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SELF-PACED RESPIRATION IN RATS: THE EFFECT OF FEEDBACK DELAY

DISSertation

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Robert P. Morgan, B. S., M. S.

The Ohio State University
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Lastly, the author would like to thank the Office of Naval Research for financial support (NR 101-733).
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>VITA</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>HISTORICAL BACKGROUND</td>
<td>5</td>
</tr>
<tr>
<td>PURPOSE</td>
<td>8</td>
</tr>
<tr>
<td>EXPERIMENTAL PROCEDURE</td>
<td>10</td>
</tr>
<tr>
<td>Stereotaxic Instrument</td>
<td>10</td>
</tr>
<tr>
<td>Microelectrodes</td>
<td>10</td>
</tr>
<tr>
<td>Measurement of Electrodes</td>
<td>11</td>
</tr>
<tr>
<td>Stimulus Signal</td>
<td>11</td>
</tr>
<tr>
<td>Delay Circuit</td>
<td>11</td>
</tr>
<tr>
<td>Spirometer</td>
<td>11</td>
</tr>
<tr>
<td>Recorders</td>
<td>12</td>
</tr>
<tr>
<td>Circuitry</td>
<td>12</td>
</tr>
<tr>
<td>METHODS</td>
<td>12</td>
</tr>
<tr>
<td>RESULTS</td>
<td>19</td>
</tr>
<tr>
<td>Direct Stimulation</td>
<td>19</td>
</tr>
<tr>
<td>Pulse Stimulation Triggered from Respiratory Pattern</td>
<td>29</td>
</tr>
<tr>
<td>Autostimulation</td>
<td>35</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>PAGE</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Direct Stimulation</td>
<td>47</td>
</tr>
<tr>
<td>Autostimulation</td>
<td>49</td>
</tr>
<tr>
<td>Phase Relation during Autostimulation</td>
<td>53</td>
</tr>
<tr>
<td>Remaining Questions</td>
<td>61</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>63</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>65</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Experimental Arrangement</td>
<td>12</td>
</tr>
<tr>
<td>2. Typical Experimental Record</td>
<td>15</td>
</tr>
<tr>
<td>3. Direct Stimulus, Open-looped Pacing</td>
<td>20</td>
</tr>
<tr>
<td>4. Direct Stimulus, Open-looped Pacing</td>
<td>22</td>
</tr>
<tr>
<td>5. Direct Stimulus, Open-looped Pacing</td>
<td>23</td>
</tr>
<tr>
<td>6. Direct Stimulus, Open-looped, Not Paced</td>
<td>24</td>
</tr>
<tr>
<td>7. Direct Stimulus, Open-looped, Not Paced</td>
<td>26</td>
</tr>
<tr>
<td>8. Direct Stimulus, Open-looped, Not Paced</td>
<td>27</td>
</tr>
<tr>
<td>9. Direct Stimulus, Open-looped, Not Paced</td>
<td>28</td>
</tr>
<tr>
<td>10. Direct Stimulus, Open-looped, Not Paced</td>
<td>30</td>
</tr>
<tr>
<td>11. Direct Stimulus, Open-looped, Not Paced</td>
<td>31</td>
</tr>
<tr>
<td>12. Direct Stimulus, Open-looped Pacing</td>
<td>32</td>
</tr>
<tr>
<td>13. Pulse Stimulation, Not Paced</td>
<td>33</td>
</tr>
<tr>
<td>14. Pulse Stimulation, Not Paced</td>
<td>34</td>
</tr>
<tr>
<td>15. Pulse Stimulation, Not Paced</td>
<td>36</td>
</tr>
<tr>
<td>16. Autostimulation. Verification of Pacing</td>
<td>37</td>
</tr>
<tr>
<td>17. Autostimulation</td>
<td>39</td>
</tr>
<tr>
<td>18. Period versus Delay</td>
<td>40</td>
</tr>
<tr>
<td>19. Period versus Delay (5 Animals)</td>
<td>41</td>
</tr>
<tr>
<td>FIGURE</td>
<td>PAGE</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>20. Period versus Delay, Slope</td>
<td>50</td>
</tr>
<tr>
<td>21. Period versus Delay, Slope (5 Animals)</td>
<td>51</td>
</tr>
<tr>
<td>22. Phase Relationship</td>
<td>54</td>
</tr>
<tr>
<td>23. Stimulus Lead-lag versus Delay</td>
<td>55</td>
</tr>
<tr>
<td>24. Stimulus Lead versus Period</td>
<td>57</td>
</tr>
<tr>
<td>25. Stimulus Phase Angle versus Period</td>
<td>58</td>
</tr>
<tr>
<td>26. Schematic Diagram of Experimental Arrangement</td>
<td>59</td>
</tr>
</tbody>
</table>
INTRODUCTION

The control and regulation of body systems has occupied physiologists since the middle of the nineteenth century when Claude Bernard wrote of the stability with which the body maintains the constant chemical composition of its fluids. This "homeostasis" of the internal environment is maintained despite a continuous exchange with the external environment. The maintenance of these constant steady state levels simultaneously with continuous external exchange is made possible by exquisite regulation and control. The study of regulation and control may be facilitated by a research method which determines an output for a given input and attempts to elucidate the law or relationship which would produce this output.

The study of pulmonary ventilation is well suited for this approach. Yamamoto and Edwards (14) showed that when an input of CO₂ is artificially introduced into the blood (or as the metabolic rate is increased), the rate of pulmonary ventilation increases proportionally. The extra input of CO₂ is eliminated from the lungs as fast as it is produced, yet the partial pressure of CO₂ in the arterial blood does not rise.

The straight line relationship between the amount of carbon dioxide added and the pulmonary ventilation reveals the relationship (law) for the exchange of carbon dioxide. The output is directly
variable with input while the content (arterial CO₂) remains constant, and regulation is accomplished. Regulation, in this context, is not to be confused with control. Control describes management of a variable such as heart rate, rate of heat production, etc. while regulation denotes an attempt to maintain a constant value such as blood pressure, partial pressure of blood gases, or constant body temperature.

The work of Yamamoto and Edwards (14) suggests that chemical fluctuations in arterial $P_{CO_2}$ or pH may be translated into non-propogated electrical potentials in the central nervous system, and that these oscillating potentials can modulate respiratory activity.

This led to further research (Davies and Yamamoto, 1966 (7) in which a successful search was made of the brain stem (rat) for respiratory areas responsive to physiological levels of electric stimulation ($\pm$ 75 uA about a DC offset of -30 uA). Slow sinusoidal polarization of these areas with a Ag-Ag Cl microelectrode elicited a synchronized pulmonary ventilation. The range of frequency control was from 0.1 to 8.0 cycles/sec. Synchronization of ventilation with stimulation frequency was accompanied by changes in tidal volume. An increased tidal volume occurred at slower frequencies of stimulation. The change in volume was always due to a change of end inspiratory level. End expiratory level remained constant. The stimulus site eliciting this synchronized response was limited to a distinct, small volume of the medulla which was anatomically mapped. Of 1359 points probed, 167 gave a positive response. This responsive region was
found to be in the ventral reticular formation of the medulla, overlying the rostral part of the inferior olive. It extends from the level of the obex 1.2 mm rostral, from the midline 2 mm lateral on each side, and is about 0.5 mm from the ventral surface and 2 mm from the dorsal surface of the medulla. The boundaries of this region are sharp and constant. Blood gas samples taken after hyperventilation showed a lowering of arterial CO$_2$. The experiments of Davies and Yamamoto (7) made no mention of an attempt to verify a one to one relationship of stimulus to breath (pace); although they did note a generalized synchrony of rate of stimulation to breathing rate. At high CO$_2$ levels, synchrony was difficult to maintain and occurred only at stimulus frequencies close to the natural respiratory frequency. This characteristic was determined by adding CO$_2$ to a respiratory gas of pure oxygen. Synchrony was usually poor when breathing CO$_2$ concentrations greater than 5%. Davies and Yamamoto apparently used only sinusoidal stimuli and did not examine the effect of other waveforms (e.g. triangular, square, ramp, etc.) upon the ventilatory pattern. No closed-loop, self-paced preparations were attempted.

Work with the unidirectional chicken (Kunz, et al.), has suggested respiratory control models postulated by Yamamoto. In this preparation a constant, high flow of gas is passed across the exchange surface of the chicken lung. This is possible because of the air sacs which are characteristic of the avian species, and which lie distal to the lung. Air directed into a tracheostomy passes through the lung and can be vented to the outside by means of exhaust ports in the
air sacs. By maintaining a high air flow (9 times resting ventilation), neither the birds respiratory movements nor its CO₂ elimination significantly change the CO₂ concentration in the lungs. If this unidirectional glow of gas has its CO₂ concentration varied sinusoidally within normal physiologic limits, the bird will synchronize its ventilatory pattern with this CO₂ stimulus frequency. Breathing can then be paced: as CO₂ pulses are frequency modulated, the rate of breathing will increase or decrease in synchrony. This open-looped preparation can be made closed-loop. By using the electrical signal from a full bodied plethysmograph as a feedback signal to control the CO₂ level of the inspired gas, each breath triggers the CO₂ change which, in turn, triggers the next breath. This results in a self-paced preparation with the CO₂ stimulus dependent upon the respirations. By then controlling the length of time between the breath and the CO₂ oscillation, delay can be introduced as a variable in the control loop.

These two techniques (Davies and Yamamoto, and Kunz, et al) have been combined in this research (7, 9). The purpose is to investigate the respiratory pacemaker mechanism of the rat.
HISTORICAL BACKGROUND

The investigation of neural tissues which are in the respiratory control system and which respond to electrical polarization have been an active area of research for many years. Loeb and Maxwell in 1896 with experiments on shrimps were the first to observe that antagonistic systems are activated when central nervous system is exposed to currents of opposite direction (10). In 1938, Barron and Mathews (2) found that vertebrate motor neurons respond rhythmically to direct current stimulation. Their work showed that any sensory stimulation of a limb results in slow fluctuations of neuronal electrical potential in its central end. They did not appear to be summed potentials, but to be due to spread by electrons of potential changes occurring in the gray matter of the spinal cord. The sign (+) of these potential changes was always the same, and no stimulus of any kind would hinder or prevent these slow potential changes in the spinal dorsal root which always followed the arrival of impulses from the periphery.

In 1939, Pitts (11) located areas in the medulla oblongata of the cat which effected respirations when electrically stimulated. High frequency stimulation (270 cycles/sec.) produced apnea but had limited effect on inspirations and expirations. His work took origin from earlier studies of Adrian and Bronk, 1928 (1) and Ferguson, 1935 (8): the amount of inspiratory activity of the diaphragm is proportional to
the frequency of impulses over phrenic nerves, and the activity of intercostal muscles is proportional to the frequency in any one fiber and the number of fibers. Also, in 1939, Worznak and Gesell found evidence that vagal stimulation augments the action potential discharges of both inspiratory and expiratory muscles.

In 1940, Brookhart (4) succeeded in varying both rate and tidal volume in the dog by stimulation of the reticular formation of the medulla. He utilized voltage and currents lower than previous workers but still exceeded the physiologic range. In 1943, Bullock (5), in reviewing the work that had been done in this area of research was able to state, "...indications are now numerous that the frequency of firing of a nerve cell depends upon the DC electrical field in which it lies ("electrotonus current or steady state potential"). Such DC fields are known to exist in nerve cell masses...".

Other motor sensitive areas of the brain stem (cat) were located by Skoglund (12) in 1947. A chlorided silver wire 500 μ in diameter implanted into the medulla oblongata and an indifferent electrode in the soft tissues of the neck elicited leg movements. Stimulation by current in one direction caused flexor responses whereas extensor responses predominated after a reversal of current.

Bindman in 1962 (3) found that slowly varying potentials applied via surface electrodes on the forepaw resulted in an increased firing of neurons within the gray matter of the central nervous system. This occurred in the absence of any other external stimulation. It was found that a surface positive current increased central firing of
neurons and were diminished by surface negative currents. In addition, the polarization of nerve terminals in muscle spindles alters membrane potential: depolarization caused an increased rate of discharge of nerve impulses while hyperpolarization decreases discharge frequency. In experiments on the spinal cord, it was found that the discharge of ventral roots was altered by passage of small currents across the thickness of the spinal cord.

Comroe, 1943, (6) removed the vermis of the cerebellum, exposed the floor of the 4th ventricle, and inserted a hollow needle into the respiratory sensitive anatomical areas previously developed by Pitts (11), and utilized by Davies and Yamamoto (7). He injected various chemicals at the loci with no reproducible results.

It appears, then, that there is ample evidence that electrotonus potential changes have a profound effect on the nervous system; and that these changes are unique, and probably not directly influenced by local chemical changes.
PURPOSE

There is much that is not understood about the mechanism of respiration. It is difficult to determine the regulation and control mechanisms because the inputs and outputs are difficult to delineate and measure. The problem is further compounded by the realization that the law is not unique to but one set of measurement parameters. It becomes very difficult to know which variables to correlate.

There have been many methods utilized in the study of respiratory pacemakers. Present concepts postulate neuronal pathways between the pons and the medulla. Respiratory rhythmicity is claimed to result from pneumotaxic and apneustic centers in the pons which control respiratory and expiratory centers in the medulla (19). Kunz, Weissberg, and Miller (9) have recently found evidence that the respiratory pacemaker in the chicken is a reverberating circuit between the medulla and the CO2 receptors in the lung.

The purpose of this present research was to investigate the possibility that respiratory pacing in the rat also consisted of a physiologic feedback mechanism. A closed-loop, autostimulation preparation utilized the electrical signal from an electro-spirometer to feedback the respiratory pattern as a stimulus to the medullary cells. A controlled delay in the feedback circuit produced changes in the respiratory period.
An essential requirement of this experimental technique was to assure a one to one synchrony of the stimulus signal and its resultant breath. The method of Davies and Yamamoto (7) was utilized with the additional refinement of autostimulatory pacing combined with a stimulus delay capability.

An analysis of the experimental results provided an insight into the intricacies of the respiratory pacemaker.
EXPERIMENTAL PROCEDURE

Apparatus and Materials

Stereotaxic instrument was Model 900 small animal instrument, David Kopf Instruments (DKI). It consists of a "U" frame affixed with two ear bars and a nose clamp. These three points rigidly fix the rat skull in space. The microelectrode is affixed to a three dimensional manipulator (Model 960) which is secured to the U frame. There is 80 mm of ventrical, lateral, and anterior-posterior movement, a forward-backward angle rotation, and a swivel capability.

Microelectrodes: Electrodes were fashioned from glass capillary tubes. A pulling device was devised consisting of two springs with clamps which grip the ends of the capillary tube and pull with opposing tension. A heating wire was coiled at the center of the tube. The heater current was adjustable with an auto transformer which attenuated 110 V, 60 cycle alternating current. When the heater coil was energized, the spring pull was activated. The heat plasticizes the center of the tube and the springs draw it out to a small diameter. A silver wire served as connector between the Ag-Cl contents of the electrode and the stimulus circuitry. A silver wire also served as the indifferent electrode which was inserted in the fascial sheets between the rats scapula and rib cage,
Measurement of electrodes: The tip diameter of 70-80 microns was verified by viewing under a light microscope against a scale with divisions of 40 microns.

Stimulus signal: The stimulus signal for pacing was provided by a 100 V EAI operational amplifier. Its signal is further conditioned to the desired 75 microamp peak to peak amplitude about a -30 microamp bias by other electronic components.

Delay circuit: An analog to digital conversion of the ventilatory pattern of the rat was accomplished by a PDP-12 digital computer which was programmed with the option of a variable delay. Circuitry then provided for digital to analog reconversion, and a further conditioning by operational amplifiers for its use as a stimulus of 75 microamp peak to peak amplitude about a -30 microamp bias.

The above delay technique was utilized during the acquisition of all the data as described in the Results and Discussion. Another delay mode, however, was successfully used which consisted of a series of monostable triggers which could be set to any variable delay before triggering the stimulus signal. This configuration is referred to in Results as "pulse stimulation triggered from the respiratory pattern".

Spirometer: Respiratory movements were recorded by means of a bag-in-bottle spirometer (Fig. 1). A four liter bottle with stoppered openings at top and bottom was filled with 100% oxygen. Utilization of two one-way valves allowed inspiration of 100% oxygen from the bottle through the lower opening, and expiration through the upper stopper into
Figure 1. The electrospirometer provides a ventilatory signal which is recorded and also is returned to the rat's medulla as a respiratory stimulus. With this autostimulation mode, each breath, after an appropriate delay, becomes the stimulus for the next breath. With the switch on direct mode, a variety of stimulus waveforms are possible.
a plastic bag suspended in the bottle. In this manner, a pressure-volume balance was maintained. Every inspiratory breath was replaced by the next expiration. To prevent baseline drift of the ventilatory pattern due to temperature changes, the spirometer was "AC coupled" by providing a small adjustable vent in the lower stopper. The pressure oscillations in the spirometer (breathing pattern) were picked up by a Grass PT 5A pressure transducer placed in the upper stopper.

**Recorders:** Pen write-outs were obtained by use of a four channel Grass Model 7 polygraph. A visual record was constantly available from a Hewlett-Packard oscilloscope. Tape records were taken by a Data Tape Magnetic Recorder, Type 5-752.

**Circuitry:** A combination of operational amplifiers, Schmidt triggers, monostable triggers, potentiometers, variable resistors, and switches provided the various experimental modes and provided a proper recording of the results. The combination of switches allowed an immediate change from calibration, to pacing, to delay circuit, etc.

**Methods**

The Ag-Ag Cl glass microelectrodes were filled with 0.9% Na Cl in 2.0% agar. This gel was melted in a large test tube immersed in a water bath. The electrode was suspended, tip down, in the solution. An air space was provided above the solution, and a slight vacuum applied through a one hole stopper. The rate of filling was dependent upon the amount of vacuum. The electrode was filled just prior to each rat experiment.
Male albino rats (Sprague-Dawley strain) of 350-400 grams were used. They were anesthetized with sodium pentobarbitol administered intraperitoneally (1.5 mg/100g rat). Surgery consisted of a tracheal cannulation, and a posterior craniotomy with removal of the caudal portion of the cerebellum exposing the obex, and the floor of the fourth ventricle. A suction device with a sensitive control permitted removal of tissue with virtually no loss of blood.

This preparation was placed in the stereotaxic instrument and the tracheal cannula connected to the bag-in-bottle spirometer, and its pressure transducer connected to the polygraph recorder. The microelectrode, fastened to the vernier controlled arm, was maneuvered so its tip touched the obex. This established a benchmark from which anatomical locations (points of stimulus) in the medulla could be referred. The respiratory sensitive neurons are in a volume of medullary tissue which is distinct and constant, yet considerable probing was often required to locate the neuronal network which resulted in pacing of respirations.

Four channels of the polygraph were utilized for a pen record of the experiments (Fig. 2):

1. A calibrated ventilatory pattern from the spirometer (Fig. 2, second from top). The calibration was accomplished by injecting a known volume of air into the spirometer from a syringe.

2. A calibrated stimulus current pattern. (Fig. 2, third from top).
Figure 2. Five polygraph channels provided the experimental record. In the top channel, the height of each pulse is proportional to the period. Second from the top is the spirometer record of ventilation. Pulses on the middle channel are at one second intervals. The record second from the bottom is the stimulus signal. The bottom channel is a composite of the spirometer and a pulse for each stimulus cycle, and verifies synchrony.
3. A signal which combined the ventilatory and stimulus patterns on one record (Fig. 2, bottom). This provided a breath by breath verification that the stimulus undulations and the respirations were (or were not) synchronized. This was accomplished by superimposing a "sync pulse" onto the ventilatory pattern marking the time of each upslope of the electrical stimulus.

4. A circuit was devised to visually display period in a quantitative manner (Fig. 2, top). This was necessary because much of the purpose of the experiment was to determine the period/delay relationship on a breath by breath basis. This was accomplished by electronically converting each ventilatory signal to a pulse. The time between pulses was then electronically integrated, and the value of this integration was displayed as the height of a pulse on the polygraph record. This pulse height was recalibrated prior to each experiment. The oscilloscope was utilized to give a constant visual calibration of the stimulus amplitude and its bias.

By either of two stimulus modes could be chosen: direct open-looped stimulus from the signal generator, or closed-loop autostimulation through the digital computer with a variable delay (Fig. 1). Direct stimulation is open-looped and the rat cannot influence the stimulus frequency or waveform. Autostimulation is closed-loop: each breath provides the stimulus signal for the next breath. The autostimulation stimulus signal originates from the spirometer ventilatory pattern.
The voltage waveform of each breath becomes the current stimulus waveform of a later breath. This conversion from voltage to current is produced by the 100 volt operation amplifier driving the current through the combined resistance of the electrode and the tissue. Variable delay of the stimulus over a range of 0.0 to 10.0 seconds in increments of 0.01 seconds is accomplished by a PDP-12 digital computer.

The experiment is conducted by first setting up the open-looped, direct stimulus configuration. The electrode is positioned in the respiratory responsive area of the medulla, and pacing attempted. Usually several locations must be tried before adequate pacing is attained. Pacing is confirmed when one breath occurs for each oscillation of the current. A further criteria is that this synchronization can be maintained over a range of about one octave. This response occurred over the full range of normal, spontaneous respiratory rates observed which was from about .75 to 2.2 cycles per second.

After open-looped, direct stimulus pacing had been attained, closed-loop, autopacing was initiated. This was done by simply switching from one circuit to the other. Delay could then be introduced by turning the vernier dial of a potentiometer. A digital voltmeter was calibrated to read the delay time in increments of 0.01 seconds.

The data during the experiment were recorded on the polygraph. The various stimulus inputs have parameters of shape, amplitude, bias,
frequency, and phase relationship. The output consists of the ventilatory pattern which also has these same parameters. By utilizing a controlled disturbance as the input and then observing the output, an insight is gained in how these respiratory neuronal networks may effect these same results.

Also, during autostimulation, to prevent a sigh from producing an overstimulus to the next breath, the signal was "clipped by saturation of an operational amplifier at the desired maximum signal strength."
Results

The pacing technique allowed a variety of stimulus patterns to be utilized. The ventilatory response to many of these waveforms showed characteristic patterns. Typical examples are shown in Figures 3 through 16.

Direct Stimulation

In Fig. 3, the lower two patterns are a continuation of the upper two. The numbers below the sinusoidal stimulus pattern indicate its frequency in cycles per second (Hz). The beginning stimulus at 1.15 Hz paced respirations. When the stimulus frequency was increased to 1.4 Hz, pacing was retained but at a reduced tidal volume. At 1.6 Hz, pacing continued; and, again, at a reduced tidal volume. A further increase to 1.8 Hz resulted in a sigh, a loss of pacing, and a varying tidal volume. A varying tidal volume was a consistent characteristic of all experiments in which pacing was lost and the stimulus and respiration waveforms were of different frequencies. An exception to this appeared to occur when the stimulus and breathing pattern were out of phase by some exact multiple. This effect is illustrated in Fig. 3. Pacing occurred at 1.15 Hz, but reoccurred at 2.3 Hz which is exactly two times the initial pacing frequency.

It also appeared to be a consistent characteristic of pacing that tidal volume remained constant, and that this constant value was
Figure 3. Direct stimulus, open-looped pacing. The lower two records are continuations of the top two. Pacing at 1.15, 1.4, and 1.6 Hz showed constant but different tidal volumes.
dependent upon the pacing frequency. This is evident from Fig. 3 which shows a constant but different tidal volume at 1.15, 1.4, and 1.6 Hz respectively.

That tidal volume is not the only criteria for pacing is also illustrated in Fig. 4. At 2.2 and 1.3 Hz, pacing did not exist, and the tidal volume pattern was erratic. Pacing did occur at 1.7 Hz; and when the stimulus frequency was increased by an exact multiple (X10), pacing appeared to continue with no change in tidal volume. Yet, when the 1.7 Hz stimulus was increased by 100, apnea resulted. When the 1.7 Hz stimulus frequency was multiplied by 1000, a near normal breathing pattern occurred.

The range of pacing consistently attained was approximately one octave (the highest frequency being two times the lowest). A typical example of this range is shown in Fig. 5. The stimulus frequency just right of center is 2.0 Hz. The spirometer record shows a constant tidal volume at this paced frequency. When the stimulus frequency was halved to 1.0 Hz, the spirometer tracing indicates that the rats respirations followed within two breaths and continued at an increased but constant tidal volume.

Additional insight into how the sinusoidal current may effect respiratory movements can be observed in Fig. 6. Here, pacing was not achieved; respirations were 48 per minute while stimulus frequency was 67 per minute. The paper speed had been increased to show the effect of the sinusoidal stimulus falling at different times in the respiratory cycle. The spirometry record shows interruptions in the
Figure 4. Direct stimulus, open-looped pacing. A constant tidal volume is not the only criteria for pacing. Pacing exists at 1.7 Hz but tidal volume remains same at X 10, apnea at X 100, near normal at X 1000.
Figure 5. Direct stimulus, open-looped pacing. Range of pacing about one octave. Stimulus frequency just right of center shows from 2.0 to 1.0 Hz, and the respiratory pattern follows within a few breaths.
Figure 6. Direct stimulus, open-looped, but not paced. At fast paper speed, shows effect of the sinusoidal stimulus falling at different times in the respiratory cycle.
normal inspiratory patterns (marked by arrows) and which interruptions appear to coincide with the ascending limb of the current.

Another example of this effect is shown in Fig. 7. The stimulus signal was at a slower frequency than the natural breathing rate of the rat, and effected the individual breaths differently. This resulted in a wide variability of both tidal volume and period.

The erratic breathing pattern of Fig. 7 resulted from a stimulus signal which is at a slower frequency than the breathing frequency. The left side of the spirometer tracing in Fig. 8 illustrates that a similar variability may occur when the stimulus frequency is faster than the breathing rate. However, the right side of the record shows how the breathing pattern again became regular when the stimulus frequency was an exact multiple (X 2) faster than the breathing frequency.

That the phase relationship of stimulus to respiration has a profound effect is further illustrated in Fig. 9. The spirometer pattern shows a random variability of period and tidal volume of over 50%. As in the previous examples described in Figures 6, 7, and 8, this effect is the result of the phase relationship of stimulus and breath. Observation of how the relative position of stimulus and breath effect period during direct pacing gave an insight into how a delayed stimulus during autostimulation may effect the breathing pattern.

Most of the effects of stimulus on ventilatory pattern appeared to occur during inspiration. That expiration may also be effected is
Figure 7. Direct stimulus, open-looped, not paced. The stimulus signal at a slower frequency than the natural breathing rate of the rat, and effected individual breaths differently.
Figure 8. Direct stimulus, open-looped, not paced. Stimulus frequency at faster frequency than
the natural breathing rate, and effects individual breaths different. The right side
of the record shows how the breathing pattern again became regular when the stimulus
frequency was an exact multiple (X 2) faster than the breathing frequency.
Figure 9. Direct stimulus, open-looped, not paced. Both tidal volume and period vary over 50% when respiration and stimulus are out of synchrony.
illustrated by Fig. 10. This stimulus pattern is a series of sine waves which can be delivered at regular intervals.

A Cheyne-Stokes-like breathing pattern resulted when the stimulus signal was at a slightly slower frequency than the natural breathing cycle. This is illustrated in Figure 11.

The rat preparations would sigh spontaneously at intermittent intervals if no stimulation was applied. During pacing, however, a sigh would rarely occur except when the pacing frequency was changed. An example of this is illustrated in Figure 12. The spirometer record shows three sighs; and after each, there is a change of tidal volume, or frequency, or both. This was a consistent observation, and possibly implies that the sigh is utilized by the rat to resynchronize its breathing rate with the stimulus frequency.

**Pulse Stimulation Triggered From Respiratory Pattern**

A variety of stimulus wave forms were possible. Figure 13 shows the effect of individual stimulus pulses. Each breath triggered a fixed, single cycle stimulus for the next breath after a variable delay. In this pattern, every third stimulus pulse was omitted. The ventilatory response is again dependent upon the phase relationship of the stimulus to the natural breathing frequency. It appears that each breath is effected on an individual basis, and that either inspiration or expiration may be effected.

The effect on individual breaths is more clearly evident when intermittently spaced pulses are utilized. Figure 14 illustrates how single cycle, sine wave stimuli effect a simultaneously occurring
Figure 10. Direct stimulus, open-looped, not paced. Expiration interrupted because of stimulus waveform.
Figure 11. Direct stimulus, open-looped, not paced. A Cheyne-Stokes-like breathing pattern as result of a stimulus frequency at a slightly slower frequency than the natural breathing cycle.
Figure 12. Direct stimulus, open-looped, paced. Sighs rarely occur during pacing except when the pacing frequency was changed. The record shows three sighs; and after each, there is a change of tidal volume, or frequency, or both.
Figure 13. Pulse stimulation triggered from respiratory pattern. Each breath triggered a fixed, single cycle stimulus for the next breath. Each pulse effects an individual breath.
Figure 14. Pulse stimulation triggered from respiratory pattern. Single cycle, sine wave stimuli effect a simultaneously occurring breath.
breath. The exact phase relationship appears to determine whether inspiration or expiration is modified.

It was also possible to alter the stimulus signal by making it much more narrow than the breathing pattern, but to have it occur in about the same time interval. This effect, an abrupt interruption of the breath, is illustrated in Figure 15.

**Autostimulation**

Prior to the autostimulation experiments, the direct stimulus technique was utilized to verify that the microelectrode was in a respiratory sensitive location where pacing could be accomplished. An example of this verification of pacing is shown in Figure 16. The top tracing is the period. The height of each pulse is proportional to the time between each breath. The second tracing is the spirograph of tidal volume with inspiration upward. The third tracing is the timer record with a pulse after each one second interval. The fourth record from the top is the sinusoidal electric stimulus (75 uA peak to peak about a -30 uA bias). The bottom tracing is a composite of two signals: the spirograph tracing, and a marker pulse produced at the beginning of each stimulus cycle.

After the correct location of the electrode was assured with direct sinusoidal pacing, the switch was thrown (Figure 1) which changes the stimulus from a pure sine wave to the spirometer waveform. In this autostimulation, or self-paced mode, the animal originates the stimulus waveform. Each breath becomes the stimulus for the next breath. This stimulus for the next breath may be delayed before being
Figure 15. Pulse stimulation triggered from respiratory pattern. Stimulus signal is much more narrow than the breathing pattern.
Figure 16. Autostimulation. Verification of pacing.
fed back to the medulla. The range of this delay can arbitrarily be
chosen at any value from zero to several seconds.

It was found that as the stimulus signal was delayed, the
respiratory rate was effected in a characteristic manner. Figure 2
shows that as the delay is increased, the period is increased until,
at a critical amount of delay, the period abruptly decreases. To
show this pattern more clearly, Figure 17 is at a slower recorder
paper speed. At 1/4 speed, the progressive changes which occur with
added delay are more clearly illustrated. It is evident that as
delay was added from left to right, the period gradually increased to
a maximum, abruptly decreased, and then started to increase again.

When period vs delay is plotted, the regular pattern of Figure 18
results. Delay has been independently increased from zero to 3.6
seconds in increments of 0.05 seconds. The period is plotted for each
delay. Period was calculated from the number of breaths taken over a
twenty second interval. The periods appear to have a minimum and
maximum value. The maximum period appears to be about twice the
minimum. Furthermore, except for very short delays, the data tend to
fall in ramps. The slopes of these ramps appear to progressively
decrease from left to right. Figure 18 is from the best experimental
run, but this pattern holds for a composite of five rats as shown in
Figure 19.
Figure 17. Autostimulation. Slow paper speed to show the effect on period as the stimulus is delayed.
Figure 18

PERIOD (sec)

DELAY (sec)
Figure 19
The phase relationship between the stimulus and its paced breath during the autostimulation experiments was measured by examination of the patterns recorded at a fast paper speed. The graphs of this relationship are shown in Figures 23, 24, and 25. Phase is illustrated in Figure 22.
Discussion

The experimental results make certain facts clear. It was confirmed that the pacing of respiration, after the method of Davies and Yamamoto (7), provides an experimental technique with great utility for the study of respiration. The results described in the direct pacing experiments were greatly facilitated by the variety of stimulus waveforms available, by the capability to modifying and delay these stimuli, and by recording techniques which allowed a comparison of an individual stimulus and its effected breath.

The compliment of stimulus waveforms utilized included sine wave, square wave, triangular, and pulses of several shapes. The responses of the respiratory waveforms to these stimuli were consistent, and showed that all ventatory parameters could be altered. Whether tidal volume and/or period were effected appeared to depend upon the timing relationship of stimulus and breath. A nonvarying phase relationship appeared to be essential for the maintenance of a steady, nonvarying respiratory pattern, and this also appeared to be a requirement for pacing. It was an unexpected result that pacing could be maintained with a 2:1 ratio of stimulus to respiration. No effort was made to examine higher ratios. However, Figure 4 suggests a 10:1 ratio will retain pacing. This may be due to the stimulus frequency being a multiple of the normal unstimulated respiratory rate of the animal. It may also be a plausible explanation for the return to the pacing rate.
at a 1000:1 ratio. The apnea which occurred at 170 Hz in Figure 4 suggests the criteria Davies and Yamamoto (7), and Pitts (11) used for verification of proper electrode placement. They found that either sine wave or squarewave stimulus at 270 Hz would produce apnea when applied at the respiratory sensitive areas of the medulla.

The experiments which utilized pulses or individual stimulus waveforms showed that either inspiration or expiration could be effected separately. Here, also, it appeared to be the timing, or phase relationship of stimulus to breath that determined the effect. It also appeared that pulses or waveforms would not effectively pace respirations if they were too narrow. The greatest range of pacing occurred when the stimulus was a continuously changing waveform such as a sine wave or when the respiratory pattern itself was utilized as a stimulus.

It is unclear from these direct stimulus experiments what parameters of the stimulus waveform produce the observed changes in the respiratory response. There are many possible factors which exert an effect. It may be the rate of change (slope) of the stimulus, it may be the amplitude, it may be the duration, it may be the amount it leads or lags the respiratory event, it could be the shape of the stimulus waveform. The delay may effect the respiratory response. It has not been the mission of this research to investigate all these possibilities. Many of the findings reported in this thesis are at the descriptive level. Further experimentation can add greater precision to the description, and offer a statistical estimation of its validity.
The closed-loop mode in which each breath becomes the stimulus for the next breath provides a stable, self-paced preparation. The experimental set-up shows that autostimulatory pacing is possible, and the experimental data provides further insight into the many variables which control ventilation.

The data plotted in Figure 18 confirms that period is a function of the delay in the external circuit (Figure 26). The data forms ramps which appear to have a harmonic nature. Figure 20 illustrates that the data tends to fall along lines passing through the origin with slopes of 1, 1/2, 1/3, etc. This means that if the data are replotted with the y-values of the points on the 2nd ramp doubled, those of the 3rd ramp tripled, the 4th quadrupled, etc., they all should fall on a continuation of the first ramp. This harmonic relationship is verified by a "least squares" calculation of the slope of this line which is equal to 1.022. Figure 20 represents data from the best experimental run. Figure 21 shows that this same harmonic pattern occurs for a composite of five rats. Applying the same least squares statistical analysis to this data gives a slope of 1.074.

The harmonic nature of the ramps may be discussed by reference to Figure 26, a schematic diagram of the experimental set-up. Period may be considered the time required for one action pulse to traverse the natural breathing loop of the rat. This sequence of events includes: the respiratory center in the medulla fires a pulse of action potentials which activates the muscles of respiration, the elastic lung tissue effects alveolar gas exchange which changes CO₂ tension in the blood,
chemoreceptors sensitive to CO₂ are activated to send a pulse of action potentials back to the medulla, and the cycle continues to repeat. The external loop may preemt this natural loop and alter the breathing period. The data (Figure 18) shows that as delay was added in the external circuit, breathing period was lengthened a like amount and the first ramp was formed. The data shows, however, that the period is never lengthened to more than twice its minimal period. When the delay in the external loop was increased to a point where the breathing period was approximately doubled, the period abruptly reverted to its initial, minimal value. It appears that after a delay equal to about twice the minimal period and the medulla has not received back a pulse from the external circuit, a pulse is fired spontaneously. Now there are two pulses occupying the same loop, and the period of each is decreased by 1/2. Because the plot of data points consistently are harmonics of one (1/1, 1/2, 1/3, etc.), it would appear that the breath pulses equally space themselves around the breathing loop. The mechanism for the generation of the spontaneous pulse is not evident from these experiments. However, we know that it never occurred when the period between breaths was below a certain minimal value. It appeared to automatically occur when the period was greater than twice this minimal value.

The data points which occur prior to the first ramp of Figure 18 have been given little attention in this experiment. During this initial interval, the response (breath) leads the stimulus. This is a consequence of the experimental arrangement but provides an
unphysiological phase relationship. Sufficient delay must be provided before the stimulus can pace the next breath. This relationship apparently does not occur until the beginning of the first ramp after which added increments of delay result in a corresponding increase of period.

The fact that the first ramp of Figure 18 does not begin until the appropriate relationship of stimulus to breath existed, suggested that their phase relationship was important. To more clearly view this relationship, the polygraph record of the experiment was recorded at a fast paper speed. From this record, precise, breath by breath measurements were made. The legend for these experiments is illustrated in Figure 22. As noted, the time in seconds that the stimulus leads the breath is denoted m. The time in seconds that the breath leads the stimulus is n. One cycle is equal to m + n.

Figure 23 is a plot of m-lead versus delay. It shows that as delay is increased from zero, n is increased a like amount until the end of the first ramp. This would be expected from the experimental arrangement in which the respiratory waveform is used for the stimulus of the next breath. Therefore, as delay is increased, n will increase a like amount.

Figure 23 reveals that as ramp one begins at a delay of approximately 0.3 seconds, the stimulus leads the breath by about 0.2 seconds; and that this m value remains constant at about 0.2 seconds throughout the first half of ramp one. Interval m then gradually diminishes to
Figure 23
zero at which point the stimulus neither leads nor lags the breath. At this point, ramp two begins. A similar response occurred for the formation of each ramp.

Knowing that both period and phase are functions of delay, suggested an investigation of the relationship between period and phase. A graph of this (Figure 24) shows that the data for all three ramps superimpose. This suggests that period is determined by when, in the respiratory cycle, the stimulus arrives (m-lead); and is independent of which previous breath actually caused the stimulus. This graph (Figure 24) has an inflection which separates it into two different ranges for period. For low values of period (P), the m-lead appears to remain constant (for this single experimental run at 0.2 sec.):

\[ m = 0.2, \quad (P \leq 0.7) \quad (1) \]

However, in the range of periods above 0.7 seconds, the m-lead decreases proportionally to the increase in period:

\[ m = -1.34 P, \quad (P > 0.7) \quad (2) \]

When the interval that the stimulus leads the respiration (m-lead) is normalized by its period, it becomes a measure of the phase angle which we call \( \theta \), and express in fractions of a cycle:

\[ \theta = \frac{m}{P} \quad (3) \]

When the graph of \( \theta \) versus period (Figure 25) is plotted, the relationship is: monotonic for both variables (i.e. for each value of P, there
Legend:

- 1st Ramp
- 2nd Ramp
- 3rd Ramp

Figure 24
Legend:
+ 1st Ramp
○ 2nd Ramp
△ 3rd Ramp

Figure 25
Figure 26
is one and only one \( \theta \); and conversely, for each value of \( \theta \) there is one and only one value of \( P \). This was not the situation in Figure 24 which shows that during the portion that the m-lead holds constant at 0.2 seconds, the period has a range of values from 0.5 to 0.7 seconds.

This relationship reveals that possibly the constant (0.2 seconds) value of stimulus leading breath is not the controlling factor as suggested in Figures 23 and 24. Instead, the relationship determining period may be as suggested in Figure 25, the phase angle the stimulus leads the respiratory response. This data suggests that at the beginning of any ramp, the stimulus leads breath by about half a period. As the ramp develops further, this m-lead gradually diminishes as period increases. At the end of the ramp, there was no phase lead between the stimulus and the breath, and the next ramp begins abruptly. The stimulus again leads the breath by half a cycle.

This phase data also provides insight into a departure noted in the period/delay data of Figure 18. The data forms a first order approximation of ramps with slopes of 1, 1/2, 1/3, etc. The latter half of each ramp appears to have a reduced slope. This effect may be explained by the abrupt change in slope of the m-lead data in Figures 23 and 24. At this point, the stimulus no longer continues to lead the breath by a constant 0.2 seconds; but instead, the lead gradually decreases as delay and period are further increased. Delay added to the system in the latter half of each ramp will not all be added to the period. A portion of this delay will be absorbed by the decrease in the lead.
One of the questions which remains unanswered is how were Davies and Yamamoto able to pace respirations over such a much greater range. They report a range of pacing from 0.1 to 8 Hz; a range of greater than 6 octaves. Not once in our hundreds of attempts on scores of rats were we able to exceed a range of one octave. We have tried to reconcile this discrepancy without convincing success. We used the same size electrodes, filled with the same solution, implanted with the techniques. We used the same anesthetic agent (Sodium Pentabarbital), and the same electrical waveform. However, we were unable to extend the range of pacing. A possible explanation may involve some combination of the following:

1. A biological difference in the rats used. Davis and Yamamoto used Charles River CD strain we used Sprague Dawley.

2. The level of anesthetic may have been different. However, we did experiment with this over a wide range of anesthetic levels.

3. The amplitude of the AC sinusoidal stimulus we eventually chose was only one half the amplitude they report (75 micro-amperes). We did try this and even greater amplitudes and still were unsuccessful.

4. The only criteria for pacing Davies and Yamamoto used appeared to be a gross change in tidal volume as stimulus frequency was changed. Although they present no records showing a synchrony or one to one correspondance of stimulus to breath, we have little doubt that this was achieved. Because we found that
tidal volume, by itself, is not sufficient verification of pacing (see Figures 3 and 4), we developed a four point criteria:

1. **Period (top tracing):** period must hold a constant value for the particular frequency at which it is being stimulated.

2. **Tidal Volume (second from top):** tidal volume must hold constant with no intermittent sighs, and must change immediately to a new constant value when the stimulus frequency is changed.

3. **Stimulus (second from bottom):** the stimulus must show a range of frequency change of about one octave (X 2). In Figure 9, stimulus frequency was changed from 2.0 to 1.0 Hz, and respirations followed within a few breaths.

4. **Sync. Signal (bottom tracing):** when pacing occurs, this composite pattern of breath and stimulus marker must hold a constant phase relationship. Any irregularity represents an "escape" from pacing.

A second question which we regret we were unable to resolve was the exact cause and effect relationship of stimulus shape and breath response. Although tedious, it has always appeared that one could systematically vary the parameters of the shape of the stimulus and categorize the changes in the respiratory response. This, however, will need to be relegated to a future time.
Summary

Respiratory control in the rat was studied by application of physiologic level currents to discrete areas of the medulla oblongata. The method of Davies and Yamamoto (7), was utilized to locate the respiratory responsive areas, and a stimulus current of various waveforms was selectively delivered by means of a microelectrode. These stimulus waveforms elicited a variety of respiratory responses which provided insight into respiratory control. It was found that all respiratory parameters could be effected. Tidal volume or period could be increased or decreased. Inspiration or expiration could be interrupted. Bizarre and irregular breathing patterns could be produced. The respiratory response could also be locked into a pacing mode in which its pattern was highly regular and repetitive with a minimum of sighs. There appeared to be a correlation of the current increasing or decreasing with the observed changes in breathing pattern.

A stable, self-paced preparation provided further information on stimulus-response relationships. The ventilatory waveform was converted into an electric signal, and then returned to the medulla as a stimulus for the next breath. A further refinement, provided for variable delay of this stimulus signal. As a consequence, the breathing period was effected in a characteristic manner. The period versus delay relationships provided a pattern of ramps which appeared to have a harmonic
nature, and suggested a pacemaker mechanism in which an action pulse oscillates around a respiratory pacemaker ring postulated to be: medulla, phrenic-intercostal nerves, inspiratory muscles, change in alveolar gas concentration, chemoreceptor, return to medulla, and the cycle repeats.

A study was also made of the phase relationship between the electrical stimulus and the ventilatory response during autostimulation. It appeared that the effect on the breath depended upon the timing of the arriving stimulus. Two postulates were formulated for this effect. One postulate suggests that a constant time difference between stimulus and breath is the essential control feature. The other suggests that stimulus-response phase angle is the essential parameter. Additional research is required before formulating a more definitive model.
BIBLIOGRAPHY


