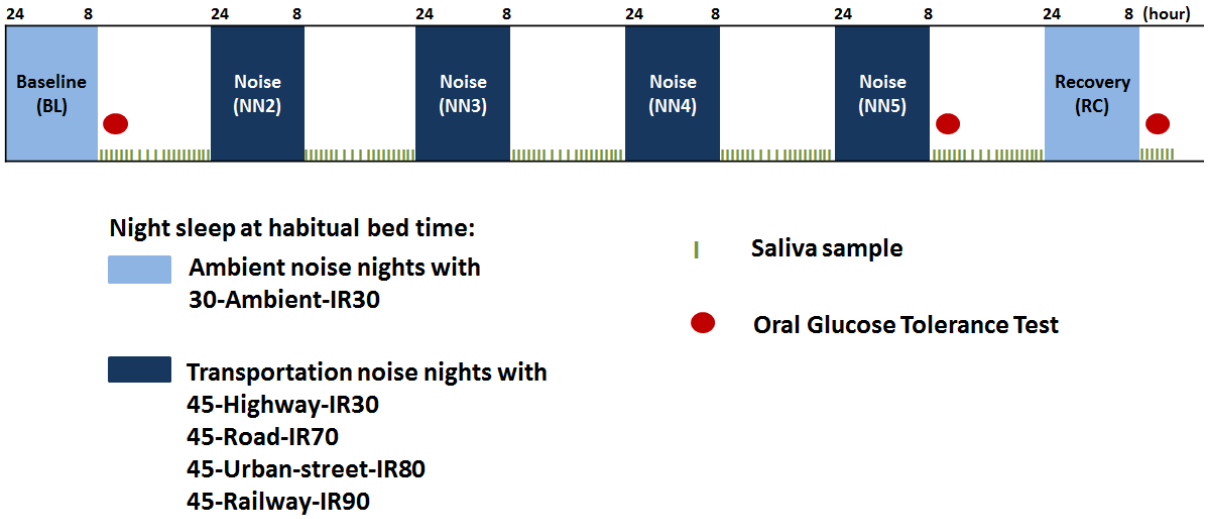

Figure VI.1: Schematic illustration of the study protocol. *Time in bed was scheduled according to each participant's habitual bedtimes (e.g. 24-8h). The order of exposure to the four different transportation noise scenarios was randomized between participants. Noise scenario: «LAeq,1h-Noise source-IR (intermittency ratio)».*

2.3. Noise exposure

Noise exposure characteristics were extensively described in previous publications with this dataset (Rudzik et al., 2018; Thiesse, Rudzik, Spiegel, et al., 2018). In short, all participants started and ended the study respectively with a noise-free baseline (BL) and recovery (RC) night during which a quiet ambient scenario (30-Ambient-IR30) was applied (*Figure VI.1*). In between, participants were randomly exposed to four different noise scenarios which had eight identical hourly equivalent continuous sound pressure levels (*L*Aeq,1h=45 dB at the ear of the sleeper) during lights-OFF to lights-ON. The noise scenarios differed with respect to the noise source (road or railway) and IR, a metric which describes the share of individual noise events compared to the overall sound exposure (Wunderli et al., 2015) - for details see *Table VI.*1. During the last morning of the study, participants were asked to retrospectively evaluate noise annoyance during each night on a scale of 0 to 100 with the question "How annoyed were you during the night by the noise"?

Scenario	Source	LAeq,1h [dB]	LAFmax [dB]	IR
30-Ambient-IR30	ambient	30	39	0.3
45-Highway-IR30	road	45	53	0.3
45-Road-IR70	road	45	60	0.7
45-Urban-street-IR80	road	45	62	0.8
45-Railway-IR90	rail	45	62	0.9

Table VI.1: Acoustical characteristics of the noise scenarios. *30-Ambient-IR30 represents the noise-free scenario of BL and RC nights. The four transportation noise scenarios were randomly introduced during the noise nights. LAeq,1h: equivalent continuous sound level, LAFmax: maximum sound level, IR: intermittency ratio (Wunderli et al., 2015).*

2.4. Sleep-related measurements

Subjective sleep quality (SSQ) was assessed 5-10 min upon awakening with the LEEDS Sleep Evaluation Questionnaire (Parrott & Hindmarch, 1978).

Polysomnographic sleep recordings (PSG) included 12 electroencephalographic, 2 electro-oculographic and 2 electromyographic derivations (Vitaport-3 digital recorder). Sleep efficiency (SE, percentage of total sleep time (TST: time spent in REM and Non-REM sleep) between lights-OFF and lights-ON), was scored according to standard criteria (Berry et al., 2016). Further details on sleep measurements and sleep variables are reported in Rudzik et al. (2018).

2.5. Glucose metabolism

Glucose metabolism was assessed via a two hours long 75-g oral glucose tolerance test (OGTT) administered one hour after awakening from BL, NN5 and RC, 30 min after inserting the venous catheter (*Figure VI.1*). Two fasting (t-15 and t0) and six post-load (time points: t10, t20, t30, t60, t90 and t120) blood samples were collected.

Assays Blood was distributed in Na-fluorid tubes and immediately centrifuged for plasma glucose measurement. Serum was obtained from another tube after 30 min clotting at room temperature for insulin measurement. Tubes were centrifuged at 4°C for 10 min at 3500rpm. All samples were then stored at -80°C until assay. Plasma glucose was assayed via the hexokinase method (Glucose GOD-PAP test, Roche) with a limit of sensitivity of 0.11 $mmol.L^{-1}$ and an intra-assay variation coefficient of 0.9%. Serum insulin was measured with an ELISA test (80-INSHU-E01.1; ALPCO) with a limit of sensitivity of 2.78 $pmol.L^{-1}$ and an intra-assay variation coefficient of 6%.

Measures The area under the curve (AUC) for glucose and insulin was calculated using the trapezoidal rule. Glucose tolerance was assessed by calculating $glucose_{AUC}$ and via glucose concentration at t120 (G_{120}). Insulin sensitivity was estimated using the the Stumvoll ISI index ($0.226 - 0.0032 \times BMI - 0.0000645 \times I_{120} - 0.00375 \times G_{90}$, where I_{120} and G_{90} represent insulin concentration at t120 and glucose concentration at t90 respectively (Stumvoll et al., 2000)).

2.6. Salivary cortisol

Saliva samples were obtained every 30 min during the first 3h after wake-up, 4h30 before lights-OFF and every 2h in-between. Samples were kept at -20°C until assay. Cortisol was measured using a direct salivary enzyme-linked immunosorbent assay (ELISA) with a limit of sensitivity of 1.0 ng/mL and a mean intra-assay variation coefficient (CV) <10% (ALPCO Diagnostics, Salem, NH, USA). The daytime trend in salivary cortisol was quantified using the best-fit curve based on periodogram

calculations (Van Cauter, 1979). The AUC during the last 2h before lights-OFF was calculated to assess evening cortisol levels.

2.7. Autonomic arousals

AA, defined as a sudden increase in HR followed by a return to initial values, were detected via the Somno-Art methodology, based on an algorithm developed by Muzet et al. (Muzet et al., 2016). The algorithm used 1Hz HR and 1Hz wrist actimetry obtained from the SOMNOtouch^{TM} device. AA were detected and quantified as changes in the steady state of average HR to higher or lower values and were often co-occurring with body movements. AA occurring during wakefulness were excluded.

2.8. Statistical analysis

Statistical analyses were performed using the SAS statistical software package (SAS Institute Inc., Cary, NC; version 9.4). Baseline characteristics were compared between the young and older group with a t-test. Two types of mixed model analysis of variance were carried out for each outcome variable (noise annoyance, SSQ, SE, cumulative AA duration and evening cortisol) separately depending on the repeated within-participant factor "noise scenario" (BL, 45-Highway-IR30, 45-Road-IR70, 45-Urban-street-IR80, 45-Railway-IR90, RC) or "night" (BL, NN2, NN3, NN4, NN5, RC). The outcome variables $glucose_{AUC}$, ISI, G_{120} were only analyzed with the repeated within-participant factor "night" (BL, NN5, RC). All the models included the fixed factors "age" (young/older) and "sex" (men/women) and the random factor "participant" with a compound symmetry covariance structure. Interactions "noise x age", "noise x sex" and "noise x age x sex" as well as "night x age", "night x sex" and "night x age x sex" were also analyzed. Contrasts were assessed with the LSMEAN statement and p-values were based on Kenward-Roger's corrected degrees of freedom (Kenward & Roger, 1997). Multiple post-hoc comparisons were corrected using the Tukey-Kramer method. Data were log-transformed when residuals were not normally distributed. Correlations were calculated with the Pearson or Spearman correlation coefficient, as appropriate. Correlation outliers were detected and removed according to the interquartile method (Tukey, 1977). Data are presented as mean ± standard error and statistical significance was set at $p<0.05$. We excluded one older woman and one older man from analysis, since they quit the experiment due to medical reasons (severe back pain that required pain medication and general discomfort and headache). One older man was excluded from glucose metabolism analysis because of impaired glucose tolerance already at baseline (WHO, 2016). For all pre-menopausal women, except two, the entire lab protocol was conducted during the follicular phase; the two remaining women did not influence statistical significances. Missing values, mainly due to technical reasons are summarized in the following table as well as the exact number of individuals and samples for each variable (*Table VI.2*).

Variable	N participants	N samples per participant	N total samples
Annoyance	42	6	248
SSQ	42	6	248
SE	42	6	242
AA duration	40	6	234
$Glucose_{AUC}$	37	3	111
G_{120}	37	3	111
ISI	37	3	108
Evening $cortisol_{AUC}$	42	5	199

Table VI.2: Number of participants and samples for each outcome variables analyzed. *Annoyance: noise annoyance, SSQ: subjective sleep quality, SE: sleep efficiency, AA duration: cumulative AA duration, $Glucose_{AUC}$: area under the curve of the OGTT glucose profile, G_{120}: glucose concentration 2 hours after glucose intake, ISI: Stumvoll ISI index and evening cortisol.*

3. RESULTS

3.1. Study population

Twenty-six young and sixteen older lean volunteers participated in the study. Their baseline characteristics are summarized in *Table VI.3*. Older participants were significantly more noise sensitive, and their sleep efficiency was significantly reduced in comparison to the younger volunteers.

	Young	Older	T-test (p-value)
Demographics			
N (w, m)	26 (12, 14)	16 (8,8)	
Age (range)	24.6 ± 0.69 (19-33)	60.8 ± 1.48 (52-70)	**<0.0001**
Baseline BMI (kg/m^2)	22.2 ± 0.41	22.0 ± 0.53	0.86
Noise sensitivity			
LEF-K (0-27)	11.0 ± 0.79	14.4 ± 0.89	**0.007**
NoiSeQ Global (0-3)	1.29 ± 0.08	1.64 ± 0.09	**0.01**
NoiSeQ Sleep (0-3)	1.12 ± 0.13	1.39 ± 0.14	0.17
Baseline sleep efficiency (%)	93.9 ± 0.86	89.2 ± 1.40	**0.004**
Baseline fasting glucose (mmol.L-1)	5.04 ± 0.08	5.23 ± 0.12	0.16
Baseline fasting insulin (pmol.L-1)	32.9 ± 3.48	30.4 ± 5.92	0.73
Baseline evening cortisol (AUC)	298 ± 29	387 ± 38	0.07

Table VI.3: Baseline characteristics of the study population corresponding to the first-night in the laboratory. *P-values were calculated between young and older using a T-test.*

3.2. Nocturnal noise annoyance, sleep quality and AA duration

Effect of noise exposure

For all participants, noise annoyance was significantly higher during the transportation noise nights compared to the noise-free BL and RC nights and SSQ was better during RC compared to 45-Highway-

IR30, 45-Road-IR70 and 45-Urban-street-IR80 (see *Table VI.4a*). SE was lower for older compared to younger participants and tended to be higher for women than for men. However, irrespective of age and sex, noise exposure did not differentially influence SE (*Table VI.4a*). Older volunteers had shorter cumulative duration of AA compared to the younger volunteers, and men's AA duration was in general longer compared to the women (*Table VI.4a*). Irrespective of age and sex, the cumulative duration of AA increased during 45-Railway-IR90 compared to BL (*Figure VI.2*).

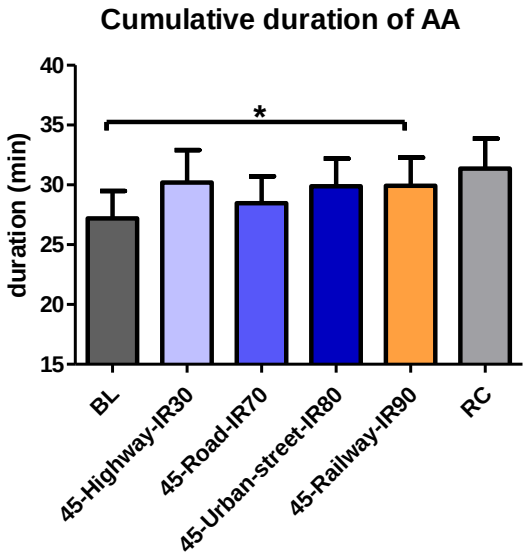

Figure VI.2: Mean cumulative duration of autonomic arousals for each noise scenario. During BL (baseline) and RC (recovery) nights participants slept with an ambient noise scenario (30-Ambient-IR30). *Four different transportation noise scenarios were randomly presented during the nights in-between. *p<0.05. Mean + SEM.*

Temporal order effect of laboratory nights

Noise annoyance was lower during BL and RC compared to the other nights and lower during NN2 compared to NN5 (*Table VI.4b*). Irrespective of age and sex, SSQ was higher during NN3 compared to NN4 ($p=0.04$) and during RC compared to NN4 ($p<0.001$) and NN5 ($p<0.001$). SE showed a "night x age" interaction; only older participants had higher SE during NN2 and NN3 compared to NN4, NN5 and RC. Cumulative AA duration was higher during NN3 compared to BL, probably because 15 out of the 40 were exposed to 45-Railway-IR90 during NN3.

Variable	Annoyance	SSQ	SE	AA	$Glucose_{AUC}$	G_{120}	ISI	Evening $cortisol_{AUC}$
Noise scenario	**<0.0001**	**0.01**	0.17	**0.03**	NA	NA	NA	0.31
Age	0.37	0.43	**<0.0001**	**0.03**	NA	NA	NA	0.17
Sex	0.10	0.29	0.05	**0.0002**	NA	NA	NA	0.96
Noise x age	0.39	0.78	0.42	0.17	NA	NA	NA	**0.03**
Noise x sex	0.65	0.70	0.06	0.60	NA	NA	NA	0.25
Noise x age x sex	0.20	0.71	0.70	0.14	NA	NA	NA	**0.02**
b. Mixed model with the repeated-within factor "night"								
Variable	Annoyance	SSQ	SE	AA	$Glucose_{AUC}$	G_{120}	ISI	Evening $cortisol_{AUC}$
Night	**<0.0001**	**<0.0001**	**0.0004**	**0.01**	**<0.0001**	**<0.0001**	**0.001**	0.24
Age	0.39	0.41	**<0.0001**	**0.03**	**0.04**	0.32	0.47	0.19
Sex	0.10	0.29	0.05	**0.0002**	0.06	0.15	0.25	0.96
Night x age	0.65	0.90	**0.006**	0.66	0.19	0.20	0.70	0.55
Nigth x sex	0.65	0.75	0.40	0.67	0.73	0.58	0.68	0.89
Night x age x sex	**0.04**	0.97	0.59	0.46	0.80	0.70	0.15	0.07

Table VI.4: P-values of the main and interaction effects of the mixed models for the outcome variables *Annoyance: noise annoyance, SSQ: subjective sleep quality, SE: sleep efficiency, AA: cumulative AA duration, $Glucose_{AUC:}$ area under the curve of the OGTT glucose profile, $G_{120:}$ glucose concentration 2h after glucose intake, ISI: Stumvoll insulin sensitivity index and evening $cortisol_{AUC:}$ area under the curve for evening cortisol. a. mixed models with the repeated within factor "noise scenario" b. mixed models with the repeated within factor "night".*

3.3. Morning glucose metabolism

Figure VI.3 illustrates glucose and insulin profiles in response to an OGTT in the morning after BL, after NN5 and after RC. The glucose response to an OGTT ($glucose_{AUC}$) was higher for the older compared to younger participants and for the men compared to the women (*Table VI.4b*). However, $glucose_{AUC}$ and G_{120} increased irrespective of age and sex after NN5 and RC compared to BL. Stumvoll ISI index decreased irrespective of age and sex in the morning of NN5 and RC compared to BL.

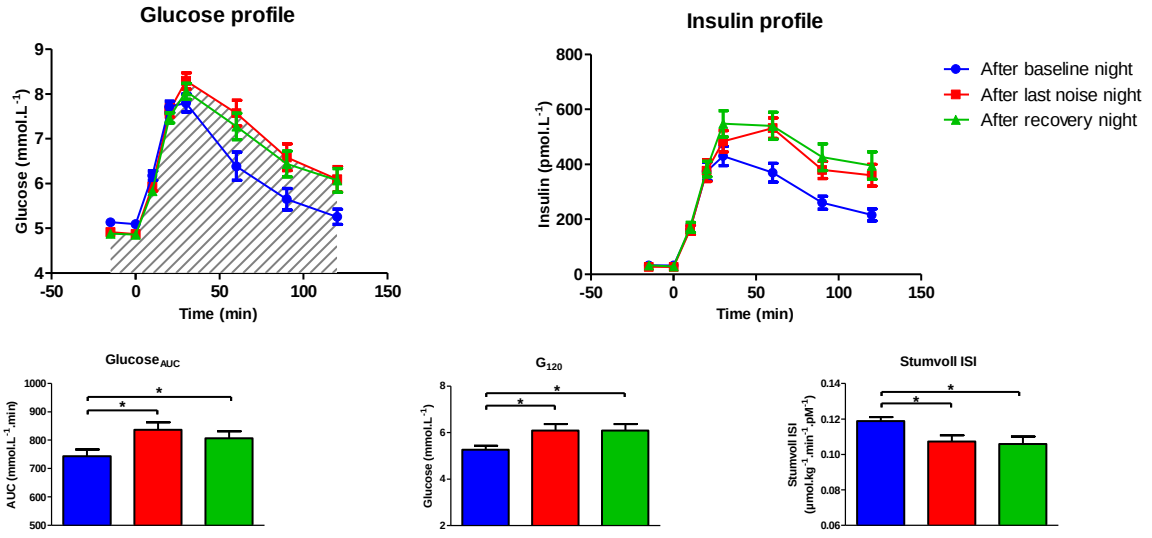

Figure VI.3: Mean glucose and insulin profile during an oral glucose tolerance test (OGTT) in the morning after BL (blue), after NN5 (red), and after RC (green) in the young and older participants (pooled). *Histograms represent $Glucose_{AUC}$ (a), G_{120} (b) and Stumvoll ISI (c) for each OGTT condition. *p<0.05. Mean ± SEM.*

3.4. Evening cortisol

Effect of noise exposure

The main factor "noise scenario" did not yield significance for evening cortisol levels (*Table VI.4a*). However, the interaction term "noise x age", illustrated in *Figure VI.4*, exhibited a different response between the age groups with unaffected evening cortisol levels in the older, while the younger participants presented higher levels after sleeping with 45-Urban-street-IR80 compared to BL (p=0.04). Furthermore, *Figure VI.A* in the appendix illustrates the interaction term "noise x age x sex" which did not yield any significant post-hoc comparisons.

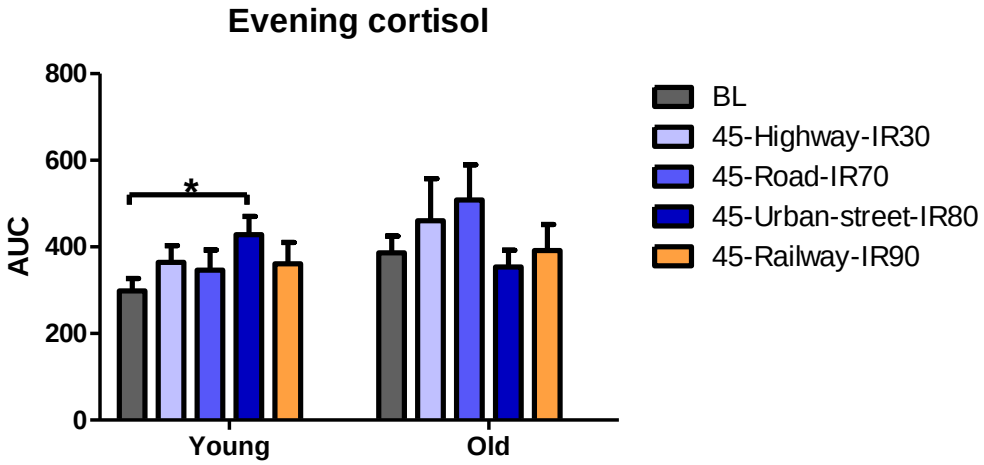

Figure VI.4: Mean evening cortisol level after the four different nocturnal noise scenarios for the young and older group. **p<0.05. Mean + SEM.*

Temporal order effect of laboratory days

We could not observe significant temporal order effects of laboratory days on evening cortisol levels in the course of the experiment (*Table VI.4b*).

3.5. Age and sex differences in the association between cumulative AA duration, glucose regulation and evening cortisol

The observed impairment in glucose regulation variables $glucose_{AUC}$, G_{120} and ISI were not related to changes in preceding evening cortisol levels (respectively, $r_{pearson}$=-0.11 p=0.54, $r_{pearson}$=0.04 p=0.84, $r_{pearson}$=0.19 p=0.29). Irrespective of sex and age, the increase in G_{120} observed on NN5 compared to BL was associated with changes in cumulative AA duration (*Figure VI.5*). Cumulative AA duration did not correlate with $glucose_{AUC}$ ($r_{pearson}$=0.01, p=0.96) and ISI ($r_{pearson}$=0.02, p=0.92). Finally, next-day evening cortisol level was not related to changes in cumulative AA duration ($r_{pearson}$=0.30, p=0.11) or $glucose_{AUC}$, G_{120} and ISI (respectively, $r_{pearson}$=0.21 p=0.24, $r_{pearson}$=0.34 p=0.06, $r_{pearson}$=0.24 p=0.16).

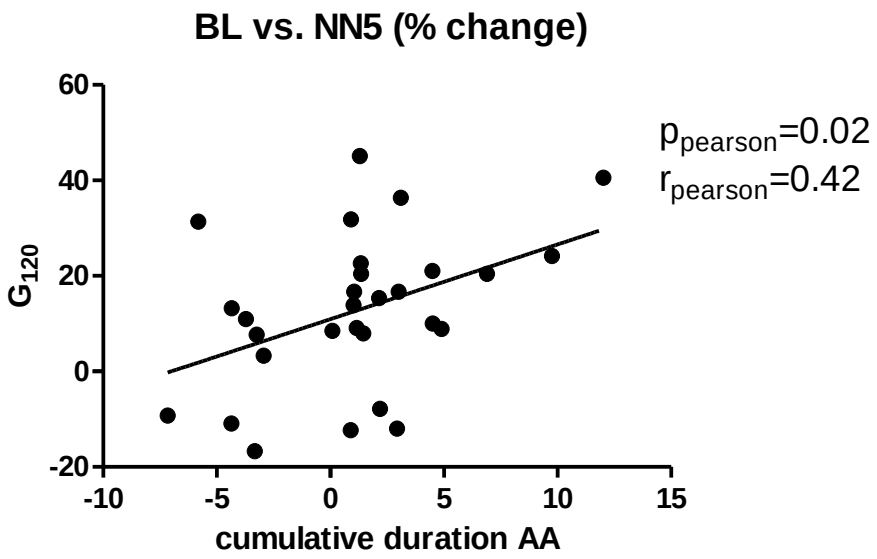

Figure VI.5: Correlation between the relative change in sleeping cumulative AA duration and next-morning G_{120} during NN5 compared to BL.

4. DISCUSSION

We confirm an age-related reduction in glucose tolerance and sleep efficiency in healthy volunteers without sleep problems, which was unexpectedly paralleled by an age-dependent shortening of AA during sleep. Men had significantly longer duration of AA than women, who tended to sleep more efficient than men in our laboratory study. We observed a significant increase in AA duration during exposure to nocturnal transportation noise which did not show age- or sex dependency. Furthermore, next-evening cortisol levels did not yield sex differences but increased only in the young group after exposure to highly intermittent road noise. Interestingly, the increase in cumulative AA duration during exposure to nocturnal traffic noise correlated with the increase in glucose concentration the next-morning at t120 for the entire study group.

Age effects

As expected, older healthy participants had a lower sleep efficiency and lower glucose tolerance compared to the young (Mander et al., 2017; Shimokata et al., 1991). AA were shorter in the older than young participants most likely because the overall heart rate variability decreases with age (Antelmi et al., 2004) due to an imbalance of the sympatho-vagal regulation, leading to difficulties of coping dynamically with environmental stressors (Struzik et al., 2006). Moreover, AA duration significantly correlated with the SDNN (standard deviation of normal to normal R-R intervals) heart rate variability index (outcome described previously in (Thiesse, Rudzik, Krämer, et al., 2018)) ($r_{pearson}$=0.44 p=0.01). Surprisingly, baseline evening cortisol levels only tended to be higher for older volunteers (*Table VI.4*, p=0.07), although aging has been reported to be associated with increased

evening cortisol levels (Van Cauter et al., 1996). This could be related to the fact that we only included very healthy older participants in our study. Furthermore, we could not observe significant changes in evening cortisol levels across the entire six-day stay in the laboratory in both age groups, which points to a rather stress-free laboratory environment. Irrespective of age, nocturnal exposure to transportation noise increased cumulative AA duration, decreased glucose tolerance and insulin sensitivity. The absence of age differences was also reported for transportation noise induced changes in sleep macrostructure (Saremi et al., 2008). Even though older participants reported to be more noise sensitive, only the young individuals exhibited increased evening cortisol levels in response to nocturnal highly intermittent road noise exposure in our study. As cortisol levels tended to be higher in older individuals, the magnitude of the response to a stressor may be smaller. Evening cortisol also yielded a three-way interaction (noise x age x sex) without any significant post-hoc comparisons, but with a trend for a higher response to nocturnal transportation noise exposure in younger men and older women than in younger women and older men (*Figure VI.A* of appendix). This sex-dependent age difference has been observed in response to acute daytime stress with stronger responses for younger men compared to younger women and for older women compared to older men (Seeman et al., 2001). The authors suggest that women experience a greater age-related increase in HPA axis response to a stressor than men (Seeman et al., 2001).

Sex effects

No sex differences were visible neither for glucose tolerance and insulin sensitivity nor for evening cortisol. Sleep efficiency was marginally lower for men than for women, a tendency also observed in the literature (Mong & Cusmano, 2016). Moreover, women had cumulative shorter AA than men, probably because men have a preponderance of the sympathetic over vagal control of cardiac function compared to women (Dart et al., 2002). The autonomic and metabolic responses to nocturnal transportation noise exposure were not different between sexes, a result that does not corroborate some epidemiological findings. The HYENA study estimated higher morning cortisol levels among women than men exposed to higher aircraft noise (Selander, Bluhm, et al., 2009), and Eriksson et al. (2014) reported a stronger association between aircraft noise exposure and T2D among women. Potentially, aircraft noise elicits a different response than road and railway noise, it could also be that the age range plays a role. Unlike Selander, Bluhm, et al. (2009) (age range: 45-70 years), the present study included younger women who may counteract the higher cortisol response observed in older women (Seeman et al., 2001). Indeed, Lefèvre et al. (2017) who included participants >18 y did not observe age differences in aircraft noise related evening cortisol levels.

AA during the night predict next morning glucose tolerance irrespective of age and sex

Interestingly, the increase in cumulative AA duration correlated with the increase in glucose concentration two hours after glucose intake, a marker of glucose tolerance. Beside the known deleterious effect of sleep restriction (Spiegel et al., 1999), decreased slow-wave sleep (Tasali et al., 2008) and sleep fragmentation (Stamatakis & Punjabi, 2010) on glucose regulation, increased sympathetic activity has also negative effects on glucose regulation (Nonogaki, 2000). The present result suggests autonomic activation as a mediator of nocturnal transportation noise induced glucose impairment.

Limitations

This laboratory study, investigating healthy and medication free (inclusive hormonal substitutes) young and older participants, has the advantage of controlling many potential covariates that could influence results in the field (sleep and eating hygiene, physical activity, room temperature and ambient light intensity). However, the present results cannot be extrapolated to field conditions where individuals may use coping strategies in response to noise annoyance. Glucose tolerance and insulin sensitivity did not return to baseline values after the recovery night. Probably one noise-free night of 8h rest is not enough to fully recover from 4 noise nights, but it could also be that the stay in the laboratory *per se* led to decreased glucose tolerance and insulin sensitivity. Results from our previous study investigating the young group of the same data set, observed different recovery response depending on the intermittency of the noise scenario, comforting the implication of the noise exposure rather than the laboratory conditions (Thiesse, Rudzik, Spiegel, et al., 2018). Finally, one main limitation of this study is the modest sample size. A bigger field study is still needed to clarify age and sex differences in response to nocturnal transportation noise exposure on AA, cortisol, glucose tolerance and insulin sensitivity.

Conclusion

In conclusion, irrespective of age and sex, nocturnal exposure to transportation noise increased cumulative AA duration, decreased glucose tolerance and insulin sensitivity. Evening cortisol levels were only increased for the younger individual after exposure to highly intermittent road noise. This study highlights autonomic activation as a key mediator of next-day impaired glucose tolerance.

Acknowledgments

We thank our volunteers for their participation, our staff team, students, civil servants, nurses, and interns for their help in conducting the study, Dr. Martin Meyer and Dr. Corrado Garbazza for the medical screening.

5. APPENDIX

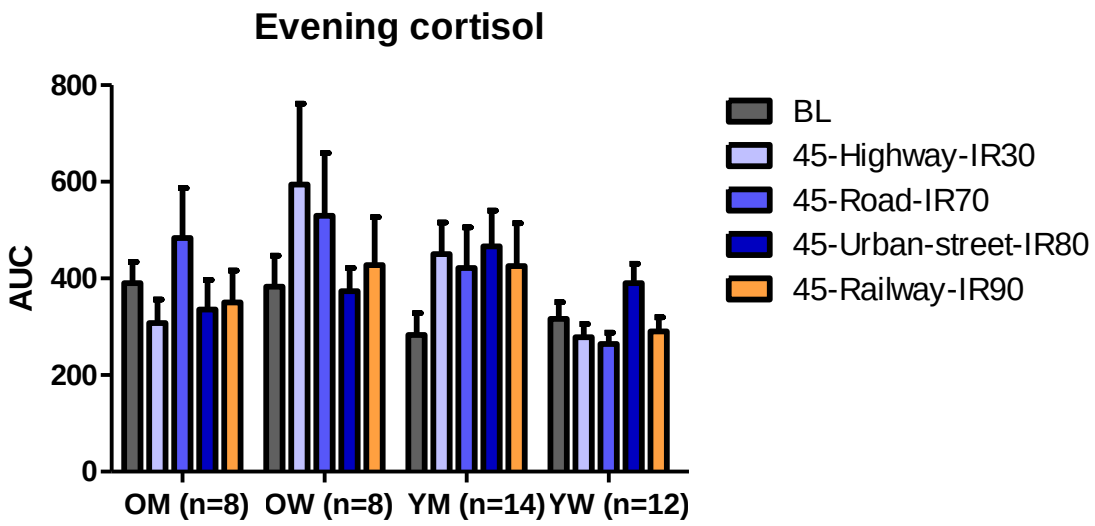

Figure VI.A: Mean evening cortisol level after the four different nocturnal noise scenarios separated in age and sex. *OM: older men, OW: older women, YM: young men, YW: young women. Mean + SEM.*

Chapter VII

GENERAL DISCUSSION

The present thesis aimed at investigating the short-term effects of nocturnal transportation noise on precursors of cardio-metabolic disorders such as stress markers and glucose regulation, and to determine the association to sleep disturbances. Already four nights of nocturnal transportation noise decreased glucose tolerance and insulin sensitivity. Moreover, highly intermittent noise exposure increased next-evening cortisol levels in the young volunteers. However, no associations between the different nocturnal noise scenarios and sleep macrostructure or cortical arousals were observed. In the young group only, the cumulative AA duration was associated with an evening cortisol increase. Moreover, the change in cumulative AA was associated with next-morning glucose tolerance (G_{120}) for all participants.

1. Summary of the main findings

The main findings of this thesis are summarized below, answering the three main research questions (see Chapter III). An overview of the main results is illustrated in *Figure VII.1*.

1. Does short-term exposure to different nocturnal transportation noise scenarios impair glucose regulation, and is this related to concomitant sleep changes? (Chapter IV)

Epidemiological studies reveal associations between long-term exposure to transportation noise and T2D (Sorensen et al., 2013) and suggest night time hours, when people sleep, to be more deleterious than daytime noise exposure (Eze, Foraster, et al., 2017; Eze, Imboden, et al., 2017). However, no studies so far investigated the effect of nocturnal transportation noise exposure on sleep and on next-day glucose regulation in combination. Therefore, our first aim was to determine if nocturnal transportation noise exposure may already affect glucose regulation after a few days, and whether this is caused by impairments in sleep. Moreover, we wanted to test if the intermittency of the noise scenario modulates metabolic responses with hypothesizing to observe stronger effects for more intermittent noise.

We first detected a decrease in glucose tolerance ($glucose_{AUC}$ and G_{120}) and insulin sensitivity (Stumvoll ISI and Matsuda indexes) after sleeping four nights with transportation noise in the healthy young participants. Exposure to more intermittent noise during the last noise night strengthened the harmful effect on the metabolism. Indeed, in contrast to participants sleeping with less intermittent noise during the last noise night, $glucose_{AUC}$ and ISI of participants sleeping with more intermittent noise did not recover to baseline levels after one noise-free night. Interestingly, the deterioration in glucose regulation was not associated with the amount SWS or CA, our first candidates for sleep disturbed effects on glucose regulation (Stamatakis & Punjabi, 2010; Tasali et al., 2008). Another possible pathway linking transportation noise exposure and deteriorated glucose regulation may be the stress axes. Indeed, high cortisol levels (Mazziotti et al., 2011; Plat et al., 1996) and increased SNS

(Lembo et al., 1994) are known to lead to insulin resistance. This brings us to the second main research question of the present thesis.

2. Does short-term exposure to different nocturnal transportation noise scenarios impair the stress axes and is it related to concomitant sleep changes? (Chapter V)

Using the same data set as for the first experiment, we were interested to determine if nocturnal transportation noise exposure affects the two main stress pathways, the SAM and HPA axes. There is some evidence for an activation of a stress response in presence of nocturnal transportation noise (Griefahn et al., 2008; Héritier et al., 2018; Jarup et al., 2008; Selander, Bluhm, et al., 2009); however, no study so far investigated in a same setting the effect on the HPA, the SAM and on sleep outcomes, which is needed to elucidate possible interrelations.

In our setting, nocturnal urinary epinephrine and norepinephrine levels, all night-spectral analysis of HRV, systolic and diastolic BP, HR as well as morning inflammatory markers were not affected by the exposure to nocturnal transportation noise. Even if transportation noise exposure increased noise annoyance, sleep macrostructure as well as the number of CA and AA were not significantly affected. CAR was not significantly increased after transportation noise exposure, but with p=0.05, showed a marginal trend for higher levels after sleeping with 45-Railway-IR90 compared to BL. More interestingly, nocturnal exposure to highly intermittent road noise (IR=0.8) increased the cumulative AA duration and next-evening cortisol level compared to the baseline ambient noise exposure. These two outcomes were positively correlated, suggesting that the increase in next-evening cortisol level could be the consequence of noise-induced increase in total AA duration. Finally, it seemed relevant to study the impact of individual covariates on the observed effects.

3. Do age, sex and PER3 polymorphism influence the impact of nocturnal transportation noise exposure on cardio-metabolic health? (Chapter VI)

Most of the long-term studies investigating the effect of nocturnal transportation noise on cardio-metabolic health included a broader age range as our first two studies with young and older individuals. As physiological aging impacts on cardio-metabolic health (Brewer et al., 2016; North & Sinclair, 2012), we reanalyzed the main outcomes of our previous studies for their age dependency. Neither cumulative AA duration, nor glucose tolerance and insulin sensitivity exhibited a different response to transportation noise exposure between younger and older individuals. Interestingly, the increase in cumulative AA duration was associated with the increase in next-morning glucose tolerance (G_{120}) for all participants. However, older participants did not show the increase in evening cortisol levels after exposure to highly intermittent road noise as observed in the younger group. With aging baseline cortisol levels are higher and may explain the lack of a further increase in response to a stress.

Some epidemiological studies also noticed sex differences in response to transportation noise exposure with increased cortisol levels (Selander, Bluhm, et al., 2009) and higher risk of T2D incidence in women (Eriksson et al., 2014) and higher risk of CVD in men (Eriksson et al., 2010; Vienneau, Schindler, et al., 2015). However, we did not observe sex differences in response to nocturnal transportation noise for glucose regulation, evening cortisol or the cumulative AA duration. It has been reported that PER3$^{5/5}$ carriers were more vulnerable to sleep loss (Groeger et al., 2008) and had higher sympathetic and lower parasympathetic tone compared to PER3$^{4/4}$ (Viola et al., 2008). PER3 polymorphism could therefore influence parameters of sleep and of the ANS such as BP, HR and HRV. However, this covariate did not show significant differences for the outcome variables, and thus was not reported in Chapter VI.

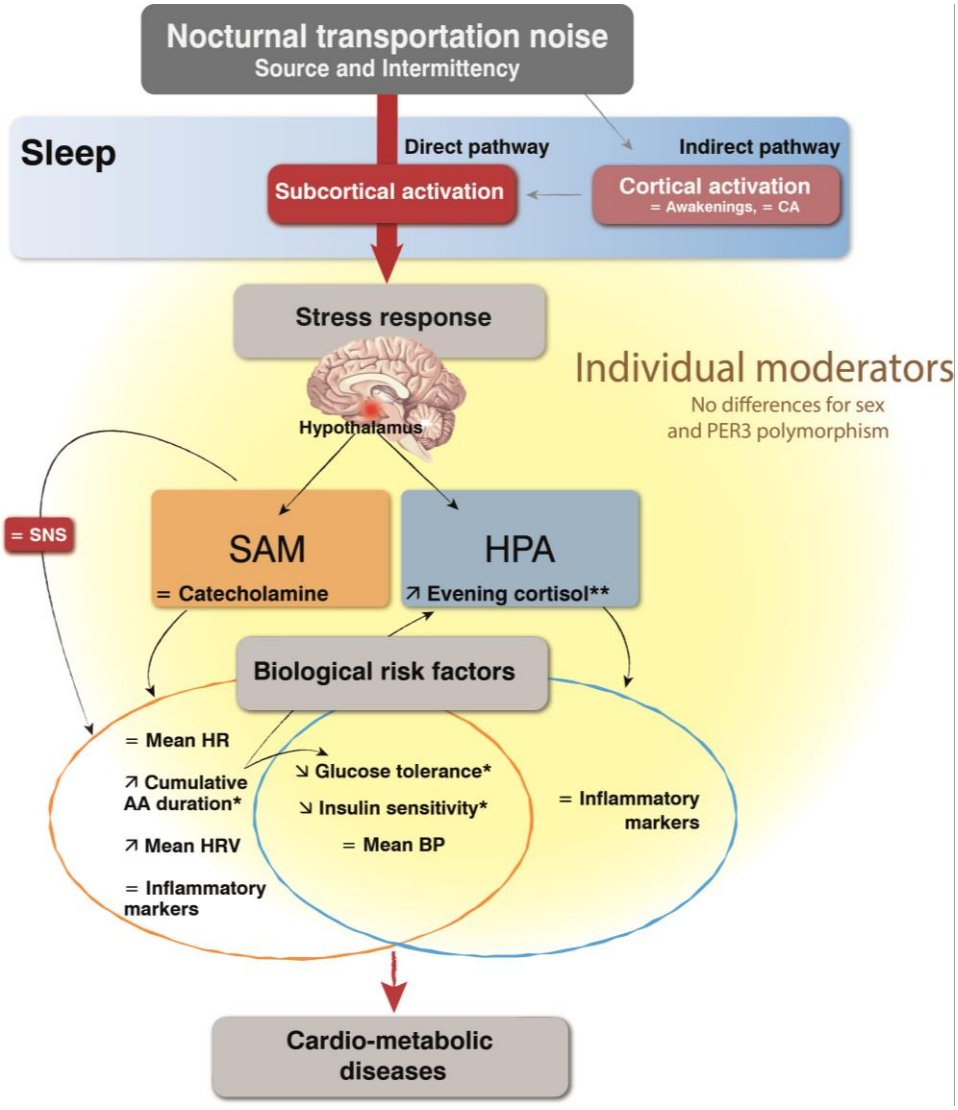

Figure VII.1: Summary figure of the possible mechanistic pathways linking nocturnal transportation noise exposure in sleeping individuals and cardio-metabolic diseases. *CA: cortical arousal, SAM: Sympathetic-Adrenal-Medullary, HPA: Hypothalamic–Pituitary–Adrenal, SNS: sympathetic nervous system, HR: heart rate, AA: autonomic arousals, HRV: heart rate variability, BP: blood pressure.* studied in the young and older participants. ** significant only for the young subgroup.*

2. General discussion and relevance

One aim of the SiRENE project was to try to cross the bridges between the short- and long-term effects of nocturnal transportation noise exposure on cardio-metabolic health. In this context, a new acoustical metric, the IR, has been proposed and tested in our laboratory study and in the long-term epidemiological studies. It was suggested that this metric is a better predictor of noise effects during sleep compared to average energetic dose metrics (e.g. LAeq or LAmax) (Wunderli et al., 2015). Foraster et al. (2017) and Héritier et al. (2017) observed stronger deleterious effects on arterial stiffness and CVD and mortality for highly intermittent noise compared to lower intermittent situations. This thesis aimed to study the short-term effects of different nocturnal transportation noise scenarios (varying in source: rail or road and intermittency: low/medium/high) on sleep and cardio-metabolic regulation. Chapter IV suggests stronger impact of highly intermittent transportation noise (45-Urban-street-IR80 and 45-Railway-IR90) on next-morning glucose regulation than lower intermittent noise scenarios (45-Highway-IR30 and 45-Road-IR70). This result is strengthened by the fact that older participants showed a similar result. Indeed, older participants sleeping with the less intermittent noise during the last noise night were able to recover better from decreased glucose tolerance (increased $Glucose_{AUC}$) than participants sleeping with the more intermittent noise scenarios (see *Figure VII.2*).

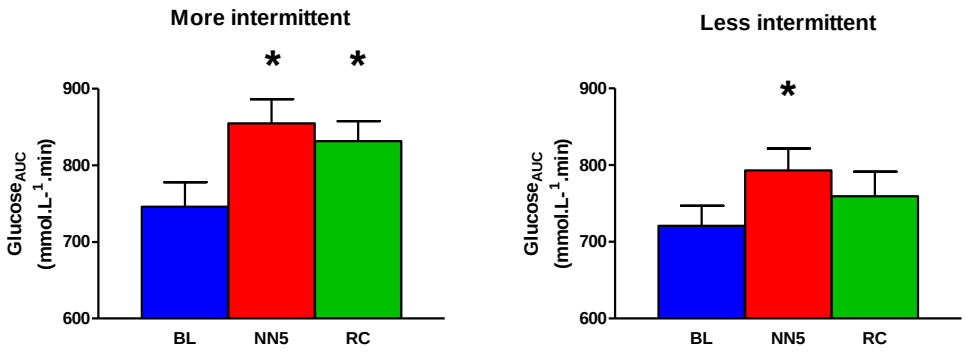

Figure VII.2. Area under the curve of the OGTT glucose profile ($Glucose_{AUC}$) for participants sleeping with a more intermittent noise scenario (n=18) vs. a less intermittent noise scenario (n=19) on NN5. ** p<0.05. BL: baseline night, NN5: last noise night, RC: recovery night.*

As explained in Chapter IV, the four transportation noise scenarios were incompletely counterbalanced between noise nights NN2 to NN5. Therefore, the effect observed was essentially driven by the difference between 45-Urban-street-IR80 and 45-Road-IR70, suggesting a more deleterious effect of 45-Urban-street-IR80 compared to 45-Road-IR70. In Chapter V, only the noise scenario 45-Urban-street-IR80 increased next-evening cortisol level. These results go in line with Héritier et al. (2017) observations of a higher hazard ratio for mortality from CVD for nocturnal IR

between 80 and 87%, corresponding to our 45-Urban-street-IR80 noise scenario. Above this IR level (90-100%) each single noise event can be distinguished with periods of silence in-between. The authors proposed that the silent periods could help to recover from the noise exposure and therefore be less harmful than lower IR (80-87%). This may explain why 45-Railway-IR90 exposure did not exhibit changes in evening cortisol levels for the young group. However, for the pooled group (young and older), a higher increase in cumulative AA duration during 45-Railway-IR90 compared to BL was observed (Chapter VI).

To try to bridge the gap between short- and long-term effects, we used ecological noise scenarios in the laboratory. Taken together, we could observe changes in metabolic and stress markers already after four nights of nocturnal transportation noise exposure in healthy individuals. In comparison to pathological conditions, the effects observed on glucose regulation and sleep were not clinically significant (see discussion of Chapter IV) and correspond rather to a physiological activation of the stress system. Our results would therefore suggest, as also observed by others (Basner et al., 2011; Griefahn & Robens, 2010; Smith et al., 2017) that nocturnal noise exposure at ecological noise level (LAeq, inside=45dB), has mild short-term effects on sleep and cardio-metabolic outcomes in healthy participants. Effects which, in a chronic state could potentially become deleterious on long-term. This result may appear contrary to epidemiological findings (Münzel, Sorensen, et al., 2017b). Nevertheless, comparisons are delicate as the study design, the noise characteristics as well as the study populations differ largely between studies. In long-term epidemiological studies other factors may influence the results, such as the health status, but also coping strategies and behavioral changes, factors that will be discussed below. Moreover, chronic exposure may lead to an apparent habituation.

As reported by Héritier et al. (2017) populations highly exposed to road and railway noise have a lower socioeconomic status and education than the general population. It is also known that they present more risk factors for CVD than the general population (Winkleby et al., 1992). Therefore, noise exposure may be an additional stressor tipping the scale to harmful consequences.

Short- and long-term studies may also differ because of possible coping strategies in the field. In psychology, coping is defined as “constantly changing cognitive and behavioral efforts to manage specific external and/or internal demands that are appraised as taxing or exceeding the resources of the person" (Lazarus, 1996). Therefore, coping strategies can be physical or psychological. Probably one of the most evident coping strategies related to noise exposure is closing the window to reduce efficiently noise intensity; however, it can have negative consequences such as decreasing ventilation or increasing room temperature which could be more deleterious than the noise exposure *per se* for sleep and health in general. Exposure to noise may also lead to sedentary life, unhealthy food intake

or delaying the sleep period to avoid evening transportation noise exposure and shorten the sleep period. All these physical coping strategies, which can be deleterious for health were controlled in our laboratory study and could explain the diverging results with long-term studies.

Resilience could be categorized as a psychological coping strategy. Resilience defines the way to cope successfully with a crisis to return quickly to the pre-crisis status. Our participants may have been very resilient to our noise exposure scenarios.

Finally, our results may show habituation to noise exposure. Our first hypothesis was to observe cortical activation which would trigger cardio-metabolic disorders. Nonetheless, our results suggest that nocturnal transportation noise exposure may disrupt the cardio-metabolic system without impairing sleep *per se* but in activating subcortical networks. The fact that we did not observe any changes in the amount cortical arousals or awakenings may be related to habituation to the noise exposure which has been shown to occur, based on the thalamic gating, at least for cortical but not for autonomic arousals (Basner et al., 2011; Griefahn et al., 2008; Muzet, 2007). However, only 6 out of the 42 participants reported living in a rather noisy environment. Another possible explanation may be that the noise level was not high enough to exhibit cortical activation; indeed, there is the idea that low noise level may only activate vegetative circuits while with increasing noise level cortical activation may occur (Muzet, 2007).

Lastly, our results suggest methodological adjustments for future studies. Evening cortisol seems to be a better indicator than morning levels for nocturnal transportation noise exposure on next day cortisol impairment. Most of the studies investigating the effect of nocturnal transportation noise exposure on cortisol levels, sampled saliva only in the morning (Griefahn & Robens, 2010) or collected only two or three samples per day (Lefèvre et al., 2017; Selander, Bluhm, et al., 2009), most probably due to logistical reasons. However, we propose that evening cortisol levels may be a better marker than CAR for nocturnal transportation noise effect on cortisol. Indeed, evening hours corresponds to cortisol circadian nadir, a time-window where small effects may be better detectable (Babisch, 2003), a result also observed in sleep restriction studies (Leproult et al., 1997). Moreover, the general believe that a low HRV is deleterious for the health has to be discussed. Indeed, on long-term low HRV is associated with increased risk of CVD (Hillebrand et al., 2013). However, on short-term, in our case in response to nocturnal noise exposure, high HRV may reflect AA (as suggested in Chapter V).

3. Strengths and limitations

This thesis has been undertaken into the framework of the national interdisciplinary SiRENE research project and as such took advantage from high-level noise and health epidemiological and acoustical

knowledge of collaborators. Within this project, high quality real-word acoustical scenarios could be applied in very controlled sleep laboratory conditions. These noise scenarios had the particularity to differ in their IR, a new acoustical metric proposed to better mirror noise-related health effects (Wunderli et al., 2015). The main strength of a laboratory setting is the possibility to control for many confounders, which is of high importance for this study as sleep and cardio-metabolic homeostasis depend on sleep hygiene, food intake, physical activity, light exposure, temperature; factors that are difficult to control in the field. Taking into account habitual sleep time is another strength of this study and distinguishes it from sleep studies (Basner et al., 2011; Griefahn et al., 2008; Saremi et al., 2008). As seen in the introduction, the circadian rhythm is implicated in sleep and cardio-metabolic regulation. This study has also the advantage of the high number of outcomes assessed, building a big data set and giving the possibility to detect associations between single effects.

However, this study presents limitations. By pre-analyzing first glucose profiles it became clear that probably one recovery night would not be long enough to return to baseline levels and would therefore not be sufficient to discard a possible lab effect on the results. Indeed, one week laboratory stay may be long enough to influence sleep and cardio-metabolic health. In the laboratory, participants were allowed to walk and exercise mild physical activity only within the room or the 20m long corridor. *Figure VII.3* illustrates wrist activity (black bars represent amplitude of the activity), obtained from the actiwatch device, of one subject during Monday and Tuesday of the week prior the laboratory stay and the same week days during the laboratory stay. Wrist-worn accelerometer is essentially used to determine sleep-wake cycle but has been moderately associated with hip-worn accelerometer which can represent physical activity (Kamada et al., 2016). *Figure VII.3* clearly shows a decrease in physical activity during laboratory stay.

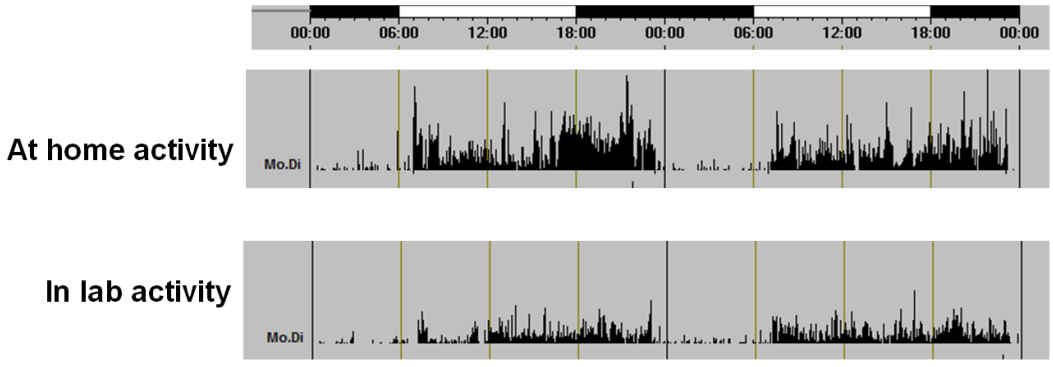

Figure VII.3: Wrist activity of one participant during two days at home and during the same week days in the laboratory.

This decrease was expected and therefore daily caloric intake was individually adjusted, using the Mifflin et al. (1990) formula with the minimal coefficient corresponding to low physical activity. However, mathematical formula to assess daily caloric intake can only approximate needed value

and in our case was not totally efficient as our young participants lost in average 500 gr and the older 360 gr. Thus our study was carried out in a mild negative energy balance state, suggesting that the harmful effects of transportation noise during sleep on glucose regulation would have been even stronger without this weight loss.

To control for a possible lab effect on glucose regulation we decided, add-on, to study a control group of six young men, who underwent exactly the same laboratory conditions and was instructed the same way but slept only with the ambient noise scenario. Because of the small sample size only explorative analyses were possible. Even if the effects were weaker, a small deterioration of glucose tolerance and insulin sensitivity was also noticeable for the control group after six days stay in the sleep laboratory.

Another lab effect is the potential first and last night effect observed for different variables. One adaptation night was probably not enough to habituate the participants to the sleep conditions. For future studies it would be worth having several baseline and recovery nights or scheduling a noise-free night in-between the noise nights to avoid these possible lab effects. It is still not clear why REM sleep increased in the course of the week, a result also observed for a lab study where participants were allowed to leave the lab during the day (Basner et al., 2011). Increased REM sleep may be the consequence of stress, however no correlation between evening cortisol and REM could be observed. The increase in REM duration probably mirrors a better sleep hygiene during the laboratory stay.

4. Conclusion and perspectives

This thesis suggests that sleeping four nights with nocturnal transportation noise, at LAeq=45dB, already increased cumulative AA duration and evening cortisol levels, decreased glucose tolerance and insulin sensitivity without cortical activation or changes in sleep macrostructure in healthy individuals. The impairment in glucose tolerance and in evening cortisol levels seems related to the increase in cumulative AA duration. It suggests that nocturnal transportation noise exposure may disrupt the cardio-metabolic system without impairing sleep *per se* but in activating subcortical networks. Additionally, intermittent noise may elicit stronger effects on the cardio-metabolic system. However, the effects were not clinically significant and would need replication to be validated. We were additionally not able to observe changes in mean HR, spectral analysis of HRV, nocturnal catecholamine, BP and inflammatory markers between the different noise exposures for the young volunteers; parameters that still have to be investigated for the older group. Moreover, analyzing event-related AA would be of interest to define the specific effect of the noise event on AA duration. The association of other risk factors of CVD such as oxidative stress (Münzel, Daiber, et al., 2017),

endothelial dysfunction (Schmidt et al., 2013) or adiposity, with nocturnal transportation noise exposure and sleep impairment will have to be investigated in future studies.

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