Wireless Monitoring for Animal Wellbeing

ECG, HR and acceleration in minipig: a feasibility study

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GENERAL

1. Wireless sensor nodes and Animal 3Rs

Recent progress by researchers at imec and Holst Centre comprises the realization of ultra-low power wireless body area networks (WBAN) used for communication among sensor nodes operating on, in or around a man's body to monitor vital body parameters and movements (EEG, ECG, EMG, skin conductance, Skin temperature, and more) *[1]*. In biomedical research the application of these wireless sensor nodes -so far- was restricted to human individuals. We argued that smart application of such wireless sensor nodes in animals could contribute considerably to the reduction and refinement of (mandatory) animal use in biomedical research. Animal use for biomedical research is under intense societal debate which -to some extent- is in conflict with the *mandatory* use of animals for research to protect man against undesired effects of new drugs. With current societal interest in replacement, reduction and refinement of animal experimentation (Animal 3Rs), authorities, industry and regulatory agencies work together to accomplish reduced animal use in (regulatory) safety testing.

2. Minipig and Safety pharmacology

Legislation to protect man from adverse effects of drugs, chemicals and food ingredients *demands* the conduct of animal studies which are outlined in regulatory test guidelines. At some point in the past, rodents were chosen for this kind of research. However, predictivity of the rodent model for man appeared not always sufficient and so, regulatory authorities demand that safety evaluation studies during development of a new drug are *additionally* carried out in a *non-rodent* model, mostly dog or non-human primate, or -since recently- minipig. Anatomical, biochemical, physiological and genetic similarities to man, and available knowledge on e.g. diseases and immunology, warrant the minipig a potent model for safety pharmacology testing *[2]*. This model can contribute to Animal 3Rs for *better information* will be obtained with the minipig model when it comes to predictivity and translation of animal results to man, ultimately leading to reduction in animal use.

The use of minipigs could *further* contribute to the refinement and reduction of animal testing (3Rs) when the testing *per se* is non-invasive and animal friendly. Thinking of Holst Centre and imec ultra-low power wireless body area networks and safety pharmacology testing the ideal situation would be that the three main organ systems (cardiovascular, respiratory and nervous systems) *with* accompanying behaviour (locomotion, location, body posture) are addressed *simultaneously* and measured *longitudinally/repeatedly* in an *animal-friendly* way by smart combinations of multiple *wireless* sensor nodes. Such measurements will increase the (statistical) power -multiple physiological parameters and organ systems addressed simultaneously, and repeated measurements within one and the same individual- and so, fewer animals will be needed.

FEASIBILITY STUDY: ECG, HEART RATE, ACCELERATION WIRELESS MONITORING IN MINIPIG

1. Introduction

Cardiovascular safety pharmacology in freely moving minipigs

Especially for cardiovascular safety pharmacology research the minipig was proven to be an attractive model *[3]*. Moreover, the consideration of data improvement has led to successful measurements in freely moving minipigs using 'non-invasive' telemetry applications, either with pre-implanted or jacketed transponders. For the first approach surgery and anaesthesia is required which may affect study outcome; for the second pre-training of the animal to wear and accept the jacket without stress.

Here we explored –as a first example– the applicability of the Holst Centre wireless ECG Necklace sensor node and X, Y and Z-acceleration node in minipig as the test subject, focussing especially on animal (dis)comfort when wearing the sensor nodes, and on quality and usefulness of the signals.

2. Methods *[Figures 1A-E]*

Principle of the ECG necklace and application on minipig

The ECG necklace *[4]* is easy to use and characterized by a low power consumption ensuring 7 days autonomy.

It contains imec's proprietary ultra-low power analogue readout ASIC (Application-Specific Integrated Circuit), and relies on a low power commercial radio and micro-processor platform. A wavelet-based heart beat detection algorithm is embedded in the processor that ensures the accurate computation of the instantaneous heart rate, even under high level of noise. The microcontroller controls the wireless transmission of the ECG data to a receiving base station connected to a computer within a range of 10m. An optional non-volatile memory module enables continuous data logging for applications in case the receiving computer is not in the neighbourhood. This was also used in the feasibility study reported here.

To attach the sensor to the minipig, the pig's skin was shaved around the left fore paw / heart region and cleaned with anti-sceptic solution. Electrode patches were sticked to the skin, one left-sided below the heart; the other right-sided above the heart. 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 belly. Belt and electrodes were covered by a commercially available bandage. Both fore-paws perforate the bandage allowing the animal to freely move around, whereas belt and other equipment stay in place. Care was taken to register and note down the orientation of the sensor on the pig, allowing translation of acceleration signals of the X, Y and Z axes.

3. Results of the feasibility study *[Figures 2A-E]*

The ECG signal turned out well (the signal quality was comparable with a wired solution) and so did the signal produced by the X, Y, Z-acceleration node. Changes in direction of the sensor nodes resulting from changes in the pig's body orientation -like turning around during walking while standing on 4 paws- were observable as an interchange of the X, Y, and Z-axis signals. Intense movements of the minipig were visible by complimentary changes in the X, Y, and Z signals as well as an increase in Heart Rate (HR).

4. Discussion / conclusions

The results of the feasibility study indicate that the Holst Centre and imec wireless sensor technology could perfectly fit-in to contribute to the principles of animal 3Rs: the minipig accepted wearing the sensor (*refinement*), and the signals of ECG, Heart rate and acceleration were acquired and analysed without any problem and with good results. Continuous and repeated, *simultaneous* monitoring with *multiple* sensor nodes addressing multiple organ systems seems to be within reach and so, more information can be obtained from fewer animals (*reduction*). Since the minipig is considered a very predictive model to extrapolate results to man, it is the ideal model to further explore the relevance of the Holst Centre and imec wireless sensor technology for safety evaluation studies; all of this in the context of animal 3Rs.

PROSPECTS

The promising results on ECG/HR/acceleration in minipig encourage further exploration of the Holst Centre and imec ultra-low power wireless sensor technology for monitoring of physiological characteristics of minipig in an animal-friendly way (*refinement*). For safety pharmacology, an *integrative* system of sensor nodes addressing *simultaneously* the cardiovascular, respiratory and nervous systems *with* accompanying behaviour is a primary goal. This will lead to animal *reduction* since more information is obtained from an individual animal, saving also time and costs. Such an integrative system for multimodal physiological assessments will find broader application in biomedical animal research (e.g. juvenile toxicology, disease models, *in vivo* imaging and mechanisms).
Miniaturization of the sensors –another goal- is a challenge by itself, but a prerequisite for non-invasive application in small animals like rats and mice.

In the *longer-term*, the data base on toxicological information on minipig will expand and –slowly but surely– information in rodents will not add new information to the data in the non-rodent species examined with such a highly sophisticated, animal-friendly, information increasing and cost reducing *integrative test system* for simultaneous, multimodal physiological assessments. Rodent studies may become needless. In this context, it should be borne in mind that the development of *regulatory* requirements (outlined in testing guidelines) always depends on the state of the science and the state of the art of test methodology, and hence 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 that at least has the same –but most likely better– predictivity of effects and from which the results can readily and reliably be translated to humans.

REFERENCES

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INFORMATION ON COLLABORATIVE INSTITUTIONS

1. Holst Centre

Holst Centre (*www.holstcentre.com*) is an independent open-innovation R&D centre that develops generic technologies for Wireless Autonomous Sensors and for Flexible Electronics. A key feature of Holst Centre is its partnership model with industry and academia in shared roadmaps and programs. Holst Centre was founded by imec (*www.imec.be*) and TNO (*www.tno.nl*) and is located on High Tech Campus Eindhoven, The Netherlands.

2. TNO Innovation for life

TNO is the largest Dutch public-private R&D organization. TNO has over 75 years of experience in generating knowledge and turning it into practical applications, contributing to the innovative power in the Netherlands and abroad. TNO's seven research themes are Healthy Living, Industrial Innovation, Safety, Energy, Mobility, Built Environment and Information Society. In 2005, TNO assigned one of its business units to coordinate its activities in Holst Centre.

3. imec

imec performs world-leading research in nanoelectronics and leverages its scientific knowledge with the innovative power of its global partnerships in ICT, healthcare and energy. imec delivers industry-relevant technology solutions. In a unique high-tech environment, its international top talent is committed to providing the building blocks for a better life in a sustainable society. imec is headquartered in Leuven, Belgium, and has offices in Belgium, the Netherlands, Taiwan, US, China and Japan.

In 2005, imec setup a legal entity 'imec-nl' to coordinate its activities in Holst Centre. imec offers several interactive programs, which give companies and institutes the opportunity to directly access imec's core expertise in leading-edge technology. Only by joining forces, it is possible to tackle tomorrow's technological challenges and to share the rising R&D costs.

LEGENDS TO THE FIGURES

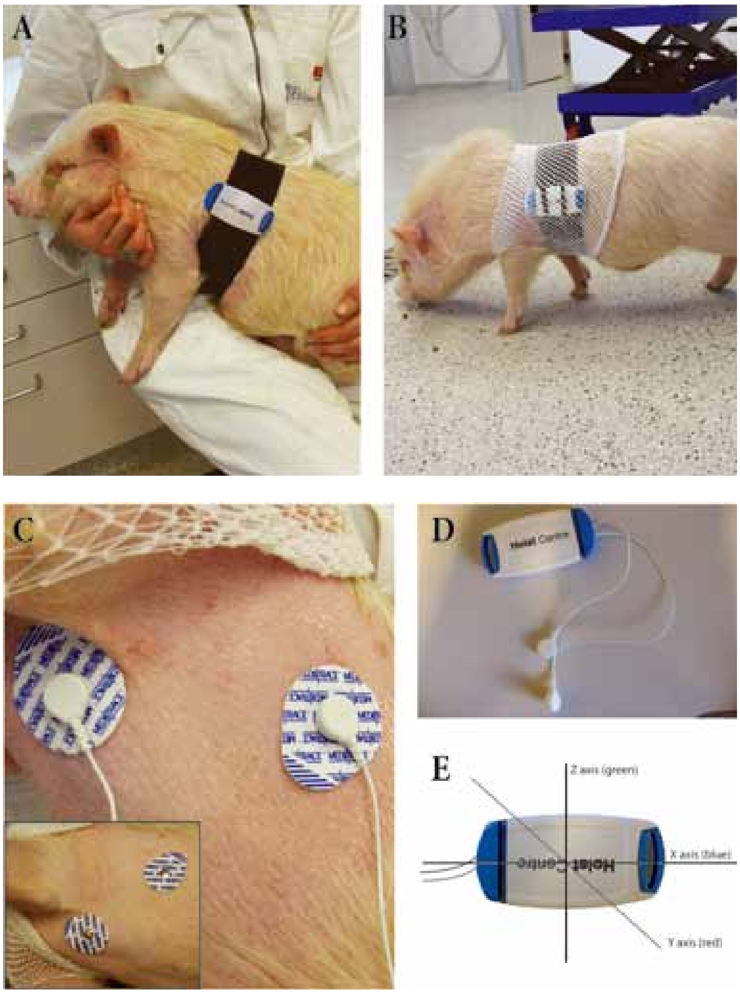

***Figure 1**. Minipig test subject (male; age: 5-6 Months; body weight 11.1 kg). Figs.1A, 1B) The Holst Centre ECG necklace was clamped in the pre-prepared 'pocket' of an elastic belt worn around the pig's belly. The orientation of the ECG necklace is noted down to be able to interpret acceleration changes in the X, Y and Z axes, since acceleration information is acquired simultaneously with the ECG. [Notice that in Fig.1A, the 'pocket' on the belt is 'deleted' (Photoshop) for the sake of illustration]. Fig.1C) Attachment of the sensor leads to the Meditrace ECG electrodes [cf. insertion], which are attached to the pig's shaved and cleaned skin. Fig.1D) Image of the HOLST Centre sensor. Fig.1E) The Holst Centre sensor node with the orientation of the different axes relative to the sensor [compare Figure 1A].*

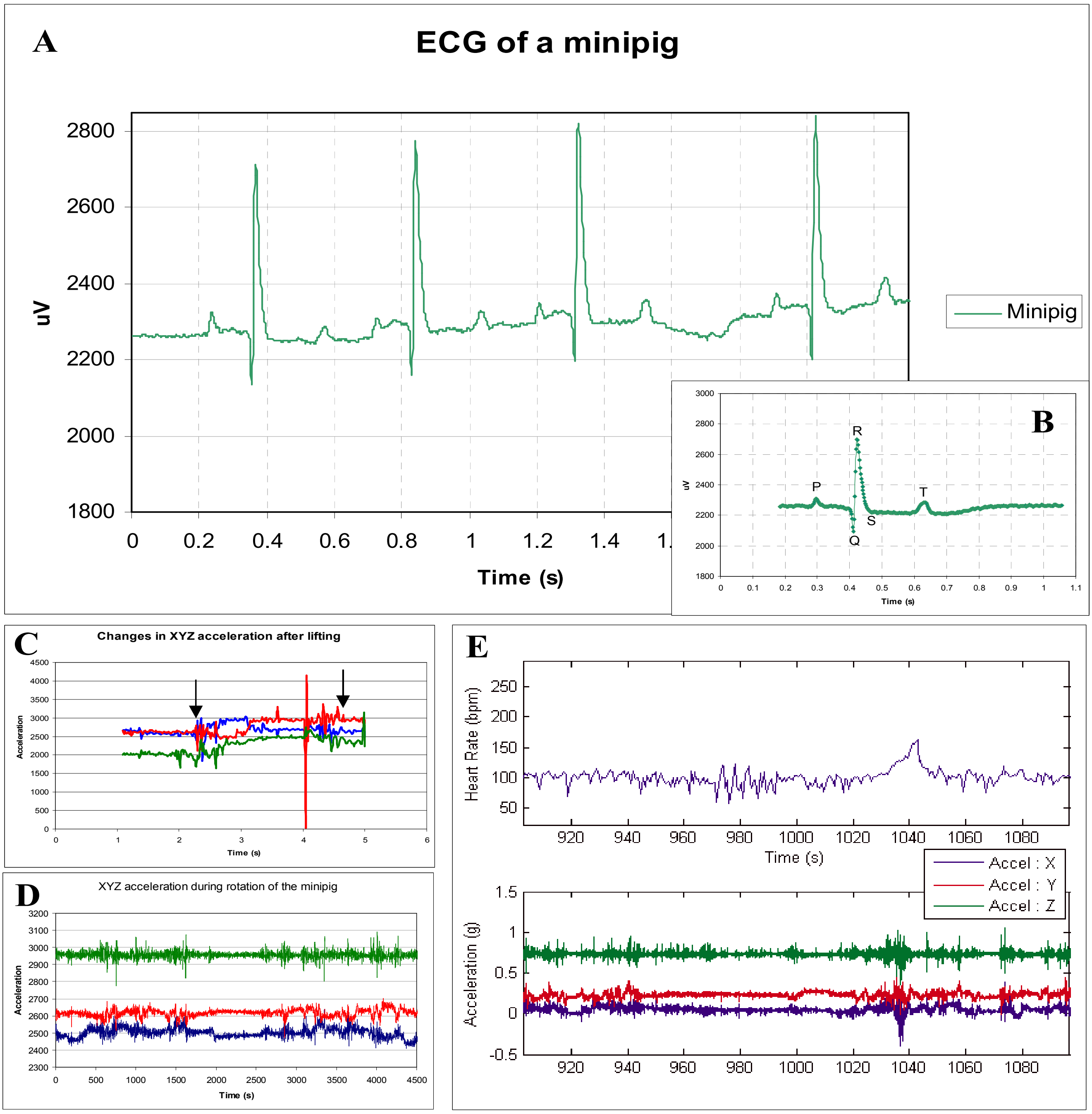

***Figure 2**. Minipig test subject (male; age: 5-6 Months; body weight 11.1 kg).*
Fig.2A) Multiple ECG complexes demonstrating a clear signal, acquired with the ECG necklace [Fig.1A]. Gain and filter setting were optimized for use in man and might be slightly adapted to acquire optimal recordings of the minipig ECG. Fig.2B) ECG complex with characteristic waves and peaks (P, Q, R, S, T) measured with Holst Centre wireless ECG Necklace sensor node. Fig.2C) Changes in XYZ acceleration (arrows) during lifting of the animal. [Compare sensor orientation of Fig. 1B (horizontal) and 1A (under slight change of angle), and Fig. 1C]. Fig.2D) Acceleration of the minipig during moving. Notice that at around 1500 seconds the animal rotates around its own axis while standing on 4 paws, resulting particularly in changes in the x-axis (blue curve); same behaviour and accompanying changes in x-axis can be observed at 2200 and 3200 seconds. Fig.2E) Notice similarities between Acceleration (lower panel) and Heart Rate patterns (upper panel).