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EFFECTS OF OCCLUSION OF THE THORACIC AORTA
ON HABITUATION OF THE FLEXOR WITHDRAWAL REFLEX
IN THE RAT

by

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ABSTRACT

Experiments were carried out to investigate the extent to which habituation of the flexor reflex depended on mechanisms operating at spinal interneurons. An attempt was made to cause selective degeneration of interneurons in the spinal cord of the rat by subjecting the cord to a period of ischaemia. Ischaemia was produced by temporary occlusion of the thoracic aorta. The flexor withdrawal reflex was tested 3, 7 or 14 days after occlusion. When compared with data from control animals it was found that ischaemia had resulted in both a qualitative change and a quantitative diminution in the amount of habituation which occurred during the presentation of 400 uniform stimuli. It was concluded that this impairment of the habituation process was a consequence of degeneration of interneurons which normally cause progressive inhibition of the excitatory flexor reflex pathway.

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PART I.

INTRODUCTION

Habituation may be defined as an animal's loss of responsiveness to an inconsequential change in its environment, or a gradual quantitative diminution of response to repeated uniform stimuli. Habituation may result in complete loss of response but the definition of habituation excludes any qualitative change in the response or any situation where there is a quantitative change in the stimulus (Griffin and Pearson, 1968). Peckham and Peckham (1887) published the first observation of this phenomenon (habituation), noting that a spider stimulated to drop from its web by the sounding of tuning fork ceased to respond as the soundings were repeated. Dodge (1925) introduced the term "habituation" in preference to the term "adaption" which had been the most common of the names previously applied to describe this type of phenomenon. More recently experiments have been undertaken to investigate the general characteristics of habituation (Prosser and Hunter, 1963; Spencer, Thompson and Neilson, 1966a, 1966b, 1966c; Thompson and Spencer, 1966). Spencer, Thompson and Neilson (1966a, 1966b, 1966c) specified in great detail the parameters of the overall input - output function of habituation. Their extensive work has provided nine general characteristics of habituation:

- 1) Given that a particular stimulus elicits a response, repeated applications of the stimulus result in decreased response (habituation). The decrease is usually a negative exponential function of the number of stimulus presentations.

- 2) If the stimulus is withheld, the response tends to return gradually to control levels (spontaneous recovery).

- 3) If repeated periods of habituation and spontaneous recovery are permitted, habituation becomes progressively more rapid (this might be called potentiation of habituation).

- 4) Other parameters being equal, the higher the frequency of stimulation, the more rapid and/or more pronounced is habituation.

- 5) The weaker the stimulus, the more rapid and/or more pronounced is

habituation.

6) Iterated stimuli may continue to affect the neuronal circuit even though the response has habituated to zero. In this case the repeated stimuli continue to enforce the habituated state.

7) Habituation of response exhibits "stimulus generalization" between independent inputs often referred to as "transfer" of habituation (transfer is a term for the phenomenon which results in habituation to cutaneous stimuli from site "B" when a site "A" had been used as the point of cutaneous stimulus application; "A" and "B" must be close together on the skin or may be points on branches of the same cutaneous sensory nerve).

8) Presentation of another (usually strong) stimulus results in recovery of the habituated response (dishabituation).

9) Upon repeated application of the dishabitulatory stimulus, the amount of dishabituation produced habituates (this might be called habituation of dishabituation).

These nine common characteristics now serve as the detailed operational definition of habituation replacing the more general definition given above (characteristic 1).

The study of habituation may yield information which could be of importance to the elucidation of the physiology of some types of behaviour. Speculation as to the usefulness of habituation as a paradigm of classical conditioning and hence as a simple analogue of learning has been extensively presented by the authors of several reviews (Galambos, 1967; Kandel, 1967). Actual classical conditioning situations, that is those involving the use of natural stimuli and behavioural (effector) responses, bear direct relationship to analogues of classical conditioning situations, that is those involving artificial (electrical) stimuli and/or responses (Collier, 1899).

There has recently been interest in finding a model neuronal circuit which could be observed in the hope that, 1) the occurrence of change in

response to iterated stimuli at the level of a simplified tissue preparation would correspond to a more gross, perhaps behavioural change in the whole organism, 2) the model would already be sufficiently well studied by classical methods so as to facilitate the study of the model with respect to its changing character, and ultimately 3) the model would yield knowledge that might explain the mechanism of the change in response to repeated stimuli. One such model which has been widely studied is the flexor reflex response in vertebrates.

The Flexor Withdrawal Reflex

A convenient paradigm of habituation is provided by the flexor withdrawal reflex in which Sherrington (1897) noted decrementing responses to closely spaced stimuli in the spinal cat. The reflex is functional in moving a limb away from a stimulus. Each flexor motoneurone can be said to have a receptive field with respect to the flexor reflex stimulus. A wide range of afferents can evoke the reflex, including cutaneous, joint, and smaller diameter muscle afferents. Single motoneurons are subject to both spatial and temporal summation of stimuli. The threshold for the reflex is low in the spinal animal and higher in the decerebrate. Habituation of the reflex may be regarded as a purposeful reaction in the sense that the organism suppresses a spinal withdrawal reflex to a light skin stimulus which has proved to be innocuous.

The locus of the process of habituation of the flexor withdrawal reflex has been under investigation. Because habituation can be obtained by the stimulation of cutaneous nerves, it cannot be due to changes at the sensory periphery i.e. receptor adaptation (Spencer et al., 1966a; Buchwald, Halas, and Schram, 1965; Groves, Lee, and Thompson, 1969). Similarly habituation cannot be due to changes in the threshold of cutaneous afferents for Wickelgren (1967a) has shown that habituation is not due to a change in the threshold of the peripheral nerve for habituation was greater than might be accounted for by undetectable changes in the afferent volley. Decrement then does not occur

in either the primary afferent fibers or their terminals because both the afferent volley and the large negative dorsal root potential are unchanged during habituation (Wickelgren, 1967a). The polysynaptic component of the glabella reflex has been shown to habituate whereas concomitantly the monosynaptic component has been shown to remain unchanged (Kregelberg, 1962); indeed it may be potentiated (Wickelgren, 1967a). Spencer et al. (1966c) have shown that excitatory post synaptic potentials (EPSP's) can be evoked in a spinal motoneurone by monosynaptic stimulation, even after EPSP's in the same motoneurone, excited by a polysynaptic route, have undergone habituation. Furthermore, Griffin and Pearson (1968) have shown that the rate of habituation of the polysynaptic flexor reflex is not influenced by manoeuvres which increase the motoneurone excitability. Habituation is not the result of an increase in presynaptic hyperpolarization as this occurs only following a C fiber volley (Mendell and Wall, 1964) and habituation occurs when only large fibers are stimulated (Spencer et al., 1966a).

The possibility remains that habituation may be caused by local dendritic post synaptic changes in the motoneurone membrane which do not affect distant synapses. This thesis is difficult to prove or disprove. The intracellularly recorded membrane potential of motoneurons has been measured at the soma (Spencer et al., 1966c), and shown to remain unaltered even though the flexor reflex has habituated. Spencer et al. (1966c) were able to record habituating EPSP's in motoneuronal cell bodies suggesting that at least some of the habituated motoneurone synapses are quite near the cell body. This implies that because of the evidence that membrane potential at the cell soma remains unaltered, habituated motoneurone synapses possess post synaptic membranes with unaltered membrane potential. However, relatively large PSP's will involve many synapses in a motoneurone and they may lie up to 0.5 mm from the soma and still be detectable there. Hence there is little reason to believe that such distant synapses could not involve a change in local membrane potential

even though the membrane potential at the soma appears constant. In short the evidence is inconclusive. Additionally it could well be that in spite of constant membrane potential, membrane conductance could vary and thus explain habituation but such evidence has not been investigated.

Since habituation does not occur in the primary afferents, in their terminals or in the motoneurons it must occur in some population of interneurons. Evidence suggests that the polysynaptic component (the interneurone pathway) rather than the monosynaptic component (the sensory afferent neurone and the efferent motoneurone) is the more likely site of changes responsible for habituation.

Two theories have been put forward to explain interneurone action 1) synaptic depression; fatigue at any or all synapses between the primary afferent endings and the interneurons, and 2) inhibitory build-up theories; increased activity at synapses which inhibit the excitatory pathways to the motoneurons. A comparison of both low frequency depression and post tetanic potentiation (PTP) with habituation indicates that although the inhibitory mechanism is more likely to be correct, synaptic depression cannot be completely ruled out (Wickelgren, 1967b). Interneurons in the dorsal horn of the rostral L7 spinal segment were studied by Wickelgren (1967b). Interneurons were located in the cat rostral L7 spinal segment in laminae IV, V and VI of Rexed (1954) using the criteria for identification of interneurons proposed by Wall (1967). Irregular spontaneous activity and very long bursts of spikes (8 or more) following a single volley were sufficient to characterize a unit as an interneurone but additional criteria proposed by Wall (1967) were applied in doubtful cases. Wickelgren (1967b), on the basis of several lines of evidence, suggested that habituating interneurons in the dorsal horn provide input to flexor motoneurons and that habituation of the former is responsible for the habituation of the latter. The three supporting lines of evidence are 1) transfer of habituation between skin and the superficial peroneal (SP) nerve is similar in

interneurones and in motoneurones, 2) the stimulus frequency which produces the maximum habituation (50-100/sec) is the same for both interneurones and flexor motoneurones, and 3) the duration of habituation is about the same in both interneurones and motoneurones, recovery being half complete 3 minutes after cessation of stimulation. In view of the evidence of habituation of interneurones and motoneurones it is likely that habituating interneurones are components of the flexor reflex pathway.

Groves, De Marco and Thompson (1969) have, in the cat, located interneurones of the dorsal horn within L6 to S1 in laminae II to IV of Rexed (1954), which habituated to 2/sec shocks (single pulses 0.1-0.3 msec. duration) applied to the skin of the hind paw at intensities well above threshold. In addition Groves et al. (1969) located 9 cells within layers VII to VIII of Rexed (1954) which showed a pattern of sensitization (increasing responsiveness) followed by subsequent habituation to cutaneous stimuli. It would appear that the role played by interneurones in the process of habituation demands examination.

Spinal Ischaemia

There is a considerable body of evidence which indicates that temporary spinal cord ischaemia can result in degeneration of interneurones, with little concomitant change in the motoneurone population (Davidoff, Graham and Shank, 1967; Murayama and Smith, 1969; Van Harreveld and Shadé, 1962).

Van Harreveld and Schadé (1962) investigated the effect of asphyxiation of the spinal cord of cats upon the nerve cell population of L7 segments. Under nembutal narcosis the dura was ligated at Th₁₀ isolating the caudal part of the dural sac and severing the spinal cord. The next day a needle was introduced into the dural cavity between the 6th and 7th lumbar vertebrae. By introducing Ringer's solution through the needle at a pressure of 20 cm. of mercury the pressure in the dural cavity was increased above blood pressure causing asphyxiation. The animals were allowed to recover and at the end of

a two week period reflex activity in the hind limbs was investigated. The spinal cord was then exposed with the animal under ether narcosis. The preparation was immobilized with Squibb's Intocostrin and the reflex action potentials were recorded by stimulating a dorsal root and leading off from the ventral root at the same segmental level (usually S1). After the physiological experiment the cord was fixed for histological investigation using the methods of Schade' and Van Harreveld (1962). A survey of the spinal cords asphyxiated for 28 to 50 minutes showed a nearly complete destruction of the interneurons in the centrally located gray matter, a very severe loss of nerve cells in the dorsal horn and an extensive destruction of nervous elements in the ventral horn, particularly in its central and medial portions. In the peroneus tibialis (PT) neurone pool, which is situated in the lateral, better preserved region of the ventral horn, most of the nerve cells with a volume larger than $1600 \mu^3$ survived. There was almost complete destruction of neurones whose volume was less than $1600 \mu^3$. This is in agreement with previous observations that small neurones are more susceptible to asphyxial damage than large ones (Matsushita and Smith, 1970; Prosser and Hunter, 1963; Groves et al., 1969; Wickelgren, 1967a, 1967b). Asphyxiation of the cord for 15 to 20 minutes did not result in marked alterations of the reflex activity nor did histological examination reveal gross alterations. Asphyxiation of the cord for 25 to 35 minutes generally resulted in preparations with considerable extensor tone in the legs and brisk tendon reflexes, often with clonus. Marked neuronal destruction had taken place in the PT neurone pool for 93 per cent of the nerve (including motoneurons) cells in the PT neurone pool had been destroyed. In the cords of preparations asphyxiated for 50 minutes which did not show tone or reflexes in the legs, 95.5 per cent of the cells in the PT neurone pool had been destroyed.

Davidoff et al. (1967) obtained similar results by occlusion of the thoracic aortae of mature cats just below the origin of the great vessels for

periods of 15 to 60 minutes while the animals were under Nembutal anesthesia and artificial respiration. After 11 to 35 days 5 μ serial sections of L7 were made and neurones with diameters of less than 20 μ were designated as interneurones. This criterion was introduced by Gelfan and Tarlov (1963). Atrophy of the spinal cord manifested by dilatation of the central canal and widening of the ventral fissure was noted. The most severe and statistically significant losses of interneurones (comparing control cats with those subjected to ischaemia) occurred in the central portion of the spinal gray matter. The motoneurones were relatively unaffected. Cats subjected to longer periods of aortic occlusion exhibited more severe neurological deficits and more extensive loss of neurones. These findings are similar to those of Van Harreveld and Schadé (1962). As had been noted earlier by Van Harreveld and Schadé (1962), Davidoff et al. (1967) have shown that interneurones are more susceptible to ischaemia than are motoneurones.

Van Harreveld and Khattab (1967) ligated cat spinal cords at T10 and asphyxiated the spinal cord for 50 minutes by increasing the pressure in the dural cavity above the blood pressure. The spinal cord was fixed by intravenous glutaraldehyde infusion from 30 minutes to 2 hours after asphyxiation. Electromicroscopic examination of the cord revealed that the structure of most of the presynaptic terminals was normal. However, when fixation was undertaken 2 to 7 hours after asphyxiation, increasingly severe degeneration of terminals, endoplasmic reticulum, and ribosomes became evident. These changes in the fine structure of the spinal cord were related to the transient rigidity (secondary tone) which is observed after spinal cord asphyxiation of such duration. In a separate series of experiments asphyxiation time was 30 to 35 minutes. The spinal cords were fixed 2 weeks later when permanent rigidity (late tone) has developed. In these spinal cords the fine structure of the cytoplasm of the surviving neurones was almost normal. However, there was a marked decrease in the number of boutons on the motoneurone bodies. Glial

processes instead of synaptic terminals were the main contacts to the cell surfaces. This is consistent with the severe destruction of interneurons which is found in these preparations. The micrographs were characterized by relatively wide spaces, mainly between glial elements.

Matsushita and Smith (1970) using the method developed by Gelfan and Tarlov (1963) were able to produce extensor rigidity of the hind limbs of the rat following the spinal cord ischaemia produced by clamping of the thoracic aorta for 15 to 30 minutes. The rigidity was investigated with respect to the nature of the rigidity produced, and the technique for its production. The method of Gelfan and Tarlov (1963) was employed since it achieved a high incidence of rigidity and a rather selective destruction of interneurons. Reasoning that the inhibition of the monosynaptic reflex response of extensor muscles by stimulation of a sectioned posterior biceps nerve in the rat follows the same mechanism as described for the cat by Eccles, Schmidt and Willis (1962), Matsushita and Smith (1970) measured the degree of inhibition of the spinal reflex system. In the cat (Eccles et al., 1962) the mechanisms underlying this inhibition are two-fold: postsynaptic inhibition at a short interval between posterior biceps stimulation and extensor reflex testing (tibial nerve stimulation) and presynaptic inhibition lasting for a much longer time. It was shown that the integrity of the postsynaptic inhibition is more vulnerable to spinal cord ischaemia than are the presynaptic systems. Rigid rats had noticeably less pre- and post-synaptic inhibition. Reflexes mediated via interneurons such as the segmented polysynaptic reflex discharge and inhibition of ankle extensor motoneurons by flexor afferent excitation were reduced in rigid rats. It would appear that there is good correlation between degree of ischaemia, degree of rigidity and degree of interneurone damage.

Such findings have suggested a different approach which can be used to study the involvement of interneurons in habituation. By producing relatively selective damage of interneurons in the spinal cord, it should be possible to

observe whether this impairment of neural function is associated with a change in the rate of habituation of the flexor reflex. If the locus of the habituation process resides at the interneurone level, ablation of interneurons should alter the habituation. It is to this end that this study has been undertaken.

PART II.

METHODS

AORTIC OCCLUSION

Male rats (of the Long Evans strain, body weight 300-400 g) were anesthetized with ether and spinal cord ischaemia achieved by temporary occlusion of the thoracic aorta at the level of the first intercostal artery. Ether anesthesia was used exclusively since barbiturate anesthetics tended to produce excessive respiratory depression. An incision about 2 centimeters long was made between the hind and fourth ribs on the left side and the upper part of the lung pushed aside with a small cotton ball. The tissue enclosing the thoracic aorta between the end of the aortic arch and the first intercostal artery was carefully parted by blunt dissection exposing the thoracic aorta. The thoracic aorta was subsequently clamped with a serra-fin about 2 centimeters long. After applying the clip to the aorta, the cotton ball used to retract the left lung was removed and the thorax was closed temporarily with haemostats. The animal respired spontaneously during the 22 minute period of aortic occlusion. The success of the occlusion could be judged to some degree from color changes in the skin of the feet. After removal of the aortic clamp, the rib cage, musculature and skin were closed separately and the animal was permitted to recover. Experiments were carried out at a room temperature which ranged from 22°C to 25°C. The rectal temperature of the animals was constantly monitored. In the test rats the aortic clamp was applied for 22 minutes, while in control rats the clamp was applied for only 1 minute but in this case the thoracic cavity was not sutured for a further 21 minutes but was closed with haemostats to simulate a clamp period (mock clamping).

ASSESSMENT OF HABITUATION OF THE FLEXOR REFLEX

The effects of spinal cord ischaemia on habituation of the flexor withdrawal reflex were examined by testing the capability of rats to habituate 3, 7, or 14 days after clamping of the thoracic aorta. Control rats were examined

3, 7, or 14 days after sham occlusion. One day before reflexes were assessed, silver EMG recording electrodes (Johnson Matthey Metals Limited, Grade 5, Diamel, Silver, .007 inches diameter) were implanted in the caudal head of the right biceps femoris muscle and bipolar silver stimulating electrodes were inserted into the skin of the right ipsilateral hind paw while the rat was under ether anesthesia. Figure 1 p. 15 illustrates the musculature of the right hind paw of the rat. Figure 2 p.16 illustrates the EMG discharge during flexion elicited by stimulation of the skin of the ipsilateral hind paw. Recordings made from the rostral head of the biceps femoris (B.F. 1) and from the caudal head of the biceps femoris (B.F. 2) are shown. It can be seen that the EMG response obtained from electrodes implanted in B.F. 2 (caudal head) is greater than the response obtained from electrodes implanted in B.F. 1. In order that the approach to the problem under investigation could be as consistent as possible, recordings were taken from the caudal head of the biceps femoris with electrodes implanted in a manner that minimized variations from preparation to preparation. Similarly, stimulating electrodes were always placed as consistently as possible between the first and second toes of the hind paw. After insertion of the electrodes, the rats were placed in Bollman restraining cages with free access to food and water, and were tested one day later. Thus, rats were implanted with electrodes 2, 6, or 13 days after aortic occlusion and tested 3, 7, or 14 days after aortic occlusion. The rat in its Bollman cage was placed in a dark soundproof box, in which it remained for a 30 minute acclimatization period prior to testing and for the subsequent test period.

During the test period 400 stimuli (30 V, 20 mA, 5 msec) with an inter-stimulus interval of 10 sec.were applied to the paw. Quantitative assessment of EMG discharge occurring in response to each stimulus was achieved by means of an integration unit. This unit was programmed to begin integration 10 msec. after the stimulus, to eliminate the stimulus artefact, and to continue inte-

gration for 500 msec. The integrated response was displayed on a digital voltmeter and recorded. Figure 3 p. 17 illustrates the electronic system used during the experiment. Prior to the test period, values for background EMG activity integrated over 500 msec. were obtained. The mean value for background activity was subtracted from each value obtained in response to the stimuli, to give the net flexor reflex response. The mean flexor reflex responses to successive groups of 10 stimuli were calculated.

The reflexes of the rats, as assessed by visual inspection of the reflexes in response to a pinch, were tested before and after the occlusion of the thoracic aorta. The degree of muscle tone was noted and a daily record was kept of any changes in tone and reflex behaviour of the rats.

The data accumulated for all the rats was examined statistically. The data from rats tested 3 days after occlusion of the thoracic aorta (hereafter referred to as "3 day clamped rats") were compared to data from control rats tested 3 days after mock occlusion of the thoracic aorta (hereafter referred to as "3 day control rats"). Similarly 7 day clamped rats were compared to 7 day control rats and 14 day clamped rats were compared to 14 day control rats.

The data were examined in the following manner. The integrated EMG response of the rat was read off the digital voltmeter. The responses to the first 10 stimuli were summed after background EMG activity was subtracted from each response. Then a mean response was calculated. This new value was designated as 100 percent response for it was the response to the first 10 stimuli and assumed to be the response of non-habituated reflex. Subsequently, each following mean response to the next 10 stimuli was calculated in the same manner, and then expressed as a percentage of the response of the first 10 stimuli (percentage of initial response). This procedure was followed for all rats from stimuli 1 to 400. Thus a percentage of the initial response was obtained for each succeeding group of 10 stimuli.

Figure 6 p. 33 depicts the graph obtained by testing a control rat. Percentage of the initial response for groups of 10 stimuli are illustrated. Similar graphs were determined for clamped rats as shown in figures 7 and 8 p. 34 and 35.

STATISTICAL ANALYSIS OF DATA

Statistical analysis was performed on responses to stimuli 1-10, 41-50, 91-100, 141-150, 191-200, 241-250, 291-300, 341-350, and 391-400, which were obtained for each rat. These individual response values were pooled within each of the 3 clamped and 3 control groups. Thus for a single group of rats a mean response to stimuli 41-50 etc. was calculated. Standard deviation and standard errors were determined for the mean response. Using the Student's t test the mean responses of the control groups were then compared to the mean responses of the clamped group at the same stimuli period. These data are illustrated in figures 9, 10, 11, p. 36, 37, 38. As a result of the t test, t values and standard errors of the combined data were derived. Tables 1a, 1b, 2a, 2b, 3a, 3b p. 25, 26, 27, 28, 29, 30 present these data as described above. The calculations were carried out using an IBM 1130 computer. Finally, all of the data within each group were pooled and a regression line determined using the computer (figures 12, 13, 14 p. 39, 40, 41). Determination of whether a line through the points provided by the responses of all rats within a clamped group differed from those of a control group was made.

Figure 1. Diagrammatic representation of the anatomy of biceps femoris muscle of the rat. (B.F. 1, rostral head of the biceps femoris; B.F. 2, caudal head of the biceps femoris; S.T., semitendinosus; Q.F., quadriceps femoris; G.M., gluteus maximus; G., gastrocnemius; T.A., tibialis anterior) The gluteus maximus had been completely removed to expose the underlying muscles.

Figure 2. EMG response of the right biceps femoris muscle.

B.F. 1 is a photograph of the oscilloscope tracing of an EMG response from the rostral head of the right biceps femoris muscle while B.F. 2 is the photograph of the EMG response from the caudal head of the same muscle under identical conditions. The two recordings were made simultaneously during the flexor withdrawal reflex. Stimulus 30V 1msec. duration.

Figure 3. Schematic diagram of the stimulating and recording equipment.

PART III.

RESULTS

POST-OPERATIVE CHARACTERISTICS

A. Control Animals

The voluntary control, and reflex characteristics of the musculature of the hind limbs of control rats were examined before, during, and after thoracotomy, and after habituation and did not appear impaired. Breathing was normal and the rats were able to walk normally immediately after recovery from anesthesia. The flexor withdrawal response to a pinch was present (often accompanied by vocalization). Posture and tone as assessed by resistance offered to passive movement were normal. There was no impairment of bladder or bowel function. Operations were carried out at room temperature (22°C). The rectal temperature of the control animals dropped from 37°C to approximately 35.5°C by the time the thorax was closed. The survival rate of control animals was close to 100%. Three control animals died during thoracotomy but none died post operatively. Thirty control rats are presented in this study, ten in each category (3, 7, or 14 days after thoracotomy).

B. Clamped Animals

The rats subjected to aortic occlusion for 22 minutes exhibited characteristics different from control rats. Ten rats were examined three days after aortic occlusion, eleven rats seven days after aortic occlusion and thirteen rats fourteen days after aortic occlusion. The clamped rats recovered from anesthesia with greater difficulty than the control rats. Recovery was not complete until 45-60 minutes after closure of the chest wound. The rats displayed rapid breathing and/or dyspnea. It was necessary to apply artificial respiration to many of the clamped rats during the period of their recovery from anesthesia. Dyspnea often persisted for 1 or 2 hours after partial recovery of alertness. The recovery period was the period of highest post-operative mortality. In such cases a pink foamy fluid was noted draining from the nostrils and mouth of moribund rats. A suspected cause of mortality

was pulmonary oedema due to an elevated pulmonary capillary pressure caused by aortic occlusion. The temperature of the clamped rats which survived, dropped from 37°C to approximately 34°C by the end of the thoracotomy procedure and rose very slowly again to the 37°C normal temperature over a period of several hours.

Clamped rats exhibited flaccid paralysis of the hind limbs for a period of 1 to 2 hours after recovery from anesthesia. During this period of flaccidity, a pinch applied to the paw resulted neither in vocalization nor reflex withdrawal. After recovery from the flaccidity the hind limb tone was either (a) slightly greater in both flexor and extensor muscles or (b) much greater than normal in extensor muscles only. Rats in category (a) initially exhibited difficulty in walking. Movements were slow and hind limbs were held in a rather lateral position. This abnormal gait gradually improved over a period of two or three days. Often the hind limbs appeared swollen and inflamed and this subsided by the third post-operative day. Flexor reflex responses could always be elicited from rats in category (a) but vocalization was usually not present (c.f. table 4 p.20). This lack of vocalization when testing for flexor reflex was noted in all but one of the 3 day clamped rats, in all but five of the 7 day clamped rats, and in all but eight of the 14 day clamped rats.

Table 4 p.20 notes the characteristics of all the clamped rats. Those animals which vocalized upon testing for the flexor reflex either developed vocalization several days after the operation or indeed had never lost the ability to vocalize. The rats in category (b) exhibited extensor rigidity which persisted until the end of the experiment. Three rats fell into this category. The rigid rats had no ability to walk on their hind legs and dragged their legs "foot-pad" upward. The rats remained rigid until the end of the investigation and in one rat the rigidity was maintained for 22 days (at which time the rat was sacrificed). In total two 3 day and one 14 day

RAT NO.	COMMENTS
3 day rats (10)	
3-1C	NV R curled toes and walks on knuckles, definite rigidity, slightly in flexor position
3-2C	- - walks poorly and slowly, increased sensitivity to touch (painful vocalization), legs held together and up when picked up by tail
3-3C	NV W
3-4C	NV - walks poorly and slowly, clumsy, slightly rigid in flexor position
3-5C	NV W
3-6C	NV W
3-7C	NV W
3-8C	NV W
3-9C	NV - walks poorly and slowly
3-10C	NV R extensor rigidity, drags hind limbs
7 day rats (11)	
7-1C	NV W
7-2C	- W
7-3C	NV W
7-4C	NV W loss of bladder function; rat urinated over itself
7-5C	NV W
7-6C	NV W developed some extensor rigidity post-operatively but lost this 1½ hrs. later
7-7C	- W
7-8C	NV W 2 hrs. post-operatively developed some extensor rigidity, lost this the next day
7-9C	- W slight vocalization to painful stimulus
7-10C	- W
7-11C	- W
14 day rats (13)	
14-1C	- - walks poorly
14-2C	- W
14-3C	NV - walks poorly, fur is easy to pull out, walking had improved with time
14-4C	NV W
14-5C	NV W
14-6C	- W
14-7C	NV W
14-8C	- W
14-9C	- W
14-10C	- W
14-11C	- W
14-12C	NV R extensor rigidity, drags hind limbs
14-13C	- W

NV: no vocalization upon presentation of painful pinch
 W: walks well
 R: extensor rigidity

Table 4. POST OPERATIVE CHARACTERISTICS OF CLAMPED RATS

rigid rat were investigated. The incidence of complete rigidity was very low, lower than that reported by Matsushita and Smith (1970). All the clamped rats except one 7 day clamped rat had normal bladder and bowel function.

EFFECT OF AORTIC OCCLUSION ON HABITUATION

The results from the habituation experiments are presented in the following 10 figures. Figure 4 p.31 shows typical habituation obtained in a 7 day control animal. It can be seen from the figure that the right flexor reflex habituates when iterated stimuli are presented. Photographs of the EMG trace appearing on the oscilloscope screen were taken during an unstimulated state, and after 2, 100, 200, 300 and 400 stimuli. The representative responses to the individual stimuli are 100%, 40.8%, 38.5%, 32.5% and 28.3% initial response. Beneath each EMG trace is a record of the corresponding integrated EMG. Note that both the duration of the EMG discharge and the time integral of the EMG decrease.

Figure 5 p. 32 illustrates results of a similar experiment performed in a 7 day clamped animal. It is evident that 1) basal activity during the period prior to stimulation is greater than in the control, 2) the absolute level of response is lower and it habituates more slowly than in the control, and 3) the flexor reflex of the clamped rat does not habituate to the same degree in terms of asymptotic values as the control rat. These are general findings in all experiments.

Figure 6 p. 33 illustrates a typical experiment on one rat, in this case the response to 400 stimuli in the 7 day control rat presented in figure 4, p. 31. Figure 6 illustrates the integrated EMG responses expressed as percentages of the initial response recorded as mean responses for stimuli 1-10, 41-50, 91-100, 141-150, 191-200, 241-250, 291-300, 341-350, 391-400. At stimuli 391-400 the mean response expressed as the percentage of the initial response is

30 percent.

Figure 7 p. 34 presents a similar experiment, in this case the response to 400 stimuli in the 7 day clamped rat presented in figure 5 p. 32. The response to stimuli 391-400 is 70 percent. It is evident that habituation was not as marked as that shown in figure 6 p. 33 for the 7 day control animal.

Figure 8 p. 35 illustrates the response of a single 3 day clamped rat which exhibited hind limb extensor rigidity. These animals did not habituate and because of the low evidence of rigidity a sample experiment is presented (figure 8). The data from these 3 rigid animals was included in the data of the groups to which the individual animals belonged (one rat belonged to 3 day rats and two belonged to 14 day rats). The mean response for any group of stimuli expressed as a percentage of the initial response was always well above 100% of the initial response. The response at the end of 400 stimuli was 100 percent initial response. The findings were peculiar to the three rats that exhibited hind limb extensor rigidity.

Ten control rats and ten clamped rats were examined three days after aortic occlusion. Figure 9 p. 36 and tables 1a + 1b p. 25, 26 present the data obtained for these two groups of rats. All responses in the clamped group except those to stimuli 91-100 proved to be significantly greater ($p < 0.05$), using the Student's *t* test, than responses in the control group. It can be seen from the graph in figure 9 p. 36 that the responses in the clamped animals remain at approximately 75% of the initial response while in the control animals habituation to 20% of the initial response occurred. In summary the 3 day control rats habituated to a mean of 20.04% initial response $SE \pm 5.88$ at 400 stimuli while the 3 day clamped rats habituated to 53.86% initial response $SE \pm 17.08$ after 400 stimuli. In all but one period of stimulation (91-100) analysed, a significant difference between the two groups of 3 day rats ($p < 0.05$) was obtained.

Comparison of the 7 day control and 7 day clamped rats revealed no signif-

icant differences between the two groups, for all periods of stimulation. The control rats habituated to a mean of 20.3% initial response $SE \pm 3.72$ at 400 stimuli while the clamped rats habituated to 41.35% initial response $SE \pm 13.55$ at 400 stimuli (fig. 10 p. 37, tables 2a + 2b p. 27, 28).

Comparison of the 14 day control rats and 14 day clamped rats revealed a significant difference ($p < 0.05$) between the 10 control animals and 13 experimental animals. The control rats habituated to a mean of 13.7% initial response $SE \pm 3.10$ at 400 stimuli while the clamped rats habituated to 62.29% initial response $SE \pm 18.03$. All periods of stimulation show a significant difference between the two groups except the 41-50 and 141-150 stimuli periods (fig. 11 p. 38, tables 3a + 3b p. 29, 30).

The reflex responses of rats tested 3 or 14 days after aortic occlusion showed a significantly ($p < 0.05$) lesser degree of habituation than the responses to the corresponding stimuli in control rats. A statistically significant impairment of habituation could not be demonstrated when comparison was made between the responses of rats tested 7 days after occlusion and their controls. The greatest impairment of habituation was seen in rats which exhibited extensor rigidity. In some cases an increase in response rather than habituation was seen (figure 8 p. 35). Only 3 rats exhibited extensor rigidity and none of these rats were in the 7 day group.

In the control and in the ischaemic preparation, the duration of EMG discharge in response to the stimulation of the paw, fell within the same range (150-250 msec.). The responses did differ in amplitude and frequency of the EMG spikes. In the control animals there is an initial high frequency burst followed by a discharge of smaller spikes (50-300 μV) at a frequency of about 50/sec. The responses in animals which had undergone aortic occlusion did not have the early high frequency component, but consisted solely of a low amplitude, low frequency discharge (figures 4 and 5 p. 31, 32). In the control rats the reflex response habituated rapidly during the presentation

of the first 50 stimuli and more gradually thereafter.

In control animals there is a significant negative linear correlation ($p < 0.001$) between the logarithm of the reflex response and the number of stimuli presented. On the other hand the relationship between log response and stimulus number in the ischaemic rats was not a linear one (see Fig. 12, 13, 14, p.39, 40, 41).

The graph for the clamped animals reveals that for the first 100 stimuli the response of the clamped rats decreases at a rate similar to the exponential function of the control rats. However, the decremental relationship changes at the 100 stimuli point and the line approaches a slope of zero from that point onwards. This observation led to an examination of the slopes of the graphs for control rats compared to the slopes of the graphs for clamped rats. This comparison was undertaken to determine if indeed there was a difference between the two lines and thus strengthen the proposal that there is a difference between clamped and control animals. Control rats fit an exponential function in all cases. In all cases clamped rats did not have a slope significantly different from zero and thus did not fit an exponential function. The rats were examined statistically by pooling for example all 3 day control rats into one group and then performing a linear regression coefficient determination. The same procedure was executed for all other groups. These data are presented below.

The probability of the slope of the relationship being zero in the case of 3 day control rats, 7 day control rats and 14 day control rats was 0.0050, 0.0003, and 0.0000 respectively. The probability of zero slope for the lines for clamped rats of 3, 7, or 14 days was 1.0000, 1.0000, and 1.0000 respectively. Clearly the control animals have a function relationship that has a measurable slope and the clamped animals do not.

It would appear that both qualitative and quantitative changes in habituation process had been brought about as a result of spinal ischaemia.

3 day rats - control response (% of initial response)

		STIMULUS NUMBER								
		1-10	41-50	91-100	141-150	191-200	241-250	291-300	341-350	391-400
Rat	3-1	100	57.5	40.8	36.7	26.5	32.3	14.6	23.1	5.4
	3-2	100	35.5	26.9	49.6	24.1	29.8	23.0	9.6	23.7
	3-3	100	52.4	45.4	30.8	42.3	20.7	3.5	6.6	4.8
	3-4	100	47.7	68.4	30.2	41.9	31.0	32.9	29.2	19.8
	3-5	100	72.9	81.2	89.2	105.9	97.7	103.6	61.4	68.3
	3-6	100	62.2	61.3	50.8	36.9	36.9	45.8	30.2	21.8
	3-7	100	63.9	51.7	44.4	27.8	19.5	14.1	22.4	15.1
	3-8	100	80.5	22.0	45.7	23.1	19.9	9.8	29.8	2.8
	3-9	100	85.3	40.0	23.9	32.2	16.8	24.6	16.3	20.2
	3-10	100	76.2	41.9	38.8	36.5	30.3	26.1	37.3	18.5
degrees of freedom		9	9	9	9	9	9	9	9	9
mean response		100	63.41	47.96	44.08	39.72	33.49	29.80	26.59	20.04
SD			15.66	18.23	18.37	24.27	23.54	28.61	15.57	18.61
SE			4.95	5.76	5.81	7.68	7.44	9.05	4.92	5.88
log mean		2.0	1.802	1.681	1.644	1.599	1.525	1.474	1.425	1.302

Table 1a. 3 DAY RATS. RESPONSE VALUES EXPRESSED AS % OF INITIAL RESPONSE HAVE BEEN EXAMINED BY STUDENT'S t TEST ($p < 0.05$).

3 day rats - clamped response (% of initial response)

STIMULUS NUMBER

	1-10	41-50	91-100	141-150	191-200	241-250	291-300	341-350	391-400
Rat 3-1C	100	101.0	21.5	63.8	44.5	46.2	72.5	80.6	100.4
3-2C	100	176.3	63.2	65.9	56.8	39.2	44.0	43.1	49.0
3-3C	100	102.0	92.2	77.1	77.1	48.6	21.6	33.5	27.3
3-4C	100	80.0	79.2	100.7	100.7	120.0	135.4	75.3	136.1
3-5C	100	125.7	108.6	76.6	50.0	49.3	32.4	38.8	28.5
3-6C	100	60.6	34.4	68.8	104.9	129.5	173.7	201.6	180.3
3-7C	100	76.5	57.4	46.3	44.6	36.6	34.9	30.9	21.1
3-8C	100	109.0	100.6	96.9	96.9	122.4	115.4	133.6	137.5
3-9C	100	69.6	108.7	56.5	86.9	73.9	86.9	82.6	108.7
3-10C	100	72.1	24.6	64.9	32.9	100.9	47.8	73.6	65.7
degrees of freedom	9	9	9	9	9	9	9	9	9
mean response	100	97.28	69.04	71.89	69.53	76.66	76.46	79.36	75.46
SD		34.48	33.96	21.22	26.80	37.76	50.69	53.08	53.86
SE		10.90	10.74	6.71	8.47	11.94	16.03	16.76	17.03
log mean	2.0	1.988	1.839	1.856	1.842	1.885	1.883	1.900	1.878
combined data (control compared with clamped)									
SE	-	12.58	12.80	9.32	12.00	14.77	19.33	18.36	18.92
t	-	2.69	1.64	2.97	2.47	2.92	2.40	2.87	2.93
d.f.	-	18	18	18	18	18	18	18	18
Significance (p 0.05)		S	NS	S	S	S	S	S	S

Table 1b. 3 DAY RATS. CONTROLS COMPARED WITH CLAMPED RATS.

7 day rats - control response (% of initial response)

	STIMULUS NUMBER								
	1-10	41-50	91-100	141-150	191-200	241-250	291-300	341-350	391-400
Rat 7-1	100	67.3	88.0	58.8	52.9	36.4	28.2	33.4	19.0
7-2	100	60.8	62.9	73.0	55.9	57.3	53.0	37.1	37.2
7-3	100	89.5	61.5	52.4	32.2	21.5	31.8	22.0	10.3
7-4	100	51.7	34.3	30.0	34.3	35.1	33.3	32.2	32.6
7-5	100	59.3	58.1	46.5	29.5	42.7	32.4	30.1	26.9
7-6	100	41.6	63.3	54.6	51.7	34.1	32.3	30.3	34.0
7-7	100	28.8	20.5	20.6	13.1	8.2	8.6	7.8	7.7
7-8	100	55.4	44.6	15.1	10.5	0.0	11.5	3.3	3.7
7-9	100	80.1	44.3	30.0	19.1	28.9	14.9	22.4	16.4
7-10	100	73.2	52.3	48.4	34.2	27.0	28.2	19.4	15.1
degrees of freedom	9	9	9	9	9	9	9	9	9
mean response	100	60.77	52.98	42.94	33.34	29.12	27.42	23.8	20.3
SD		17.95	18.50	18.36	16.26	16.47	13.01	11.17	11.77
SE		5.68	5.85	5.80	5.14	5.21	4.13	3.53	3.72
log mean	2.0	1.784	1.724	1.633	1.523	1.464	1.438	1.377	1.307

Table 2a. 7 DAY RATS. RESPONSE VALUES EXPRESSED AS % OF INITIAL RESPONSE HAVE BEEN EXAMINED BY STUDENT'S t TEST ($p < 0.05$).

7 day rats - clamped response (% of initial response)

STIMULUS NUMBER

	1-10	41-50	91-100	141-150	191-200	241-250	291-300	341-350	391-400
Rat 7-1C	100	64.4	55.5	47.6	44.9	47.2	34.7	32.4	50.8
7-2C	100	75.1	48.4	41.1	38.0	37.7	19.5	12.7	7.5
7-3C	100	40.2	37.0	30.5	32.5	26.8	15.9	17.4	16.8
7-4C	100	53.9	33.8	34.2	54.4	56.6	51.7	63.1	54.4
7-5C	100	93.3	89.0	85.2	100.0	90.4	129.2	124.9	166.0
7-6C	100	57.9	31.6	31.6	17.5	29.8	28.1	31.6	31.6
7-7C	100	39.7	44.2	25.9	17.8	17.4	20.1	17.4	10.7
7-8C	100	62.2	49.1	47.5	34.4	26.1	25.6	39.5	32.3
7-9C	100	51.2	48.2	54.3	54.3	52.2	67.0	59.6	53.4
7-10C	100	59.2	47.3	34.2	25.7	58.5	6.8	5.4	10.3
7-11C	100	43.3	44.3	38.4	37.5	34.8	41.2	28.5	21.0
degrees of freedom	10	10	10	10	10	10	10	10	10
mean response	100	58.22	48.04	42.77	41.55	43.41	39.98	39.32	41.35
SD		15.90	15.36	16.45	23.05	20.60	34.21	33.68	44.93
SE		4.80	4.63	4.96	6.95	6.21	10.31	10.16	13.55
log mean	2.0	1.765	1.684	1.631	1.619	1.637	1.602	1.595	1.616
combined data									
SE	-	7.76	7.76	7.96	9.22	8.61	12.10	11.74	15.40
t	-	.32	.62	.02	.88	1.64	1.03	1.29	1.35
d.f.	-	19	19	19	19	19	19	19	19
Significance (p<0.05)		NS	NS	NS	NS	NS	NS	NS	NS

Table 2b. 7 DAY RATS. CONTROLS COMPARED WITH CLAMPED RATS.

14 day rats - control response (% of initial response)

		STIMULUS NUMBER								
		1-10	41-50	91-100	141-150	191-200	241-250	291-300	341-350	391-400
Rat	14-1	100	106.0	69.3	70.1	24.8	35.8	18.2	6.1	12.2
	14-2	100	86.3	42.9	49.4	47.4	26.1	10.4	13.1	11.6
	14-3	100	71.3	53.3	47.5	33.8	26.1	11.3	12.9	5.2
	14-4	100	36.7	33.4	45.6	27.9	8.2	10.7	6.2	10.4
	14-5	100	70.7	50.6	33.8	28.7	22.6	11.0	16.9	18.3
	14-6	100	54.3	69.5	42.3	36.8	38.7	38.5	38.5	31.6
	14-7	100	55.1	48.5	33.2	19.4	5.0	12.1	3.1	3.9
	14-8	100	58.0	47.8	38.5	41.6	39.4	33.0	33.6	29.6
	14-9	100	44.9	34.4	25.5	37.1	16.7	15.1	17.9	8.1
	14-10	100	43.9	25.0	26.4	22.5	8.9	-	14.3	6.3
degrees of freedom		9	9	9	9	9	9	9	9	9
mean response		100	62.72	47.47	41.23	32.00	22.75	17.75	16.26	13.72
SD			21.26	14.54	13.14	8.88	12.82	9.96	11.55	9.81
SE			6.72	4.60	4.16	2.81	4.05	3.15	3.65	3.10
log mean		2.0	1.797	1.676	1.615	1.505	1.357	1.249	1.211	1.138

Table 3a. 14 DAY RATS. RESPONSE VALUES EXPRESSED AS % OF INITIAL RESPONSE HAVE BEEN EXAMINED BY STUDENT'S t TEST ($p < 0.05$).

14 day rats - clamped response (% of initial response)

	STIMULUS NUMBER								
	1-10	41-50	91-100	141-150	191-200	241-250	291-300	341-350	391-400
Rat 14-1C	100	24.2	23.4	21.7	18.4	16.9	12.3	22.7	23.6
14-2C	100	148.0	124.0	126.0	138.0	100.0	356.0	198.0	254.0
14-3C	100	67.0	55.1	29.6	30.1	25.6	20.7	3.6	15.7
14-4C	100	69.3	52.7	87.3	42.9	43.4	52.0	59.1	77.8
14-5C	100	90.8	62.1	58.3	29.4	24.0	42.5	36.8	18.3
14-6C	100	57.2	45.6	32.7	27.3	28.0	15.2	15.4	17.0
14-7C	100	81.3	82.0	72.1	74.1	74.8	71.9	64.7	60.3
14-8C	100	43.8	27.8	11.7	36.1	23.4	4.8	5.2	1.6
14-9C	100	90.4	86.9	85.6	90.4	75.0	70.0	65.2	58.2
14-10C	100	74.1	65.5	36.5	54.8	27.9	17.2	63.4	54.8
14-11C	100	144.5	200.0	74.3	101.3	243.2	364.8	16.2	87.8
14-12C	100	128.2	119.9	173.5	110.1	185.2	121.6	111.6	98.0
14-13C	100	96.3	71.7	65.4	66.3	60.4	58.1	38.7	42.2
degrees of freedom	12	12	12	12	12	12	12	12	12
mean response	100	85.82	78.21	67.44	63.02	71.37	92.85	53.89	62.29
SD		36.98	47.45	45.13	37.49	69.29	125.02	53.04	65.02
SE		10.26	13.16	12.52	10.40	19.22	34.12	14.71	18.03
log mean	2.0	1.939	1.893	1.829	1.799	1.853	1.968	1.732	1.794
combined data									
SE	-	13.91	16.54	15.66	12.90	23.64	41.56	18.18	22.10
t	-	1.65	1.85	1.66	2.39	2.04	1.80	2.05	2.19
d.f.	-	21	21	21	21	21	21	21	21
Significance (p<0.05)		NS	S	NS	S	S	S	S	S

Table 3b. 14 DAY RATS. CONTROLS COMPARED WITH CLAMPED RATS.

Figure 4. Electromyographic discharge (e.m.g.) and simultaneous output from the integrator (i.e.m.g.) recorded under basal conditions and in response to the 2nd, 100th, 200th, 300th, and 400th stimuli in a control rat. Sham occlusion of the thoracic aorta had been carried out 7 days earlier. Habituation of the reflex is demonstrated and the responses to the respective stimuli are 100%, 40.8%, 38.5%, 32.5%, and 28.3% initial response.

Figure 5. Electromyographic discharge (e.m.g.) and simultaneous output from the integrator (i.e.m.g.) recorded under basal conditions and in response to the 2nd, 100th, 200th, 300th, and 400th stimuli in a rat which had undergone occlusion of the thoracic aorta for 22 minutes, 7 days earlier. Note that the basal activity is higher but the responses to stimuli are lower than in the control experiment in Fig. 4. Minimal habituation occurred. The responses to the respective stimuli are 100%, 80.5%, 70.5%, 72.0%, and 75.0% initial response.

Figure 6. Flexor reflex responses to stimuli presented at 10 sec. intervals to a 7 day control rat. The response is plotted as a mean response to stimuli 1-10, 41-50, 91-100, 141-150, 191-200, 241-250, 291-300, 341-350, and 391-400. Photographs of EMG discharge shown in Fig. 4 were taken at the points indicated by the arrows.

Figure 7. Flexor reflex responses to stimuli presented at 10 sec. intervals to a 7 day clamped rat. The response is plotted as a mean response to stimuli 1-10, 41-50, 91-100, 141-150, 191-200, 241-250, 291-300, 341-350, 391-400. Photographs of EMG discharge shown in Fig. 5 were taken at the points indicated by the arrows.

Figure 8. Flexor reflex responses to stimuli presented at 10 sec. intervals to a 3 day clamped rat with hind limb extensor rigidity which developed following aortic occlusion. Points plotted are mean responses to consecutive periods of 10 stimuli as noted on the abscissa. Habituation of the reflex did not occur.

Figure 9. Three day rats. Mean $\pm$ S.E. Flexor reflex responses to stimuli presented at 10 sec. intervals for control rats in which the thoracic aorta was occluded for 1 min. (crosses) and rats in which the thoracic aorta was occluded for 22 min. (filled circles). The mean response to each successive group of 10 stimuli is expressed as a percentage of the mean response to the first 10 stimuli in each animal.

Figure 10. Seven day rats. Mean $\pm$ S.E. Flexor reflex responses to stimuli presented at 10 sec. intervals for control rats in which the thoracic aorta was occluded for 1 min. (crosses) and rats in which the thoracic aorta was occluded for 22 min. (filled circles). The mean response to each successive group of 10 stimuli is expressed as a percentage of the mean response to the first 10 stimuli in each animal.

Figure 11. Fourteen day rats. Mean $\pm$ S.E. Flexor reflex responses to stimuli presented at 10 sec. intervals for control rats in which the thoracic aorta was occluded for 1 min. (crosses) and rats in which the thoracic aorta was occluded for 22 min. (filled circles). The mean response to each successive group of 10 stimuli is expressed as a percentage of the mean response to the first 10 stimuli in each animal.

Figure 12. The relationship between the logarithm of the mean flexor reflex response to successive groups of 10 stimuli, and the number of stimuli presented. This relationship is linear in the case of control rats. Regression data is shown for control rats only, because the relationship described in the case of clamped animals is not exponential. There is a significant negative correlation between these parameters for control rats but not for clamped rats. The groups of rats compared in the graph are animals examined 3 days after thoracotomy.

Figure 13. The relationship between the logarithm of the mean flexor reflex response to successive groups of 10 stimuli, and the number of stimuli presented. This relationship is linear in the case of control rats. Regression data is shown for control rats only, because the relationship described in the case of clamped animals is not exponential. There is a significant negative correlation between these parameters for control rats but not for clamped rats. The groups of rats compared in the graph are animals examined 7 days after thoracotomy.

Figure 14. The relationship between the logarithm of the mean flexor reflex response to successive groups of 10 stimuli, and the number of stimuli presented. This relationship is linear in the case of control rats. Regression data is shown for control rats only, because the relationship described in the case of clamped animals is not exponential. There is a significant negative correlation between these parameters for control rats but not for clamped rats. The groups of rats compared in the graph are animals examined 14 days after thoracotomy.

PART IV.

DISCUSSION

The severity of neurological impairment obtained after aortic occlusion was much less than that observed in an earlier study by Matsushita and Smith (1970). These authors reported that after occlusion of the aorta for 21-23 minutes, 52% of rats exhibited extensor rigidity. In the present work, marked extensor rigidity occurred in only 12% of rats after occlusion for 22 minutes. This disparity may be, at least partly, due to the fact that a different strain of rat was used. It is possible that subtle differences exist in the vasculature of the spinal cord between Wistar and Long Evans strains and this could manifest itself as a difference in susceptibility to asphyxial insult. It is possible that variations with the species have some bearing on the outcome of aortic occlusion. Murayama and Smith (1969) report that some cats appeared completely normal postoperatively after occlusion of the thoracic aorta for up to two hundred minutes.

Even though ischaemia did not usually result in rigidity it did cause significant changes in spinal cord function. The basal activity in the unstimulated muscle was increased, but the magnitude of the response to an electrical stimulus to the ipsilateral hind paw was less than in control animals. This decrease in magnitude of the response is not likely to be due to damaged or destroyed afferent fibers. Van Harreveld (1940) has shown that afferent and spinal ganglia are not histologically damaged by asphyxiation. Van Harreveld and Niechaj (1970) have noted that components of the dorsal root potential (DRP) survived such cord asphyxiation. In the present study the responses of rigid rats to repeated stimuli either did not habituate or habituated more slowly than the responses of normal rats.

Two hypotheses have been advanced to explain the development of rigidity following spinal ischaemia. Gelfan and Tarlov (1959) consider that rigidity is due to increased excitability of motoneurons which occurs as a result of their partial denervation by the selective destruction of interneurons which normally innervate them. It was proposed that the neurons destroyed include

those relaying impulses to supraspinal centers as well as to spinal motoneurons, and that this must be the basis for the sensory paralysis in rigid preparations. It was also assumed to be the basis for the motor paralysis. This chronic rigidity was not considered to be due to an exaggerated stretch reflex, since it was neither abolished nor prevented by section of dorsal roots. The destruction of interneurons was considered responsible for the loss of normal regulation of motoneurone activity and consequent muscle responses. It was proposed that denervation (destruction of interneurons also denervates motoneurons) increases the excitability of motoneurons to the point of discharging "spontaneously". It was also proposed that the "spontaneous" discharges of such denervated motoneurons are directly responsible for the unremitting and enduring rigidity. This work had been carried out on dogs. Van Harreveld and Marmont (1939) have suggested that the rigidity produced by ischaemia is reflex in origin and arises from an increased discharge in fusimotor fibers. The rigidity of spinal cats was considered to be a "high extensor tone" due to the damage of the "tone inhibiting system" by the temporary anoxia of the lumbosacral cord. The excitatory component of spinal cord was proposed to be more resistant to anoxia than the inhibitory component. Gelfan and Tarlov's (1959) demonstration of functional failure of interneurons relaying reflex excitations as well as inhibitory impulses in rigid dogs cannot be fitted into Van Harreveld and Marmont's (1939) thesis. In contrast to Gelfan and Tarlov, Van Harreveld showed that the strong extensor tone in rigid cats is of reflex origin since transection of the dorsal roots of the lower cord abolishes it. Oka and Van Harreveld (1968) stated that Gelfan and Tarlov (1959) based their postulate mainly on the observation that dorsal root section did not abolish the rigidity permanently. Yet Oka and Van Harreveld presented evidence that after section of the relevant dorsal roots of a rigid muscle group, nerve endings in contralateral muscles and perhaps in other segments can supply the sensory input for the rigidity

developing after radiculotomy. Only total deafferentation and isolation of a cord segment led to complete and permanent absence of rigidity. It should be pointed out that Van Harreveld and Marmont (1939) using histological preparations noted that only 3 to 75 percent of the normal number of anterior horn cells were present 14 days following asphyxia. The number surviving diminished with increasing duration of asphyxia. Both Gelfan and Tarlov (1959) and Van Harreveld and Marmont (1939) indicated that interneurons were more severely damaged than motoneurons. Gelfan and Tarlov (1959) revealed that most of the cells destroyed were interneurons and that all large and small motoneurons could survive when 80% of the interneurons were destroyed.

Murayama and Smith (1969) confirmed the studies of Gelfan and Tarlov (1959) with respect to the spinal origin of the rigidity, decrease in polysynaptic reflexes, increased response to repetitive stimulation, presence of spontaneous alpha and fusimotor neurone activity, and Renshaw cell activity. The precise nature of spinal rigidity however remains difficult to explain.

In view of the fact that rigidity occurred so rarely in the present experiments it is not possible to add any information which could help explain its origin. It would seem unlikely that the excitability of the flexor motoneurone had increased, as the magnitude of flexor responses was lower than in control rats. This however could have been due to partial loss of neuronal elements e.g. afferent fibers and relay cells in the excitatory flexor reflex pathway. It is of interest to note that habituation never occurred in rigid preparations and perhaps related neuroanatomical alterations are common to both characteristics.

Rats that have undergone occlusion of the thoracic aorta were pinched on the hind paw to test for the presence of a flexor reflex. The reflex could always be elicited but compared to the control rats, the rats that have undergone aortic occlusion did not vocalize (in the majority of cases) when a painful pinch was presented. This finding implies possible impairment of

pathways by which the animals identify a painful stimulus applied to the hind paw. In view of the paucity of information concerning this finding it is difficult to speculate as to the underlying mechanism. Murayama and Smith (1969) reported that stimulation of the skin by pinching or inserting needles rarely caused any response in acute rigid preparations: there was neither a withdrawal reflex nor evidence of any sensation of pain by the cat. No explanation for this finding was presented.

In the present study only three rats exhibited extensor rigidity and none of these rats were in the 7 day group. The inability to demonstrate a quantitative impairment of habituation in this group may have simply been a consequence of an unequal distribution of rigid rats between the three groups.

The results of this study indicate that aortic occlusion renders an animal incapable of habituation of the hind limb flexor reflex. It is proposed that aortic occlusion caused ischaemia of the spinal cord and this ischaemia resulted in selective destruction of a portion of the spinal neurone pool.

In addition to the phenomenon known as habituation a phenomenon opposite to habituation and known as sensitization has been described. An increased flexor reflex response to repeated stimuli is known to occur under ischaemic conditions such as those described by Murayama and Smith (1969). Sensitization is elicited after some procedure is applied to alter the normal neurophysiological condition of an organism. Experiments recently carried out on cats by Groves and Thompson (1970) in which habituation or sensitization of reflexes have been recorded at the same time as changes in the activity of interneurons have led to the so called "dual-process" theory for habituation. A tentative distinction was made between two inferred systems, the "S-R pathway", which is the most direct route through the central nervous system from stimulus to response, and "state", the collection of pathways, systems, and regions that determines the general level of responsiveness of the organism. Habituation is assumed to occur in the S-R pathway and sensitization in the state system.

The two processes interact to yield the final common behavioral outcome. Neurophysiological evidence for this dual-process theory is strikingly clear. Three categories of interneurons responding to cutaneous stimuli have been found with approximately equal frequency: a "nonplastic" type showing no changes in response, and two types of "plastic" interneurons, one showing habituation (type H) and one showing sensitization (type S). The nonplastic types of interneurons do not change with repeated stimulation and do not appear to have any characteristic type of firing pattern. The type H interneurons exhibit significant habituation even during the period of maximum muscle sensitization as shown by Groves, De Marco and Thompson (1969). Type H interneurons have a characteristic high-frequency short latency firing pattern and respond within a restricted range of latencies (5-12.5 milliseconds). Type S interneurons exhibit sensitization followed by habituation to, or below, initial control level. Type S cells characteristically respond with a longer latency than type H cells (6-180 milliseconds). The plasticity of interneurons parallels exactly the two hypothetical processes suggested by Groves and Thompson (1970). Type H interneurons lie within laminae I-V of Rexed (1952). Type S interneurons are situated in laminae V-VII. Several inferences can be drawn. First, the latencies of type H neurons to stimulation of cutaneous nerves are all sufficiently short that these neurons could participate in the most direct S-R reflex pathway. Type S neurons, on the other hand, usually exhibit latencies which are too long for them to participate in the most direct reflex paths. Type S neurons, therefore, might act from outside the direct S-R reflex pathway onto final interneurons or motoneurons to yield the ultimate behavioral output.

Wickelgren (1967b) has completed an analysis of habituation of interneurons in the lumbosacral region of spinal cord in cats. It was reported that interneurons habituated and that units with response latencies of more than 6 milliseconds to single shocks were more likely to habituate than units

with response latencies of less than 6 milliseconds. Wall (1967) has described "novelty detectors" in lamina V of the lumbosacral cord of decerebrate cats. These neurones respond vigorously to the first few presentations of cutaneous stimuli but soon cease responding if the stimulus is repeated. Mendell (1966) and Mendell and Wall (1967) described "wind-up" of cells of the spinocervical tract. This phenomenon was simply a progressive increase in evoked activity with repetitive stimulation of C (small diameter) fibers, and resembles sensitization very closely. Frank and Fuortes (1956) described a similar phenomenon for interneurons of the lumbosacral cord.

These studies point out the basic distinction between sensitization and habituation and suggest that there may be separate populations of interneurons, the functional properties of which are able to account for the behavioral plasticity observed in the flexor reflex of acute spinal cats.

A synaptic hypothesis consistent with the general dual-process theory of response habituation and the known types of interneurons needs to be developed. The process as postulated by Groves and Thompson (1970) must occur at some region of synaptic action on interneurons, either at the first synapse formed by afferent fibers or at some subsequent synapse. The synaptic hypothesis, for convenience is classified into two categories: extrinsic and intrinsic. Postsynaptic and presynaptic inhibition are the two known forms of extrinsic action that can induce decrements in synaptic transmission. Additionally some other as yet unknown form of extrinsic synaptic action may exist. Two types of intrinsic action have been forwarded: 1) "Monosynaptic low frequency depression" (Thompson and Spencer, 1966) which could be a consequence of alterations in mobilization and/or release of transmitter, and 2) "membrane desensitization" (Sharpless, 1964) which would involve decreased responsiveness of the postsynaptic membrane to a transmitter as a result of repeated activation by the transmitter. Which of the alternatives is in fact the operative process remains to be elucidated. Indirect evidence for the

existence of postsynaptic inhibition as the process on which habituation is based has been provided by Wickelgren (1967b). She reports that in three dorsal horn cells studied, generalization or transfer of habituation occurred in only one direction (i.e. generalization to stimulus applied to a second site "B" after habituation had been established by stimuli applied to an original site "A" but not vice versa). Wickelgren (1967b) argues that if habituation were due to "synaptic depression", common habituated interneurons ought to show generalization of habituation to both stimuli, whereas postsynaptic inhibition could operate on only one interneurone channel and hence would yield generalization of habituation in only one direction. The argument is indirect and rests entirely on the demonstration of asymmetry of generalization of habituation. Wickelgren (1967b) has demonstrated three interneurons which show transfer of habituation in only one direction. Glanzmann (1972) has shown bidirectional generalization of habituation on seven type H interneurons. It is significant that at least some interneurons exist which show unidirectional generalization and hence postsynaptic inhibition could well be the *modus operandi*. However the situation remains unsolved.

Evidence against the proposal that inhibition is the process on which habituation depends is not strong. This evidence relies on the report by Spencer, Thompson and Neilson (1966c) that strychnine and picrotoxin do not prevent habituation. This work is confused however by the fact that the stimulus strength during control periods and during periods of pharmacological intervention with strychnine or picrotoxin was not constant. Indeed the stimulus strength was lowered during the later period and this alone could lead to decreased habituation as pointed out by Thompson and Spencer (1966) in an earlier review. Altered stimulus strength could mask the blocking effects of the drugs. Inspection of records, even for periods where the same stimulus strength was used reveals that with application of strychnine or picrotoxin and combined application of strychnine and picrotoxin there is a

small but real diminution of habituation apparently ignored by Spencer, Thompson and Neilson (1966c).

Habituation of the discharge of interneurons situated in lamina V of the dorsal horn of the cat has been demonstrated by Wall (1967) and Wickelgren (1967b). The characteristics of habituation of activity in these interneurons were very similar to those obtained from motoneurons (Wickelgren, 1967a). Interneurons in lamina IV, which like those in lamina V respond to low threshold cutaneous afferents, do not habituate. Gradual failure of transmission of activity from neurons in lamina IV to those in lamina V may partly be the cause of habituation (Wickelgren, 1967b). Wall (1967) has suggested that habituation at this site may be the result of activity in a side chain of inhibitory interneurons. Thus, stimulation of cutaneous afferents may cause (a) excitation of lamina V cells, both directly and via cells in lamina IV, and (b) initiate activity in a parallel pathway which functions to depress the excitability of neurons in lamina V. It has been suggested that the nerve cells of the substantia gelatinosa may be good candidates for such a side chain (Wall, 1967).

Heimer and Wall (1968) indicated histologically (using the Fink-Heimer technique) that lamina II or the substantia gelatinosa proper, receives a massive termination of dorsal root fibers. Ralston (1965) has shown that the marginal cells (15-20 μ diameter) which are confined to the surface of dorsal lamina II, are spindle shaped with their long axes parallel to the surface of the lamina, and appear to receive dorsal root small fiber terminations. Large cutaneous fibers were noted to track medially to the dorsal horn to enter lamina IV and synapse with large neurons, also sending collaterals to synapse more dorsally in laminae II and III. It could well be that cells of the substantia gelatinosa and possibly also the marginal cells both of which have been shown to receive cutaneous input (Heimer and Wall, 1968) are components of an inhibitory side chain which may be responsible for producing a decrement

of activity in the direct flexor reflex pathway. The cells of the substantia gelatinosa are small and small neurones are the most susceptible to asphyxial insult. It is possible that the impairment of habituation observed in this study may have been due to degeneration of substantia gelatinosa cells which had been components of an inhibitory side chain. It is likely that degeneration of interneurones forming the direct excitatory flexor reflex pathway had also occurred, as the magnitude of responses in the nonhabituating rats was lower than in control rats. The higher basal activity seen with EMG recordings of clamped rats prior to testing could be due to the same as yet unsolved, but in these cases attenuated, mechanism responsible for the extensor rigidity seen in a few preparations.

PART V.

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