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DEVELOPMENT OF SMOOTH MUSCLE ELECTROMYOGRAPHIC METHOD: DETECTION AND INTERPRETATION OF SLOW WAVE ACTIVITIES IN GASTROINTESTINAL TRACT AND PREGNANT UTERUS

PH.D THESIS

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CONTENTS

List of publications

This thesis is based on the following publications:

I. Szucs KF, Nagy A, Grosz G, Tiszai Z, Gaspar R: **Correlation between slow-wave myoelectric signals and mechanical contractions in the gastrointestinal tract: Advanced electromyographic method in rats**. *Journal of Pharmacological and Toxicological Methods* 2016, **82**:37-44.

[IF: 2.238]

II. Szucs KF, Grosz G, Sule M, Nagy A, Tiszai Z, Samavati R, Gaspar R: **Identification of myoelectric signals of pregnant rat uterus: new method to detect myometrial contraction**. *Croatian Medical Journal* 2017, **58**(2):141-148.

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III. Szucs KF, Grosz G, Sule M, Sztojkov-Ivanov A, Ducza E, Marki A, Kothencz A, Balogh L, Gaspar R: **Detection of stress and the effects of central nervous system depressants by gastrointestinal smooth muscle electromyography in wakeful rats**. *Life Sciences* 2018, **205**:1-8.

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Other publication unrelated to this thesis:

I. Berko S, Szucs KF, Balazs B, Csanyi E, Varju G, Sztojkov-Ivanov A, Budai-Szucs M, Bota J, Gaspar R: **Electroporation-delivered transdermal neostigmine in rats: equivalent action to intravenous administration**. *Drug Design, Development and Therapy* 2016, **10**:1695-1701.

[IF: 2.822]

List of abbreviations

AUC:	area under curve
cpm:	cycles per minute
EEG:	electroencephalography
EMG:	electromyography
ENS:	enteric nervous system
FFT:	fast Fourier transformation
GI:	gastrointestinal
i.p.:	intraperitoneal
i.v.:	intravenous
ICC:	interstitial cells of Cajal
PsD:	power spectrum density
SEMG:	smooth muscle electromyography
SG:	strain gauge

1. INTRODUCTION

1.1 Gastrointestinal motility disorders

The detection of motility problems of smooth muscle organs has not yet been solved. Gastrointestinal (GI) motility disorders are among the most frequent forms of GI motility problems, such as oesophageal reflux disease, gastroparesis, ileus, and colon obstruction in intensive care [1], but the daily clinical practice is additionally faced with gastric arrhythmia, heartburn, reflux, irritable bowel syndrome, chronic constipation, etc. [2]. It is currently considered that these motility disorders are idiopathic in origin; their diagnosis, prognosis and treatment are therefore not satisfactory [3].

The gut-brain axis creates a two-way communication network created by the complex system of the enteric nervous system (ENS), cerebral nerves and the humoral system. As a part of the ENS the GI smooth muscle has its own pacemaker cells, called the interstitial cells of Cajal (ICCs), which play a key role in the generation and propagation of the electric signal in GI contractility. ICCs generate slow-wave electric impulses to induce action potentials for contractions and determine the frequency of the signals [4, 5]. Functional GI malfunctions can be the consequences of dysregulation in the gut–brain axis. The altered gut-brain interaction can be in the background of functional GI disorders and other motility diseases, such as gastroesophageal reflux disease and irritable bowel syndrome. The investigation of the gut-brain axis and the enteric plexus activity helps to promote the understanding of GI diseases and the therapies for GI disorders and stress-induced GI malfunctions [6-8].

1.2 Uterine motility disorders

The other major unsolved problem is premature delivery, which is defined as delivery occurring before 37 completed weeks of gestation also caused by smooth muscle overactivity. Premature labour is the major contributor to perinatal mortality

and morbidity, with an average rate of 10-12% in developing and developed countries and accounts for 75%–85% of all neonatal deaths.

The physiology of pregnant uterine contractility is very complex and not yet fully understood. Myometrial contraction is regulated by sexual and stress hormones, the autonomic nervous system, ion channels and transmitters. Dysregulation of the myometrial contractility can lead to either preterm or slow-to-progress labour [9].

ICCs have been immunohistochemically detected using c-kit/CD 117 antibodies in a variety of smooth muscle tissues as well, including the myometrium. These myometrial CD 117-positive cells could behave as sensors, controlling myometrial contractility, depending on sexual and also the stress hormone levels. Myometrial ICCs exhibited spontaneous electrical and pacemaker activity and responsibility for the generation of slow myoelectric waves. It has been suspected that CD 117-positive cells are associated with myometrial motility disorders, which may have a role in the pathogenesis of endometriosis and the regulation of labour in the pregnant uterus. [10, 11]

Besides, preterm delivery is related to stressful life events, anxiety, depression, lack of psychosocial support and physical abuse. Stress triggers through three different mechanisms: hypothalamus-pituitary-adrenal axis, activation of the inflammatory processes, and ischemic mechanisms, all can affect the development of premature births. In response to maternal emotional and physical stress, the endocrine system (i.e., the hypothalamus-pituitary-adrenal axis) is activated, releasing hormones such as adrenocorticotropin, cortisol, cytokines and prostaglandins. Patients with anxiety and depressive disorders also have a higher incidence of functional GI disorders that are frequently unrecognized, these organs respond to psychosocial distress through smooth muscle contractions [12-14].

1.3 Problems of *in vivo* detection of smooth muscle electric activity

Non-invasive GI electric mapping (multi-electrode method) and the electroenterogram (ring electrode method) as new techniques have been tested to

characterize the GI motility, and especially the gastric activity in humans [15, 16]. Although these methods are promising, it cannot be guaranteed that the recorded signals originate solely from the putative GI tracts. The signals can obtain the GI slow- and fast-waves along with noises such as respiratory and motion artefact [17].

In the other hand, a sensitive method to predict the early signs of term or preterm pregnant uterine contraction would have also a great importance in obstetric practice. Although a few clinical studies have been published about the application of electromyography in obstetrics [18, 19], the characterization of slow wave uterine signals and their comparison to other smooth muscles waves have not been described yet.

The possibility of slow-wave myoelectric signal interference, or even masking with fast-wave signals from the brain, cardiac muscle or skeletal muscle, is very high, and an effort is made to reduce this through the special design of the sensors [20]. Identification of the signals from the various smooth muscle organs is therefore an essential requirement for the development of non-invasive clinical methods for the diagnosis of motility disorders of given parts of the GI tract and for the prediction of premature birth. On the other hand, such a model may serve as an excellent method for smooth muscle pharmacology *in vivo*.

Motion artifacts are major obstacles to the various electromyographic examinations in wakeful subjects. Studies have demonstrated that the results of electroencephalographic (EEG) records can be modified by muscle work during the record because the frequencies of the brain and myogenic activity are overlapping. The electromyographic (EMG) signals can be eliminated by computerized screening from EEG records [21]. The skeletal muscle induced motion artifacts have higher amplitude compared to the basal activity, which may cause large distortion in the measurement. Therefore motion artifacts have to be filtered out, however, in the case of overlapping frequencies, filtering can result in significant data loss [22, 23].

2. AIMS

There are very few equipments available to follow smooth muscle functioning and detect the effect of different drugs *in vivo*. Therefore, we have set the goal of developing an *in vivo* measurement method to better and more specifically interpret the information from highly complex electromyography signals in the simple determination of the area under curve (AUC).

The first aim of our study was to identify the slow wave frequency parameter of the gastrointestinal tract, the gastric, small intestine and the colon, and the identification and characterization of the uterine electrical activity of the pregnant rat and to separate these signals from each other. To attain this goal, we have developed a method with which to follow up the changes in the myoelectric activity of gastrointestinal tract and pregnant uterus in parallel with the mechanical contraction in anaesthetized rats. The recording software has been equipped with effective electronic filters to separate the slow-waves of the smooth muscle signals from the cardiac, brain and skeletal muscle activity.

We hypothesized that our smooth muscle EMG method - accomplished with a digital cutter for removing motion artifacts - can be applicable in wakeful rats under normal and stress conditions. The second aim of our study was to follow up the stress condition induced alteration in the GI tract and to measure the effects of central nervous depressants by SEMG in wakeful rats.

To achieve these goals we initiated in vivo experiments in male and female rats.

3. MATERIALS AND METHODS

3.1 Housing and handling of the animals

The animals were treated in accordance with the European Communities Council Directives (86/609/ECC) and the Hungarian Act for the Protection of Animals in Research (Article 32 of Act XXVIII). All experiments involving animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: IV/198/2013 and IV/3796/2015).

Sprague-Dawley rats (Charles-River Laboratories, Budapest, Hungary) were housed at 22 ± 3 °C and a relative humidity of 30-70%, under a 12 h light/12 h dark cycle. Standard rodent pellet food (Charles-River Laboratories, Budapest, Hungary) and tap water were provided ad libitum. Each animal was fasted for two hours before the experiments.

3.1.1 Mating of the animals

The pregnant uterus study was conducted on sexually mature, pregnant, female rats (body mass: 140–160 g, 50–60 days old). They were mated in a special mating cage with male rats (240-260 g) in the early morning hours. An electric engine-controlled, movable door separated the area between the male and female rats. Since rats are usually active at night, the separating door was opened before dawn. Within 4 hours after the possibility of mating, copulation was confirmed by the presence of a copulation plug or spermatozoa in the vaginal smears. In positive cases, the female rats were separated and the day of copulation was considered to be the first day of pregnancy.

3.2 Detection of gastrointestinal myoelectric activity

Male rats (10-12 weeks old, body weight: 260-300 g) were anaesthetized intraperitoneally (i.p.) with a combination of ketamine and xylazine solution (36 and 4

mg/kg, respectively). The jugular vein was cannulated for later intravenous (i.v.) drug administration.

After laparotomy, the total GI tract was resected with the exception of one segment (stomach, small intestine or large intestine, n=6 for each group) from the abdomen under deep anaesthesia. A bipolar thread electrode pair (SEN-15-1; MDE GmbH, Walldorf, Germany) was inserted into the serosal surface of the target organ (the distance between the two electrodes was 8 mm), while a bipolar disk electrode pair (SEN-15-2; MDE GmbH, Walldorf, Germany) was placed subcutaneously above the specific segment of the GI tract (the distance between the two electrodes was 20 mm). An implantable strain gauge (SEN-04-FSG2; MDE GmbH, Walldorf, Germany) was sutured onto the surface of the stomach, ileum or caecum, along the long axis of the muscle fibres, in order to detect the mechanical contractions (**Fig. 1**). So as to cover the incision, the surfaces of the abdominal wall were constricted and the abdominal skin was replaced after the positioning of the sensors.

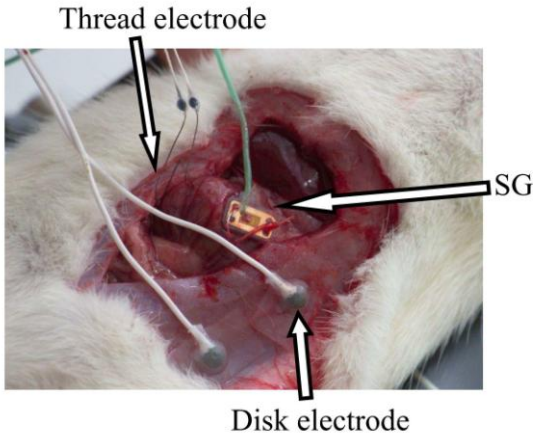

Figure 1. Representative picture of the positioning of the electrodes and strain gauge (SG) for recording the cecal myoelectric and mechanical signals in a rat with a resected stomach and small intestine. The thread electrode pair and the SG were positioned on the caecum, while the disk electrode pair were positioned on the abdomen under deep anaesthesia.

The animals were then placed immediately onto a heatable operating table (EXP-D-TC/MA-02; MDE GmbH, Walldorf, Germany) in order to maintain the body temperature (set to 37 °C). The basal activity was detected for 60 min. The electric signals were recorded and analysed by an on-line computer and amplifier system by

the S.P.E.L. Advanced ISOSYS Data Acquisition System (MDE GmbH, Walldorf, Germany). Electromyographic (EMG) signals were amplified by using a custom-made amplifier designed by MDE Ltd., Budapest, Hungary. In order to reduce the artefacts we used a double-filter system. All analogue signals were pre-filtered with a first-order Bessel-type lowpass filter and were converted to digital signals at a sample rate of 2 Hz with a slope of 80dB/decade. The pre-filtered myoelectric signals were then filtered further by Bessel-type bandpass filters with a frequency of 0-30 cycles per minute (cpm) with a slope of 140dB/decade. Each filter was a digital IIR filter. The recorded signals were analysed by fast Fourier transformation (FFT). The frequency of the electric activity was characterized in cpm, and the magnitude of the activity was described as power spectrum density (PsD). When more than one peak was found in the spectrum, only the highest peak was considered.

The mechanical contractions were evaluated by area under the curve analysis of the primary contractility curves. Before the pharmacological studies, both the mechanical (strain gauge) and electric (thread and disk electrodes) signals were recorded for 30 min (n=8 for each segment).

In the case of anaesthetized, non-GI tract-resected rats (n=9), a bipolar disk electrode was placed under the abdominal skin, 1 cm right from the midline of the laparotomy, and 3 strain gauges were sutured one by one onto the surface of the stomach, ileum and caecum (**Fig. 2**). The abdominal incision surfaces were closed by surgical staples after the placement of the sensors.

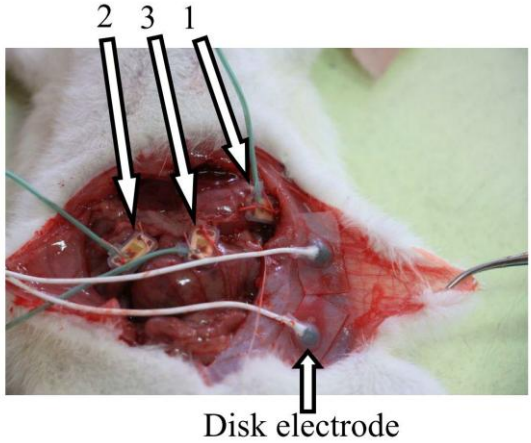

Figure 2. The positioning of the disk electrode and strain gauges (1: stomach; 2: ileum; 3: caecum) for recording of the gastrointestinal (GI) myoelectric and mechanical signals in a non-GI tract-resected rat under deep anaesthesia.

Both the mechanical (strain gauges) and electric signals (disk electrode) were recorded for 30 min before the administration of the investigated drugs. The myoelectric signals were recorded with the above-mentioned equipment, but the analysis and filtering of the signals were carried out according to our findings in the partially GI tract-resected animals (see in the Results section).

3.2.1 Pharmacological investigations

A dose of neostigmine (20 μg/kg) was administered and after 30 min a dose of atropine (300 μg/kg) i.v. for the GI tract-resected and intact rats. Following the addition of each drug, the AUC and FFT of 30-min periods were evaluated. The effects were expressed as percentages of the spontaneous activity. The AUC, cpm and PsDmax values were determined and compared statistically (unpaired t test) by using the computer program Prism 5.0. (GraphPad Software, USA).

3.3 **Detection of myoelectric activity**

Female, full term pregnant (21st and 22nd day of pregnancy) rats were anaesthetized intraperitoneally with a combination of ketamine and xylazine solution

and the jugular vein was cannulated for later intravenous drug administration as previously done.

After laparotomy, the total GI tract was resected from the abdomen under deep anaesthesia. A bipolar thread electrode pair was inserted into the myometrium, while a bipolar disk electrode pair was placed subcutaneously above the uterus. An implantable strain gauge was sutured onto the surface of the left uterine horn, along the long axis of the muscle fibres, in order to detect the mechanical contractions (**Fig. 3**). So as to cover the incision, the surfaces of the abdominal wall were constricted and the abdominal skin was replaced after the positioning of the sensors.

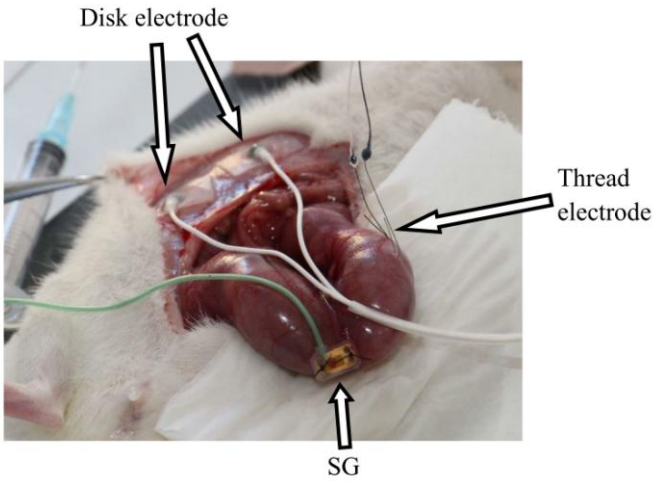

Figure 3. Representative picture of the positioning of the electrodes and strain gauge (SG) for recording the myometrial myoelectric and mechanical signals in a rat with a resected gastrointestinal (GI) tract.

The animals were then placed immediately onto a heatable operating table in order to maintain the body temperature. The basal activity was detected for 60 minutes. The electric signals were recorded and analysed by the same S.P.E.L. Advanced ISOSYS Data Acquisition System. Electromyographic signals were amplified and filtered with frequency of 0-30 cpm and were converted to digital signals at a sample rate of 2 Hz. The recorded signals were analysed by fast FFT. The highest peak of PsD was considered during the evaluation. The mechanical contractions were evaluated by AUC analysis of the primary contractility curves.

Before the pharmacological studies, both the mechanical and electric (thread and disk electrodes) signals were recorded for 30 min (n=8).

In the case of anaesthetized, non-GI tract-resected rats (n=12), a bipolar disk electrode was placed under the abdominal skin, 1 cm right from the midline of the laparotomy, and 2 strain gauges were sutured one by one onto the surface of the uterus and caecum (**Fig. 4**). The abdominal incision surfaces were closed by surgical staples after the placement of the sensors.

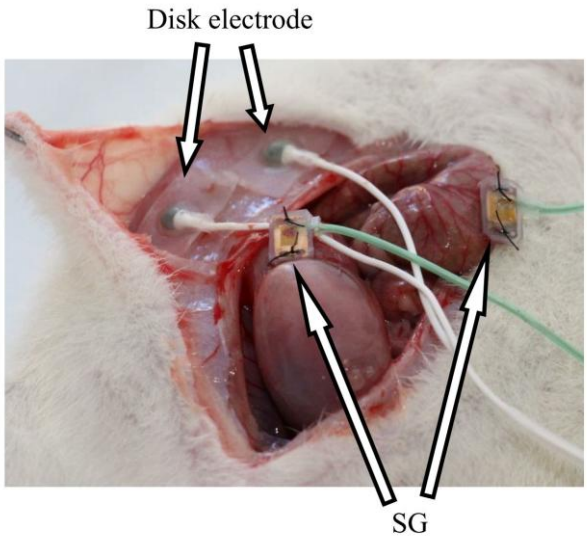

Figure 4. The positioning of the disk electrode and strain gauges (uterus, caecum) for recording of the uterine and gastrointestinal (GI) myoelectric and mechanical signals in a non-GI tract-resected rat under deep anaesthesia.

Both the mechanical and electric signals were recorded for 30 min before the administration of the investigated drugs. The myoelectric signals were recorded with the above-mentioned equipment. The analysis and filtering of the signals were carried out according to our previous findings (see in the Results section).

3.3.1 Pharmacological investigations

Two doses of oxytocin (1 μg/kg) were administered after recording the basal activity, 15 min. apart. After 30 min, a dose of terbutaline (50 μg/kg) was injected i.v.

both for GI-resected (n=8 on the 21st day of pregnancy) and non-GI-resected (n=10 on the 22nd day of pregnancy) rats. One dose of neostigmine (20 μg/kg) and one dose of atropine (300 μg/kg) i.v. were administered 30 min apart for non-GI-resected rats on the 22nd day of pregnancy. Following the administration of each drug, the AUC and FFT of 30-min periods were evaluated. The effects were expressed as percentages of the spontaneous activity. The AUC, cpm and PsD_{max} values were determined and compared statistically (unpaired t test) by using the computer program Prism 5.0.

3.4 **Stress measurements**

3.4.1 EMG monitoring

Male rats were anaesthetized with isoflurane inhalation, then a bipolar disk electrode pair was fixed subcutaneously 1 cm right from the midline above the gastrointestinal tract. The connecting cable of the sensor to the swivel was led subcutaneously and the terminal was led out through the skin of the neck. After the placement of the sensor, the abdominal and cervical incision surfaces were closed by surgical sutures and staples, respectively.

The basal activity was detected the day after the placement of the sensors. Food and water were withdrawn 2 hours before and during the detection. The animals were placed one by one in cages with high-pitched walls, with a transparent wall at the front side. The animals were not restricted in their movements for 30 min while recording basal GI tract activity (control). Then the rats were anaesthetized with 3.5 % isoflurane inhalation and placed and fixed onto a glass plate by strong sticky belts. The rats were laid on the abdomen and were not able to move or turn around. After full awakening (3-5 min), the GI activity was recorded again for 30 min under this stress condition. When diazepam (5 mg/kg) or haloperidol (1 mg/kg) was administered intraperitoneally for the given groups of rats, the treatments were done after recording the basal activity. Then 30-min recording was carried out for each drug to determine their actions before and during stress condition.

The myoelectric signals were recorded and analyzed by the S.P.E.L. Advanced ISOSYS Data Acquisition System. Electromyographic signals were amplified and filtered with a first-order bandpass Bessel-type filter with a frequency of 0-30 cpm. The FFT of 30-min periods were evaluated. When more than one peak was found in the spectrum, the highest peak was considered as characteristic for the given GI tract segment. During the evaluation, the EMG spectrum of basal activity was compared to the activities after drug treatment or during stress period. The stress-induced alterations were expressed as percentage of the spontaneous activity. The PsD_{max} values were compared statistically (one-way ANOVA) by using the computer program Prism 5.0.

To remove the motion artifacts, a digital cutter was built into the software. The edge values of the limiter were set by the motion artifact-free sections of the records. Thereby, we were able to cut the artifact signals by their obviously high outlier amplitude.

3.4.2 Collection of plasma and organ samples

At the end of each period of 30 min, samples of 0.5 ml blood were collected from the tail veins into 1 ml tubes containing K_3EDTA (0.6 mg/tube) and centrifuged (1700 ×g, 10 min, 4° C) to separate plasma. The plasma samples were stored at -20° C until hormone assay and HPLC analysis. The organ samples for haloperidol determination were collected after termination by CO_2 inhalation. Brain, lung and liver tissue samples were homogenized in 0.01 M KH_2PO_4 (pH=4.3):methanol = 75:25 mixture (1:4 w/v) with a tissue blender. Tissue homogenates were stored at –70 ºC until HPLC analysis.

3.4.3 Plasma corticosterone analysis

The plasma concentration of corticosterone was measured by enzyme-linked immunosorbent assay (ELISA) from the collected samples. A Mouse/Rat Corticosterone ELISA (BioVendor, Bio-Kasztel Ltd, Hungary) kit was used for the quantification of corticosterone. After the preparation of microplate wells, we

dispensed 10 µl of each calibrator, sample and control solutions in duplicates. We added the incubation buffer and 50 µl of enzyme conjugate into each well, and incubated the plate for 2 hours at room temperature. After the washing procedure we added the substrate solution to each well, and incubated for 30 min in the dark. We stopped the reaction by adding the 50 µl of stop solution, and then we determined the absorbance of each well at 450 nm within 15 min.

3.4.4 HPLC analysis

An aliquot of plasma or tissue homogenate sample (200 µl) was combined with 20 µl of 50 µg/ml internal standard solution and 100 µl 1 M glycine buffer (pH=10.5), then 1.5 ml acetonitrile was added. The mixture was vortexed for 1.5 min and centrifuged at 12,000 rpm for 10 min at 4 ºC. The supernatant was evaporated to dryness under a stream of nitrogen at 40 ºC. The residue was reconstituted in 100 µl mobile phase and a volume of 20 µl solution was injected for analysis.

The chromatographic separations were performed on a Kromasil Eternity C18 (5 µm, 150 mm × 4.6 mm) analytical column at 35 ºC. The mobile phase used for the separation consisted of acetonitrile : water : isopropyl alcohol : ammonium hydroxide = 40 : 50 : 10 : 0,025 (v/v/v/v) pumped at a flow rate of 1 ml/min. The detection wavelength was 245 nm.

3.4.5 Contractility studies in isolated organ bath

Before measurement, rats were fasting for 24 hours. The animals were terminated by CO_2 inhalation. The stomach, ileum and cecum were dissected, rinsed with Tyrode solution (composition in mM: 137 NaCl, 3 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 12 $NaHCO_3$, 0.4 NaH_2PO_4, 6 glucose, pH 7.4) and cleaned from fat. Strips from the stomach were prepared as described earlier [24]. The ileum and the cecum were cut into 5-mm-long muscle rings. The smooth muscle samples were mounted vertically in an organ bath containing 10 ml, 37°C Tyrode buffer and carbogen (95% O_2 + 5% CO_2) was bubbled through it. The initial tension of the tissues was set to 1.5 g. The

samples were incubated for 60 min, the buffer solution was changed in every 15 min. After the incubation period, the control contractions of smooth muscle were evoked by KCl (25 mM) and a cumulative dose-response curve of 10^{-4}–10^{-9} M diazepam or haloperidol was obtained. The activity of gastric strips, ileal and cecal rings was measured with a gauge transducer (SG-02, MDE GmbH, Walldorf, Germany) and recorded with a SPEL Advanced ISOSYS Data Acquisition System. The haloperidol or diazepam effects were characterized by the percentage of the AUC values of recorded contractions before and after adding them into the organ bath. The analyzed periods were 5 min both for the control and the drug-altered contractions.

4. RESULTS

4.1 Gastrointestinal smooth muscle myoelectric activity

The separation of the primary EMG curve from the caecum obtained by serosal thread electrodes reveals that our system detected both slow- and fast-wave (spike) signals. In parallel, the mechanical contractions were also detectable by strain gauge (**Fig. 5**).

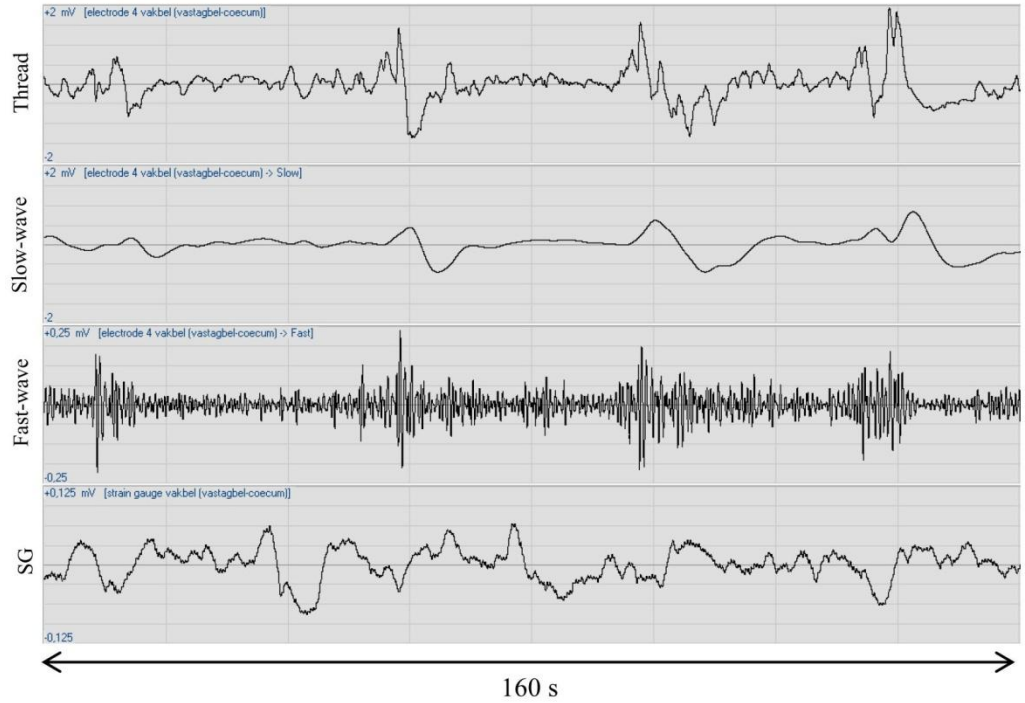

Figure 5. Representative primary myoelectric and mechanical signals of the caecum in rat. The signal was detected by serosal thread electrode pair. The primary signal was separated to slow-wave and fast-wave signals. The strain gauge (SG) detected the mechanical contractions.

Representative primary EMG curves and mechanical contractions from partially GI tract-resected rats revealed the different characteristics of the stomach (**Fig. 6A**), ileum (**Fig. 6B**) and caecum (**Fig. 6C**). The thread and disk electrodes recorded similar myoelectric signals. When the primary EMG curves were transformed by FFT, the resulting cpm values differed for each individual GI tract segment.

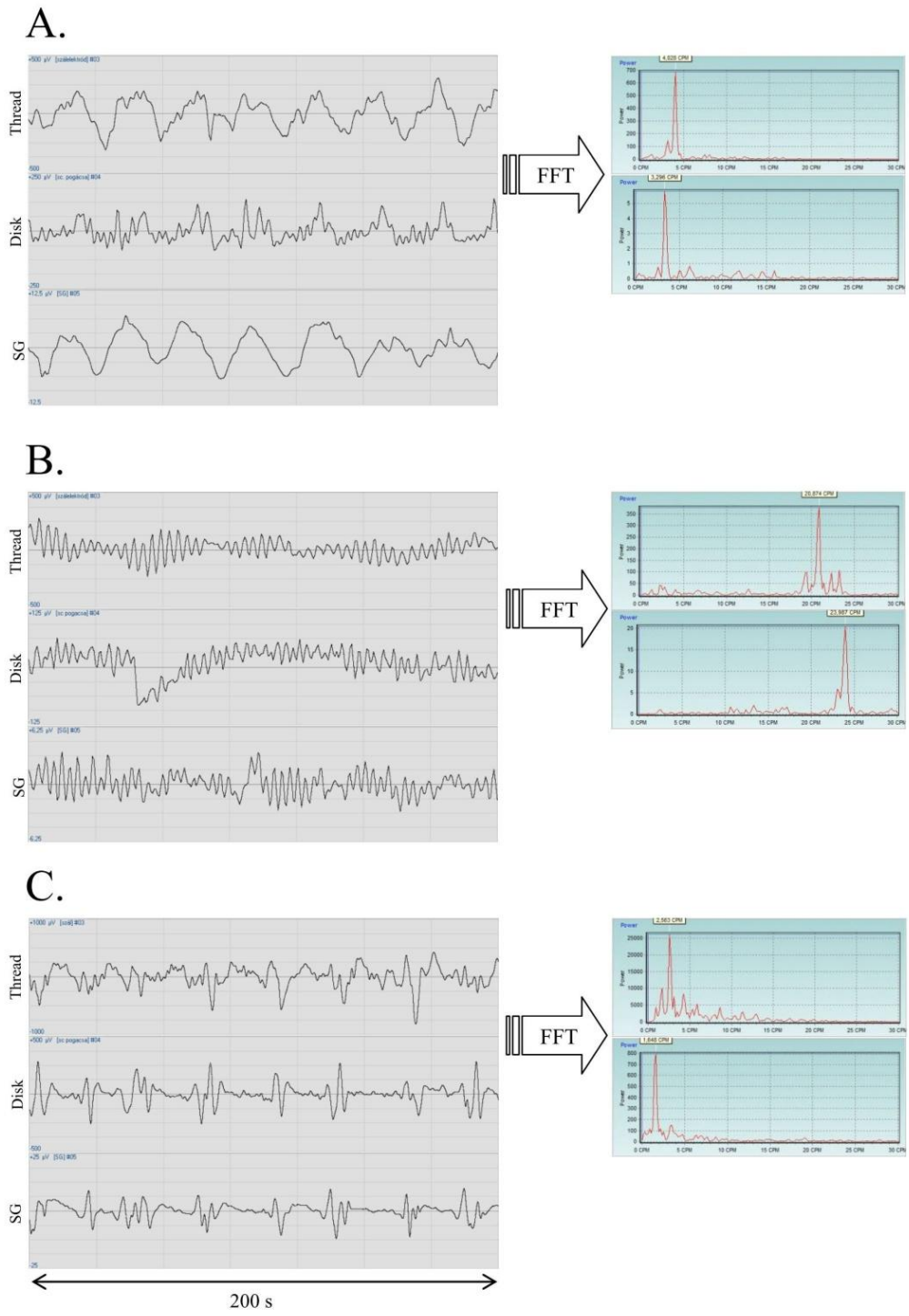

Figure 6. Primary myoelectric and mechanical signals of the stomach (**A, left**), ileum (**B, left**) and caecum (**C, left**) from partially gastrointestinal (GI) tract-resected rats. The myoelectric signals were detected with silver thread and disk electrodes, while the mechanical contractions were measured with the strain gauge (SG). Both the myoelectric and the mechanical signals were found to be typical of the given GI segment. By means of fast Fourier transformation (FFT), the segment specific spectra were gained from the electromyographic signals (**A, B** and **C right**). Each GI

segment has the characteristic frequency expressed in cycles per minute (cpm), determined by the highest peak in the spectrum.

The characteristic cpm values for the stomach, ileum and caecum were 3-5, 20-25 and 1-3 cpm, respectively (**Fig. 7A**). The maximum intensity of the signals (PsD_{max}) was higher with the thread electrodes in the given cpm ranges (**Fig. 7B**).

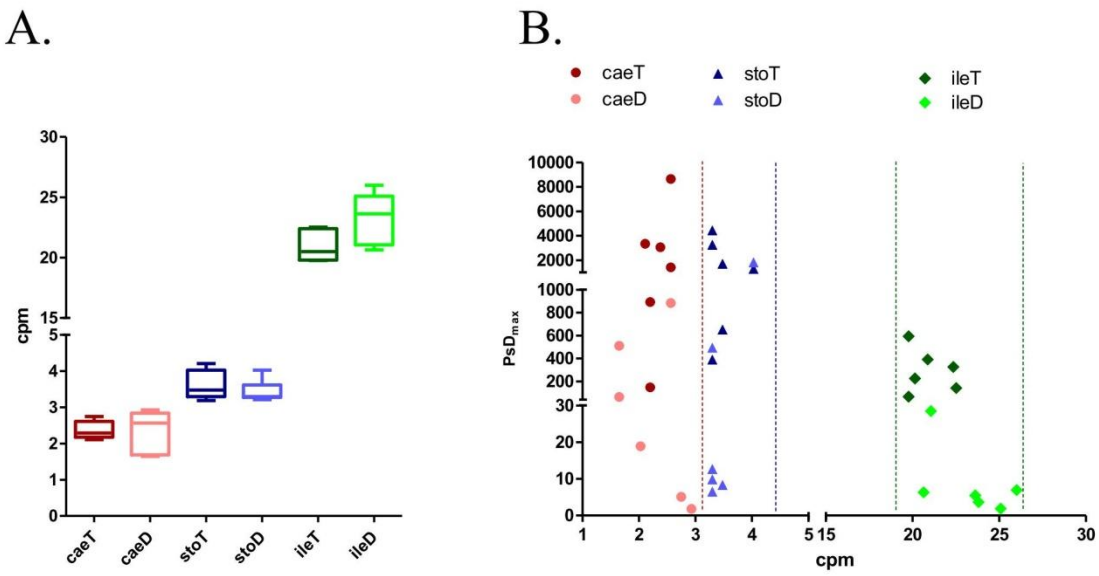

Figure 7. Cycles per minute (cpm) values of the spectra obtained by Fast Fourier transformation for the stomach (sto), ileum (ile) and caecum (cae), measured with thread (T) and disk (D) electrodes. The data are presented as whiskers plots (**A**). The intensity of the signals is expressed as the maximum of the power spectrum density (PsD_{max}), which corresponds to the peak in the spectrum. In the given cpm ranges, the PsD_{max} values detected with the thread electrodes were usually higher than those with the disk electrodes (**B**).

Neostigmine increased, while atropine decreased both the electrical and mechanical signals in the partially GI tract-resected rats. The FFT analysis revealed significant changes in the PsD_{max} values, while the AUC analysis demonstrated similar changes in the contractions in each investigated GI tract segment (**Fig. 8A-C**).

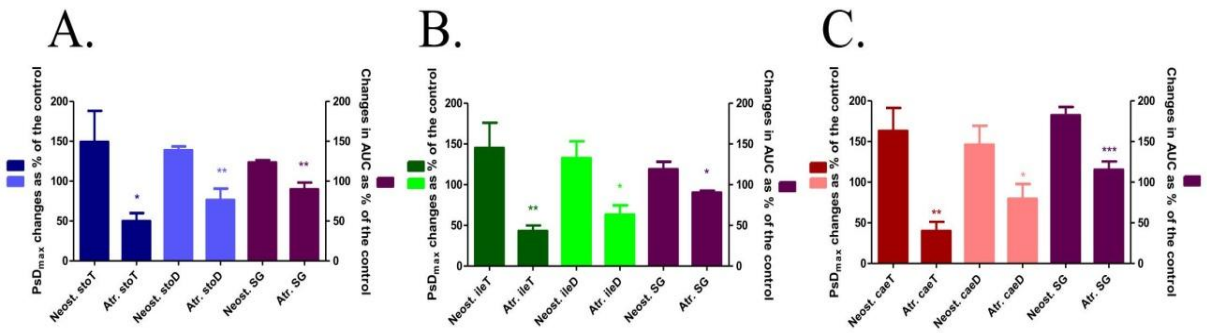

Figure 8. Changes in myoelectric and mechanical gastrointestinal (GI) activity after neostigmine (Neost.) and atropine (Atr.) treatments in partially GI tract-resected rats. The electric signals were detected with thread (T) and disk electrodes (D) and analysed by fast Fourier transformation with GI tract segment specific filtering. The contraction was measured with a strain gauge (SG). The intensity of electric signals was expressed as the maximum of the power spectrum density (PsD_{max}), while the mechanical contraction was evaluated by area under curve (AUC) analysis. In the stomach (sto) (**A**), ileum (ile) (**B**) and caecum (cae) (**C**), neostigmine increased, while atropine reduced the electric and mechanical activities of the smooth muscles relative to the basic activity (100%). ($p<0.05$: *; $p<0.01$: **; $p<0.001$: ***)

However, the pharmacological treatment did not significantly influence the cpm values as compared with the control (**Fig. 9**).

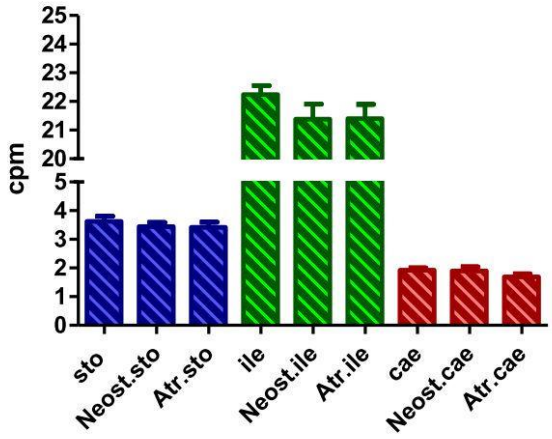

Figure 9. Influence of neostigmine (Neost.) and atropine (Atr.) treatments on myoelectric frequency values in partially gastrointestinal tract-resected rats. The electric signals were detected with both thread and disk electrodes. Neither neostigmine nor atropine altered the characteristic cpms of the stomach (sto), ileum (ile) or caecum (cae) significantly.

In the non-GI tract-resected rats, the subcutaneous abdominal disk electrode recorded the complete electric signals from the GI tract with the basic 0-30 cpm filter to cut out fast-wave signals from other organs. In parallel, the implanted strain gauges in the stomach, ileum and caecum measured the real mechanical contractions of these GI segments (**Fig. 10A**). FFT analysis with a 0-30 cpm filter revealed 3 main maxima in the PsD values, corresponding to the 3 main frequency ranges determined for the stomach, ileum and caecum in the partially GI tract-resected animals (**Fig. 10B**). The individual organ-specific filtering highlighted the PsD_{max} values for the given GI tract segments (**Fig. 10C**).

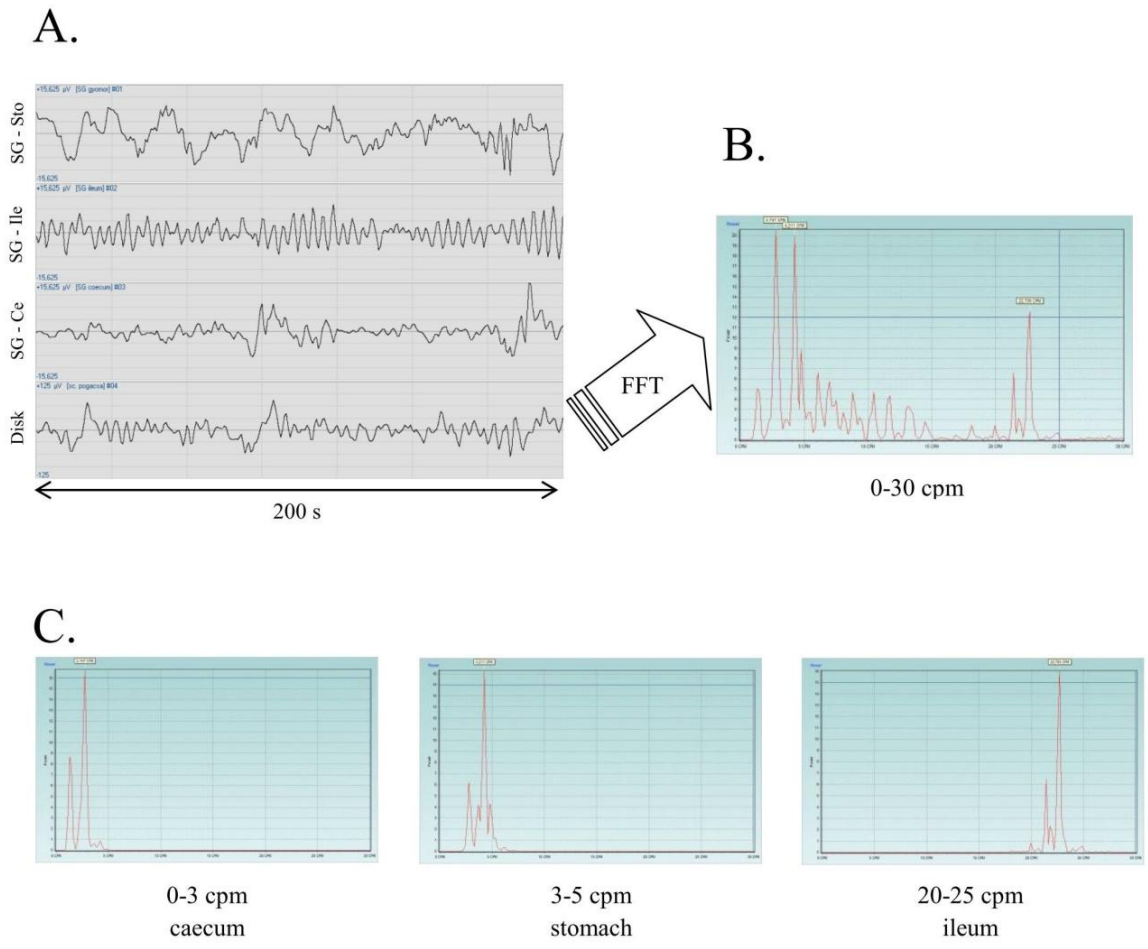

Figure 10. Primary myoelectric and mechanical signals of the gastrointestinal (GI) tract from non-GI tract-resected rats. The myoelectric signals were detected with silver disk electrodes positioned on the abdomen, while the mechanical contractions were measured with strain gauges (SG) sutured onto the stomach (sto), ileum (ile) or caecum (cae) (**A**). Through FFT of the primary myoelectric signals, all 3 maximum power spectrum densities (PsD_{max}) were revealed in the

characteristic cpm ranges for the stomach, ileum and caecum (**B**). The individual specific filters (0-3, 3-5 and 20-25 cpm for the caecum, stomach and ileum, respectively) gave the PsD_{max} values in the typical cpm ranges for each segment (**C**).

We also determined the pharmacological response in the non-GI tract-resected rats. Neostigmine increased, while atropine decreased the filtered electric and mechanical signals in all the GI tract segments. The extents of stimulation or inhibition were similar in the stomach, ileum and caecum (**Fig. 11A-C**).

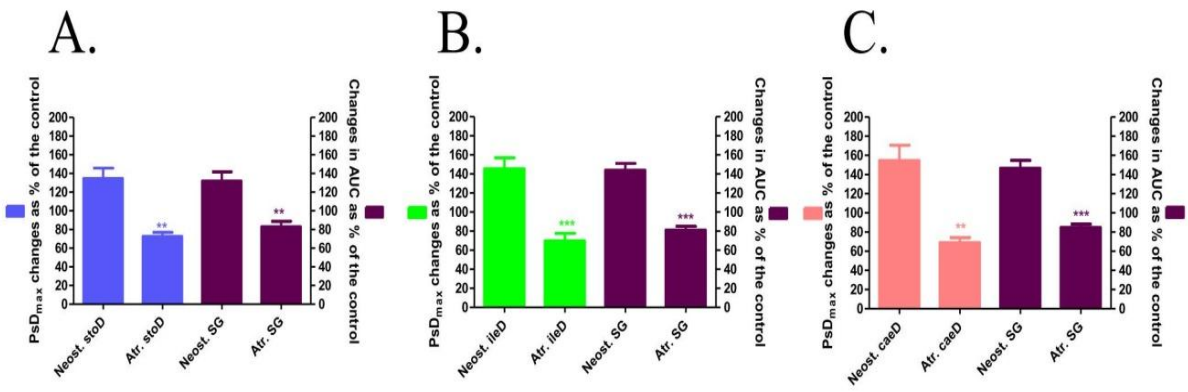

Figure 11. Changes in myoelectric and mechanical gastrointestinal (GI) activities after neostigmine (Neost.) and atropine (Atr.) treatments in non-GI tract-resected rats. The electric signals were detected with abdominal disk electrodes (D) and analysed by fast Fourier transformation with GI tract segment-specific filtering. The contraction was measured by strain gauge (SG). The intensity of electric signals was expressed as the maximum of the power spectrum density (PsD_{max}), while the mechanical contraction was evaluated by area under curve (AUC) analysis. In the stomach (**A**), ileum (**B**) and caecum (**C**) neostigmine increased, while atropine reduced the electric and mechanical activities of the smooth muscles relative to the basic activity (100%). ($p<0.01$: **; $p<0.001$: ***)

A very good correlation was found between the changes in the PsD_{max} and AUC values in all the GI tract segments (**Fig. 12A-C**).

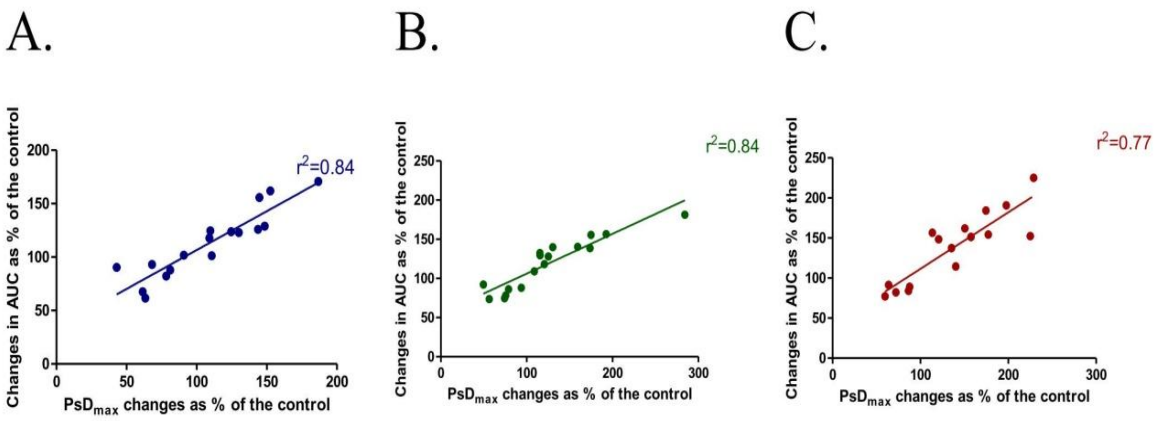

Figure 12. Correlations between myoelectric and mechanical responses induced by neostigmine and atropine treatments in non-gastrointestinal (GI) and GI tract-resected animals. The myoelectric response is expressed as the maximum of the power spectrum density (PsD_{max}), which corresponds to the highest peak in the spectrum gained by fast Fourier transformation. The mechanical response is expressed as the area under the curve (AUC) of the recorded smooth muscle contractions. Linear regression analysis revealed a very good correlation between the changes in PsD_{max} and AUC values in the stomach (**A**), ileum (**B**) and caecum (**C**).

4.2 Pregnant uterus smooth muscle myoelectric activity

Primary EMG curves from GI tract-resected rats represent characteristic myoelectric signals recorded by thread or disk electrodes. When the primary EMG curves were transformed by FFT, the resulting spectra had sharp and dominant peaks (PsD_{max}) at low cpm values, and terbutaline reduced the PsD_{max} of the spectra (**Fig. 13**).

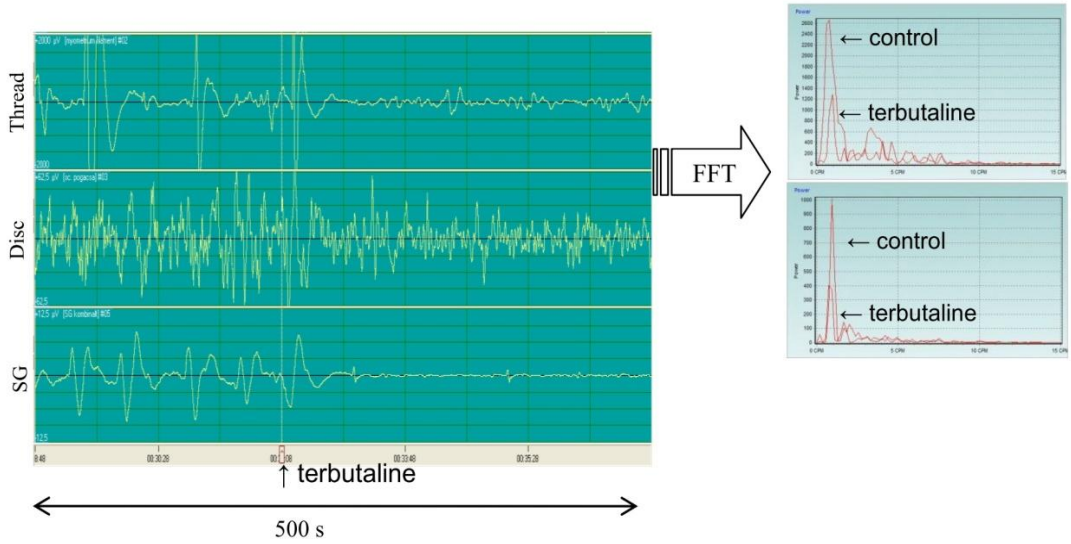

Figure 13. Primary myoelectric and mechanical signals of the pregnant uterus (**left**), from gastrointestinal (GI) tract-resected rats. The myoelectric signals were detected with silver thread and disk electrodes, while the mechanical contractions were measured with the strain gauge (SG). Fast Fourier transformation (FFT) reveals specific spectra from the electromyographic signals (**right**). Each spectrum has the characteristic frequency expressed in cycles per minute (cpm), determined by the highest peak in the spectrum.

The characteristic cpm value for the uterus was found between 1-2.5 cpm measured by thread or disk electrodes (**Fig. 14A**). The PsD_{max} representing the maximum intensity of the signals was higher with the thread electrodes in the cpm range 1-2.5 (**Fig. 14B**).

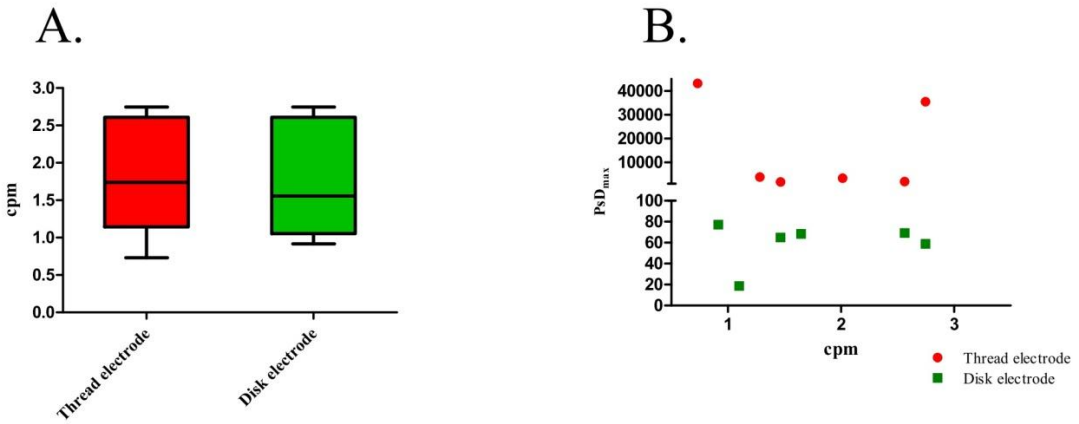

Figure 14. Cycles per minute (cpm) values of the spectra obtained by Fast Fourier transformation, measured with thread and disk electrodes. The data are presented as whiskers plots (**A**). The

intensity of the signals is expressed as the maximum of the power spectrum density (PsD_{max}) which corresponds to the peak in the spectrum. In the given cpm ranges, the PsD_{max} values detected with the thread electrodes were usually higher than those with the disk electrodes (**B**).

In pharmacological experiments oxytocin increased, while terbutaline decreased both the electrical and mechanical signals of the uterus in the GI tract-resected rats (**Fig. 15**).

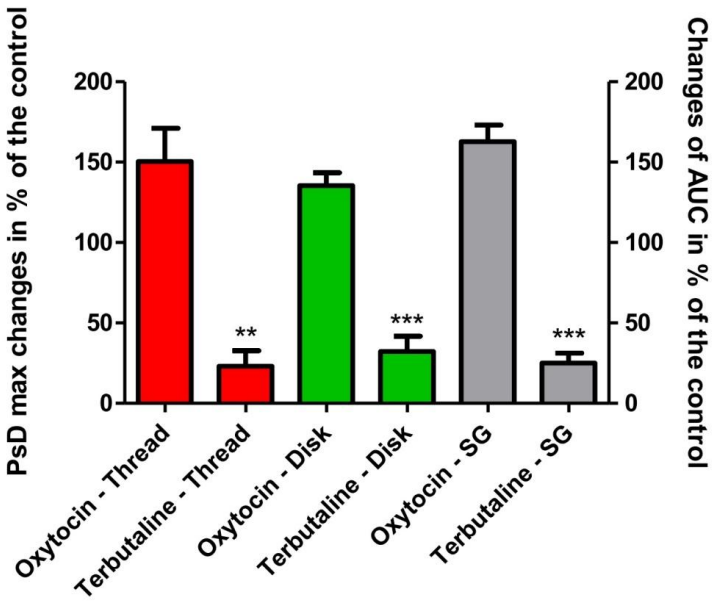

Figure 15. Changes in myoelectric and mechanical uterine activity after oxytocin and terbutaline treatments in GI tract-resected rats. The electric signals were detected with thread and disk electrodes and analysed by fast Fourier transformation with specific frequency filter. The mechanical contraction was measured with a strain gauge (SG). The intensity of the electric signals was expressed as the maximum of the power spectrum density (PsD_{max}), while the mechanical contraction was evaluated by area under curve (AUC) analysis. Oxytocin increased, while terbutaline reduced the electric and mechanical activities of the smooth muscles relative to the basic activity (100%). ($p<0.05$: *; $p<0.01$: **; $p<0.001$: ***)

The FFT analysis revealed significant changes in the PsD_{max} values, while the AUC analysis demonstrated similar changes in the mechanical contractions. The extents of stimulation or inhibition were similar in both mechanical and electrical changes. A very good correlation was found between the changes in the PsD_{max} and

AUC values measured by thread (**Fig. 16A**) or disk electrode (**Fig. 16B**) in comparison with SG signals.

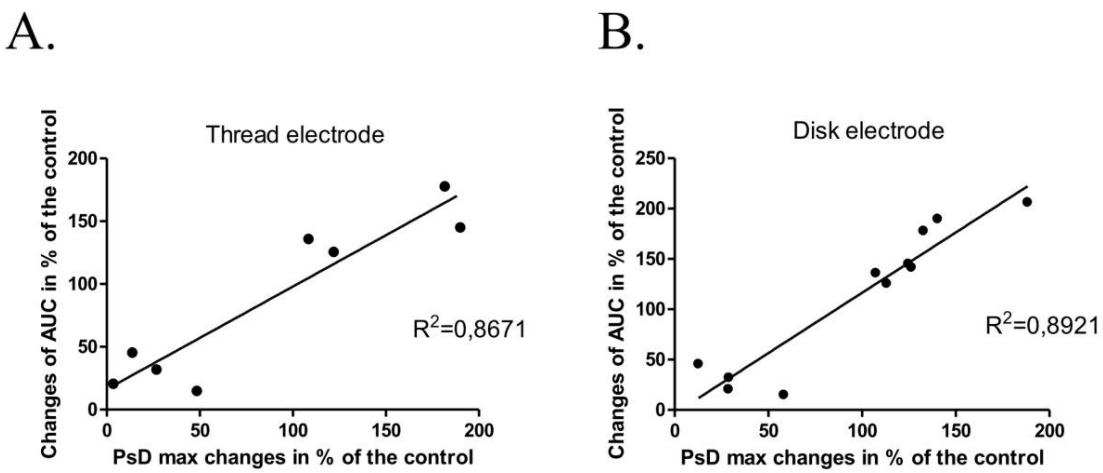

Figure 16. Correlations between myoelectric and mechanical responses induced by oxytocin and terbutaline treatments in GI tract-resected animals. The myoelectric response is expressed as the maximum of the power spectrum density (PsD_{max}), which corresponds to the highest peak in the spectrum gained by fast Fourier transformation. The mechanical response is expressed as the area under the curve (AUC) of the recorded smooth muscle contractions. Linear regression analysis revealed a very good correlation between the changes in PsD_{max} and AUC values in the uterine signals recorded by thread (**A**), or disk electrode (**B**).

The myoelectric and contractility responses of caecum and pregnant myometrium have been recorded for different drugs in parallel. We have administered neostigmine and atropine, which mainly act on the GI tract, and oxytocin and terbutaline, which have effects on the myometrium. Neostigmine and atropine treatment caused significant changes in the myoelectric signal of the caecum (**Fig. 17A**), while oxytocin and terbutaline had actions both the electrical and mechanical signals of the uterus only (**Fig. 17B**).

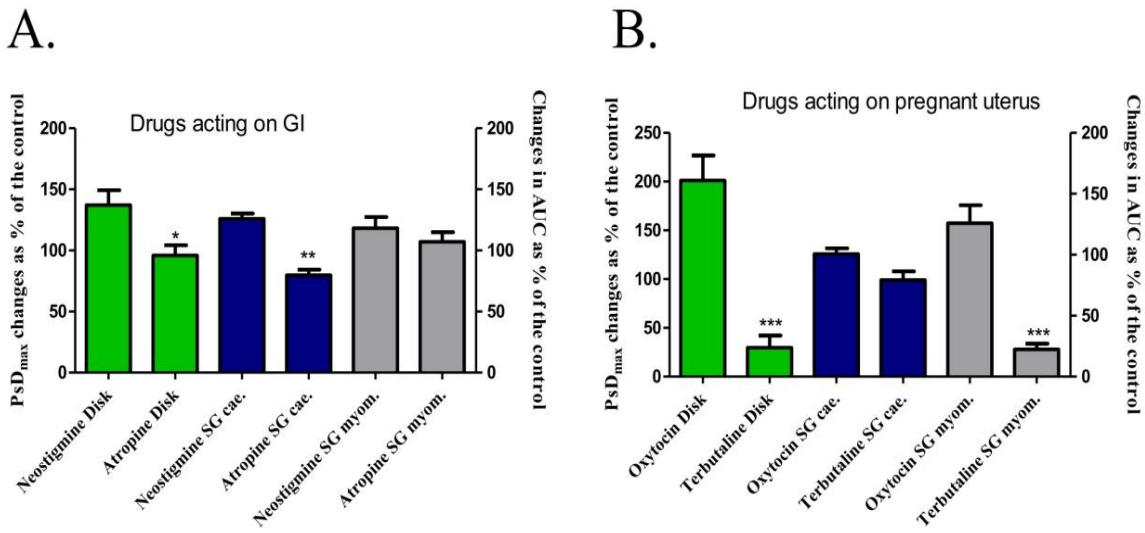

Figure 17. Changes in myoelectric and mechanical gastrointestinal (GI) and myometrial activities after neostigmine and atropine (**A**), or oxytocin and terbutaline (**B**) treatments in non-GI tract-resected rats. The electric signals were detected with abdominal disk electrodes and analysed by fast Fourier transformation with 0-3 cpm frequency filter. The contraction of caecum (cae.) and myometrium (myom.) was measured by strain gauge (SG). The intensity of electric signals was expressed as the maximum of the power spectrum density (PsDmax), while the mechanical contraction was evaluated by area under curve (AUC) analysis. Significant changes were found in case of neostgimin-atropine treatment in cecal signals, while oxytocin-terbutaline treatment affected the uterine smooth muscle signals relative to the basic activity (100%). ($p<0.05$: *; $p<0.01$: **; $p<0.001$: ***).

4.3 **Detection of stress in wakeful rats**

The myoelectric activity of the GI tract was detected in awake rats. The motion artifacts had approximately 4-fold higher signals on the primary record as compared with the GI tract activity. The motion artifact was separated from the basal electric activity of the GI smooth muscle by the cutter built into the software. With this modification, the motion artifact-induced peaks were cut in the primary curves (**Fig. 18A-B**). When the modified EMG curves were transformed by FFT, the PsD_{max} values on the modified spectra were reduced, but the cpm frequencies of the PsD_{max} values remained in the same range (**Fig. 18 C**).

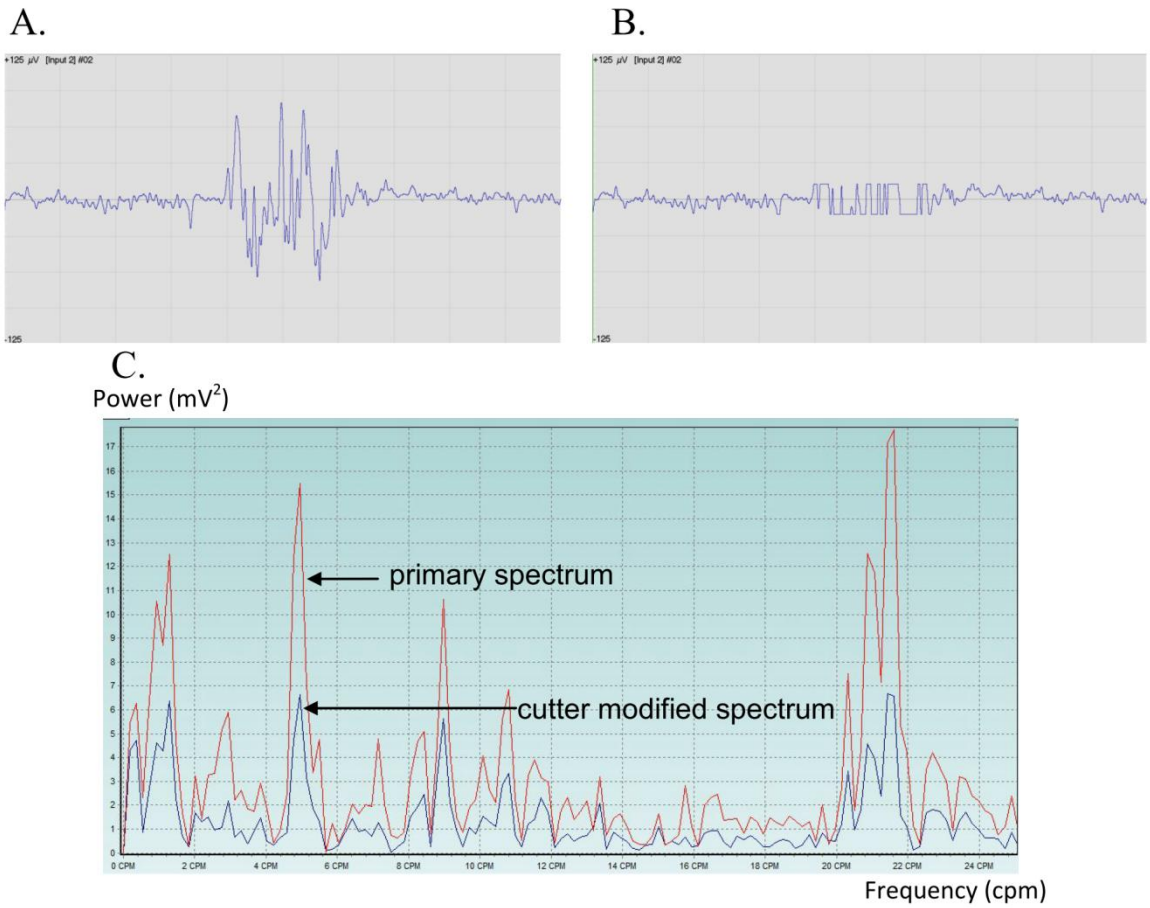

Figure 18. The primary myoelectric signal of the gastrointestinal tract in rat with outlier peaks of motion artifact (**A**) was cut by the software in-built digital cutter to remove the motion artifacts (**B**). The specific spectra were gained by fast Fourier transformation (FFT). The red curve represents the FFT spectrum of the primary spectrum with motion artifacts, while the blue curve shows the spectrum after the digital cut. The cycle per minute (cpm) frequencies of the peak values remained the same after modification, but with lower power spectrum density maximum values (**C**).

The digital cut reduced the motion artifact elevated PsD_{max} values to the range of values in anaesthetized rats, but did not further reduce the values detected in anesthetized rats (**Fig. 19**).

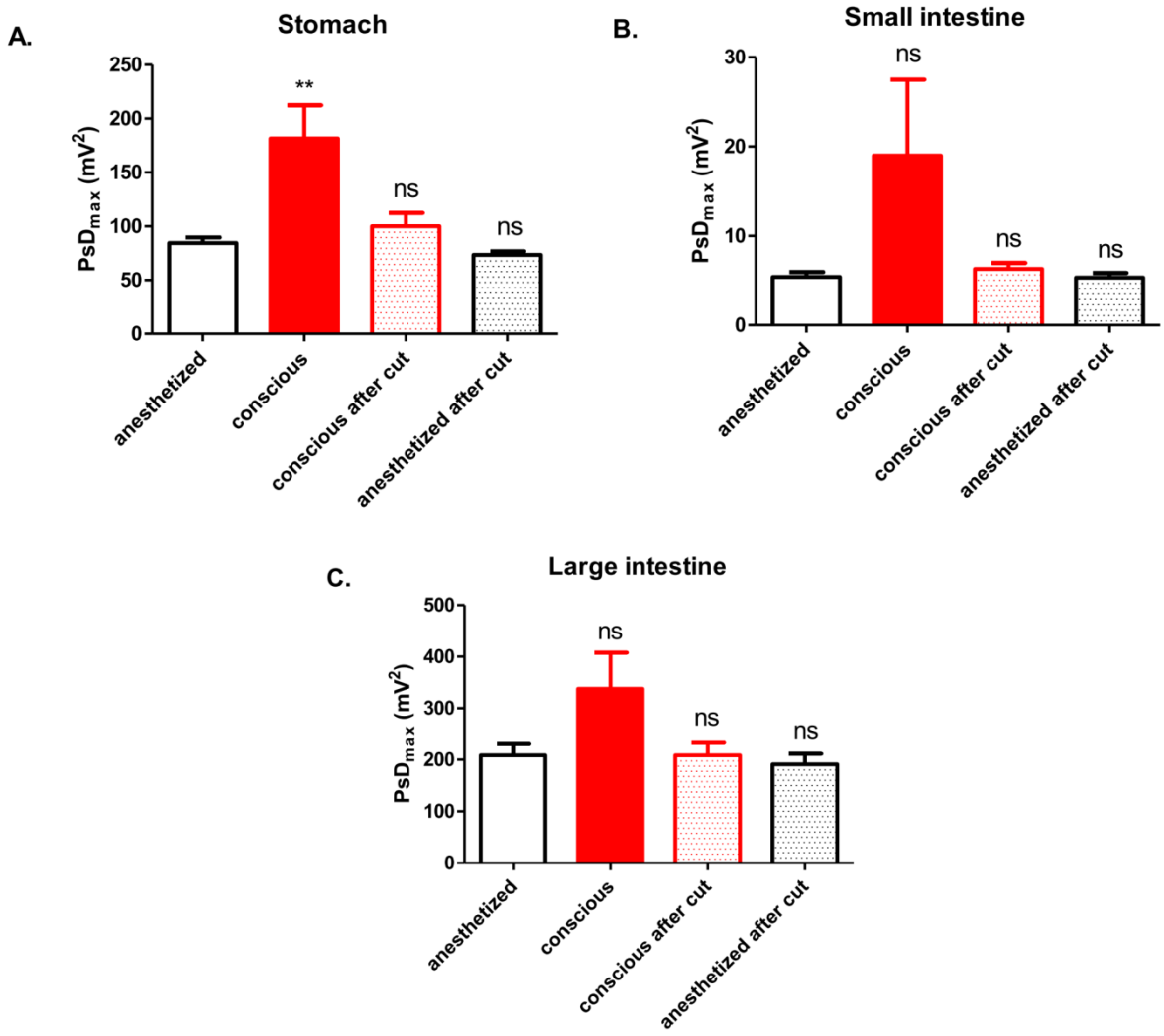

Figure 19. Changes of power spectrum density (PsD_{max}) values in stomach (**A**), small intestine (**B**) and large intestine (**C**) in rats (n=20). The motion artifact in the conscious animal group led to high PsD_{max} values, but these outliers were removed with the digital cutter. The application of the digital cutter did not modify significantly the motion artifact-free myoelectric signals from the anaesthetized rats, but reduced the PsD_{max} values of conscious rats to the anesthetized level after the cut. (ns: not significant; $p < 0.01$**)

Immobilization stress significantly increased the plasma level of corticosterone. The rate of increase was approximately 3-fold as compared with the resting value (resting value: 75.4 ng/ml, stress value: 214.5 ng/ml) (**Fig. 20A**). In parallel, the PsD_{max} values in the whole GI tract were elevated 1.5-2-fold during immobilization. The greatest increase was found in the gastric values, although the increase in the gastric PsD_{max} values was not significantly higher than the increase in the small or large intestine values (**Fig. 20B**).

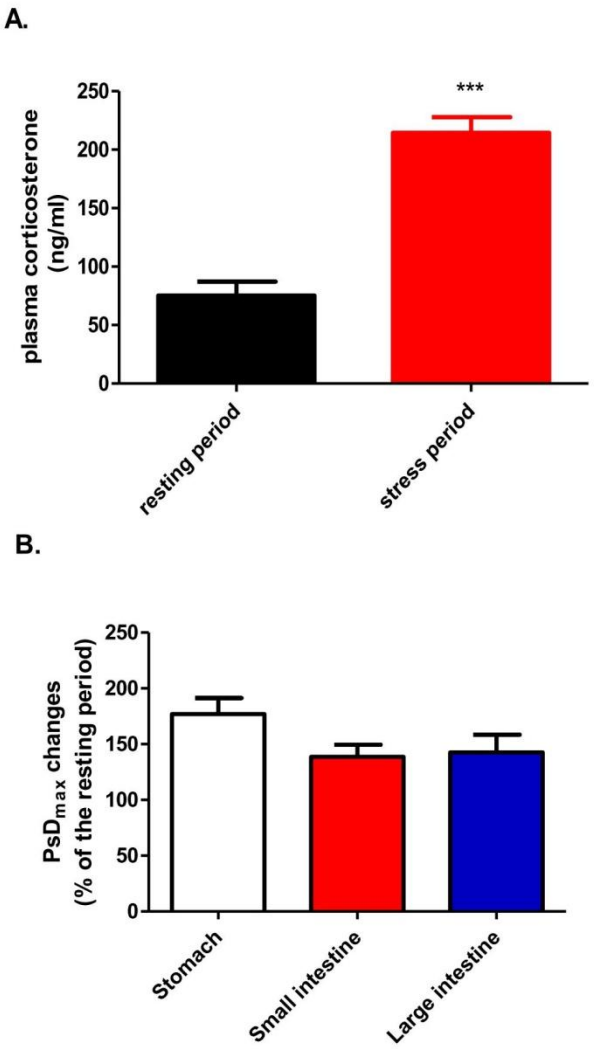

Figure 20. Immobilization stress induced a significant increase in the plasma corticosterone level of rat (n=20) as compared with the resting period (***: $p<0.001$) (**A**). The stress condition also raised the power spectrum density (PsD_{max}) values of the FFT spectra from the stomach, small intestine and large intestine as compared with the resting period. (**B**).

Diazepam treatment reduced the resting EMG activity by 40-60%, while the stress-induced PsD_{max} values were set back to the resting level, reaching around 100% in all the three GI tract sections. In the large intestine, immobilization stress did not induce a significant increase in the presence of diazepam. **(Fig. 21A)**. Haloperidol treatment reduced the resting EMG activity by 40%, but immobilization stress was not able to induce significant elevation in the myoelectric activity of the whole GI tract **(Fig. 21B)**.

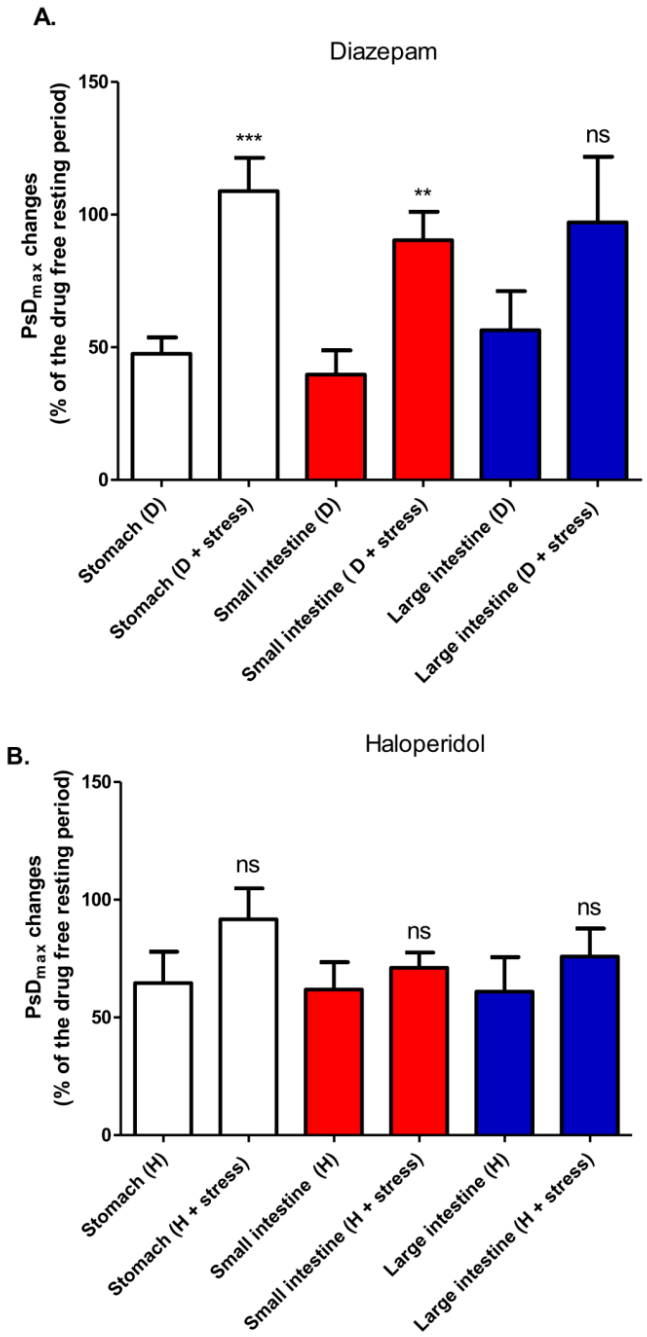

Figure 21. Changes in power spectrum density maximum (PsD_{max}) values after diazepam (**A**) or haloperidol (**B**) treatments in conscious rats (n=15 for each group) under immobilization-induced stress. The values are expressed as the percentage of the resting values without drug treatment. Both diazepam and haloperidol reduced the myoelectric activities of the gastrointestinal smooth muscles. They reduced the PsD_{max} values during the stress-free period, however, only haloperidol was able to inhibit the increase in the PsD_{max} value during immobilization stress. (ns: not significant; $p < 0.01$**; $p < 0.001$***)

Both diazepam and haloperidol treatments reduced the plasma corticosterone levels during the resting period by approximately 30 %, however, these alterations were not significant. Only haloperidol was able to blot out the stress hormone inducing effect of immobilization **(Fig. 22)**.

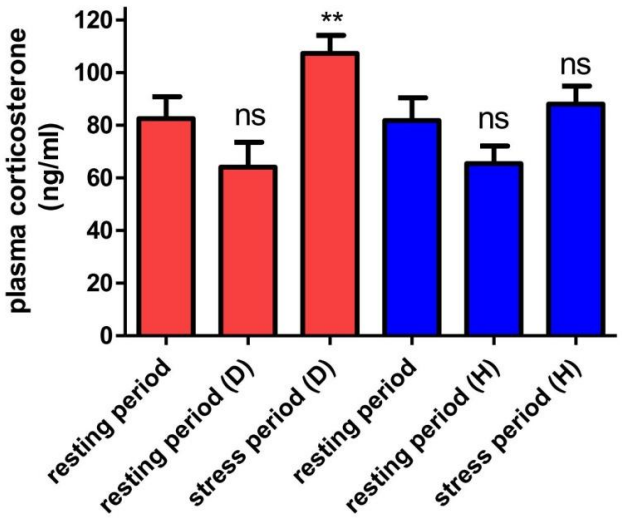

Figure 22. Alterations of plasma corticosterone levels by drug treatments (red columns: diazepam, blue columns: haloperidol) and immobilization stress in rats (n=15 for each group). The hormone level was elevated significantly by stress compared to the control level. Diazepam (5 mg/kg) or haloperidol (1 mg/kg) treatment alone reduced the plasma corticosterone values, although the alterations were not significant. Only haloperidol was able to inhibit the increase in the plasma corticosterone level during immobilization stress. (D. diazepam; H: haloperidol; ns: not significant;*: $p<0.05$; **; $p<0.01$)

The plasma level of diazepam was 0.42 μg/ml at the end of the resting period (30 min after drug administration), and its level was reduced to 0.13 μg/ml at the end of the stress period (60 min after drug administration). Haloperidol was not detectable in the plasma even at the end of the resting period, but it was found in the brain, liver and lung in a concentration of 0.30, 1.91 and 2.23 μg/g, respectively. At the end of the stress period, the levels of haloperidol were still considerable in these organs (brain: 0.16 μg/g, liver: 1.33 μg/g, lung: 0.96 μg/g). After statistical analysis, only the pulmonary level of haloperidol at the end of the stress period was significantly lower as compared with the end of the resting period **(Fig. 23)**.

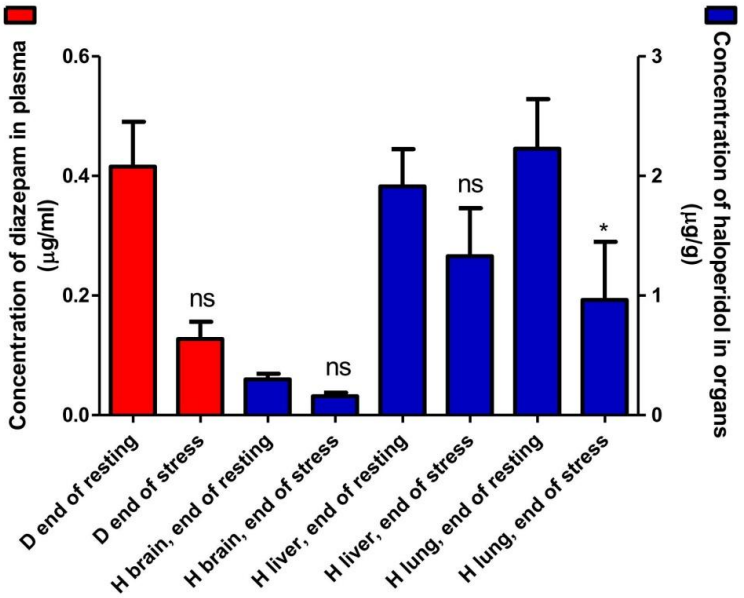

Figure 23. Plasma levels of diazepam (red columns) and organ levels of haloperidol (blue columns) after 30 min (end of resting period) and 60 min (end of stress period) of intraperitoneal administration (5 mg/kg for diazepam, 1 mg/kg for haloperidol) in rats (n=15 for each group). The drug levels were determined by the HPLC method. (D: diazepam; H: haloperidol; ns: not significant; *: $p<0.05$).

Significant correlation was found between the drug induced change in the PsD_{max} values of the stomach, small intestine or large intestine and the change in corticosterone plasma levels. The levels of correlations were the same in all the three GI tract segments **(Fig. 24)**.

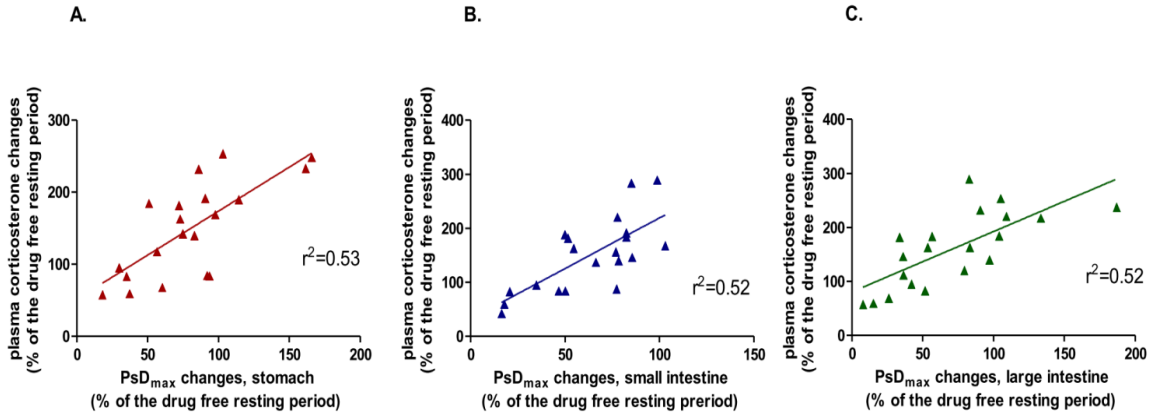

Figure 24. Correlation between the changes of the power spectrum density maximum (PsD_{max}) values in the given sections of the gastrointestinal system (**A**: stomach, **B**: small intestine, **C**: large

intestine) and the changes of corticosterone plasma levels in rats. The linear regression analysis proved a significant correlation between PsD_{max} and corticosterone alteration.

Neither diazepam nor haloperidol elicited any significant relaxing effect on gastric strip, ileal or cecal rings in isolated organ studies **(Fig. 25)**.

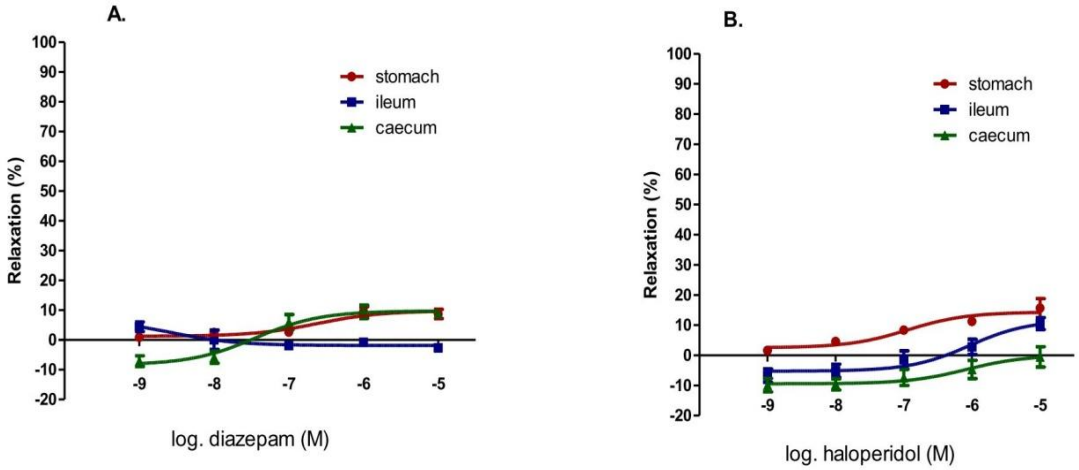

Figure 25. Effects of diazepam (**A**) and haloperidol (**B**) on KCl induced stomach, ileum and caecum contractions. The change in contraction was calculated via the area under the curve and expressed in % ± SEM. No significant effects were observed directly on the investigated GI samples induced by the drugs.

5. DISCUSSION

Several attempts have been made to record the GI tract and uterine motilities with either implanted or surface electrodes *in vivo*, but the ideal method has still not been found. Two main trends can be distinguished in the methods of recording GI tract myoelectric signals: determination of the propagation of the GI tract electric waves with a multi-electrode array [25-27] and investigation of the GI tract myoelectric activity with serosal or cutaneous electrode pairs [28-31]. The latter usually focuses on the characterization of the electric signals, searching for cycles or periods in the spectra of the myoelectric waves. The multi-electrode array method is also appropriate for the determination of the frequency of the contractions.

5.1 Gastrointestinal smooth muscle electromyography

The focus during the development of our research model was to create a smooth muscle electromyographic tool for simple and reliable measurements on these organs with pharmacological and diagnostic aims. We applied simple electrode pairs, and we used built-in digital filters in the frequency range 0-30 cpm to separate the noise-free, slow-wave smooth muscle signals.

The electric signals of the smooth muscles, including the GI tract or pregnant uterus, are known to have significantly lower frequencies as compared with brain, cardiac or skeletal muscle electric signals. FFT analysis reveals that the frequency ranges of electroencephalograms, electrocardiograms and electromyograms for skeletal muscles are 5-50 Hz, 3-20 Hz and 10-20 Hz, respectively [32, 33]. The slow waves of smooth muscle electric activity are usually characterized with cpm that is the 60-fold value of Hz. The cpm for the gastric smooth muscles in humans has been found to be 2.5-4 cpm [29, 34, 35], while that for the ileum has been described as 7.5-12 cpm in humans [28, 36, 37] and 14-17 cpm in mice [27]. The information available on the myoelectric cpms for the colon and uterus are very poor; an old study reported 2-9 cpm from *in vitro* measurements on human colon specimens [38].

The FFT calculated range for the stomach (3-5 cpm) was in harmony with earlier findings in humans [29, 34, 35]. That for the caecum (1-3 cpm) was lower,

while that for the ileum (20-25 cpm) was higher than earlier findings in humans or rodents [27, 37, 38]. The similarity only with the gastric data can be explained by the fact that the contractility force of the stomach is usually greatest among the GI tract segments [39, 40] and even with weaker filters and sensors, therefore these predominant signals can be detected quite well.

5.2 Uterine smooth muscle electromyography

In order to determine the individual myoelectric signals of the pregnant uterus and the GI tract segments, i.e. the stomach, small or large intestine, we resected non-investigated parts. Two pairs of electrodes measured the myoelectric signals from the left segment: one was inserted into the organ, while the other was positioned under the skin of the abdomen. The most intensive signals were provided by the organ-inserted electrodes, and these signals were also measured from the abdomen to allow a comparison of the detectability of the activities between the two sites. Electrical signals of the same character were obtained from the surface of the organ and from the abdomen. We found that pregnant uterine smooth muscle frequency is between 1-3 cpm. The myoelectric activity was measured in parallel with the mechanical contractions by the applied strain gauge. This triple measurement with the partial resection and electric filters gave a clear record of the electric waves of the given organs and their detectability through the abdominal wall in comparison with the contractions. The detection of the myoelectric activity from the abdomen through the disk electrodes has a special significance: the possible motion artefact caused by the muscle contraction cannot influence the disk electrode as compared with the thread electrodes implanted in the serosal surface of the muscle. Although both spikes and slow wave signals can be obtained from the serosal surface, the abdominal record reveals only slow wave because of the filtering effect of the tissues [17]. We proved that we were able to detect the spikes (Fig.5), but those are not applicable for a non-invasive measurement, therefore all the fast-wave signals have been filtered out during our further experiments.

The experiments on the partially resected animals revealed that the stomach, ileum and caecum have different mechanical and myoelectric activities, which are

well distinguishable from each other. However, the myoelectric signals of the pregnant uterus and caecum overlap.

The salience peaks for the given GI tract segments and the uterus were detected and distinguished even in non-GI tract-resected animals in the range 0-30 cpm after FFT analysis of the primary records. This means that the smooth muscle activity can be detected at the same time *in vivo* from the abdomen.

The cpm values for the given GI tract segments remained constant during the pharmacological treatments. The PsD_{max} values, however, varied depending on the intensity of the contractions. We proved this correlation via the well-known GI tract motility increasing and decreasing effects of neostigmine and atropine, respectively. We did not find significant differences among the GI tract segments in their response to either neostigmine, or atropine. This is not a surprise, because there is no information concerning any differences in sensitivity to these drugs in the main GI tract parts. The oxytocin-induced increase and the terbutaline-induced decrease were clearly detectable via the observation of the changes in the PsD_{max} intensity of the myoelectric spectra from pregnant uterus.

The PsD_{max} values usually express the intensity of myoelectric signals. We observed a very good correlation between the changes in mechanical contractile response and the changes in electric PsD_{max} values. This means that the changes in PsD_{max} values recorded even on the abdominal wall perfectly reflect the real changes in the contractions of the GI tract and uterine smooth muscle.

The frequency range for the pregnant uterus and the colon myoelectric activity are almost equals therefore we launched a series of experiments with non-GI-resected pregnant animals to investigate how we can distinguish the myoelectric signals of these two types of smooth muscles. The mechanical contractions of both organs were detected by strain gauges applied on the surface of the caecum and pregnant myometrium. It is known that the caecum and pregnant myometrium have different responses to drugs. Caecum is much more sensitive to neostigmine (contraction) and atropine (relaxation), while the pregnant uterus has a very intensive response to oxytocin (contraction) and terbutaline (relaxation) [41, 42]. We found that drugs acting on myometrial activity altered the PsD_{max} with a good correlation to

myometrial mechanical contractions, but they did not affect cecal activity. The drugs acting on the caecum elicited similar action on cecal activity without influencing the myometrial response. It means that we can follow the uterine contractions with the evaluation of PsD_{max} values when the uterine activity is predominant. During pregnancy (especially towards the end of pregnancy) the mass of the uterus is significantly increased, and its activity is predominant over the cecal contractions. Our method seems to be proper for the detection of the uterine activity in that late-pregnancy period.

5.3 **Smooth muscle electromyography in stress measurements**

On the other hand, the non-invasive and reliable measurement of stress condition by physiological parameters is still an unsolved clinical problem. Although the relationships between stress condition and organ systems like cardiovascular, dermal or GI systems are obvious, the level of stress is mostly appraised by psychological tests [43]. A new technique and instrument measuring both psychological and physiological parameters have been reported for stress evaluation. Among the physiological parameters, this instrument detects cardiovascular and dermal responses [44], however, the GI parameters are not involved in the analysis. Although the *in vivo* GI motility can be assessed by the measurement of gastric content [45] or by the detection of a coloured marker in the intestine [46], these methods evaluate the GI responses after the termination of the experimental animals.

The consequences of the function of the brain-gut axis include the stress induced alterations in the GI tract motility, which may lead to inflammatory bowel disease, peptic ulcer and many other GI tract disorders [47]. Acute stress response enhances the motility in the GI tract, which is controlled by peripheral neuropeptides and corticosterone-releasing hormone [48]. We hypothesized that our EMG method can be applicable to awake rats and to measuring the correlation between GI tract motility and stress condition.

Awake EMG measurements are always disturbed by motion artifacts, especially in experimental animals where the movements cannot be reduced by self-

control. Therefore, our first task was to identify the EMG signals of motion artifacts and then develop a digital cutter to remove them from the record without significant loss of smooth muscle data. The motion artifacts had much bigger EMG signals than the smooth muscle did, thus their identification and removal was an easy task by the newly developed digital cutter. The goodness of cut was proved by the FFT spectrum analysis, in which the characteristic peaks remained at the same frequency, only their PsD_{max} values were reduced, which could be anticipated before cut. After cut, the smooth muscle EMG record showed basal activity like in anesthetized animals, where the motion artifact is a non-significant factor. These results suggest that we have successfully kept the smooth muscle signals with the effective removal of motion artifact.

Acute immobilization, which increases the plasma corticosterone level, is an accepted stress method in rats [49]. Immobilization can increase the stress factors within quite a short period of time, 30-60 min can be enough for the significant elevation of stress hormone levels in rodents [50, 51]. Considering this fact, we applied immobilization to our rats for 30 minutes, which period is also suitable for the detection of GI tract myoelectric activity. Both corticosterone and PsD_{max} values were increased during the immobilization, and the correlation found between the two parameters indicates that the severity of acute stress can be predicted by the detection of smooth muscle EMG in awake rats. This correlation was reinforced by the results gained after treatment with central nervous system depressants.

Diazepam is a well-known anxiolytic agent, which elicits a moderate stress reducing effect in rats [52]. Haloperidol is a strong antipsychotic, which can cease the whole stress response, although it can induce oxidative stress in the brain but only in the case of chronic treatment [53]. However, in our experiments the animals were terminated after 60 min of single drug treatment, therefore the oxidative stress-inducing effect of haloperidol could not develop. The plasma levels of both diazepam and haloperidol were checked 30 min (end of resting period) and 60 min (end of stress period) after their administration. The measurement of plasma levels was important to justify the presence of effective concentrations during our experiments. In the case of diazepam, the lowest plasma level was around 0.1 μg/ml at the end of the experiments, which provides sufficient anxiolytic action in rats [54]. Haloperidol has

special pharmacokinetics with a fast distribution into different organs, thus the concentration of haloperidol in these organs can be much higher than its plasma concentration after 1 h of parenteral administration [55]. In our experiments, the haloperidol plasma level was under the limit of our detection even after 30 min, but significant amounts of drug were found in the brain, liver and lung. Since around 0.1 μg/g concentration of haloperidol is considered to have therapeutic action in rats [56], the 0.16 μg/g concentration that was found after 1 h in the brain of our rats seems to be satisfactory to provide the required neuroleptic effect.

While haloperidol blocked the rise of corticosterone during acute stress, diazepam was only able to reduce the increase in the stress hormone level. This difference between the effects of these two types of drug was clearly detectable in the smooth muscle EMG response of the GI tract, especially in the gastric and the small intestine PsD_{max} values. The isolated organ bath studies proved that neither diazepam nor haloperidol had a direct smooth muscle relaxing effect on the GI tract smooth muscles, therefore the responses found in awake rats originated from their central nervous system effects, possibly mediated through the brain-gut axis.

6. CONCLUSION

Based on our results, we believe that we have successfully developed a method for the *in vivo* detection of GI activity that serves a basis for non-invasive, reliable and specific GI tract segment measurements. We have clarified the different myoelectric activities of the stomach, ileum and caecum. Additionally, our preclinical method is able to investigate the pregnant myometrial activity, *in vivo*, and already suitable for pharmacological investigations for drugs acting on GI tract or uterine contractions.

It also can be concluded that our smooth muscle EMG instrument can measure the level of acute stress in awake rats, which shows correlation with the stress hormone plasma level. This finding is further evidence for the function of the brain-gut axis. Our technique is also applicable to the investigation of different drugs affecting the central nervous system through the GI tract myoelectric response.

The drawback of our method is that we can use the changes in PsD only as a basis for comparison. There are huge differences in electric activity between the individual experimental animals, and the deviations between the absolute values are high. We are currently seeking a way to reduce these differences.

We suppose that this method serves as a good basis for the development of a new clinical tool to investigate the pathophysiological processes in GI tract or pregnant uterus contractility in animals and assess the stress and anxiety levels of patients in different kinds of psychological disorders and during pharmacotherapy. Our method is the first one that can measure the stress response via the GI tract reactions. This kind of monitoring of smooth muscle myoelectric responses may open a new perspective for internist to follow up the changes in GI tract or even for obstetricians to detect early signs of premature contractions or predict initiation of labour. Moreover, this method is useful in the diagnosis and therapy of psychosomatic disorders.

7. REFERENCES

1. Aderinto-Adike, A.O. and E.M. Quigley, *Gastrointestinal motility problems in critical care: a clinical perspective.* J Dig Dis, 2014. **15**(7): p. 335-44.

2. Varni, J.W., et al., *PedsQL gastrointestinal symptoms module: feasibility, reliability, and validity.* J Pediatr Gastroenterol Nutr, 2014. **59**(3): p. 347-55.

3. Paine, P., J. McLaughlin, and S. Lal, *Review article: the assessment and management of chronic severe gastrointestinal dysmotility in adults.* Aliment Pharmacol Ther, 2013. **38**(10): p. 1209-29.

4. Lammers, W.J., *Normal and abnormal electrical propagation in the small intestine.* Acta Physiol (Oxf), 2015. **213**(2): p. 349-59.

5. Cryan, J.F. and S.M. O'Mahony, *The microbiome-gut-brain axis: from bowel to behavior.* Neurogastroenterol Motil, 2011. **23**(3): p. 187-92.

6. Chen, X., R. D'Souza, and S.T. Hong, *The role of gut microbiota in the gut-brain axis: current challenges and perspectives.* Protein Cell, 2013. **4**(6): p. 403-14.

7. De Palma, G., S.M. Collins, and P. Bercik, *The microbiota-gut-brain axis in functional gastrointestinal disorders.* Gut Microbes, 2014. **5**(3): p. 419-29.

8. Drossman, D.A., *Functional Gastrointestinal Disorders: History, Pathophysiology, Clinical Features and Rome IV.* Gastroenterology, 2016.

9. Hajagos-Toth, J., et al., *The effects of progesterone on the alpha2-adrenergic receptor subtypes in late-pregnant uterine contractions in vitro.* Reprod Biol Endocrinol, 2016. **14**(1): p. 33.

10. Horn, L.C., A. Meinel, and B. Hentschel, *c-kit/CD 117 positive cells in the myometrium of pregnant women and those with uterine endometriosis.* Arch Gynecol Obstet, 2012. **286**(1): p. 105-7.

11. Hutchings, G., et al., *Myometrial interstitial cells and the coordination of myometrial contractility.* J Cell Mol Med, 2009. **13**(10): p. 4268-82.

12. Van Oudenhove, L. and Q. Aziz, *The role of psychosocial factors and psychiatric disorders in functional dyspepsia.* Nat Rev Gastroenterol Hepatol, 2013. **10**(3): p. 158-67.

13. Dolatian, M., et al., *Preterm delivery and psycho-social determinants of health based on World Health Organization model in Iran: a narrative review.* Glob J Health Sci, 2012. **5**(1): p. 52-64.

14. Facchinetti, F., E. Annessi, and F. Ottolini, *The pregnant uterus as a psycho-endocrine organ.* Gynecol Endocrinol, 2012. **28 Suppl 1**: p. 33-6.

15. Yassi, R., et al., *The gastrointestinal electrical mapping suite (GEMS): software for analyzing and visualizing high-resolution (multi-electrode) recordings in spatiotemporal detail.* BMC Gastroenterol, 2012. **12**: p. 60.

16. Garcia-Casado, J., et al., *Enhancement of non-invasive recording of electroenterogram by means of a flexible array of concentric ring electrodes.* Ann Biomed Eng, 2014. **42**(3): p. 651-60.

17. Qin, S., et al., *Signal reconstruction of the slow wave and spike potential from electrogastrogram.* Biomed Mater Eng, 2015. **26 Suppl 1**: p. S1515-21.

18. Qian, X., et al., *Simultaneous Recording and Analysis of Uterine and Abdominal Muscle Electromyographic Activity in Nulliparous Women During Labor.* Reprod Sci, 2016.

19. Trojner Bregar, A., et al., *Uterine electromyography during active phase compared with latent phase of labor at term.* Acta Obstet Gynecol Scand, 2016. **95**(2): p. 197-202.

20. Prats-Boluda, G., et al., *Active concentric ring electrode for non-invasive detection of intestinal myoelectric signals.* Med Eng Phys, 2011. **33**(4): p. 446-55.

21. Gabsteiger, F., et al., *ICA-based reduction of electromyogenic artifacts in EEG data: comparison with and without EMG data.* Conf Proc IEEE Eng Med Biol Soc, 2014. **2014**: p. 3861-4.

22. De Luca, C.J., et al., *Filtering the surface EMG signal: Movement artifact and baseline noise contamination.* J Biomech, 2010. **43**(8): p. 1573-9.

23. Fratini, A., et al., *Relevance of motion artifact in electromyography recordings during vibration treatment.* J Electromyogr Kinesiol, 2009. **19**(4): p. 710-8.

24. Vane, J.R., *A sensitive method for the assay of 5-hydroxytryptamine.* Br J Pharmacol Chemother, 1957. **12**(3): p. 344-9.

25. Du, P., et al., *High-resolution mapping of in vivo gastrointestinal slow wave activity using flexible printed circuit board electrodes: methodology and validation.* Ann Biomed Eng, 2009. **37**(4): p. 839-46.

26. Lammers, W.J., et al., *Disturbances in the propagation of the slow wave during acute local ischaemia in the feline small intestine.* Eur J Gastroenterol Hepatol, 1997. **9**(4): p. 381-8.

27. Shozib, H.B., et al., *Acceleration of ileal pacemaker activity in mice lacking interleukin 10.* Inflamm Bowel Dis, 2013. **19**(8): p. 1577-85.

28. Chen, J.D., B.D. Schirmer, and R.W. McCallum, *Measurement of electrical activity of the human small intestine using surface electrodes.* IEEE Trans Biomed Eng, 1993. **40**(6): p. 598-602.

29. Chen, J.D., B.D. Schirmer, and R.W. McCallum, *Serosal and cutaneous recordings of gastric myoelectrical activity in patients with gastroparesis.* Am J Physiol, 1994. **266**(1 Pt 1): p. G90-8.

30. Zhang, J., et al., *Development of gastric slow waves and effects of feeding in pre-term and full-term infants.* Neurogastroenterol Motil, 2006. **18**(4): p. 284-91.

31. Domino, M., B. Pawlinski, and Z. Gajewski, *The linear synchronization measures of uterine EMG signals: Evidence of synchronized action potentials during propagation.* Theriogenology, 2016. **86**(8): p. 1873-8.

32. Ushiyama, J., Y. Takahashi, and J. Ushiba, *Muscle dependency of corticomuscular coherence in upper and lower limb muscles and training-related alterations in ballet dancers and weightlifters.* J Appl Physiol (1985), 2010. **109**(4): p. 1086-95.

33. Gil, Y., W. Wu, and J. Lee, *A synchronous multi-body sensor platform in a Wireless Body Sensor Network: design and implementation.* Sensors (Basel), 2012. **12**(8): p. 10381-94.

34. Obioha, C., et al., *Effect of Body Mass Index on the sensitivity of Magnetogastrogram and Electrogastrogram.* Journal of Gastroenterology and Hepatology Research, 2013. **2**(4): p. 513-519.

35. Hocke, M., et al., *Every slow-wave impulse is associated with motor activity of the human stomach.* Am J Physiol Gastrointest Liver Physiol, 2009. **296**(4): p. G709-16.

36. Pezzolla, F., et al., *Electrical activity recorded from abdominal surface after gastrectomy or colectomy in humans.* Gastroenterology, 1989. **97**(2): p. 313-20.

37. Chang, F.Y., et al., *Fasting and postprandial small intestinal slow waves non-invasively measured in subjects with total gastrectomy.* J Gastroenterol Hepatol, 2007. **22**(2): p. 247-52.

38. Chambers, M.M., et al., *In vitro electrical activity in human colon.* Gastroenterology, 1981. **81**(3): p. 502-8.

39. Tsubouchi, T., et al., *Stimulatory action of itopride hydrochloride on colonic motor activity in vitro and in vivo.* J Pharmacol Exp Ther, 2003. **306**(2): p. 787-93.

40. Jeong, S.I., et al., *Regulation of contractile activity by magnolol in the rat isolated gastrointestinal tracts.* Pharmacol Res, 2009. **59**(3): p. 183-8.

41. Arias, F., *Pharmacology of oxytocin and prostaglandins.* Clin Obstet Gynecol, 2000. **43**(3): p. 455-68.

42. Persson, H. and T. Olsson, *Some pharmacological properties of terbutaline (INN), 1-(3,5-dihydroxyphenyl)-2-(T-butylamino)-ethanol. A new sympathomimetic beta-receptor-stimulating agent.* Acta Med Scand Suppl, 1970. **512**: p. 11-9.

43. Hinz, A., et al., *Psychometric evaluation of the Generalized Anxiety Disorder Screener GAD-7, based on a large German general population sample.* J Affect Disord, 2017. **210**: p. 338-344.

44. Neureiter, E., et al., *An introduction to the 'Psycho-Physiological-Stress-Test' (PPST)-A standardized instrument for evaluating stress reactions.* PLoS One, 2017. **12**(12): p. e0187859.

45. Bulbul, M., et al., *Opposite effects of central oxytocin and arginine vasopressin on changes in gastric motor function induced by chronic stress.* Peptides, 2017. **87**: p. 1-11.

46. Keenan, C.M., et al., *AM841, a covalent cannabinoid ligand, powerfully slows gastrointestinal motility in normal and stressed mice in a peripherally restricted manner.* Br J Pharmacol, 2015. **172**(9): p. 2406-18.

47. Konturek, P.C., T. Brzozowski, and S.J. Konturek, *Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options.* J Physiol Pharmacol, 2011. **62**(6): p. 591-9.

48. Forbes, S.C. and H.M. Cox, *Peptide YY, neuropeptide Y and corticotrophin-releasing factor modulate gastrointestinal motility and food intake during acute stress.* Neurogastroenterol Motil, 2014. **26**(11): p. 1605-14.

49. Mendez-Cuesta, L.A., et al., *Early changes in oxidative stress markers in a rat model of acute stress: effect of l-carnitine on the striatum.* Basic Clin Pharmacol Toxicol, 2011. **109**(2): p. 123-9.

50. Uwaya, A., et al., *Acute immobilization stress following contextual fear conditioning reduces fear memory: timing is essential.* Behav Brain Funct, 2016. **12**(1): p. 8.

51. Bello, N.T., et al., *Dietary-induced binge eating increases prefrontal cortex neural activation to restraint stress and increases binge food consumption following chronic guanfacine.* Pharmacol Biochem Behav, 2014. **125**: p. 21-8.

52. Mendez-Cuesta, L.A., et al., *Diazepam blocks striatal lipid peroxidation and improves stereotyped activity in a rat model of acute stress.* Basic Clin Pharmacol Toxicol, 2011. **109**(5): p. 350-6.

53. Abdel-Salam, O.M., et al., *Brain and liver oxidative stress after sertraline and haloperidol treatment in mice.* J Basic Clin Physiol Pharmacol, 2013. **24**(2): p. 115-23.

54. Martin, W.R., J.W. Sloan, and E.P. Wala, *Precipitated abstinence in the diazepam-dependent rat.* Pharmacol Biochem Behav, 1993. **46**(3): p. 683-8.

55. Miyazaki, H., et al., *Disposition and metabolism of [14C]-haloperidol in rats.* Arzneimittelforschung, 1986. **36**(3): p. 443-52.

56. Ikemura, M., et al., *The blood concentration and organ distribution of haloperidol at therapeutic and toxic doses in severe fatty liver disease.* Leg Med (Tokyo), 2012. **14**(3): p. 147-53.

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