Integrative Wireless Monitoring of Minipigs

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Abstract

The applicability of the Holst Centre wireless-sensor node technology is assessed for use on animals in light of efforts to reduce the number of animals and refine the procedures of the use of animals in biomedical (preclinical) research. The Holst Centre ECG Necklace – used on minipigs as a new non-rodent model for safety pharmacology – proved to be an accurate, valuable asset for wireless monitoring of ECG/HR and motion/positioning signals. The results obtained demonstrated the reliability of the system compared to existing technology, yet with a smaller form factor. The differences in results compared to freely moving pre-implanted minipigs (TSI telemetry) are attributable to the experimental design. The ECG sensor node sampled a clear ECG signal and calculated the heart rate in real time, which was not influenced by motion artefacts. In addition, the 3D accelerometer signals were successfully used in combination with ECG/HR and could exclude ambiguous signal changes. Its applicability to the minipig appeared very animal friendly. The pros and cons are discussed in relation to animal wellbeing and relevance for the use of animals in biomedical preclinical research. The sensor node is a stable platform for future sensor extension and integration. This warrants an integrative system for simultaneous assessment of multiple organ systems, with time contributing fully to the principles of the three Rs of animal use (Reduction, Refinement and Replacement).

1. General

1.1 Introduction

Recent advances by researchers of imec/Holst Centre comprise research platforms evidencing technological breakthroughs in the areas of wireless communication, digital signal processing, energy harvesting, sensing and read-out, leading to the realization of ultra-low power wireless body area networks (WBAN). A WBAN is used for communication among sensor nodes operating on, in or around the human body to monitor vital body parameters and movements. Ideally, multiple signals sensed from the body with numerous sensor nodes – either attached to the body surface or implanted in tissue – are transmitted to a home base station. From there, the signal may be forwarded to a hospital, clinic or elsewhere, via a wireless local area network (WLAN), cellular network or public switched telephone network. Sensor nodes in the WBAN are specifically used for electroencephalograms (EEG), electrocardiograms (ECG), electromyograms (EMG), the monitoring of skin temperature and skin conductance, and electro-oculograms (EOG).[7] Apart from medical applications, the WBAN may also serve the user by providing lifestyle, assisted living, sports or entertainment functions.[15] However, to the best of our knowledge, the Holst Centre wireless sensor nodes have not been applied for use on animals in biomedical research.

1.2 Wireless sensor nodes and the three Rs of animal use

The use of animals for biomedical research is a topic of intense public debate, which to some extent conflicts with the mandatory use of animals for research to protect human beings against the undesirable effects of new drugs. Legislation to protect human beings from the adverse effects of new drugs requires that animal experiments using rodents (rats, mice) and non-rodents (dogs, non-human primates) are carried out as outlined in regulatory experiment guidelines. Public interest in replacing, reducing or refining animal experimentation (the three Rs of animal use) has led to the current situation where authorities, the industry, academia and regulatory agencies work together to refine and reduce the use of animals in (preclinical) safety testing. In this context, the minipig has recently been recognized by a group of more than 40 industrial, governmental and academic experts from all over Europe as an alternative non-rodent model which can improve drug safety for humans due to the minipig's superior predictivity and translation to man.[1,3-6,16,17] We hypothesized that this contribution to the three Rs of animal use could be further improved by deploying the Holst Centre wireless sensor technology in this area of (mandatory) safety evaluation studies.

1.3 Wireless monitoring of minipigs: ECG, heart rate, acceleration

In this paper, we report on a feasibility study[1] in which we explored – as a first initiative – an integrative application of the Holst Centre ECG necklace sensor node combined with an X, Y and Z-acceleration sensor in minipigs as test subjects. The primary focus was on 1) animal wellbeing during monitoring; 2) usefulness and quality of the signals; and, 3) relevance of the integrative simultaneous information of ECG, heart rate (HR) and acceleration (activity). For comparison, results obtained from freely moving minipigs – though pre-implanted with telemetry transponders – are reported and compared with those obtained with the Holst Centre ECG sensor node. The pros and cons of the different technologies are discussed in relation to animal wellbeing, particularly animal refinement and reduction. Finally, we speculate on the feasibility of devising an integrative animal-friendly WBAN to address multiple organ systems simultaneously. Primary focus is on safety pharmacology studies and simultaneous multimodal assessment of the cardiovascular, respiratory and nervous systems, preferably with accompanying behaviour. In the present study we focus on the minipig.

1) Part of this study appeared in the Ellegaard Newsletter no. 35, Spring of 2011

2. Methods

2.1 Holst wireless sensor nodes technology in the minipig

2.1.1 Principle of the ECG necklace sensor node

The Holst Centre ECG necklace (Figure 1A) is characterized by a low power consumption ensuring 7 days of autonomy. It contains imec's ultra-low-power analogue readout ASIC (Application-Specific Integrated Circuit) and relies on a low-power commercial radio and microprocessor platform. The ECG algorithm used in the ECG sensor node is based on Continuous Wavelet Transform (CWT) as described by Romero et al.[14] for its robustness to motion artefacts in ambulatory settings. Efficient and reliable R-peak detection is important for advanced ECG applications like arrhythmia monitoring. The algorithm has good performance: a sensitivity of 99.86% and positive predictivity of 99.91% when detecting R-peaks were noted by Romeo and colleagues.[13] A wireless connection between the ECG necklace and the receiver base station transmits ECG and HR data over a range of 10 m. An optional non-volatile memory module enables continuous data logging for applications in case the receiver base station is not in the neighbourhood. This was also used in the feasibility study reported here.

The ECG sensor node is also fitted with a 3D accelerometer to monitor the orientation and movements of the individual carrying the device (Figure 1). The sensor can be extended by using other sensors over the analogue and digital interface to enable future adaptation such as monitoring of stress/emotion in (working) environments and sweat for e.g. dehydration warning. The combination of a multisensory system with advanced analysis applications creates a future-proof device for any ambulatory environment.

2.1.2 Application of the ECG necklace sensor to minipigs: ECG, HR and X ,Y, Z-acceleration

The ECG sensor node detects a heartbeat if there is an R-peak to be distinguished. To test the applicability of the ECG sensor node to monitor the animal's biomedical signals, we attached the sensor to the minipig (Figure 1B to D). Electrode patches were attached to the skin, one on the left side below the heart; the other on the right side above the heart, after shaving and cleaning the skin (Figure 1B). Sensor leads were attached to the electrodes. The sensor itself was clamped into a pre-prepared 'pocket' of an elastic belt attached around the pig's midsection to maximize the freedom of movement for the minipig (Figure 1D).

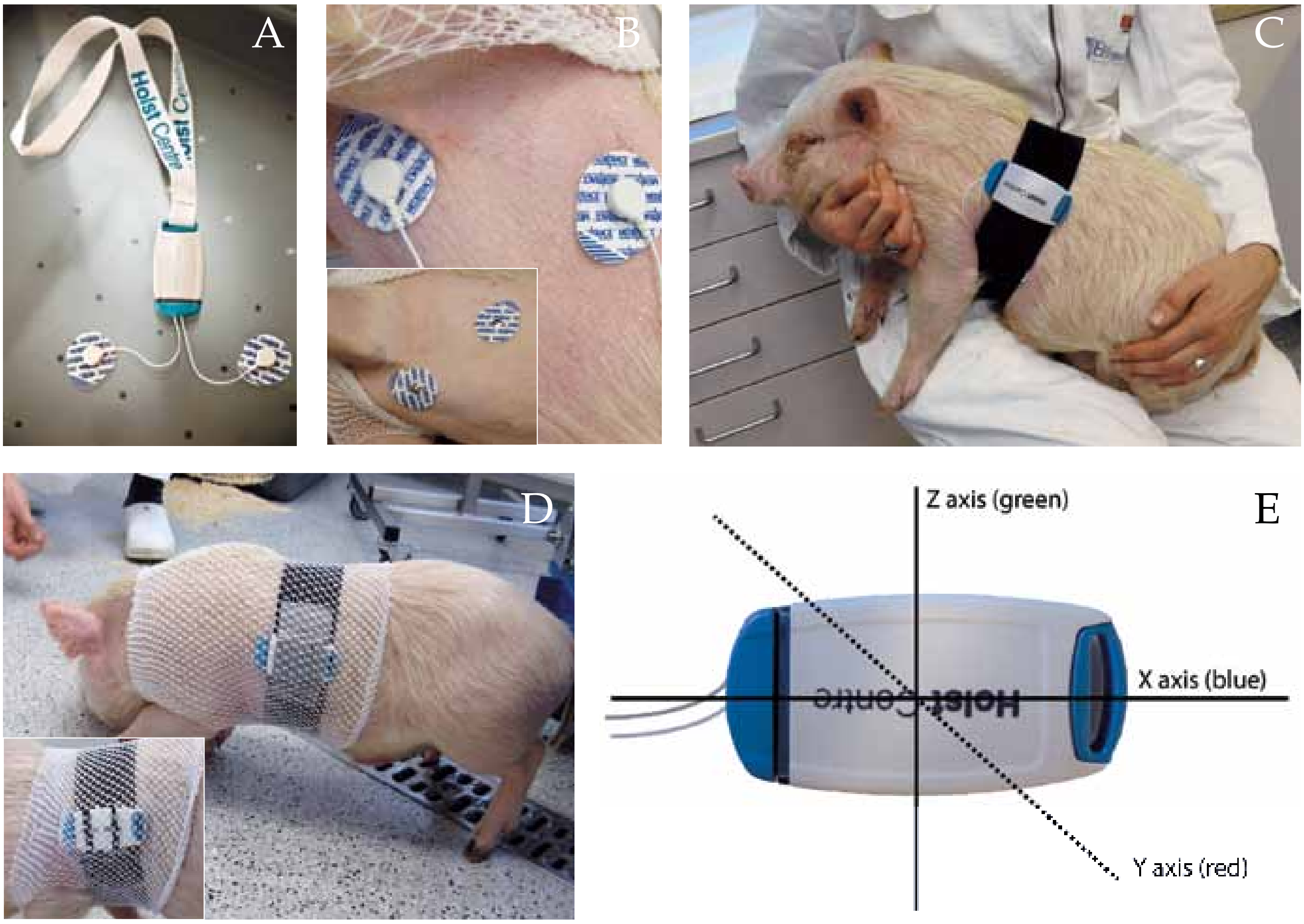


Figure 1. Application of Holst Centre ECG sensor node on Göttingen Minipig.
Fig.1A: Holst Centre ECG Necklace with ECG, HR and 3D acceleration as applied to the minipig.
Fig.1B: Attachment of the sensor leads to the Meditrace ECG electrodes [cf. insertion], which are attached to the pig's shaved and cleaned skin.
Fig.1C to Fig.1E: The Holst Centre ECG necklace was clamped in the pre-prepared 'pocket' of an elastic belt worn around the pig's midsection (see 1d with insert). The orientation of the ECG necklace is recorded (cf. 1C, 1E) to enable the interpretation of acceleration changes on the X, Y and Z axes, since acceleration information is acquired simultaneously with the ECG. (Note that in Figure 1C, the 'pocket' on the belt has been 'deleted' (Photoshop) for the sake of illustration.)

Technology	Hardware manufacturer	Software manufacturer	Invasive	Freely moving	Conscious	Parameters	Accuracy/resolution of the signal algorithm
Telemetry pre-implanted	ITS	NOTOCORD	yes	yes	yes	ECG, HR, BP, LVP, BT	Good
Telemetry	Imec/Holst	Imec/Holst	no	yes	yes	ECG, HR, X,Y,Z -acceleration	Good

Table 1. (Non)Invasive monitoring of cardiovascular parameters in freely moving couscious minipig.

The orientation of the sensor on the pig was included in the tests, allowing the translation of acceleration signals of the X, Y and Z axes in combination with the ECG and HR. The total duration of the experimental session was about 4 hours, during which the animal was continuously equipped with the Holst ECG sensor node. ECG, HR and X, Y, Z-acceleration were monitored over three periods of 30–45 minutes each. The data logged during recording were checked on a remote monitor. Changes in spatial orientation of the animal, like turning on horizontal and vertical body axes during walking or lifting of the animal – spontaneously or intentionally – were noted. Animal wellbeing was monitored by three observers; one of whom was Ellegaard's veterinarian who is accustomed to working with minipigs on a daily basis. During the data analysis, special attention was paid to the integration of the simultaneous signals of observed behaviour, ECG, HR and acceleration (activity), in relation to animal wellbeing.

2.2 Telemetry technology for minipigs' ECG and HR

2.2.1 Example of ECG and HR data in freely moving (pre-implanted) minipigs

The results of the Holst Centre feasibility study with the ECG necklace are discussed in relation to existing technologies for recording ECG in freely moving animals. For direct comparison, results from telemeterized pre-implanted freely moving minipigs (Table 1), are included, kindly provided by Boehringer Ingelheim Pharma GmbH & Co. KG. The relevance and potential power of the Holst Centre sensor node technology for biomedical research on animals is primarily focussed on – though not restricted to – safety pharmacology studies in minipigs.

3. Results

3.1 Results of the feasibility study using the Holst Centre wireless sensor nodes in minipigs

The results of the feasibility study, which – as a first example – explored the applicability of the Holst Centre ECG Necklace sensor node and X, Y and Z-acceleration node using minipigs as the test subject, demonstrated that the minipig could freely move during continuous or repeated monitoring and unambiguously accepted wearing the sensor. The animal was strolling around through the experiment room, exhibiting relaxed behaviour when offered food pellets or drinking water and did not seem to be bothered by having to wear the sensor (see Figure 1D).

The continuous ECG, HR and acceleration signals were acquired and analysed with good results. A random example of the recorded ECG is shown in Figure 2A; signal morphology, i.e. the typical P, Q, R, S, T peaks and waves, is shown in Figure 2B. Subtle irregularities were observed in the ECG signal (see arrows in Figure 2A). These irregularities appear quite small and not very problematic, but could be optimized for minipig-adapting gain and filter settings. Qualitative screening of the recorded signals with the Holst Centre BAN offline Data Analysis tool indicated that the ECG trace might move up and down with changes in activity and behaviour of the animal; polarity of the ECG signal, however, appeared constant as far as this feasibility study is concerned (see Figure 3A).

As expected, the average HR (roughly 100 bpm) measured automatically by the Holst ECG sensor node (Figure 4) is similar to the HR calculated manually in the example above from RR intervals (Figure 4C). HR variations could be explained by acceleration

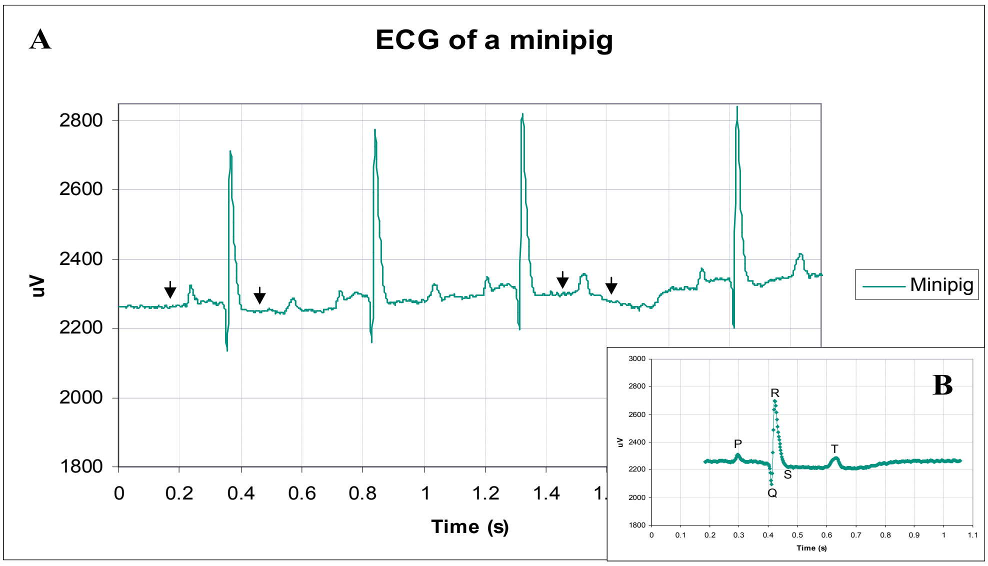
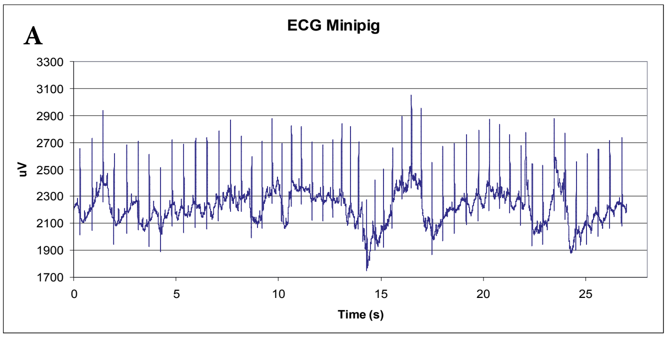


Figure 2[2]. ECG recorded with Holst ECG sensor node in freely moving Göttingen Minipig. Random example of the recorded ECG. Fig. 2A: Multiple ECG complexes demonstrating a clear signal with subtle irregularities (arrows). Gain and filter setting were optimized for use in humans and could be slightly adapted to acquire optimal recordings of minipigs. Fig. 2B: Signal morphology: ECG complex with characteristic waves and peaks (P, Q, R, S, T). Abbreviations: uV = microvolts; s = seconds.

2) This figure has previously been published in the Ellegaard Newsletter no. 35, Spring of 2011); included here for the completeness of the article.



Parameter	Mean (all timepoints)	± SD
PR (ms)	111	35
QRS (ms)	70	12
QT (ms)	247	46
RR (ms)	516	57
HR (bpm)	118	14

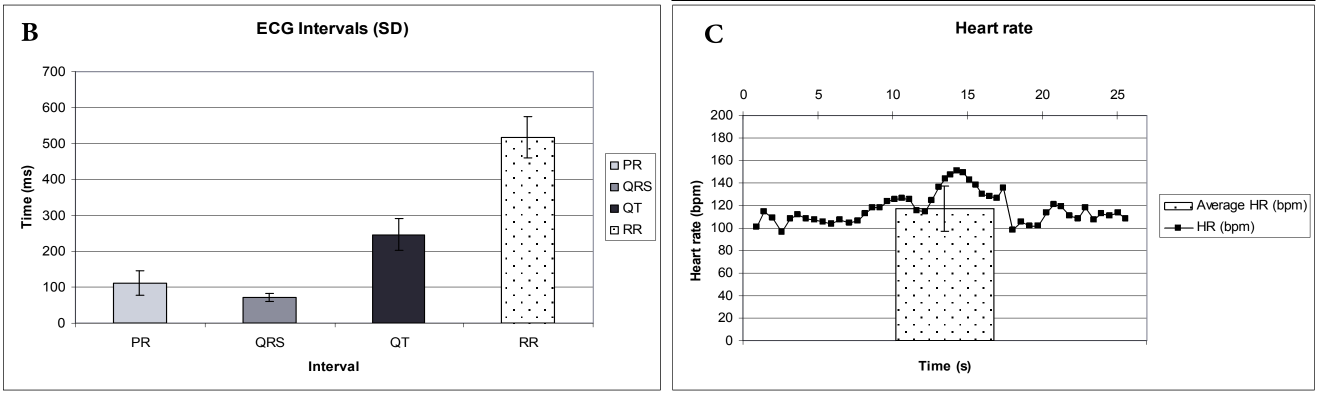
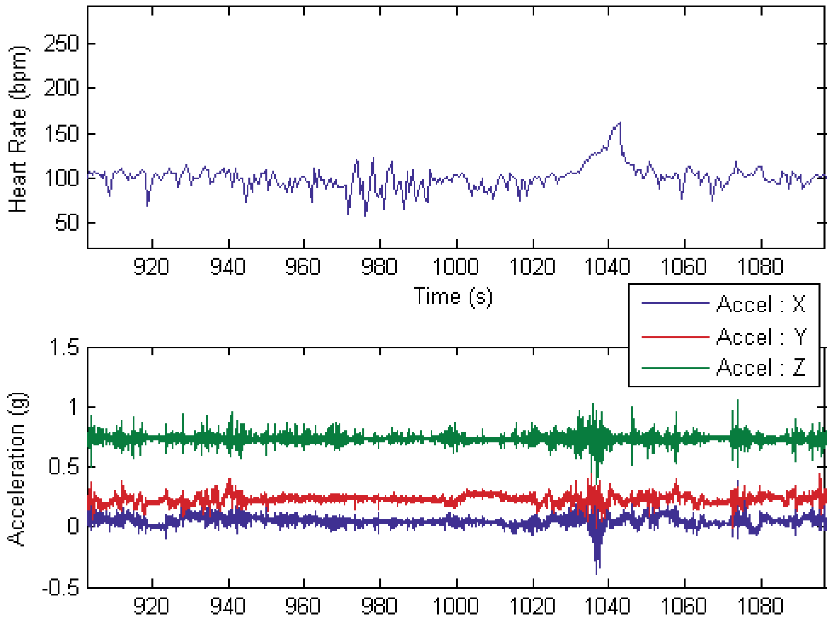


Figure 3. ECG recorded with Holst ECG sensor node in a freely moving Göttingen Minipig. Fig.3A: Random example of ECG trace obtained with Holst Centre ECG sensor node. Table 2: Summarized values for (manually) measured ECG intervals from 50 ECG complexes. Fig. 3B: Graphic representation of ECG intervals. Fig. 3C: Graphic representation of heart rate (HR) calculated from RR. Abbreviations: SD = standard deviation; ms = milliseconds; s = seconds; bpm = beats per minute.

The mean values of the different ECG intervals of the example trace of Figure 3A are shown in Table 2; these are graphically depicted in Figure 3B; HR calculated from RR intervals is shown in Figure 3C.



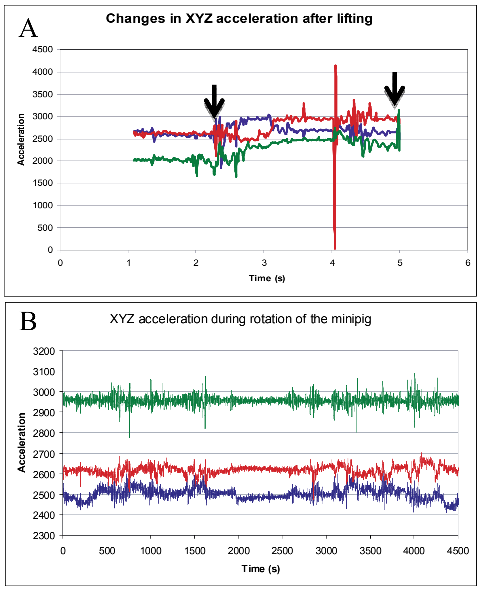


Figure 4[3]. (left panel) Note the similarities between acceleration (lower panel) and HR patterns (upper panel).

Figure 5[4] (right panel). Fig. 5A: Changes in XYZ acceleration (arrows) during lifting of the animal (compare the sensor orientation of Fig. 1D (horizontal) and 1C (under slight change of angle). Fig.5B: Acceleration of the minipig during moving. Note that at around 1500 seconds, the animal rotates on its own axis while standing on four hoofs, resulting particularly in changes on the x-axis (blue curve); the same behaviour and accompanying changes in the x-axis can be observed at 2200 and 3200 seconds.

3;4) These figures have previously been published (Ellegaard Newsletter no. 35, Spring 2011); and are included here for the sake of completeness.

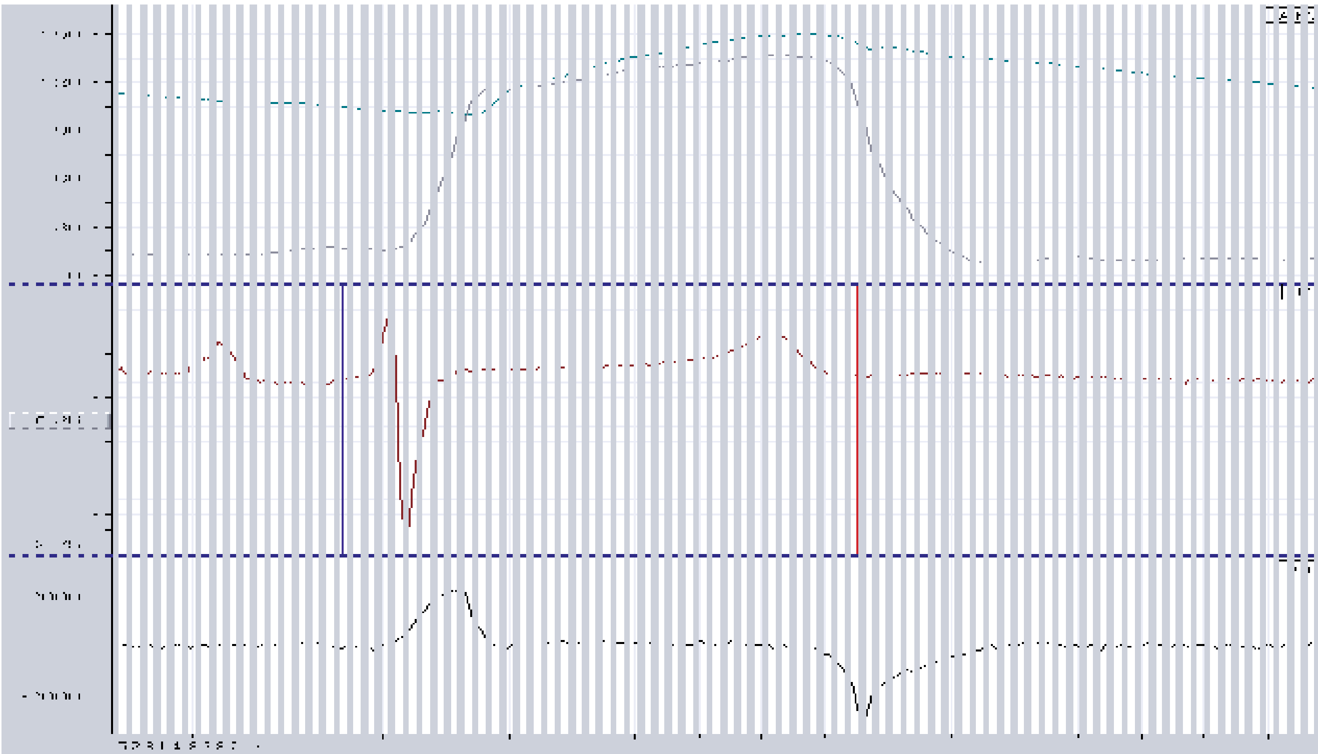


Figure 6. Overview of the cardiovascular/ECG signals obtained with a fully implantable telemetric device (ITS) in freely moving Göttingen Minipigs. Abbreviations: AP/LVP = arterial pressure/left ventricular pressure; EKG = Electrocardiogram; dP/dt = maximum rate of change in left ventricle pressure.

changes (due to the animal's activity) (see Figure 4). Such changes in orientation (X, Y, Z-position) of animal-like lifting (Figure 5A) or rotation (Figure 5B) were observed as changes in one or more of the acceleration signals leading to simultaneous HR changes (see Figure 7A, B, time: 1020–1060).

3.2 Intrinsic HR in the Göttingen Minipig (ITS telemetry)

An example of cardiovascular/ECG signals recorded in freely moving pre-implanted telemeterized minipig (ITS telemetry) is shown in Figure 6. The pre-implanted equipment allows for the simultaneous monitoring of arterial (AP) and left ventricular pressure (LVP), and also shows the maximum rate of change in left ventricular pressure (dP/dt) in addition to ECG (see Discussion).

ECG evaluation over a 24-hour period showed that the polarity of the T-wave shifted during the monitoring period without obvious reason. However, at higher HR (for example after dosing or feeding), the T-wave was positive in all animals. In summary, over the 24-hour period 45% of the T-waves were positive, 42% negative and 13% bipolar. The morphology of the P-wave was typical for each individual and never changed obviously during the experiment.

The mean values for (manually) measured ECG intervals over the entire course of 24 hours in the freely moving telemeterized Göttingen Minipig are summarized in Table 3 (means averaged over all time points, or including values at HR ≤ 80 only).

The HR-values trend over a 24-hour period is shown in Figure 7. Note that the average HR of telemeterized minipigs is low (compare Tables 3 and 2) suggesting minor, if any, stress (about 56 bpm during the 7 daytime hours; roughly 75 bpm during darkness).

Comparison of the daylight period (1–7 hours) and the darkness period (8.3–20.3 hours) (Figure 7) showed a highly significant ($p < 0.0001$) HR increase during the night (statistical key: paired t test, significant if $p < 0.05$) (Figure 8B). An additional experiment

Parameter	Mean (all time points)	±SD	Mean (HR≤80)	±SD
PR (ms)	125	21	128	22
QRS (ms)	56	9	56	9
QT (ms)	320	38	336	26
RR (ms)	861	272	998	222
HR (bpm)	77	26	63	12

Table 3: Summarized values for (manually) measured ECG intervals during 24 hours in the freely moving pre-implanted Göttingen Minipig. ECG recorded with ITS/Notocord telemetry equipment (see Figure 7). Abbreviations: HR = heart beat; bpm = beats per minute; ms = milliseconds; SD = standard deviation.

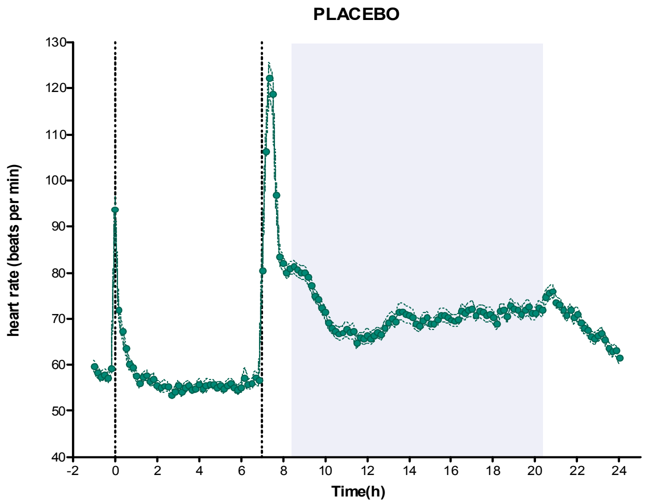


Figure 7. HR measured over a 24-hour monitoring period with a fully implantable telemetric device (ITS) in freely moving Göttingen Minipigs. Data were summarized every 10 minutes as median values ± SD (minimum 400 sequential beats). The dotted line at 0 hours indicates oral administration of a placebo whereas the dotted line at 7 hours indicates feeding. The grey shaded area represents the dark period.

(n = 4) was performed without feeding. In this study, a mean HR during daytime of 53 bpm was followed by a HR of 51 bpm at night (Figure 8A), demonstrating that the effect depends on the postprandial condition and is not merely a diurnal effect. This supports the suggestion that the higher HR values found in the minipig of the feasibility study and measured with the Holst ECG wireless sensor node indeed originate from the fact that food was available.

4. Discussion

4.1 Holst wireless sensory system studied in the Göttingen Minipig

The aim of the research presented here is to assess the applicability of HOLST wireless sensor node technology for animals in light of the reduction and refinement of the use of animals in biomedical (preclinical) research. For example, the applicability and relevance of the Holst ECG necklace sensor node in the Göttingen Minipig was studied, focusing especially on 1) animal wellbeing; 2) specificity and quality of the signals; and 3) relevance of the integrative assessment of ECG, HR and acceleration (activity). For this, the results of ECG/HR of telemeterized pre-implanted freely moving minipigs are used for direct comparison.

The Holst ECG sensor node measures ECG and HR and is also fitted with a 3D accelerometer to monitor the orientation and movements of the individual animal wearing the device. The sensor can be extended with other sensors by means of the analogue and digital interface to enable future adaptation such as monitoring of stress/emotion and sweat as measures of health status and well-being. The combination of a multi-sensory system with advanced analysis applications provides a scientific device for use in any ambulatory environment in the future. Therefore, we argue that application of Holst Centre wireless sensor technology on animals may substantially contribute to animal refinement and reduction, and eventually combinations of sensor nodes may even replace current testing once new biomarkers are discovered from smart combinations of different physiological outcomes.

4.2 Holst ECG necklace: an accurate and valuable asset for wireless monitoring of minipigs

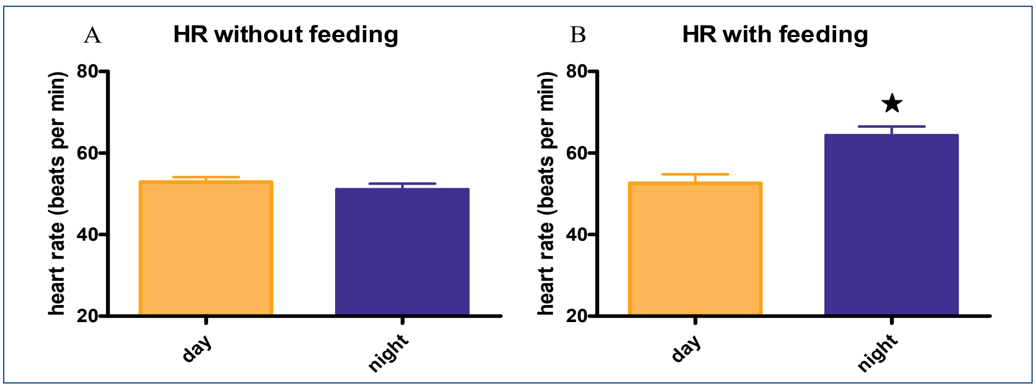
The Holst Centre ECG Necklace proved to be an accurate and valuable asset for wireless monitoring of minipigs' ECG/HR and motion/positioning signals. The results obtained demonstrated the reliability of the system compared with existing technology, yet with a smaller form factor. The ECG sensor node sampled a clear ECG signal and calculated HR in real time, which was not influenced by motion artefacts. In addition, the 3D accelerometer signals indicated the animal's movements and orientation and were successfully used in combination with ECG/HR signals; as a matter of fact, the information on X, Y, Z acceleration helped to exclude ambiguous signal changes. Overall, ECG peaks and waves were clearly distinguishable. The total ECG trace might move up and down, however, without affecting the morphology of the ECG complexes. Moreover, changes in polarity were not observed during this feasibility study. The length of the ECG intervals could clearly be measured.

4.3 Animal wellbeing and HR: What about stress, trained handling, light/darkness, surgery, feeding?

HR values of about 110 bpm were observed in minipigs with the Holst ECG sensor node. As discussed below, relative to the HR measured in the pre-implanted telemeterized minipig (ITS telemetry), the HR measured with the Holst Centre ECG sensor node is high, but may be explained by the fact that food was available during the feasibility testing of the Holst Centre sensor node.

The HR of telemeterized pre-implanted minipigs was well monitored. The HR appeared low and remained below 100 bpm even during oral administration, suggesting that the animals were well

Figure 8. HR measured with a fully implantable telemetric device (ITS) in freely moving Göttingen Minipigs. Fig.8A: HR in 4 animals in daytime (mean 53 bpm) compared to night (mean 51 bpm) without feeding 7 hours after treatment; p = 0.2101. Fig.8B: HR in 4 animals in daytime (mean 53 bpm) compared to night (mean 64 bpm) with feeding 7 hours after treatment; p = 0.0120.



trained and that the procedure of oral administration was not associated with a high level of stress. Surprisingly, however, HR markedly increased during feeding: highly significant increases in HR (up to 122 bpm) were observed which were long-lasting and never returned to the daytime level for the rest of the 24-hour monitoring period (mean of all experiments at night: 71 bpm). In the absence of feeding, it was found that the average daytime HR was 53 bpm and was followed by a similar HR of 51 bpm at night. These findings demonstrate that the effect depends upon the postprandial condition and is not merely diurnal.[11]

Together, these results suggest that for minipigs, feeding per se has a higher impact on heart rate than actions like trained handling for oral administration and probably explain the differences in baseline HR measured in the latter experiment with the ITS pre-implanted telemeterized minipigs and the Holst ECG sensor node in the feasibility study. After all, in the feasibility study, food pellets and the behaviour of approaching observers were used by the observers to test animal wellbeing.

Beglinger and Becker[2] reported HR of 103 (±14) bpm in sling-restrained Göttingen Minipigs (~ 20 kg). Although in this case, a stress-induced HR increase cannot be ruled out, there was no reason whatsoever to believe that the minipigs in the feasibility experiment, monitored with the Holst Centre ECG sensor node were under stress. Kano[8] reported values from 72 to 76 bpm in freely moving miniature pigs weighing 17 kg (but not Göttingen Minipigs). HR in resting miniature pigs was 80 (±3.5) bpm in investigations from Kuwahara.[10]

The HRs in the present study are comparable to data from freely moving, well-trained and group-housed Labrador dogs.[9] At TNO we measured baseline values of HR in Beagle dogs, respectively group-housed versus solitarily housed Beagles, of 84 bpm (daylight) and 72 bpm (darkness) versus 79 bpm (daylight) and 69 bpm (darkness).[12] Note that no differences were observed between solitarily and group-housed animals, probably because the solitarily housed animals can hear, see and smell neighbouring dogs and dogs in the animal house. Note also that HR in periods of daylight and darkness hardly differs, which is consistent with findings in telemeterized minipigs of this paper: telemeterized pre-implanted dogs in the study of Pijnappel and De Groot[12] were fed during morning hours; apparently the effect of feeding on HR was temporary and recovered during the daylight period.

Together, these findings illustrate that in minipigs, feeding schedules may be important confounders when it comes to HR measures. With regard to the results obtained with the Holst ECG sensor node, it is concluded that stress levels were low but feeding probably increased the HR and, related to that, ECG intervals. So, in an animal wellbeing perspective, stress levels are clearly low for animals wearing a Holst Centre ECG sensor node.

4.4 Wireless monitoring and animal wellbeing: pros and cons of external sensor nodes and pre-implantation

Unlike pre-implanted telemeterized animals, no surgical procedure is required for the Holst sensor nodes. Otherwise, during surgery, an animal can be equipped with different sensors to address different organ systems. For example, as demonstrated here for the telemeterized minipig (TSI telemetry), a complete test battery of cardiovascular parameters is given. The disadvantage, however, is that surgery places a rather large burden on the animal when it comes to animal wellbeing, and the necessary anaesthesia, analgesia or antibiotics may change the threshold for toxicity of a drug to an unknown extent. Therefore, additional control animals may be required. A technical disadvantage of implanted telemetry transponders is the fact that the batteries of the implanted transponder may run out of energy. Although to date, transponders can be refurbished at reasonable costs, for the animal this implies that another surgical procedure is needed (with all the disadvantages for animal wellbeing and quality of research outcomes). Sometimes, the animals can still be used for other purposes where telemetry is not required, but often the animals will have to be culled and new animals will have to be implanted.

Clearly, completely non-invasive external recording is vastly superior when it comes to benefitting animal wellbeing. To date, manufacturers of telemetry equipment focus on jacketed telemetry equipment to record ECG, implying that the transponder is to be worn on the body in jacket pockets (e.g. DSI/EMKA). So far, these transponders are quite heavy, and training is needed to accustom the animal to wearing the jacket without stress. This is deemed essential for maintaining good study data. However, it takes time to train the animals, and this is a burden for the animal, although limited. In addition, time and money are involved in the training. Good-quality data of studies with 'happy' animals contribute to reduced variation in study results meaning that fewer individuals are needed for unambiguous study outcomes. This contributes to achieving the three Rs of animal use for increased statistical viability.

4.5 Holst wireless sensor technology and the three Rs of animal use: mouse, rat and minipig predictability

The results of the present study showed that Holst technology could add knowledge to contribute to reducing and refining the use of experimental animals. In principle – and when used up until now – Holst Centre ultra-low-power wireless sensor nodes would contribute the most to the principles of the three Rs when applied to small laboratory animals (mice, rats) as they still represent by far the largest number of laboratory animals to date. An emerging issue in biomedical research remains: the predictability of rats and mice for humans. Recently, a group of more than 40 industrial, governmental and academic experts from all over Europe reported that minipigs have many advantages for drug and chemical safety testing and could improve drug safety for humans.[1] The results of the present study involving minipigs indicate that the Holst Centre wireless sensor technology could be ideal for supporting the three Rs of animal use: the minipig accepted wearing the sensor (refinement), and the signals of ECG, HR and acceleration were acquired and analysed without any problem and with very good results. Continuous, repeated and simultaneous monitoring with multiple sensor nodes addressing multiple organ systems seems to be within reach, which enables more information to be obtained from fewer animals (reduction). Otherwise, time and costs will be saved, opening up more room for further studies.

4.6. Minipig and multimodal *integrative* physiology platform: discovering new biomarkers

Development/application of an integrative multimodal test system is proposed to allow simultaneous animal-friendly, information-enhancing and cost-reducing assessments of physiological parameters indicative of the health status of specific organ systems and hence the overall health status of an individual. An additional challenge is simultaneous monitoring and analysis of physiological parameters with accompanying behaviours using smart combinations of e.g. acceleration and EMG sensor nodes with ECG or EEG, to define characteristic behaviours (specific locomotion, localization or body posture) which, in turn, may be indicative of anxiety, pain, depression, etc. Hence, characteristic new biomarkers may be discovered. In the short term, this will lead to animal reduction since more information is obtained from an individual animal, also saving time and costs (e.g. for pharmaceutical industries which can abandon unpromising leads in time). In the long term, the database of toxicological information about minipigs will expand and, slowly but surely, information on rodents will not add new information to the data for non-rodent species examined with such a highly sophisticated integrative test system for simultaneous, multimodal physiological assessments. Rodent studies may become needless. In this context, it is worth remembering that the development of regulatory requirements (outlined in testing guidelines) always depends on the state of the science and state-of-the-art testing methodology. Thus, it may be possible in the future to replace the currently required tests on single endpoints in different species by a limited test battery in a single species, like the minipig, which has at least the same, but probably better, predictivity of effects and whose results can readily and reliably be translated to humans.

4.7 Sensor platform, minipig, the three Rs of animal use: future directions

To the best of our knowledge, Holst Centre ultra-low-power wireless sensor technology has only been used in biomedical research using human subjects. Once developed, the proposed system will benefit animals in the first place (refinement and reduction of animal use), society, animal rights parties, governmental and regulatory authorities and the pharmaceutical, food and chemistry industries. The system is unique in the sense that, compared to systems already on the market, it is far more animal-friendly and/or more powerful as it can address multiple organ systems, e.g. the cardiovascular, respiratory and nervous systems with accompanying behaviour, using wireless technology; simultaneously, continuously and repeatedly. Moreover, smart combinations of physiological endpoints may lead to the discovery of new biomarkers that may be very informative in the early phase of drug discovery. Moreover, continuous data logging without a computer present is possible, as is a connection to a network for ambulatory situations.

The sensor node is a stable platform for future extension and integration of sensors. As indicated by Ellegaard et al.,[3] a variety of clinical signs (e.g. body posture, activity, behaviour, dehydration, etc.) may be observed in safety evaluation studies. These signs are supported by cardiovascular measurements such as HR, blood pressure, ECG, respiratory rate, EEG, and telemetry/remote monitoring. The Holst Centre sensor node is actually designed for human application to obtain ECG and HR signals, combined with activity sensors, and transmits the data over a telemetric connection. These measurements of biomedical signals were also obtained from the minipig, making the platform a versatile system for animal wellbeing. The next phases in system development could include SpO2, respiration, and galvanic skin response (GSR) in a small package and low power consumption for extended trials with minimum effect on animal behaviour.

Future sensors will also measure dehydration and EEG signals, which would complete major safety pharmacology measurements. These parameters will be combined to determine HR variability, and the emotion and stress of an individual, ultimately resulting in autonomous monitoring of the individual. By translating these parameter measurements and applications for the minipig, the multi-sensory system and algorithms will also assist in fully achieving the refinement, replacement, and reduction (3 Rs) objectives highlighted in the RETHINK project.[1]

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