Modulation of Respiratory Sinus Arrhythmia in Rats with Central Pattern Generator Hardware

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Abstract:
We report on the modulation of respiratory sinus arrhythmia in rats with central pattern generator (CPG) hardware made of silicon neurons. The neurons are made to compete through mutually inhibitory synapses to provide timed electrical oscillations that stimulate the peripheral end of vagus nerve at specific points of the respiratory cycle: the inspiratory phase (φ₁), the early expiratory phase (φ₂) and the late expiratory phase (φ₃). In this way the CPG hardware mimics the neuron populations in the brainstem which through connections with cardiac vagal motoneurones control respiratory sinus arrhythmia (RSA). Here, we time the output of the CPG hardware from the phrenic nerve activity recorded from rats while monitoring heart rate changes evoked by vagal nerve stimulation (derived from ECG) controlled by the CPG. This neuroelectric stimulation has the effect of reducing the heart rate and increasing the arterial pressure. The artificially induced RSA strongly depends on the timing of pulses within the breathing cycle. It is strongest when the vagus nerve is stimulated during the inspiratory phase (φ₁) or the early expiratory phase (φ₂) in which case the heart rate slows by 50% of the normal rate. Heart rate modulation is less when the same exact stimulus is applied during the late expiratory phase (φ₃). These trials show that neurostimulation by CPG hardware can augment respiratory sinus arrhythmia. The CPG hardware technology opens a new line of therapeutic possibilities for prosthetic devices that restore RSA in patients where respiratory-cardiac coupling has been lost.

Keywords: Silicon central pattern generator, neurostimulation, respiratory sinus arrhythmia, Bötzinger system.
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1. Introduction

1.1. Central Pattern Generators

Central pattern generators (CPGs) are small groups of neurons that regulate biological rhythms and coordinate motor activity. Central to rhythm generation is a pair of neurons A and B which interact through reciprocally inhibitory synapses. When neuron A fires, neuron B is hyperpolarized and vice versa. In this way neuron A and B burst out of phase with each other producing a biphasic rhythm. These neuron pairs are ubiquitous in invertebrates where they take the form of the half-centered oscillators that regulate the leech heart (Marder and Calabrese, 1996; Kristan et al., 2005; Norris et al., 2006), the dorsal and ventral swim interneurons that control the swim motion of the tritonia and the clione (Selverston, 2010; Marder et al. 2005) and the triplet of pyloric neurons that produce the triphasic rhythm of the stomatogastric ganglion of the lobster (Rabinovich et al, 2006). In mammals, central pattern generators form hierarchies of coupled oscillators located at the base of the brain that determine motor activity such as breathing, coughing and swallowing (Shiba et al. 2007; Nalivaiko et al. 2009, Nakamura and Katakura, 1995). The brainstem central pattern generator is known to couple the rate of respiration to the heart beat with the effect of making the heart beat faster during inspiration than during expiration. This phenomenon known as respiratory sinus arrhythmia (RSA) is believed to increase blood pumping efficiency and save cardiac energy (Abdala et al., 2009; Baekey et al. 2010; Ben-Tal et al. 2012; Champagnat et al. 2009; Fortuna et al., 2009; Nicholls and Paton, 2009). Respiratory sinus arrhythmia is lost in heart failure and hypertension and is a prognostic indicator for sudden cardiac death (Mortara et al. 1994). Our recent data have proposed a brainstem respiratory neural network comprising excitatory and inhibitory populations in the pre-Bötzinger and Bötzinger complexes and pons that generates a 3 phase rhythm (Rubin et al., 2009; Molkov et al., 2010; Daun et al., 2009; Smith et al., 2007) which is essential for the presence of respiratory sinus arrhythmia (Beakey et al, 2010). The dynamics of the cardiovascular and respiratory oscillators has also been analyzed using stroboscopic techniques to reveal time intervals over which these oscillators synchronize through phase or frequency locking (Schäfer et al. 1999; Landa and Rosenblum, 1995; Rosemblum et al. 2001; Rulkov et al., 1995). Our aim here was (i) to build an artificial (silicon) CPG generating 2 and 3 phase rhythms and (ii) to assess whether it could reciprocally
Modulation of sinus arrhythmia with CPG hardware interact with a rat to control its heart rate such that the heart rate could be lowered in each of the three respiratory phases. Our motivation came from the belief that neurostimulation by artificial neural networks may also provide the premise for novel therapies aimed at either retraining natural central pattern generators or substituting them with prosthetic CPG implants. We believe that this may have clinical relevance especially in control of heart rate in cardiovascular disease.

1.2. Analogue neural hardware for neuromotor control

The interfacing of neurons with transistors is a new research front which has emerged in recent years driven by advances in microfabrication techniques. Hybrid electronics has successfully imaged the electrical activity of individual neurons placed on transistor gates (Fromherz, 2002; Fromherz et al., 1991), gate arrays (Cui et al., 2001; Zheng et al., 2005; Patolsky et al., 2006) and on graphene (Hess et al., 2011). Computer models of Hodgkin-Huxley neurons have been interfaced with biological neurons to understand the function of one neuron in a network. This method has been used to drive the half centered oscillator in the leech heart (Olypher et al., 2006; Sorensen et al., 2004) and map the modes of synchronization between pairs of coupled neurons (Elson et al., 1998; Pinto et al., 2001; Varona et al., 2001). Computer models of biological neurons have the advantage of being easy to program and to modify. However the range of time scales associated with the Na, K, A, NaP, Ca (L), Ca (T), h (hyperpolarization activated cation current) and C (Ca activated K current) ion channels of real neurons invariably introduces stiffness in the systems of differential equations which makes them difficult to integrate. Accurate integration of the 14 differential equations describing one such neuron is usually achieved at the cost of minutes of computation time (Meliza et al., 2012). In contrast, an artificial CPG that interacts with biological systems must respond to a stimulus accurately and in real time. One therefore needs to follow the example of Nature and implement such a CPG using analogue neuron hardware. Interconnecting neuron pairs with reciprocally inhibitory synapses produces stable spatio-temporal pulsing patterns (Rabinovich et al., 2001; Rabinovich et al., 2006; Shilnikov et al., 2005). These patterns repeat in a cyclic manner while generating pulse sequences corresponding to the bi-phasic or tri-phasic rhythms that are needed to mimic the respiratory CPG.
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2. Methods

2.1. Artificial CPG

Here, we report on the first use of CPG hardware to control motor activity in rats. The network is built from 6 neurons that model the conductances of the sodium and potassium channels (Mahowald and Douglas, 1991; Samardak et al., 2009). These neurons are reciprocally interconnected with 30 (6×5) gap synapses that inject in the post-synaptic neuron a current proportional to the difference between the membrane voltages of the pre- and post-synaptic neurons. The synapses can be made inhibitory or excitatory however, for the purpose of the present investigation they were used in the inhibitory configuration. In essence, the network is an analogue computer that integrates in real time the 24 (6×4) Hodgkin-Huxley equations of neurons mutually coupled via their synapses. The synaptic conductances were adjusted by means of gate voltages during preliminary trials to make neuron pairs oscillate out-of-phase with each other. The external current stimulus was provided by the phrenic nerve activity which acted as a timing stimulus to the artificial CPG. The network outputs the time series voltage data representing the membrane voltages of individual neurons. To obtain a biphasic rhythmic pattern it is sufficient to use a single pair of mutually inhibitory neurons. The “membrane” voltage of artificial neurons oscillates between the 0V and the 4.4V levels which corresponds to the Na action potential (+45mV) and the K mediated after-hyperpolarization (-70mV) in real neurons. The voltage thresholds that open and close the Na ion channel (activation threshold: 1.353V; inactivation threshold: 2.853V) and K ion channel (activation threshold: 1.208V) were set by scaling the known biological thresholds to the 0-4.4V interval. The time constants of the Na and K gates together with the “membrane” capacitance were chosen to fit bursts of about 10-20 spikes in the time interval of phrenic duration (inspiration). Given that the inspiration phase lasts 0.4s, the interval between spikes had to be in the range 20ms-100ms for a firing frequency of 10Hz - 50Hz. These are the typical frequencies obtained for a current stimulus of ~ 100μA. The neurons have a firing threshold of 80μA and their firing frequency increases with increasing stimulation - up to five-fold at our maximum input current – as in real neurons (Hodgkin and Huxley, 1952). We have performed preliminary trials of synchronization to respiration by stimulating the artificial CPG with recordings of the phrenic nerve through a NI6259 DAQ card from National
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Instruments. The reciprocally inhibitory synapses connecting neuron 1 (N1) and neuron 2 (N2) required asymmetric conductances to obtain the biphasic rhythms that synchronize in phase and out of phase with phrenic activity (inspiration).

2.2. Preparation of live experiments

In situ rat preparation was employed as previously described (Paton; 1996). Following deep anaesthesia (Halothane) as evidence by loss of withdrawal responses to noxious pinching of the tail and paws, rats were bisected beneath the diaphragm and exsanguinated. The head and thorax were cooled in ice-chilled Ringer’s and the preparation decerebrated at the pre-collicular level. The descending aorta was isolated and cannulated with a double lumen catheter. The aorta was perfused with a Ringer’s solution (composition in mM: NaCl (125); NaHCO₃ (24); KCl (3); CaCl₂ (2.5); MgSO₄ (1.25); KH₂PO₄ (1.25); dextrose (10); pH 7.35–7.4 after carbogenation). All chemicals were from Sigma (UK) containing 1.25% ficoll, gassed with carbogen, filtered (25 µm screen filter) and warmed (31°C). Perfusion pressure was set to around 70-80 mmHg by increasing flow rate to 25 ml.min⁻¹ and addition of arginine-vasopressin to the perfusate (200–400 pm). Glass suction electrode recordings of centrally generated respiratory activity were made from the left phrenic nerve and the left central end of the vagus nerve. A third suction electrode was used to electrically stimulate the cut peripheral end of the right vagus nerve; this was connected to the artificial CPG. Phrenic, central vagal activities and ECG (recorded in some experiments) were amplified, filtered and recorded using AM Systems amplifiers. The signals were AC amplified (10–20 k) and bandpass filtered (80 Hz to 3 kHz). Perfusion pressure and heart rate (derived from either interpulse interval or the R-R interval) were also recorded. All data were acquired on Spike 2 software (Cambridge Electronic Design). Peripheral chemoreceptors were stimulated to increase respiratory frequency using sodium cyanide (0.05ml; 0.03%. i.a.).

The artificial CPG received input from the phrenic nerve and produced voltage oscillations that were used to stimulate the cut peripheral end of the vagus nerve (Fig.1a). The raw phrenic signal consists of fast voltage oscillations during the inspiratory phase that require amplification, rectification, smoothing and conversion to a current to stimulate the CPG. The processing is done electronically in the series of
Modulation of sinus arrhythmia with CPG hardware steps depicted in Fig.1a. The raw phrenic signal is first amplified 10,000 times by the AM Systems pre-amplifier that filters out noise. The signal is then amplified by a second amplifier that has a gain tuneable in the 1-20 range and whose purpose is to finely adjust the amplitude of the stimulus relative to the firing threshold of neuron 1. This threshold corresponds to the activation of the Na channel (1.353V). After amplification, the phrenic signal still oscillates about 0V. The next processing stage rectifies and smoothes the signal with a diode bridge and RC integrator with time constant of 10ms. The typical waveform of the rectified and integrated phrenic signal is shown in Fig.1b. This voltage ($V_{PN}$) is then converted into a current ($I_{PN}$) by a transconductance differential amplifier performing the function $I_{PN} = GV_{PN}$ where $G=0.1\text{mS}$. The current $I_{PN}$ is then injected into N1 - the leftmost neuron in the lower part of Fig.1 (photograph). N1 or N2 then modulates the heart rate through stimulation of the cut peripheral end of the vagus nerve (VN). The neuron may be directly connected to the vagus nerve as in most experiments the input impedance of the vagus nerve is higher than the leakage resistance in our artificial neurons (10k$\Omega$). Changes in conductivity of the Ringer bath may however cause the impedance of the stimulating electrode to drop below 10k$\Omega$ decreasing the amplitude of neuron spikes. To guard against attenuation of the output signal, we lower the output impedance of the neuron by fitting an emitter follower amplifier between the stimulating neuron and the vagus nerve.

This artificial circuit was designed to provide 3 phase stimulation, namely to selectively excite the vagus nerve during inspiration ($\varphi_1$), early expiration ($\varphi_2$) and late expiration ($\varphi_3$) as shown in Figure 1b. When excited by the phrenic input, N1 and N2 fire during phase 1 and phases 2+3 respectively. To stimulate phase 3 only, we introduce a time delay $\tau$ to slow the rise of the current stimulating neuron 2 (Fig.1a). In this way, we are able to tune the timing of N2 by controlling the moment at which its input current reaches the 80 $\mu$A firing threshold. To stimulate phase 2 only, we substitute the phrenic nerve with the input from central vagus nerve recording as input to N1. This is because the central vagus nerve activity contains not only central inspiratory activity but also post-inspiratory discharge. Since the latter has higher amplitude than the former this was used to trigger the firing of N1 at the onset of the early inspiratory phase.
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Although the amplitude of neuron oscillations (4.4V) is large compared to the action potentials in the vagus nerve (-70mV → +40mV) a large fraction of this signal is dissipated through resistive losses in the envelope of the vagus nerve. Unlike current clamp experiments where current is applied across the membrane, vagus nerve stimulation is mostly external hence requires stimulating voltages of about 4V.

3. Results

We first studied the natural activity of the cardiorespiratory system of two rats in the absence of external modulation (Fig.2). Motor activity was recorded via measurements of the perfusion pressure (PP), the electrocardiogram (ECG), the phrenic nerve activity (PN) and the central vagal nerve activity (VN). The instantaneous heart rate (HR) was computed either from the ECG or from the fast oscillations of the perfusion pressure. Its time average is remarkably similar in Rat #1 and Rat #2 at 4.8 beats/s. The instantaneous heart rate oscillates in phase with the rhythm of respiration to reveal the natural respiratory sinus arrhythmia (RSA) mediated by the brainstem CPG (Fig.2). The natural RSA has a period of 4.1s and amplitude of ~0.08Hz which is much smaller than the artificial RSA we obtain with the silicon CPG.

We then closed the feedback loop of the CPG in Fig.1 to excite the vagus nerve during the inspiratory phase $\varphi_1$ (Fig.3). A strong RSA is observed which manifests as a slowing of the heart rate from 4.8 beats/s to 2.5 beats/s (Fig.3a). This artificially induced RSA (AIRSA) makes the heart rate decrease almost linearly during stimulation at a rate of -3 beats/s$^2$. During the recovery period following stimulation, the heart rate returns to its resting value at a rate of +1 beat/s$^2$. In Fig.3b, the injection of sodium cyanide has the effect of increasing the phrenic burst rate. As a result, the duty cycle of neuron bursts of the artificial CPG increases which allows less time for the heart rate to recover. It follows that the heart rate of Rat #2 shows oscillations of weaker amplitude averaging at ~3 beats/s, yet they remain perfectly synchronized to the rhythm of respiration.

In Fig.4, the vagus nerve is now stimulated by N2 over the entire expiration phase ($\varphi_2+\varphi_3$). This out-of-phase synchronization occurs as the firing of N1 is triggered by the PN signal and N2 oscillates out of phase with N1. In this situation, the heart rate halves and remains constant over the time interval in which the stimulation is applied. The heart rate of Rat #1 drops to 3 beats/s (Fig.4a) and to 2.5 beats/s for
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Rat #2 (Fig.4b). This can be explained by observing that the expiratory phase lasts 4 times longer than the inspiratory phase. Hence, the vagus nerve is stimulated over a time interval 4 times longer than in the inspiratory phase as seen in Fig.3 with little time for recovery in between spike bursts. The heart rate therefore appears to have saturated at its lower value (~2.5 beats/s) which is found to be the same in both rats. Concerning the lack of recovery time, note that the heart rate shows a lone peak (Fig.4b) when N2 stays quiet for an unusually long period of time. Another interesting feature is the series of randomly timed phase slips which appear in Fig.4a. These are especially clear in the second derivative of the PP curves where heart beats appear to be missing.

Fig.5 shows the effect of stimulating the vagus nerve over a short time interval at the beginning of the expiration phase ($\phi_2$). We achieve this by using the cVN as an input to N1. Given that the cVN signal peaks at the beginning of the expiration phase, neuron N1 was made to fire during phase $\phi_2$ by tuning the amplifier gain so that the cVN signal crosses the N1 threshold near the cVN peak. Fig.5a shows the cardiorespiratory activity of Rat #2 when stimulated by short bursts of two of three pulses contained in phase $\phi_2$. Given the narrow time window (<0.4s), AIRSA is hardly detectable and the heart keeps beating at its rest pace of 4.8 beats/s. If by contrast, the spike bursts become broad enough to overlap the inspiratory phase (Fig.5b), the strong AIRSA described in Fig.3 is observed again.

From the above results it would appear that AIRSA depends mainly on the duration of spike bursts with a minimum of 3 consecutive spikes needed to evoke any response. However, we now show that the timing of spike bursts also matters. Fig.6 reports the effect of applying spike bursts in the second half of the expiratory phase ($\phi_3$) which was achieved by delaying the rise of the current stimulating N2. AIRSA is observed, however the modulation of the heart rate is comparatively weaker than in the inspiratory phase in Fig.3 despite the spike bursts lasting 2 to 3 times longer than in the inspiratory phase in Fig.3. In Fig.6a, the heart rate decreases to 2.5-4 beats/s compared to 2.5 beats/s in Fig.3. This suggests that it is more difficult to induce AIRSA in the late expiration phase than in the inspiration phase. Further evidence for the reduced sensitivity in phase $\phi_3$ is provided by reducing the duration of spike bursts (Fig.6b). The spike bursts seen after $t=5243$s have similar width to those applied in the inspiration phase in Fig.3 yet produce virtually no AIRSA at all.
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Lastly, AIRSA increases arterial pressure as seen by the greater magnitude of the PP oscillations during the time intervals of stimulation. For instance, the PP oscillations double in amplitude when stimulation is applied during the inspiratory phase (Fig.3a) and triple when it is applied in the late expiratory phase (Fig.5b).

4. Discussion

This study shows that the cardio-respiratory system of the rat can be successfully interfaced with an artificial CPG to produce artificial modulation of the heart rate. The artificial modulation of the heart rate is up to 16 times greater than the natural modulation produced by the brainstem CPG. In all experiments we have performed, vagus nerve stimulation consistently decreases the heart rate while increasing vascular pressure. An advantage of the CPG technology we have developed is that it provides natural synchronization to the respiration cycle. This is because the nonlinear response of artificial neurons represents a major improvement over established pacemaker electronics. The competition between neurons in the CPG further allows timing neuron bursts at different points of the respiration cycle to study the response of the heart outside the dynamic range of the brainstem CPG. The primary effect of stimulation by artificial neurons is to decrease the heart rate proportionally to the duration of the stimulus. However this response is not the same at every point of the respiration cycle: it varies according to the timing of stimulation. Vagus stimulation makes the heart rate drop at -3Hz/s² in the inspiration phase (Fig.3, Fig.5b) but only -1Hz/s² in the late expiration phase (Fig.5a). This explains the larger heart rate modulation seen in the former case. The reduced sensitivity in the late expiration phase might relate to interaction within the cardiac ganglion such that there is likely to be less noradrenaline released from cardiac sympathetic terminals which fire at end of inspiration and early expiration (Boscan et al., 2001).

Since noradrenaline potentiates vagal nerve responses at the level of the cardiac ganglion (Levy et al., 1966; Potter, 1987), vagal brachycardia could be reduced. Heart rate recovery during quiescent intervals appears to be the same across the respiration cycle with a recovery rate of +1Hz/s².

Under quasi-continuous stimulation (Fig.5a), the heart rate does not drop to zero but saturates at 2.5beats/s - about half the pace of natural oscillations. Without any external perturbation, respiration and
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the cardiovascular system form two coupled oscillators whose interaction produces RSA (Fig.2). The effect of constant excitation by the silicon CPG is to introduce a third oscillator which competes with the brainstem CPG to synchronize with the cardiovascular system. In this process N1 and N2 which fire 10-50Hz can lock to the heart beat at 2.5Hz. The phase slips indicated by the red arrows in Fig.5a are believed to arise from such phase locking. This behavior is known to occur when the synchronization region between two coupled oscillators is being reduced by the action of an external perturbation. In the present case this perturbation is the artificial CPG. Schäfer et al. (Schäfer et al., 1999) have modeled the heart as a noisy van der Pol oscillator whose natural oscillation frequency is modulated by respiration. In such system, the perturbation (noise) has the effect of narrowing the region of cardio-respiratory synchronization allowing phase slips to occur.

The CPG technology demonstrated here differs fundamentally from previous hybrid-neural systems (Olypher et al., 2006; Pinto et al., 2001) in that computation in performed by analogue hardware rather than software. Our analogue network integrates stimuli in real time, has lower power consumption than cardiac pacemakers and incorporates circuits that are easily scaled to less than 1mm² of silicon wafer. These are crucial advantages for medical implants. The two-phase oscillations are very robust since our experiments have only required us to adjust the gain of the second amplifier stage to compensate for small differences in PN amplitude in the two rats. To obtain multiphase phase oscillations slow potassium currents would need to be included in the neuron model to deliver spike bursts whose duration is set by competition between neurons rather than by the duration of the stimulus.

In summary, we have shown that a silicon CPG can be used to time the slowing of the heart rate within the respiration cycle of a rat, inducing artificial respiratory sinus arrhythmia. The modulation of the heart output is more efficient during the inspiratory phase than the late expiratory phase. Our silicon CPG has successfully been coupled to a rat model. This paves to way to novel medical devices which mimic biological CPGs to engineer neuromodulation synchronized to biological rhythms.

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Captions:

FIGURE 1: Modulating the heart beat with mutually inhibitory neurons

(a) A decerebrated rat is placed in ice-chilled carbogenated Ringer solution. Electrodes are attached to the thoracic phrenic nerve (PN), the central vagus nerve (VN) to record inspiratory patterns and vagus nerve activity respectively. The perfusion pressure (PP) and electrocardiogram (ECG) are also recorded to monitor the heart output and the heart rate respectively. A pre-amplifier stage magnifies the phrenic signal by 10,000. A second amplifier stage rectifies and smoothes the pre-amplified signal resulting in quasi-rectangular impulses indicative of inspiration. The voltage gain of the second stage is used to fine tune the amplitude of phrenic pulses relative to the firing threshold of neuron 1. A differential transconductance amplifier is fitted in output of the second stage to convert voltage pulses into the current pulses input to neuron 1. Neuron 1 (N1) and neuron 2 (N2) are a reciprocally inhibitory pair of silicon neurons which oscillate out of phase with each other. N1 and N2 belong to the 6 neuron central pattern generator shown in inset. The voltage oscillations of N1 (resp. N2) are used to stimulate the vagus nerve in-phase (resp. out-of phase) with the phrenic input. The PN, ECG, PP and VN outputs are recorded by the ‘spike2’ data acquisition software with a sampling frequency of 5kHz.

(b) A sample of the typical time series phrenic signal input to neuron 1. The respiration cycle is divided into three phases: the inspiration phase ($\phi_1$), the early expiration phase ($\phi_2$) and the late expiration phase ($\phi_3$) which we aim to stimulate with the CPG

FIGURE 2: Natural respiratory sinus arrhythmia. The instantaneous heart rate (HR) was calculated from electrocardiogram (ECG) recordings of Rat #1. Both Rat #1 and Rat #2 had similar average heart rate (4.8 beat/s). The natural respiratory sinus arrhythmia is observed as the slow modulation of the heart rate of period 4.1s and amplitude ~0.08Hz.

FIGURE 3: Motor activity induced by stimulating the vagus nerve in the inspiration phase - $\phi_1$. The vagus nerve is stimulated by neuron N1 (blue trace). The time series data of the electrocardiogram
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(EGC), perfusion pressure (PP), phrenic nerve input (PN), central vagus nerve output (VN) and the “membrane” voltages of silicon neurons N1 and N2 are plotted for (a) Rat #1, (b) Rat #2. The instantaneous heart rate (HR) was calculated from the ECG (a) and from the fast oscillations of the perfusion pressure (b). Panel (b) further shows the effect of changing the respiration frequency. In both rats, vagus nerve stimulation during the inspiration phase induces bradycardia and increases the amplitude of pressure oscillations. Neurons N1 and N2 remains synchronized to the respiration cycle when the respiration rate of Rat #2 changes.

FIGURE 4: Motor activity induced by stimulating the vagus nerve in the expiration phase - $\varphi_2 + \varphi_3$.

The vagus nerve is now stimulated by neuron N2 (blue trace) which fires out-of-phase with N1 and PN.

(a) Rat #1 – Neuromodulation applied during the long expiration phase reduces the average heart rate from ~4.8 beats/s to ~2.5 beats/s. The heart rate occasionally drops to ~2 beats/s during the longer bursts (487s). Neuro-stimulation by N2 remains synchronized out-of-phase during the change in respiration frequency.

(b) Rat #2 – Here also neuromodulation reduces the average heart rate to ~3.2 beat/s. The heart rate increases when N2 falls silent (471s).

FIGURE 5: Motor activity induced by stimulating the vagus nerve in the early expiration phase - $-\varphi_2$.

The vagus nerve is stimulated by neuron N1 (blue trace) whose firing is triggered by the central vagus nerve.

(a) Rat #2 - The firing threshold is set at the level of the VN peak producing a narrow spike burst at the beginning of phase 2. The average heart rate ~5beats/s is the natural heart rate. Temporal modulation can be detected but is of the same order as the error on measurement.

(b) Rat #2 – The firing threshold is set at a lower level giving wider spike bursts that overlap regions 1 and 2. Neuromodulation strongly reduces the heart rate from 5beats/s to ~3beats/s during the spike burst intervals. The PP amplitude triples during the bursts.
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**FIGURE 6: Motor activity induced by stimulating the vagus nerve in the late expiration phase - \( \varphi_3 \).**

The vagus nerve is stimulated by neuron N2 (blue trace) delayed by time constant \( \tau \).

(a) Rat #2 / small delay \( \tau \): the vagus nerve is stimulated over the full duration of the late expiratory phase.

Moderately strong sinus arrhythmia is induced which sees the heart rate occasionally dropping from 5 beats/s to little less than 3 beats/s.

(b) Rat #2 / long delay \( \tau \): the vagus nerve is stimulated at the end of the late expiratory phase (\( \varphi_3 \)). The resulting sinus arrhythmia is weaker but clearly visible at 5231s, 5235s and 5348s when the spike bursts consist of 3 or more spikes.
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Figure 1
Modulation of sinus arrhythmia with CPG hardware

Figure 2
Figure 3
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Figure 4
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Figure 5
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Figure 6
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