CHAPTER 2

MATERIALS AND METHODS

2.1 Animal preparation

Anesthesia was induced with Zoletil (4.4 mg/kg) injected intramuscularly and maintained by 1.5-3.0% Isoflurane delivered in 100% oxygen. In supine position, each pig was intubated with a cuffed endotracheal tube and ventilated with humidified room air and oxygen through a ventilator which controls of tidal volume at 12 ml/kg and respiratory rate at 10 – 15 cycles/min. The surface electrocardiogram (lead II), heart rate (HR) and femoral arterial blood pressure (ABP) were continuously monitored.

The femoral arterial blood pressure, blood gases, pH and electrolytes was monitored every 30 min and maintained within acceptable physiologic ranges (ABP=70-125 mmHg, pCO_2=35-60 mmHg and pH=7.35-7.45) by giving intravenous sodium bicarbonate or by adjusting ventilation parameters as needed (Kanlop et al., 2011). Vecuron (0.3 mg/kg bolus, 0.5 mg/kg/hr maintenance) (Doyle and Mark, 1990; Karzai et al., 1997; Perez-de-Sa et al., 2002) administer intravenously to minimize skeletal muscle contraction during the measurement of DFT.

2.2 Determination of defibrillation threshold

The determination of DFT was performed using a three-reversal up/down protocol. Briefly, the initial shock strength was chosen at 400 V. In the event of successful defibrillation, the leading edge voltage was decreased in 80 V steps per

defibrillation attempt until a first reversal from successful defibrillation to failure was achieved. If the initial shock was unsuccessful, the voltage was increased in 80 V steps per defibrillation attempt until a reversal from failed to successful defibrillation was achieved. At each reversal point the algorithm was iterated in the opposite direction except that after the first reversal the voltage step size was decreased to 40 V and 20 V respectively for a total of three reversals. The DFT was defined as the lowest energies required for successful defibrillation of VF after three reversal points, when the next lower setting failed to defibrillate the heart. Each shock was delivered 10±1 s after induction of VF.

2.3 Electrode placement

The experiment consists of 3 type of electrodes which are 2.3.1) defibrillation electrode for defibrillation, 2.3.2) can electrode; the additional returning electrode which enlarged defibrillation shock field covered all area of the heart, and 2.3.3) VNS electrode for LCVNS.

2.3.1 Defibrillation electrode placement

The right external jugular vein was exposed and isolated from the surrounding tissues. Under fluoroscopic guidance, dual-coil electrode with a 53 mm distal coil and 78 mm proximal coil, as shown in Figure 2-1A, was inserted to the heart via the right external jugular vein. The distal coil and the proximal coil were placed at the right ventricular apex and the junction between right atrium and superior vena cava, respectively as shown in Figure 2-2. The catheter was secured at the venotomy site to

stabilize the position. VF box was connected to pacing end of this electrode for VF induction by delivered the AC current via the pacing tip.

2.3.2 Can electrode placement

The cylindrical aluminum returning electrode with 1.2 cm thickness and 5.64 cm diameter (Figure 2-1B) was implanted subcutaneously at the level of the heart at the left pectoral region (Mouchawar et al., 1997) (Figure 2-2) and was connected with the proximal coil of the defibrillation electrode.

2.3.3 Electrode placement for VNS

The LC vagus nerve between left internal jugular vein and left internal carotid artery was exposed and isolated from the surrounding tissues (Figure 2-3) by VNS stainless bipolar hook electrode (Figure 2-1C). The VNS electrode is separated into the positive hook (cephalad position) and the negative hook (caudal position) with a 6-mm distance. The LC vagus nerve was stimulated by a 10-mA current, 5 and 20 Hz of frequency and 10, 15 and 20 s of duration for each frequency. A polypropylene (PP) sheath is used to prevent current shunting between the electrodes and muscle around the vagus nerve.

2.4 Nerve cuff placement formepivacaine infusion

The Nerve cuff made from PE tube (O.D.=5.2 mm, I.D.= 3.8 mm, L= 20 mm). It was slit longitudinal for nerve placement and mepivacaine infusion as shown in Figure 2-1D. It was covered around the LC vagus nerve at the portion below the VNS electrode (caudal side) and secured at the position. Plastic film was wrapped around this area prevented mepivacaine leak out.

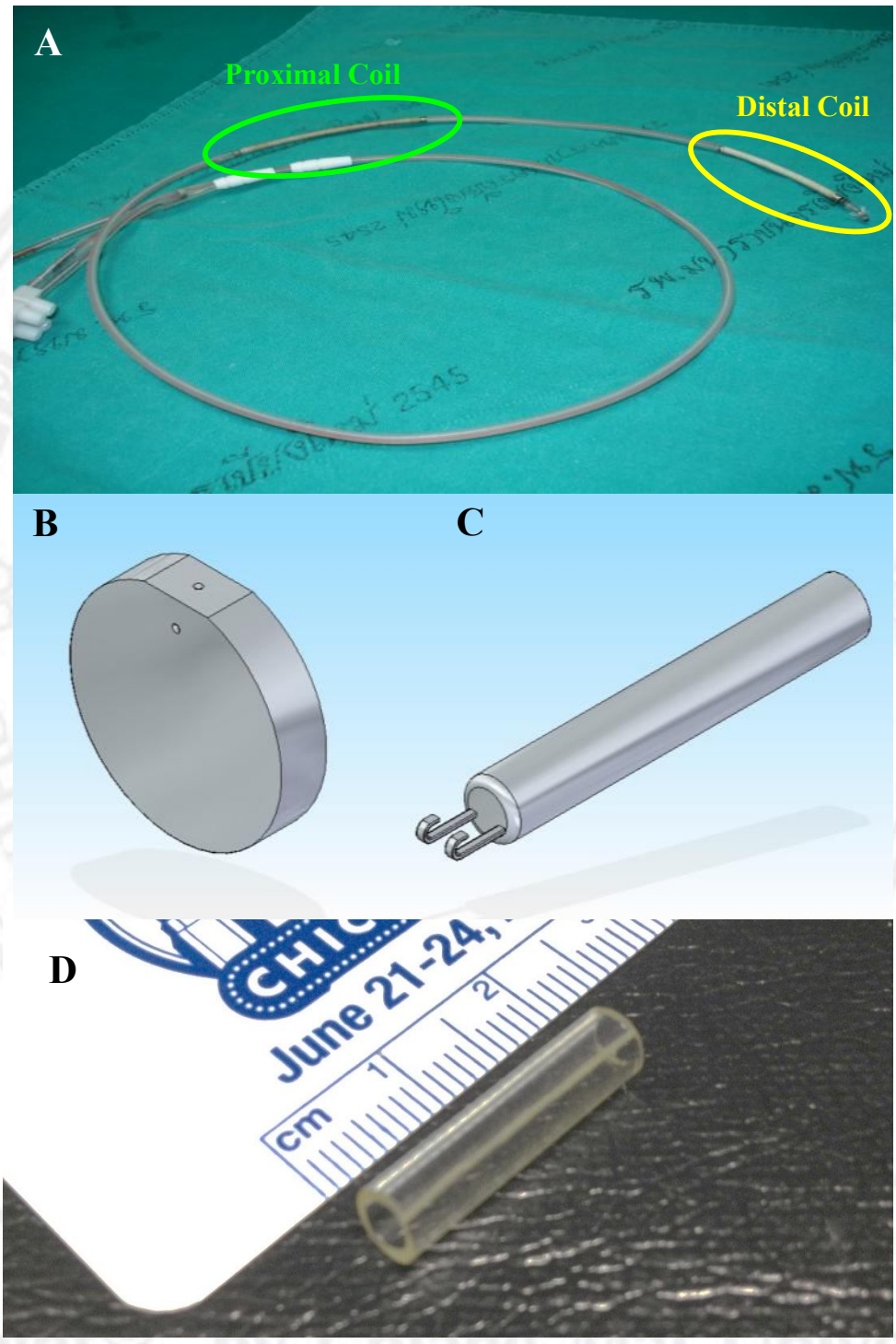


Figure 2-1: (A) Defibrillation electrode, (B) Can electrode, (C) VNS electrode, and (D) Nerve cuff for mepivacaine infusion.

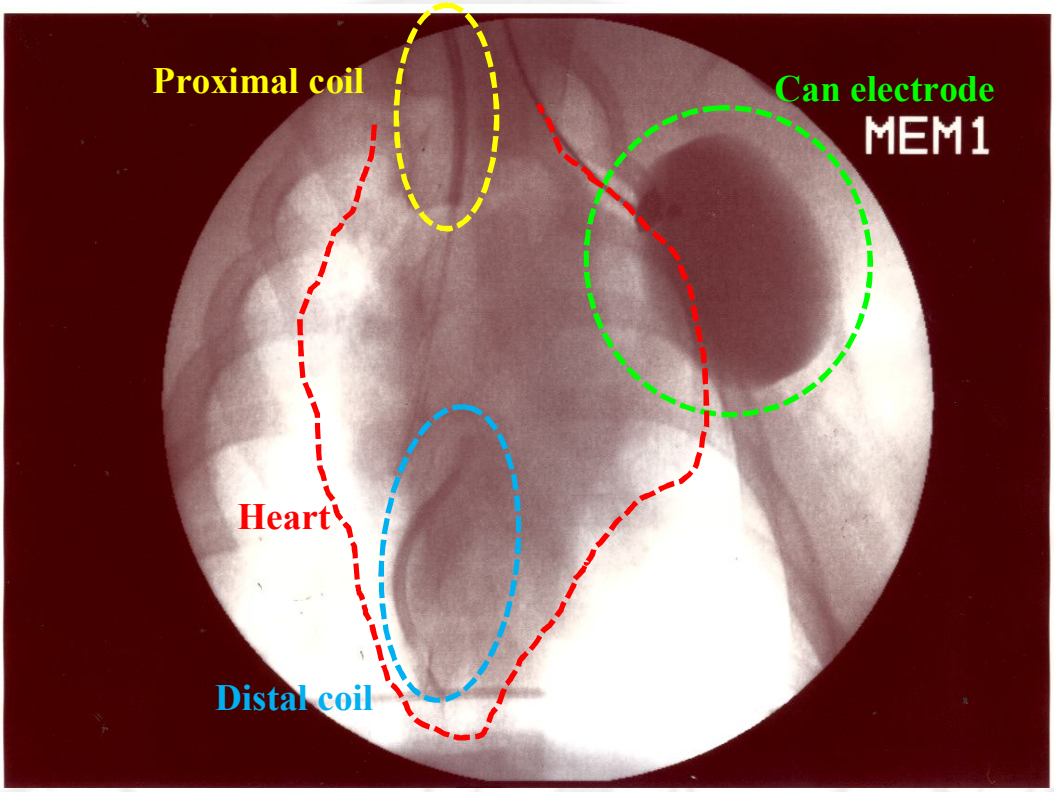


Figure 2-2: Defibrillation and Can electrode placement under fluoroscopic guidance.

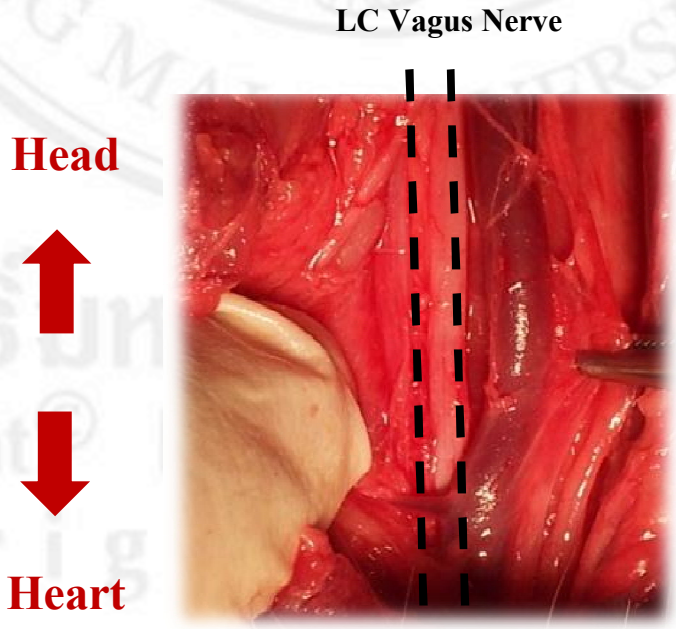


Figure 2-3: The LC vagus nerve location.

2.5 VNS setting

The LC vagus nerve was stimulated by trains of electrical rectangular pulse with1 ms pulse-width, at 5 and 20 Hz frequency and 10, 15, and 20-s stimulation period for each frequency. The electrical stimulation was set and delivered by the program electrical stimulator (PES) as shown in Figure 2-4

2.6 Experimental protocols

This experiment consists of three main protocols, 1) The effect of VNS on ECG parameters during normal sinus rhythm (NSR), 2) The effect of VNS on the defibrillation threshold (DFT), and 3) the effect of VNS blockage by local anesthetic agent on NSR and the DFT.

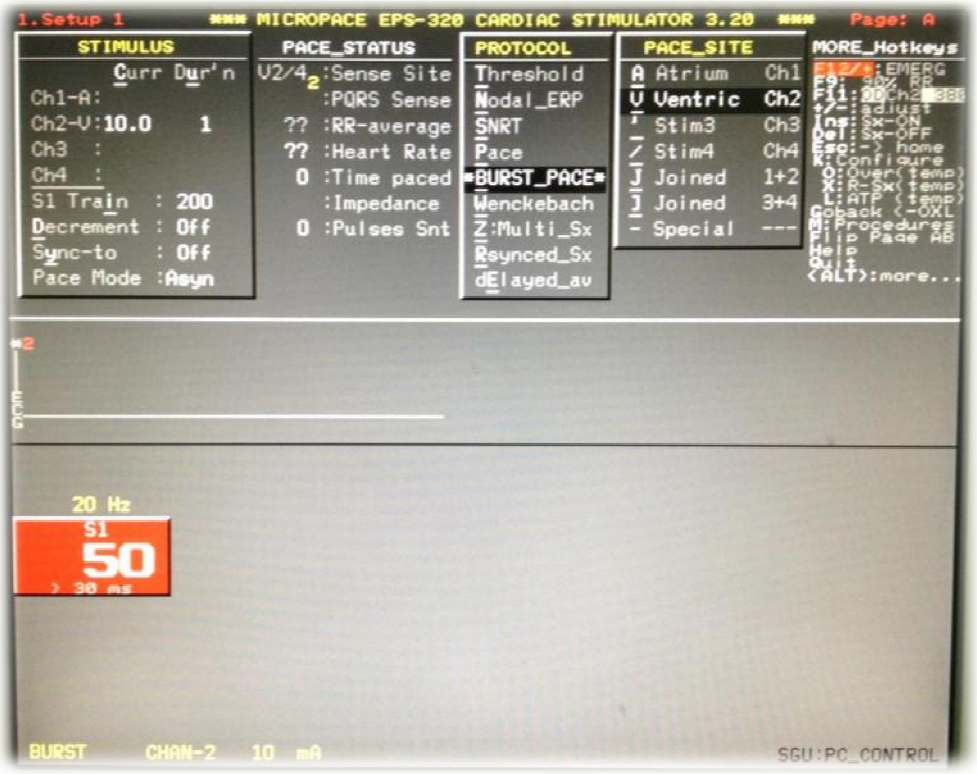


Figure 2-4: The program electrical stimulator's windows.

Protocol 1: The effect of VNS on ECG parameters during normal sinus rhythm (NSR)

ECG was recorded at the beginning of the protocol via Chart 5 for window (PowerLab) as shown in Figure 2-5. Then, LC vagus nerve was isolated from surrounding tissue and stimulated by trains of a 10-mA electrical rectangular pulse with 1 ms pulse-width, at 5 and 20 Hz for 10, 15, and 20-s stimulation periods for each frequency. After stabilized period of the subject, VNS was repeated triplications for each condition. The experimental protocol is shown in Figure 2-6. At the end of the study, P-R interval, QRS complex, R-R interval, and Q-T interval from ECG recordings were analyzed by averaging 5 intervals immediately before VNS, during VNS, and after VNS termination.

Figure 2-5: The ECG recording's windows (Chart 5 for window).

Protocol 2: The effect of VNS on the defibrillation threshold (DFT)

The study was performed following the first protocol in the same pig. The VF induction was initiated by delivering a 50-Hz AC to the subject via the tip of an electrode at the RV apex for 2 s. VF was allowed to last for 10 s, after which the defibrillation shock was delivered to terminate VF. VNS was initiated at the time of VF induction. The VNS durations were 10, 15 and 20 s for each VNS frequency (5 and 20 Hz). The baseline group was done without VNS. The rescued shocks were delivered to the heart if the shock failed to defibrillate until successful defibrillation. Each shock was separated by a resting period of at least 4 min or until ECG morphology of subject returns to normal. The three-reversal up/down protocol was used for DFT measuring (Shinlapawittayatorn et al., 2006; Strobel et al., 1998).

DFT parameters that consist of peak voltage (V), total energy (J), impedance (Ω), and pulse width (ms) were recorded if this defibrillation is successful. In each experiment, the DFT was determined as baseline (prior to VNS) and after VNS. The experimental protocol is shown in Figures 2-7. The diagram for the effect of VNS on NSR and DFT protocols is shown in Figure 2-8.

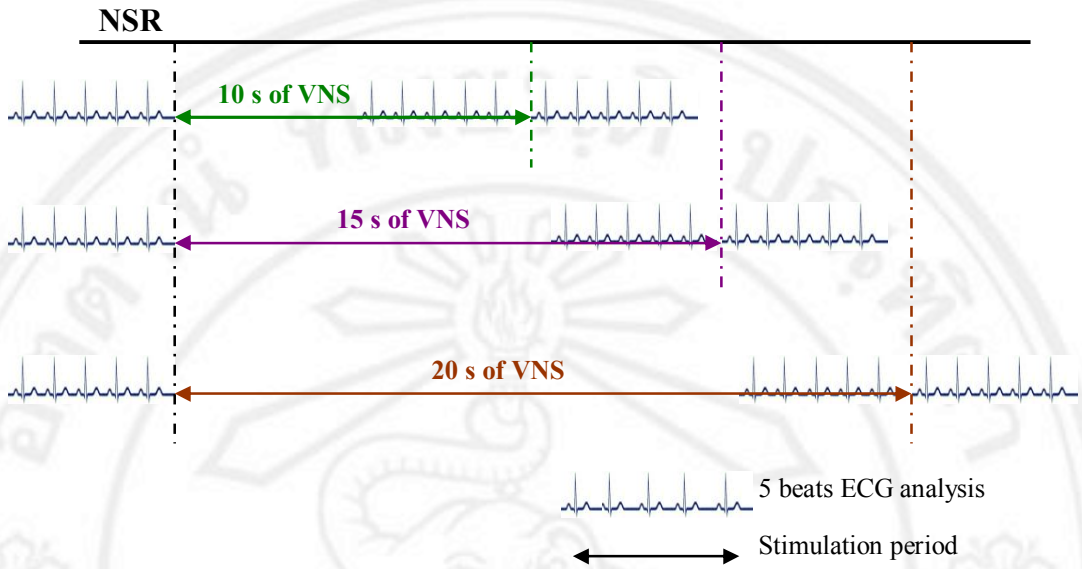


Figure 2-6: Experimental protocol for the effect of VNS on NSR at 5 and 20 Hz

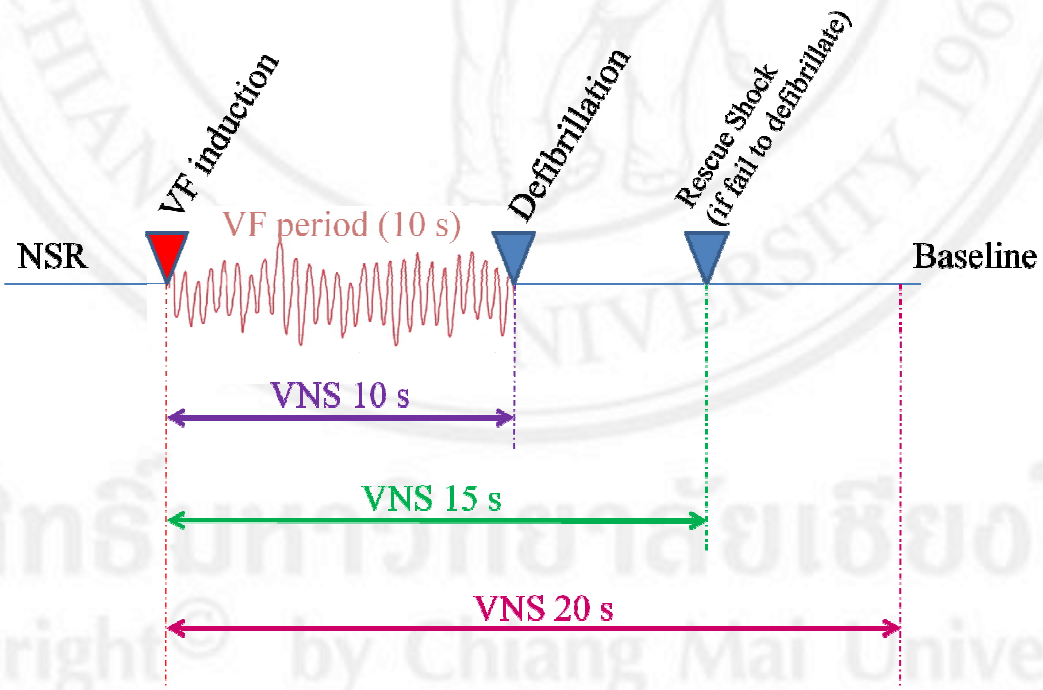
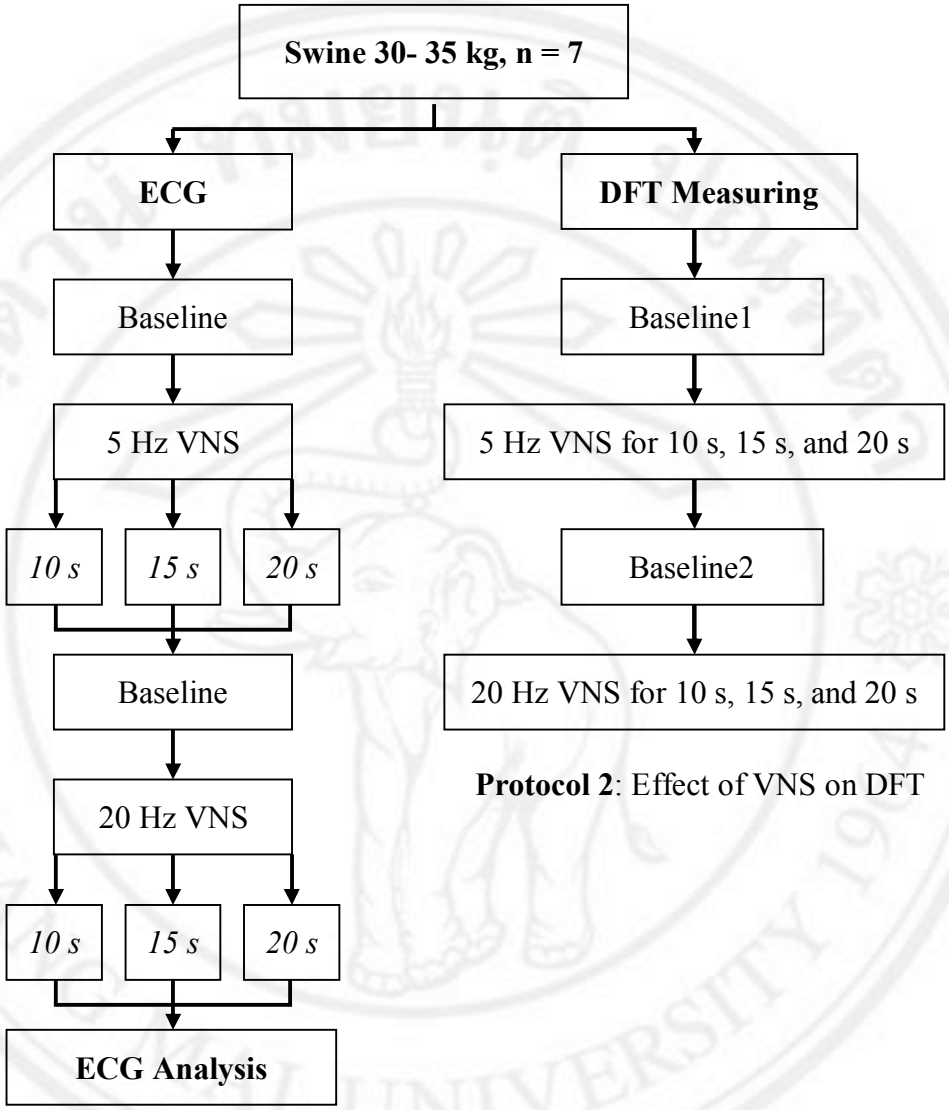


Figure 2-7: Experimental protocol for the effect of VNS on DFT at 5 and 20 Hz



Protocol 1: Effect of VNS on NSR

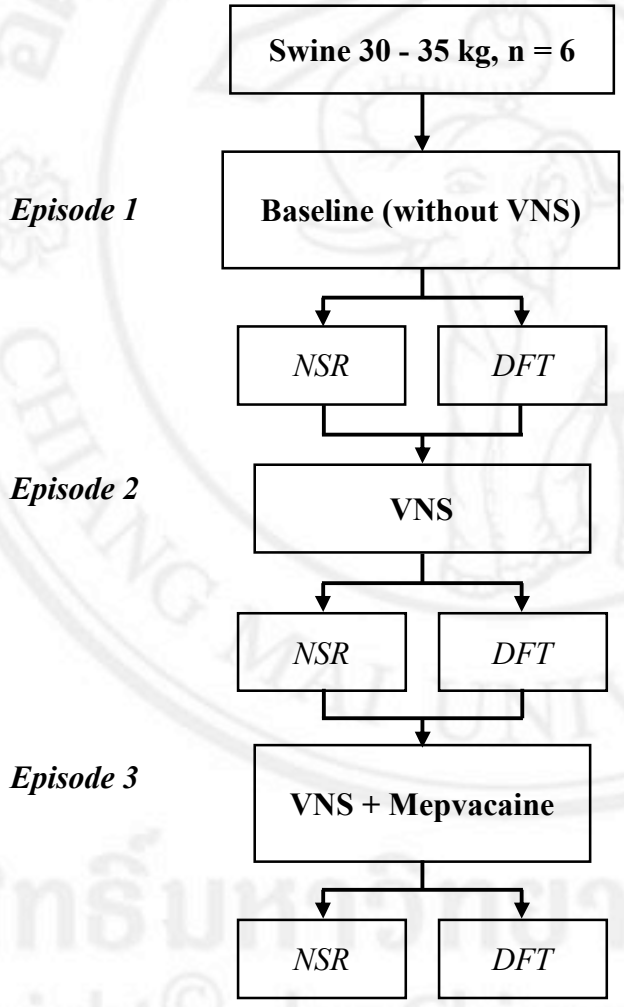
Protocol 2: Effect of VNS on DFT

Figure 2-8: Diagram for the effect of VNS on NSR and DFT protocols.

Protocol 3: The effect of VNS blockade by local anesthetic agent on NSR and the DFT

The study of VNS blockade as mepivacainewas performed by the combination process of the 1st and 2nd protocols. The VNS frequency and duration in this protocol obtained from the previous protocols that were the most effective in DFT reduction.

There were 3 episodes as follows 1) baseline without VNS, 2) VNS baseline, and 3) VNS and the blockade by mepivacaine as shown in Figure 2-9. In each episode, the ECG recording was performed before DFT measuring. The LC vagus nerve was covered by the nerve cuff which is the route for the administration of 3% mepivacaine hydrochloride. Mepivacaine was maintained in the nerve cuff along the 3rd episode.



Protocol 3: VNS blockage by Mepivacaine

NSR: ECG was recorded as described in protocol 1 (NSR protocol)

DFT: DFT was measured as described in protocol 2 (DFT protocol)

Figure 2-9: Diagram for VNS blockage protocol.

2.7 Statistical analyzed

Data were presented as mean ± SE. Comparison of the differences was performed by the Wilcoxon Matched Pairs Signed-Ranks Test. Probability value less than 0.05 ($P<0.05$) was considered statistically significant.