THE ROLE OF INHIBITORY MECHANISMS IN
HABITUATION AND SENSITIZATION OF THE FLEXOR REFLEX

by

JOHN FERGUSON MacDONALD
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Department of Physiology

The University of British Columbia
Vancouver, B.C., Canada.

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Several authors have suggested that the reticular formation may act as a generator of behavioural inhibition. Furthermore, they have implied that this inhibition may progressively reduce evoked behaviour leading to behavioural habituation (Hernandez-Peon, 1960; Sokolov, 1963; Stein, 1966). Stein (1966) proposed that recurrent inhibition of the reticular formation might be responsible for habituation of behavioural arousal.

Wall (1970) presented the hypothesis that PTP of inhibitory synapses within the flexor reflex pathway might cause habituation of this reflex. However, experiments performed upon the spinal cat have not indicated a role for inhibition in habituation of the flexor reflex (Spencer, et al., 1966c; Groves and Thompson, 1973). A more reasonable explanation for habituation of this reflex in the spinal animal is a reduction in the efficacy of excitatory synapses within the direct reflex pathways (Farel, et al., 1973).

Habituation of the flexor reflex is not identical in the spinal and intact rat (Pearson and Wenkstern, 1972). Habituation is more readily observed in the intact rat. This thesis has demonstrated that habituation of the flexor reflex is retarded but not prevented by the infusion of drugs which are known to antagonize inhibition (strychnine and bicuculline) provided the spinal cord is not transected. This was not the case for habituation of this reflex in the spinal rat. Indeed, the infusion of strychnine actually facilitated habituation in the spinal rat.
Spontaneously active spinal interneurones were progressively inhibited (inhibitory build-up) by repeated cutaneous stimulation. This build-up of inhibition was greater with intense stimuli than with weak stimuli. However, a similar build-up of inhibition was not found after transection of the spinal cord. Inhibition itself tended to habituate in the spinal rat regardless of the intensity of the stimuli. The injection of strychnine eliminated inhibitory build-up in the intact rat in half of the interneurones tested but was ineffective in the remaining interneurones.

Decerebration releases a tonic inhibition of the flexor reflex mediated by the reticular formation (Holmqvist and Lundberg, 1961). This thesis has demonstrated that habituation of the flexor reflex is much more pronounced in the decerebrate than in the spinal rat provided intense stimuli are employed. This evidence suggests that the reticular formation may be responsible for the genesis of inhibitory build-up. A similar build-up of inhibition of spinal activity has been shown following repetitive stimulation of the reticular formation (Abrahams, 1974; Haber and Wagman, 1974).

The decrement of the flexor reflex related to a build-up of inhibition was only apparent when the intensity of the stimuli was noxious (possibly painful) and it may represent a mechanism for adaptation to pain. Serotonergic systems and the raphe nuclei are associated with an inhibition of behavioural arousal and the inhibition of pain. This thesis has shown that lesions of the nucleus raphe dorsalis and pre-treatment with p-CPA facilitate habituation of the
flexor reflex relative to the intact animal (not treated with p-CPA). It is suggested that the raphe nuclei inhibit the reticular neurones responsible for the genesis of inhibitory build-up.

Habituation of the flexor reflex in the spinal rat is best explained by a mechanism of reducing synaptic efficacy. However, intersegmental inhibitory mechanisms may be capable of modulating the amplitude of the reflex which in turn may alter the degree of habituation. Furthermore, the decrement of inhibition observed in the spinal rat may be responsible for long term sensitization of the flexor reflex.
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LIST OF ABBREVIATIONS

C, centrigrade
C7, transection at the seventh cervical vertebra
cc, cubic cm.
cm., centimeter
CS, conditioned stimulus
CR, conditioned response
D.C., direct current
dia., diameter
DRP, dorsal root potential
EJP, excitatory junction potential
EMG, electromyographic
EPSP, excitatory post-synaptic potential
FRA, flexor reflex afferent
FRI, flexor reflex interneurone
FMN, flexor motorneurones
FWR, flexor withdrawal reflex
g, gram
GDEE, glutamic acid diethyl ester
GR, general response
GS, general stimulus
Hg., mercury
hr., hour
ILD, inhibition of long duration
IPSP, inhibitory post-synaptic potential
kg, kilogram
LSD, lysergic acid diethyl amide
LIST OF ABBREVIATIONS (continued)

M, motivation
mA, milliampere
min., minute
mg, milligram
ml, millilitre
mm., millimeter
msec., millisecond
MΩ, megohm
n, number
n.r.d., nucleus raphe dorsalis
n.r.g., nucleus reticularis gigantocellularis
PAD, primary afferent depolarization
PAH, primary afferent hyperpolarization
p-CPA, para-Chlorophenylalanine
PTD, post-tetanic depression
PTP, post-tetanic potentiation
R, response
S, stimulus
sec., second
T₅, T₁₀, transection at the fifth or tenth thoracic vertebrae
UR, unconditioned response
US, unconditioned stimulus
μ, micron
μA, microampere
μg, microgram
v., volt
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A central, if not intrinsic, objective of neurophysiology is the correlation of physiological mechanisms of the central nervous system with the overt and complex behaviour of the organism. Pavlov employed the conditioned reflex as a model from which he could extrapolate the laws of behaviour (acquisition, extinction, and generalization) and inferred the underlying physiological mechanisms. Sherrington, however, stressed an understanding of the physiological mechanisms underlying the behaviour of simple spinal reflexes. This thesis borrows its philosophical approach from both sources and uses a reflex (flexor withdrawal reflex) to determine if certain physiological mechanisms (inhibitions) can account for certain behavioural characteristics of the reflex (habituation and sensitization).

Russian neurophysiologists, who follow the teachings of Pavlov, suggest that inhibition plays a particularly significant role in the manifestation of behaviour. Beritoff (1965:161), for example, states that: "Widespread inhibition constitutes the basic factor in integration of the central nervous system during behavioural reactions....General inhibition arises in the central nervous system and spreads over almost all areas of the brain, with concurrent focal states of excitation in
in only limited neuronal circuits." In contrast, Sherrington and his
associates found that inhibition of spinal reflexes was manifest as a
contraction of agonist with a concomitant "reciprocal relaxation" of
antagonist muscles. Furthermore, cessation of the stimulus resulted in
"rebound contraction" of antagonist muscles according to the law of
"successive spinal induction". "Reciprocal inhibition" of extensor
muscles is blocked by administration of strychnine and this inhibition
is followed by excitation ("rebound excitation") once the stimulus is
terminated (Graham Brown, 1912; Beritoff, 1965). This inhibition would
not seem to bear much resemblance to "general inhibition". There is a
basic difference in the definition of the word "inhibition" as employed
by the Pavlovian, in comparison with the Sherringtonian, school of
thought. The Pavlovian definition of inhibition is less restrictive
and refers to the loss or reduction of a response normally elicited by
the stimulus. A requirement of a dynamic process which intervenes to
block or reduce pre-existing activity is expected by the Sherringtonian
school. This difference in definition has lead to considerable confusion
(MacIntosh, 1975).

There is, however, a point of compromise between "general inhibition"
and "reciprocal inhibition." Stimulation of the skin not only leads to
an excitation of agonist and inhibition of antagonist muscles but also
produces a background (general) inhibition of agonist muscles. Thus,
flexors and extensors are inhibited (Graham Brown, 1912; Beritoff, 1965).
Furthermore, it is recognized that stimulation of various brain structures
will lead to general inhibition of spinal reflexes (Sherrington, 1906;
Western neurophysiologists have of course extended Sherrington's concept of "reciprocal inhibition." Notable extensions are found in the development of the new concepts of "feed-back" and "feed-forward" inhibition. In addition an entirely new mechanism of inhibition has been recognized in the form of a depolarization of pre-synaptic terminals (pre-synaptic inhibition). However, the basic Sherringtonian conceptualization of the role of inhibition has not been changed. For example, compare this statement made by Eccles (1973:89): "I always think that inhibition is a sculpturing process. The inhibition, as it were chisels away at the diffuse and rather amorphous mass of excitatory performance at every stage of synaptic relay.\textquotedblright, with the earlier statement made by Beritoff.

Behaviourism and Habituation

Science has two essential aspects—the empirical and the explanatory. The empirical aspect is primarily concerned with the facts of the science as revealed by observation and experiment. The explanatory or theoretical aspect, on the other hand, consists in a serious attempt to understand the facts of the science, and to integrate them into a coherent, i.e., a logical, system. (Hull, 1952:1).

The study of behaviour has not been readily adapted to the scientific approach. It has suffered from a lack of empirical information and a plethora of unsubstantiated explanations. Central to the study of behaviour is the determination of how behaviour is acquired or how learning takes place. Behaviourists have been forced to consider the acquisition of behaviour in terms of the organism's response to experimentally controlled
stimuli but with little or no knowledge of the internal workings of the organism. The organism is assumed, largely by necessity, to be a "black box". This has resulted in the adoption of the "empty organism" approach to the study of behaviour. The psychologist can by this means study behaviour by employing theoretical constructs to explain the relationships between the stimulus and the response. Strict empiricism, on the other hand, demands a precise knowledge of these internal mechanisms and how they interact. Thus, behaviourism must be amalgamated with modern neurophysiology.

A key link has been and is being sought that would connect empirical neurophysiology with the rudimentary mechanism or mechanisms by which behaviour is acquired. In other words, can the recognizable electrophysiological properties of the nerve cell and the synapse account for the acquisition of behaviour? The principle of parsimony dictates that the isolation and characterization of a simple system of afferent and efferent connections should be correlated with a simple form of learning. Provided it is accepted that no two sets of neurones need operate in quite the same manner the process of learning can be empirically defined. Attempts to draw a vinculum between the activity of the nervous system and the acquisition of behaviour, must include definitions of both the overt behaviour and the supposed underlying neuronal mechanism or mechanisms.

Defining the process of learning might seem to be a trivial problem but finding a scientifically valid definition is an extremely difficult conundrum. Initially, it is perhaps of greatest value to use an operational definition. This can be accomplished by imposing limits upon a subjective
description. In this manner, learning can be defined as a change in performance (response) that occurs as a consequence of experience. The change in performance must be retained for some period of time and it must not be attributable to alterations in motivation nor to such phenomena as maturation or fatigue. Entelechy can play no role in this definition.

Subjective concepts of what constitutes learning can be consolidated by examining several of the theoretical formulations of Hull (1943, 1952). Behaviour was defined in terms of a series of postulates which described the mechanisms by which an organism adapts, behaviourally, to a significant change in the environment. For example, variables such as amplitude, latency, and probability of response could be functionally related to an internal "excitatory potential" or "stimulus-response continuum." This "excitatory potential" (actually a theoretical construct itself) would be determined by stimulus factors originating both from within and without the organism.

The "first major automatic mechanism" for adapting to the environment is the reflex, with its presumed rigid stimulus-response connections. Hull (1952) stated that no learning takes place at this level. The stimulus-response pathway was considered to be a device whereby the organism could respond stereotypically to a relatively homogenous groups of emergency or defensive situations. The latency of response would be small to meet the survival-threatening stimulus.

The conditioned reflex was envisaged as the "second major adaptive behaviour mechanism." The capacity to profit from past experience or simple learning would increase the number of possible response patterns,
although at the expense of response time.

To a certain extent learning is defined by the exclusion of a number of phenomena, for example fatigue. The distinction between learning and fatigue would seem to be arbitrary. The physiological counterparts of fatigue would include receptor adaptation and muscular fatigue. These processes are clearly excluded because a consensus of opinion would insist that learning is a process of the central nervous system although this may not be true for invertebrates.

The central nervous system can modify learning. For example, it is recognized that volition or the degree of motivation can subvert or facilitate the process of learning. Empirically this distinction would seem to be one of degree of complexity. To be more specific, the learning process is assumed to be located within or at least functionally associated with a specific subset of behavioural units (each unit is equated with a single neurone for the sake of simplicity). The unconditioned stimulus (US) activates a subset of neurones which in turn activate a subset of efferent neurones that produce the unconditioned response (UR). Similarly, the conditioned stimulus (CS) activates a different subset of neurones which in turn activate yet another subset of efferent neurones that produce the conditioned response (CR). The pairing of the US and the CS activate a conjoint subset of efferent neurones that produce the UR and/or the CR (Figure 1). Other non-inclusive subsets of neurones, that are activated by extraneous stimuli (originating from the environment of the organism or from the internal milieu of the organism itself), can modify the activity of the previously mentioned conjoint subsets of
Figure 1. Subsets of neurones subserving conditioning and modification of conditioning. The circles represent the subset of neurones activated by a particular stimulus (US, CS, and internal stimuli such as motivation or volition, M). Pairing of the US and CS activates and intersection of neurones (UR ∩ CR) that evokes the CR. An intersection of the neurones activated by the US, CS, and internal stimuli (UR ∩ CR ∩ M) can result in facilitation or inhibition of the CR. For the sake of simplicity the subsets subserving stimulus elements are not included in this diagram.
neurones. This accounts for the ability of neurones subserving volition and motivation to alter the process of learning. Of course there is not always a strict demarcation between the conjoint and non-conjoint subsets with a resultant possibility of generalization of the stimulus and/or response. This type of statement is made with the fundamental assumption that neurones and their connections form the basis of behaviour and that they are the vehicle by which behaviour is acquired.

The parsimonious approach requires a definition of the simplest form of learning. The Pavlovian form of learning (classical conditioning paradigm) is usually recognized as the primary event of learning in contrast to a more complex instrumental learning (operant conditioning paradigm). Stated in brief, conditioning refers to the transfer of the UR from the US to the CS. The response then evoked by the CS is called the CR. The CS is "associated" with the US and/or UR. However, other "non-associational" events may occur simultaneously with conditioning. Repetition of the US, with or without paired presentation of the CS, can evoke an UR of ever decreasing amplitude. This decrement of response following iterated US presentation is referred to as "adaptation" of the UR (Walker, 1967). An augmentation or sensitization of the CR to repetition of the CS can occur following the conditioning procedure. Neither "adaptation" nor sensitization appear to be contingent upon pairing of the US and CS. The intensity or significance of the stimulus does have a bearing upon the mutability of response amplitude. In particular, response sensitization is associated with high intensity stimulation (Hinde, 1966). Presentation of the US can produce a general activation of the organism,
in response to any extraneous stimulus, with the result that a response which resembles the CR might be evoked following the initial presentation of the CS. "Pseudoconditioning" refers to this generalized sensitization during the conditioning procedure.

"Adaptation" and sensitization are considered to be confounding variables within the conditioning paradigm. It is clear that these non-associational" components are not dependent upon the process of conditioning. "Adaptation" is similar, if not identical, to behavioural habituation (Kandel, 1967) and behavioural habituation can be defined as an attenuation of response parameters (amplitude, response duration, probability of response, etc.) following repetition of the stimulus (Harris, 1943). Peripheral events such as muscular fatigue and receptor adaptation are also excluded from this definition as they are from the definition of learning.

Response sensitization may be defined as an increase in responsiveness with repetition of the stimulus (Groves and Thompson, 1970) and it is also considered to be a property of the central nervous system. The correlation of sensitization with strong or aversive stimuli has lead to the postulate that sensitization represents a modification of the "central excitatory state" (Sherrington, 1898) or the "excitatory potential" (Hull, 1952). By this it is meant that the presentation of an intense (even painful) stimulus can evoke an alteration in the presumed tonic activity of the central nervous system. This influence is often not specific to the sensitizing stimulus (Hinde, 1966) and a considerable degree of stimulus generalization can be expected (Thompson, et al, 1973).
The terminology "central excitatory state" was used by Sherrington (1906) to describe subliminal fringe phenomena of transient duration but the concept has been extended to imply a "central motivational or drive state" (Lashley, 1938; Stellar, 1956) or a "central arousal state" (Lindsley, 1960; Duffy, 1962). Thus, a general activation of the organism might predispose the increment of response.

Sensitization may be represented within the classical conditioning paradigm (Figure 2). If either the US or CS is of sufficient intensity it can alter the level of excitability in the "state" system. The conjoint subset of neurones subserving the association of the US and CS would be facilitated by an increase in general excitability through activation of an inclusive set of neurones (UR CR GR). This facilitation would also extend to the generalized stimulus (GS) with a resultant increase in the generalized response (GR). This concept of an altering level of excitability (a decrement of excitability) has also been used to explain behavioural habituation (Jasper, et al., 1958).

The study of behavioural habituation has brought about a dichotomy of opinion as to which theoretical explanation best describes the mechanism underlying habituation. These theories can be divided into "dynamic" theories and those proposing some form of progressively reducing "central efficiency". Proponents of the latter type of theory suggest that the response occurs less strongly simply because the stimulus no longer possesses the capacity to evoke the response as a result of a reduction in the efficiency of reflex transmission. Alternatively, the stimulus might no longer possess the capacity to evoke
Figure 2. Subsets of neurones subserving conditioning and the "state" system. In addition to the subsets activated by the US and CS, a set of "state" neurones is defined (GR). A general stimulus (GS) can activate the "state" neurones which can lead to evocation of a GR which may be different or the same as the CR but that cannot be considered a CR. The CS may also cause an activation of the subset GR resulting in a CR of greater amplitude or of increasing amplitude that is independent of conditioning (sensitization). This figure differs from figure 1 in that the subsets US and CS are exclusive of the subset M but are inclusive of the subset GR.
the response because some active process intervenes. There is a "dynamic" intervention imposed upon the stimulus-response pathway. The "central efficiency" explanation is parsimonious whereas the "dynamic" explanation requires the additional postulation of an active form of dis-facilitation or inhibition. Thus, an excitatory pathway from stimulus to response is sufficient for the description of a theory of "central efficiency" but "dynamic" theories must include either a decrease in tonic facilitation of the stimulus-response pathway or it must include yet another pathway in which a progressive inhibition of the stimulus-response pathway can occur.

There are numerous examples of "dynamic" theories. The concepts of "conditioned inhibition" (Sokolov, 1965), "reactive inhibition" (Hull, 1952), and "afferent neuronal habituation" (Hernandez-Peon, 1960) are typical of such theories. These theories lack a credible degree of supportive neurophysiological evidence. However, this is not so with regard to "central efficiency" theories. Many of these theories have been discussed critically by Groves and Thompson (1970) and Thompson, et al. (1973).

Paramount among the "central efficiency" theories is the "Dual-Process" theory of habituation (Groves and Thompson, 1970; Thompson, et al., 1973) which is the most prepossessing of the various theories of habituation. This theory is based upon the culmination of scientific evidence ranging from studies of the activity of single neurones in the nervous system of simple invertebrates to complex behavioural habituation in the vertebrate. A brief ratiocination of this theory can reduce the problem of defining habituation and sensitization. The "Dual-Process" theory is stated by Thompson, et al. (1973:240-241) as follows:
1. Every stimulus that evokes a behavioral response has two properties: It elicits a response and influences the "state" of the organism. The S-R pathway is the most direct route through the central nervous system from stimulus to discrete motor response, however circuitous, redundant, and variable that pathway may be and regardless of whether the response is learned or unlearned. State is the general level of excitation, arousal, activation, tendency to respond, etc., of the organism.

2. Repetition of an effective stimulus results in an inferred decremental process in the S-R pathway which may be termed habituation.
   (a) During habituation training, habituation develops exponentially and reaches an asymptotic level.
   (b) The rate of development and degree of relative habituation are directly related to stimulus frequency and inversely related to stimulus intensity. Frequency has strong effect and intensity a weak effect on habituation.
   (c) Upon cessation of the habituating stimulus, habituation decays spontaneously (spontaneous recovery).
   (d) Repeated series of habituation training and spontaneous recovery result in progressively more habituation.
   (e) Response habituation will exhibit generalization to a test stimulus to the extent that the habituating and test stimuli activate common "habituation" elements.

3. Presentation of an effective stimulus results in an inferred incremental process in a state of excitation or tendency to respond of the organism which may be termed sensitization.
   (a) The process of sensitization occurs in state system(s) but not in S-R pathways.
   (b) During habituation training, sensitization first grows and then decays.
   (c) The amount and duration of sensitization are directly related to stimulus intensity. At higher intensities, sensitization is directly related to stimulus frequency. At low intensities there may be little or no sensitization.
   (d) Upon cessation of a stimulus that has produced sensitization, sensitization decays spontaneously.
   (e) Repeated presentations of a sensitizing stimulus result in progressively less sensitization, that is, sensitization decreases or habituates.
   (f) Response sensitization will exhibit generalization to a test stimulus to the extent that the sensitizing and test stimuli activate common sensitization elements.
   (g) Dishabituation, the increase in a habituated response following presentation of a stimulus other than the habituation stimulus, is simply an instance of sensitization.
(h) Under certain circumstances (strong stimuli presented regularly at relatively slow rate) temporal conditioning of sensitization of state may occur.

4. The two processes of habituation and sensitization occur and develop independently of one another but interact to yield the final response output function. Habituation may be primarily "phasic" in its action on response output, while sensitization may be primarily "tonic"

Spencer, et al. (1966a) have defined nine "parametric" characteristics of habituation. "Parametric" refers to various alterations of the stimulus parameters used to derive the "parametric" characteristics of habituation. Six of these characteristics are found in items 2.a to 2.e, inclusively. Dis-habituation is the eighth. The final characteristic is summarized as follows: "The effects of habituation training may proceed beyond the zero or asymptotic level." Additional stimulation after the response has habituated to the asymptotic level will result in slower recovery.

The question remains as to whether or not habituation and/or sensitization conform to current criteria used to define learning. Thorpe (1963) expressed the opinion that habituation was a form of learning and habituation has been used as a learning paradigm in the study of the behaviour of neurones in the invertebrate central nervous system (Kandel and Spencer, 1968). This acceptance of habituation as a form of learning has not been universal. For example, Miller (1967:644) sets four criteria for the acceptance of any behavioural process as an authentic learning event. Learning was defined in a restrictive manner: "Learning is a relatively permanent increase in the response strength that is based upon previous reinforcement and that can be made specific to one out of two or more arbitrarily selected stimulus situations."
"Relative permanence," Miller defines as a matter of days or months as opposed to minutes or hours. This may exclude sensitization due to its short duration (Pearson, 1974) but there are reports that habituation may last for long periods (days and months) (Nesmeinanova, 1957; Kozak, et al., 1962). Unfortunately the time sequences of altered activity in the nervous system of the vertebrate are usually measured (electrophysiologically) in orders of magnitude well below those stipulated by this requirement.

Miller's reference to an "increase in response strength" and "selected stimulus situations" implies several critical aspects of learning. These factors can be illustrated conceptually in terms of Hull's "stimulus-response continuum." For example, if it is postulated that there exists both a stimulus and response continuum for each response and stimulus, respectively, then the process of learning has occurred when a stimulus-response contiguity has been established between the stimulus and the response continuum and between the response and the stimulus continuum (Figure 3). To be more specific, each stimulus has the capacity to evoke a number of response potentialities (possibility of evoking a response as opposed to actually evoking a response) on the response continuum and each potentiality has an equal probability of being strengthened by an association with the stimulus. An "association" will have been established when the probability of producing any particular response potentiality on the continuum becomes much greater than that of any other response potentiality. In a similar manner, a specific response may be produced from a number of stimulus potentialities on a stimulus continuum.
Response Continuum

Before conditioning

\[ P(R_1) = P(R_2) = P(R_3) = \ldots = P(R_n) \]

After conditioning

\[ P(R_2) > P(R_1) \ldots > P(R_n) \]

Stimulus Continuum

\[ P(S_1) = P(S_2) = P(S_3) = \ldots = P(S_n) \]

Figure 3. Response (above) and stimulus continua (below) and the development of a stimulus-response contiguity. A stimulus can produce a number of response potentialities \((R_1 \ldots R_n)\) on a response continuum. Prior to conditioning the probability that any "association" will be developed between \(S\) and a particular response potentiality \((R_2)\) is the same as that of any other potentiality. After conditioning the probability that a \(S\) will produce \(R_2\) is much greater than that of any other potentiality. A similar argument holds for a specific response and its continuum of stimulus potentialities.
When the probability that one stimulus potentiality will elicit the response is greater than that of the others an "association" will have been established (Figure 3). A super-imposition of the stimulus and response continua and an establishment of the stimulus-response contiguity represents the process of learning.

The crux of this concept is the enforcement of an "association" of the stimulus to the response and/or the response to the stimulus, in a specific manner. Stimulus and response generalization can be defined as a failure to develop a specific stimulus-response contiguity. Furthermore, Miller requires that the stimulus-response contiguity take the form of either the classical or operant conditioning paradigms. Miller excludes habituation, sensitization, and conditioning in simple organisms from his definition. However, Miller's definition is still based upon the "empty organism" approach with imposed subjective restrictions. With no knowledge of the intervening variables which establish the stimulus-response contiguity it is perhaps too stringent in its exclusion of the phenomena of habituation and sensitization. If the objective is to analyse the physiological correlates of behaviour it is more logical to adopt a definition which allows a component by component examination of the learning process.

Eisenstein (1967:654) has presented an alternative definition of learning which he states as follows:

A system is said to demonstrate learning when its output (response) to a given test input (stimulus) is a function of the total previous input-output pattern of which the test input was a part. That is, a system can be said to have learned if its output to a given test input is a
function of the specific input-output pattern to which it has been exposed.

Several aspects are often common to the learning process. One of these is repetition of the stimulus in order to reinforce the association between the stimulus and the response. Critical to this is the temporal association of more than one set of stimulus-response elements. Habituation and sensitization often occur during the conditioning procedure. They share a relationship with learning in the sense that the acquisition of any behaviour requires a repetitive establishment of the stimulus-response pattern (at least for simple forms of learning) and in that the response shows a temporal relationship to previous stimulation. They do not necessarily demonstrate an association between sets of stimulus-response elements in the same way that the US-UR is associated to the CS-CR.

The stereotyped reflex envisaged by Hull ("first major automatic mechanism") can be considered a constant response behaviour. It fails to account for habituation and sensitization of reflexes, where the response is no longer constant in quantity, but varies as a function of experience (repeated stimulation). However, habituation and sensitization do not attain the level of the "second major adaptive behaviour mechanism" or the conditioned reflex in that the change of behaviour is not qualitative. As a consequence, habituation and sensitization appear to fit half-way between Hull's "first major automatic mechanism" and his "second major adaptive behaviour mechanism." If learning requires an association between more than one set of stimulus-response elements it is doubtful that habituation and sensitization are forms of learning. Perhaps they should be considered sub-learning phenomena.
SECTION II

Integration in the Nervous System

Acceptance of the "neurone doctrine" has placed ever increasing emphasis upon the neurone as the functional unit of behaviour. The central problem has been to determine how the coding and storage of information can be represented or circumscribed by such an anatomical structure. Attempts to answer this question have taken several directions. One approach has been to attribute information storage to a modification in electrotonic potentials which are integrated across large complex clusters or functional matrices of cells. Each element of the matrix was assumed to be an individual neurone. This conceptual approach had its origins in Gestalt psychology, when cognition was envisaged as a coding of bio-electric fields, a sort of electronic hologram of information storage. This hypothesis appeared to be confirmed by the experiments of Lashley. Lesions of the neocortex did not cause specific behavioural deficits, but rather, the volume of cortex consumed in the lesion seemed to be the significant factor. As a consequence, Lashley (1929:179) rejected the hypothesis that behaviour could be represented by specific anatomical loci within the neocortex: "Integration cannot be expressed in terms of connections between specific neurones....the mechanisms of integration are to be sought in the dynamic relationships among the parts of the nervous system rather than in details of structural differentiation."

A lack of knowledge of the topography of the sensory and motor cortex at
that time, coupled with primitive surgical procedures, are assumed to have led to Lashley's spurious conclusion (Kandel and Spencer, 1968). Although the experiments performed by Lashley cannot be used to justify his conclusion the hypothesis is not necessarily invalid. None the less, Lashley's impact upon the field of physiological psychology was significant and many psychologists questioned the practicality of attempts to correlate behaviour with neural structures.

Recent evidence has provided some support for the concept that neurones might influence each other electrotonically. Any single neurone might influence another neurone which is in close apposition, electrotonically, either by means of an electrotonic synapse or by generating an extracellular field potential of sufficient intensity to alter the activity of the adjacent neurone. The existence of electrotonic synapses is now recognized in invertebrate nervous systems and there is some evidence that such synapses may also exist in the central nervous system of the mammal. This type of electrotonic coupling between neurones seems aptly suited to the synchronization of groups of neurones. It is difficult to interpret such a mechanism of neuronal interaction in terms of complex information processing. However, it may prove to be a significant means of triggering certain stereotyped behaviours. For example, in Tritonia, swimming behaviour is triggered by a group of neurones electrotonically coupled together (Getty and Willows, 1974).

The recording of extracellular potentials in the nervous system has focused, for the most part, upon transient events associated with the propagation of potentials in the axon, cell body, dendrite, or across the synapse. Although it has not been fashionable to do so, other
long term alterations in extracellular field potentials have been studied. Some of the earliest attempts to record electrical events in the nervous system examined field potentials (Brazier, 1963; Rowland, 1968). Somjen (1973) uses the term "sustained potentials" (D. C., steady potentials) to describe these long term potentials which are recorded from the cortex and spinal cord with respect to a common (ground) electrode. "Sustained potentials" have also been correlated with behavioural events such as the expectation of response (contingent negative variation; Walter, 1968) and the reinforcement of conditional stimuli (Morrell, 1961; Rowland and Goldstone, 1963).

There is increasing evidence that "sustained potentials" recorded in the spinal cord, as a consequence of repeated afferent stimulation, are the result of a progressive increase in the concentration of extracellular potassium (Somjen and Lothman, 1974). Neither "sustained potentials" nor the simultaneous elevation in potassium concentration are necessarily related to dorsal root potentials (DRP's) (Kriz, et al., 1974; Somjen and Lothman, 1974). The duration of "sustained potentials" is far greater than that of the conventionally recorded electrical events such as primary afferent depolarization and hyperpolarization.

Increases in the extracellular concentration of potassium have been correlated with spreading depression (Lothman, et al., 1975) which suggests that increases in potassium concentration might inhibit neuronal discharge (depolarization block) and such a mechanism might account for long term inhibition of spinal reflexes (Abrahams, 1974). However, it is not yet known if the changes in local extracellular potentials are of a
sufficient magnitude to modulate neuronal activity. "Sustained potentials" are elicited with the discharge of inhibitory as well as excitatory interneurones suggesting that changes in potassium concentration may be secondary to the release of intracellular potassium during neuronal discharge (Somjen, 1973). Changes in the extracellular concentration have also been reported to alter synaptic transmission (Cooke and Quastel, 1973).

In opposition to the hypothesis that behaviour is represented as bioelectronic fields has been the conceptualization of the neurone, and specifically the synapse, as the functional unit of behaviour (Eccles, 1964; 1973; Roberts, 1966; Kosower, 1972). The synapse might be called the unit of microbehaviour. The quest has been to discover how information processing can occur in the central nervous system as the result of alterations in the function of synapses (synaptic plasticity) and in the interaction between many synapses.

The synapse has not been accessible to direct examination until quite recently. Traditionally the study of the synapse has been indirect and has relied upon the examination of reflexes. The concept of the reflex not only links stereotyped movement to the underlying microbehaviour of the synapse, but the compounding of reflexes serves to connect physiology to the study of behaviour. Sherrington (1948) credits Thomas Willis (1664) as the originator of the concept of the reflex. However, Descartes (1677) provided the first comprehensive description of the reflex as a means of explaining the agonist-antagonist relationship of the limb muscles. The realization that the behaviour of the simple
reflex was the result of the activity of synapses must be credited to Sherrington even though he did not have a specific knowledge of the chemical and electrical events that occur at the synapse. At the time that Sherrington was determining many of the properties of the synapse others were scrutinizing the relationships between reflexes and complex behaviours (Watson, 1924; Pavlov, 1927).

Behaviour has been described as a complexing of simple reflexes but with an important addendum (Pavlov, 1927:14): "The essential feature of the highest activity of the central nervous system...consists not in the fact that innumerable single stimuli do initiate reflex reactions in the animal, but in the fact that under different conditions these same stimuli may initiate quite different reflex reactions; and conversely the same reaction may be initiated." The compounding of reflex arcs does not exclude nor does it explain the development of contiguity between various response-stimulus elements. To be more specific, if two reflexes are compounded the resultant behaviour is synergistically related to the summated arcs.

The elements of the central nervous system participate in numerous dynamic processes which might be temporarily or permanently rearranged in order to code and store information. Current knowledge of neurophysiological events indicates a number of possible foci where modulation of neuronal discharge might take place. These foci and the events that occur at these foci are summarized below:

Afferents
1) blockage  2) depolarization  3) hyperpolarization
(inhibition or excitation)
Synaptic termination

1) location of the terminal with respect to the post-synaptic element (dendrite, soma, axon, etc.)

2) Anatomical plasticity
   a) distance from pre- to post-synaptic membrane
      (occurrence of synaptic spines)
   b) number of synaptic contacts and location of contacts upon the target cell.
   c) size and locality of post-synaptic receptive regions
   d) number and location of trigger zones

3) Chemical transmission
   a) modulation of synthesis, mobilization, availability for release, transfer to post-synaptic membrane, uptake, enzymatic destruction
   b) transmitter causes inhibition, excitation, and/or both in the post-synaptic cell
   c) sensitivity of post-synaptic receptors to the transmitter

4) Electrical transmission
   a) pre-synaptic membrane properties
   b) trans-cellular impedance
   c) post-synaptic membrane properties

5) Propagation and initiation
   a) dendritic (active or passive transmission)
   b) endogenous pacemaker activity (underlying metabolic processes)
   c) repetitive discharge of exogenous origin (after-discharge, reverberating circuits)
The initial event is the arrival of the afferent volley to the brain or spinal cord. Information is frequency coded within this volley and different groups of afferents carry specific information. The "all or none" property of the afferent nerves severely limits the informational content of the volley. Within the cord the afferent fibres may be subject to blockade (Wall and Johnson, 1958) or to collision and deactivation by antidromically travelling potentials (Wall and Gutnick, 1974). The structure and geometric arrangement (fine branching collaterals) of the terminal regions of the afferents may contribute functional properties to the terminal region (Lloyd, 1971). The terminals are subject to either depolarization (primary afferent depolarization (PAD) or to hyperpolarization (primary afferent hyperpolarization (PAH), and/or both (Hodge, 1972) with concomitant suppression (pre-synaptic inhibition) or facilitation (pre-synaptic facilitation) of afferent transmission (Eccles, 1964; Hodge, 1972; Mendell, 1972; Schmidt, 1973). Repetitive interneuronal discharge may account for these changes in primary afferent excitability (Lloyd, 1971; Schmidt, 1973; Yu and Avery, 1974) although primary afferent collaterals may themselves contribute (Wall, 1958; Wall and Johnson, 1958; Lloyd, 1971). The duration of PAD and PAH (200 msec.) is far too brief to account for even temporary information storage. However, it is becoming apparent that PAD and PAH are a consequence of temporary modulation of a tonic level of primary afferent depolarization and much longer periods of altered excitability may occur with repetitive stimulation (Schmidt, 1973).

The release of potassium by discharging afferents has been postulated
as a mechanism whereby one afferent terminal can depolarize another. An accumulation of extracellular potassium might account for evoked DRP's (Barron and Mathews, 1938; Krnjevic and Morris, 1972; Vycklicky, et al., 1972); however, the time course and magnitude of changes in the concentration of extracellular potassium does not correlate with the occurrence of DRP's (Liebl, et al., 1973; Kriz, et al., 1974; Somjen and Lothman, 1974).

The afferent synapses may terminate upon various elements within the spinal grey matter. The effectiveness of any particular synapse will depend upon the location of the terminal with respect to the postsynaptic neurone (i.e., dendritic versus somatic) (Jack, et al., 1971; Merrill and Wall, 1972). Complex synaptic arrangements have been observed such as the glomerular complex found in the substantia gelatinosa and clark's column (Rethelyi and Szentagothai, 1969; Rethelyi, 1970). Afferent terminals participate in these complexes and they may form the anatomical counterpart for pre-synaptic inhibition (Schmidt, 1973).

While gross structural relationships between afferent nerves and target neurones are considered to be genetically determined (Sperry, 1951, 1965; Wiesel and Hubel, 1965) more subtle anatomical changes are possible as a consequence of functional "use" or "disuse" of pathways. The total number of synapses, the involvement of synaptic spines, the width of the synaptic cleft, and other properties of synapses may be structurally altered by activity or lack of activity in the pathway (Cragg, 1972, 1974; Lund and Lund, 1972; Eccles, 1973). The significance of this form of plasticity as a means of storing information is yet to be determined.
Informational events occurring at synapses consist of coupled chemical and electrical processes. Modulation of transmission might occur with a change in the biochemical reactions of transmission, in the associated electrical events, or in the membrane properties of the pre- or post-synaptic neurones. The ability of the chemical synapse to act as an information coder and capacitor may be extensive. The transmitter may hyperpolarize, depolarize or prevent post-synaptic depolarization by increasing conductance with a resultant inhibition or excitation of the activity of the post-synaptic neurone.
SECTION III

Synaptic Plasticity (functional)

The synapse has long been considered the site of the genesis of habituation and sensitization of spinal reflexes. Sherrington (1898: 140; Sherrington and Sowtown, 1915) described both an initial sensitization and a later habituation of spinal reflexes: "In most cases a few repetitions tires out the reflex reaction, after increasing somewhat for a few repetitions at the beginning of the examination, they begin to fade out, and do so unless a rest is allowed." Sherrington (1906:218) had no direct experimental access to the synapses mediating spinal reflexes but he postulated, on the basis of indirect evidence, that the origin of habituation was a decrease in the functional efficacy of these synapses: "...the seat of fatigue [habituation] is intraspinal and central more than peripheral and cutaneous; and that it affects the afferent part of the arc inside the cord, probably at the first synapse."

A number of characteristics of habituation (see p. 14, this thesis) were also recognized by Sherrington (1906:218-219):

1) spontaneous recovery

The local fatigue of a spinal reflex seems to be recovered from with remarkable speed, to judge by observations on the reflexes of the spinal dog. A few seconds' remission of the stimulus suffices for a marked though incomplete restoration of the reaction.

2) influence of stimulus intensity

In my experience, these spinal reflexes fade out sooner under a
weak stimulus than under a strong one.

3) stimulus generalization

When the scratch reflex elicited from a spot of skin is fatigued, the fatigue holds for that spot but does not implicate the reflex as obtained from the surrounding skin. When the spot stimulated is close to the one tired out, the reflex shows some degree of fatigue, but not that degree obtained for the original spot.

The synapses of the central nervous system of the vertebrate are not nearly as accessible to study as those of the central nervous system of invertebrates. As a consequence, synaptic plasticity has been extensively studied in invertebrates. It can not be stated with certainty that synaptic plasticity occurs by the same mechanism in vertebrates as invertebrates nor can it be stated that habituation and sensitization are a consequence of synaptic plasticity. However, habituation and sensitization share many of the characteristics of synaptic plasticity and many phenomena demonstrated by both the synapses of vertebrates (peripheral and central) and invertebrates can be placed under the rubric of synaptic plasticity. Synaptic plasticity refers to an alteration in the efficacy of transmission and a large number of phenomena such as "low frequency depression", post-tetanic potentiation (PTP) and "frequency facilitation" may be included in this definition. A discussion of synaptic plasticity and habituation in invertebrates is justified if for no other reason than because these phenomena are proposed as likely mechanisms for habituation and sensitization of spinal reflexes (Sherrington, 1906; Groves and Thompson, 1970; Thompson, et al, 1973; Farel, et al., 1973).

Considerable evidence has accrued which associates synaptic plasticity
with a number of phenomena which are at least superficially similar to habituation and sensitization. It has been found that repetition of the stimulus can lead either to a subsequent post-synaptic (or post-junctional) potential of greater or lesser magnitude and duration when compared to the initial evoked post-synaptic potential. The only central synapses readily available for study have been those in the invertebrates, which demonstrate very similar phenomena.

If two stimuli are applied with only a brief (several msec.) pause between them the size of the recorded excitatory junction potential (EJP, in the case of the neuromuscular junction) or the excitatory post-synaptic potential (EPSP, central synapses of invertebrates and the post-synaptic response of peripheral ganglia) in response to the second stimulus will be greater in magnitude than that evoked by the first. An increase in the amount of transmitter released from the pre-synaptic terminal, by the second stimulus, is usually associated with this "short term facilitation" (Del Castillo and Katz, 1954; Hubbard, et al., 1971; Schlapfer, et al., 1974). Repetition of the stimulus, at rates below those used to produce "short term facilitation", is also capable of releasing subsequently larger amounts of transmitter resulting in "frequency facilitation" or "frequency potentiation" (Schlapfer, et al., 1974). Repetition of the stimulus, at rates below those used to produce "short term facilitation", is also capable of releasing subsequently larger amounts of transmitter resulting in "frequency facilitation" or "frequency potentiation" (Schlapfer, et al., 1974) of the synapse examined (Del Castillo and Katz, 1954; Liley, 1956; Dudel and Kuffler, 1961;
At even lower frequencies of stimulation the size of the post-synaptic potential may actually be less than that observed after the initial stimulation. This effect is called "low frequency depression" of the synapse (Del Castillo and Katz, 1954; Liley, 1956; Horn and Wright, 1970; Dudel and Kuffler, 1971; Schlapfer, et al., 1974). It may occur due to a decrease in the amount of transmitter released (Del Castillo and Katz, 1954; Thies, 1965; Miledi and Slater, 1966; Betz, 1970; Horn and Wright, 1970).

"Frequency facilitation" and "short term facilitation" bear some relationship to another phenomenon, post-tetanic potentiation (PTP). While changes in the post-synaptic potential may occur during the stimulation, PTP places an emphasis upon changes in the post-synaptic potential after the application of the tetanizing trains of stimuli. PTP can be defined as a relatively long term (min. to hrs.) increase in the magnitude and duration of the post-synaptic potential following the cessation of a high frequency train. PTP occurs at the neuromuscular junction, the synapses of peripheral ganglia, and within the central nervous system of both the vertebrate and invertebrates. There is a general concensus that PTP is a pre-synaptic event. If divalent calcium ions are added to the bathing medium of the neuromuscular junction, during the period of tetanus (but not afterwards), a facilitation of PTP occurs (Rosenthal, 1969; Weinreich, 1971). It has been suggested (Stinnakre and Tauc, 1973) that each spike in the train allows greater influx of calcium into the terminal than previous spikes resulting in a post-tetanic period of
increased transmitter release with subsequent stimulation.

In the spinal cord of the mammal, spinal reflexes undergo PTP (Lloyd, 1949; Eccles and Rall, 1951; Jefferson and Benson, 1953; Hughes, 1958; Wall and Johnson, 1958; Eccles and Krnjevic, 1959) and the complex EPSP recorded in the spinal motor neurone undergoes a simultaneous potentiation (Brooks, et al., 1950; Curtis and Eccles, 1960). Spinal reflexes also demonstrate "low frequency depression" (Lloyd, 1949; Wilson, 1958; Eccles, 1964; Kuno, 1964). The EPSP recorded in spinal motor neurones demonstrates "frequency facilitation" as well (Kuno, 1964; Kuno and Weakly, 1972a). Other regions of the vertebrate brain also demonstrate phenomena similar to "frequency facilitation" (Richards, 1972; Bliss and Lømo, 1973; Douglas and Goddard, 1975).

The application of a tentanotic train of stimuli to an afferent nerve is followed by a period of augmentation of reflex excitability that may range from several seconds (Lloyd, 1949; Eccles and Rall, 1951) to periods of hours (Beswick and Conroy, 1964, 1965; Fentress and Doty, 1966; Spencer and April, 1970; Bliss and Lømo, 1973). The duration of the period of PTP is dependent upon the frequency and duration of the tetanizing trains (Lloyd, 1949; Spencer and April, 1970) but brief intermittent periods of tetanus yield more prolonged periods of PTP (Granit, 1956).

If the period of tetanization is increased from a duration of minutes to one of hours the tetanization is not followed by PTP but rather by a period of depression (PTD) which may last up to five hours (Hughes, 1958; Bishop, et al., 1959; Fentress and Doty, 1966; Spencer and April, 1970;
Richards, 1972).

The previously described phenomena are defined, to a large extent, by the stimulus parameters used to evoke them. If two different stimulus parameters are employed to evoke a facilitatory (or depression) phenomenon there has been a tendency to define two phenomena even though the same mechanism may underlie the observed phenomena. For example, can a decision be made as to what relationship exists between the mechanism of PTP and that of "frequency facilitation"? Such a question has not yet been answered. It is likely that any particular system of neural connections displays a number of these phenomena. Richards (1972) provided an example of the complexity of the relationship between the frequency of stimulation and the changing size of the post-synaptic potential. The system employed by Richards was an indirect measure of the complex EPSP's evoked in the olfactory cortex of the guinea pig during repetitive stimulation of the olfactory tract. Stimulating this tract at various frequencies resulted in these alterations in the size of the EPSP:

1) Potentiation of EPSP's occurred following tetanic trains however if the train was continued long enough a depression of the EPSP's occurred.

2) A progressive facilitation of the size of the evoked EPSP's with stimulus repetition at frequencies of stimulation from 5 to 40 stimuli per second was found.

3) At a lower frequency of stimulation (0.5 to 2 stimuli per second) the EPSP's were progressively reduced in size. Therefore, the stimulus parameters determined if PTP, PTD, frequency facilitation, or low frequency depression would occur in this particular pathway.
Kandel and Tauc (1965a, b) described a form of facilitation in the abdominal ganglion of Aplysia that occurred when two consecutive stimuli were applied to heteronymous pathways. This facilitation was not dependent upon properties of the post-synaptic neurones suggesting that this facilitation was also pre-synaptic in origin. However, processes such as "frequency facilitation" and PTP are homosynaptic, that is they can be demonstrated even for a single pre-synaptic terminal. The "heterosynaptic" facilitation described by Kandel and Tauc (1965a, b) is believed to occur as a consequence of a synaptic action upon the pre-synaptic terminal. Pre-synaptic inhibition (heterosynaptic inhibition) was also observed in the ganglion (Tauc, 1965). "Heterosynaptic facilitation" develops after fewer stimulus repetitions than PTP and it has a much longer time course than PTP in the "homosynaptic" pathway (Kandel and Tauc, 1965b; Tauc and Epstein, 1967). "Heterosynaptic facilitation and inhibition" both demonstrated "low frequency depression" (they became less effective with repetition of the stimulus).

Many of the previous forms of "synaptic plasticity" bear a resemblance to habituation and sensitization. However, it is not sufficient to assume that "low frequency depression" is the underlying mechanism of habituation and that sensitization is the result of "frequency facilitation" or "heterosynaptic facilitation". These properties of synapses must be shown to occur in the reflex arc during simultaneous habituation and sensitization of that reflex. A reflex ideally suited for such an examination would be a monosynaptic reflex with large and accessible pre- and post-synaptic neurones. The monosynaptic EPSP recorded from the post-
synaptic neurone should demonstrate the parametric characteristics of habituation and sensitization. If this were found to be the case, then it might be assumed that habituation and sensitization occurred by some form of synaptic depression or facilitation, respectively. A degree of caution must be exercised, however, because no extrapolation can be made about the mechanisms of habituation and sensitization in this reflex to the same behavioural phenomena in more complex systems. Furthermore, if synaptic plasticity is a general characteristic of chemical synapses it is self-evident that habituation and sensitization of a monosynaptic reflex will be the result of synaptic plasticity (assuming the synapse is the site of habituation and sensitization). Therefore, the next question is whether or not synaptic plasticity underlies habituation and sensitization in more complex systems of neurones. In particular, can inhibition play a role in habituation?

Invertebrates are obvious candidates for such studies of habituation and sensitization as the accessibility of their neurones is far greater than that of the vertebrate central nervous system. Habituation and sensitization have been extensively studied in invertebrates (Eisenstein and Peretz, 1973; Pakula and Sokolov, 1973; Wyers, et al., 1973). The most complete study of habituation and sensitization has been performed using the gill withdrawal reflex of Aplysia. Mechanical stimulation of the siphon (mantle, shelf, purple gland, and siphon) caused a reflexive withdrawal of the gill and siphon. Repeated elicitation of this withdrawal resulted in habituation of the response which demonstrated eight of the nine parametric characteristics of habituation (there was a lack
of generalization which is to be expected in a monosynaptic reflex). Habituation of this reflex lasted for periods of several minutes to periods of three weeks (Carew, et al., 1971; Carew, et al., 1972; Carew and Kandel, 1973).

The activation of tactile receptor neurones in the skin of the siphon evoked putative monosynaptic EPSP's in the appropriate motor-neurones, and although some interneurones were activated during stimulation of the reflex the monosynaptic component is likely the most significant component contributing to habituation of this reflex. During habituation of the reflex these EPSP's underwent a decrement in size which also demonstrated eight of the characteristics of habituation. Furthermore, these EPSP's were likely monosynaptic as they were consistently short in latency, persisted in bathing solutions high in divalent ions (known to block the activity of interneurones), and the injection of tetra-ethyl ammonium into the pre-synaptic neurone increased their magnitude and duration (this drug when injected intracellularly increases the duration of the pre-synaptic spike and presumably increases the amount of transmitter released) (Bryne, et al., 1974).

The post-synaptic membrane showed no long term change in its conductance which might have accounted for the decrement of the EPSP (Castellucci, et al., 1970) and there was a one to one relationship between the spikes in the sensory neurone and the EPSP's in the motorneurones. Repeated intracellular stimulation of the sensory neurone evoked constant amplitude spikes, but the EPSP underwent a simultaneous decrement (Bryne, et al., 1974). Therefore, it has been proposed that habituation of this
reflex is a consequence of a form of "low frequency depression" or "synaptic depression" (Carew and Kandel, 1973; Castellucci and Kandel, 1974).

It was found that dishabituation and sensitization of the gill-withdrawal reflex occurred by a mechanism independent of habituation. Dishabituation appeared to be identical with "heterosynaptic facilitation", and a strong stimulus presented to a heteronymous pathway potentiated (dishabituated) the EPSP's evoked in the motorneurones by stimulation of a habituated pathway, and the reflex itself (Carew, et al., 1971). Elicitation of this reflex with noxious stimulation induced periods of response sensitization which lasted up to three weeks (Pinsker, et al., 1973).

There is some evidence to suggest that the gill pinnule of Aplysia is capable of performing a reflexive withdrawal independently of the central nervous system. For example, neurones which may be the sensory and motor components of the reflex are located within the pinnule itself (Peretz and Estes, 1974). Peretz and Moller (1974) demonstrated that the amplitude or general excitability of the pinnule withdrawal was dependent, in part, upon excitatory activity originating from the anterior gill ganglion (a peripheral ganglion). In ganglionectomized preparations (abdominal ganglion removed) the further removal of the anterior gill ganglion resulted in a decrease in response amplitude, a reduced rate of habituation, and prevented dishabituation. This implies that this ganglion may play some role in the establishment of habituation of the pinnule withdrawal response.
Intracellular recording from neurones of the anterior gill ganglion (during habituation of the response) revealed a long latency and polyphasic potential (initial depolarization followed by hyperpolarization) evoked by tactile stimulation of the pinnule. The hyperpolarization phase became progressively greater in duration with successive stimuli. This resulted in a gradual reduction in the tonic activity of some of the neurones in the ganglion. Stimulation of the connective, from ganglion to pinnules, did not cause an observable muscular response.

On this basis, Peretz and Moller (1974) postulated that the anterior gill ganglion contributed to pinnule withdrawal response amplitude by a mechanism of "heterosynaptic facilitation". As well, they suggested that a progressive inhibition of the tonic activity of the gill ganglion leads to a progressive dis-facilitation of the pinnule response which contributed to habituation of this response. The concept that habituation might occur as a consequence of build-up of inhibition was also proposed by Holmgren and Frenk (1961). They recorded a progressive increase IPSP's recorded from neurones in the parietal ganglion of Helix following repetitive stimulation.

Inhibitory synapses are also capable of demonstrating facilitatory phenomena. Tauc (1969) reported that hyperpolarization evoked in certain ganglionic neurones underwent a progressive increase in amplitude and duration with stimulus repetition. There was a direct relationship between the extent of this build-up of inhibition (ILD, inhibition of long duration) and the size of the initial stimulus. A more extensive examination of the facilitation of inhibitory synapses was performed on
neurones in the abdominal ganglion of Aplysia by Waziri, et al. (1969), and it was shown that inhibitory synapses were capable of "frequency facilitation" and PTP. Although this mechanism was considered to be a possible contributory factor to habituation it was rejected because strong stimuli were more effective than weak stimuli in producing the build-up of inhibition and because it was not possible to demonstrate dis-inhibition following a strong shock to a heteronomous pathway. Employing the parametric characteristics of habituation it would seem that if inhibition were to account for habituation, the build-up of inhibition should be greater with weak than strong stimuli and dis-habituation should occur by dis-inhibition. However, there is some latitude in the interpretation of the relationship between stimulus intensity and degree of habituation (see p. 92, this thesis). Furthermore, if dishabituation is in fact a super-imposed "heterosynaptic facilitation" or sensitization of the decremented pathway it is not necessary for dis-inhibition to occur. In fact, it might be argued that this is why the rate of habituation is greater following a dishabituatory stimulus.

Recently, Farel, et al. (1974) have demonstrated that repetitive stimulation of the lateral column of the spinal cord (frog) evoked a monosynaptic EPSP which demonstrated eight of the parametric characteristics of habituation. This decrement of the EPSP suggests that "synaptic depression" occurs in a similar manner in the vertebrate spinal cord. Spencer, et al. (1966c) found that monosynaptic EPSP's evoked in flexor motorneurones of the spinal cord (cat) did not demonstrate decremental behaviour when the flexor reflex was undergoing simultaneous habituation.
They concluded that the polysynaptic component of the flexor reflex habituates but monosynaptic EPSP's evoked in flexor motorneurones do not. However, habituation of monosynaptic spinal reflexes has been reported in man (Dimitrijevic and Nathan, 1973). It seems rather surprising that monosynaptic EPSP's evoked in flexor motorneurones did not display some form of synaptic plasticity. This may be attributable to the stimulus parameters employed by Spencer, et al (1966c) or perhaps it suggests that plasticity is confined to select synapses. Electrotonic synapses apparently do not demonstrate synaptic plasticity (Martin and Pilar, 1964), and Bennett (1968) has suggested that synaptic plasticity is a distinguishing property of the chemical synapse.

In conclusion, it must be stressed that the properties of invertebrate synapses may not be the same as those of vertebrate synapses. However, the many similarities between synapses in invertebrates and vertebrates must justify at least a discussion of these properties. Furthermore, the similarities between habituation of simple reflexes in invertebrates and habituation of spinal reflexes in the vertebrate suggest that a similar mechanism may underlie both forms of habituation (Farel, et al., 1974).
Inhibition: Habituation and Sensitization of the Flexor Reflex

Habituation and sensitization have been demonstrated in numerous sensory systems (Buchwald and Humphrey, 1973), but the flexor withdrawal reflex has been used as a model in order to develop the parametric characteristics of habituation and sensitization. After Sherrington's original description of habituation and sensitization, spinal reflexes continued to be the preferred models by which to study behavioural habituation (Prosser and Hunter, 1936; Spencer et al., 1966a, b, c; Wickelgren, 1967a, b). The central origin of habituation was confirmed either by stimulating afferent nerves directly (Wickelgren, 1967b) or by recording motor responses from the ventral rootlets (Buchwald, et al., 1965), thereby demonstrating that in the mammal neither peripheral receptors nor the neuromuscular junction are critical foci for habituation.

Few studies have utilized the intact preparation when examining habituation of the flexor reflex. The almost exclusive use of the spinal preparation was encouraged by a desire to reduce the reflex to its simplest anatomical components. The objective was to isolate a single site of the origin of habituation (presumably synaptic). The flexor reflex is not an ideal reflex for such a study. For example, studies of the monosynaptic gill-withdrawal reflex of Aplysia or of the disynaptic plantar reflex (Egger and Wall, 1971) seem far more suited to such an approach.

The flexor reflex undergoes habituation in the intact rat (Griffin
and Pearson, 1968b), and man (Dimitrijevic, et al., 1972). Habituation of this reflex differs in the intact animal when compared to the spinal animal. In particular, the amplitude of the flexor reflex is initially greater in the intact animal than in the spinal with a concomitant alteration in the initial rate of sensitization (Dimitrijevic, et al., 1972; Pearson and Wenkstern, 1972). This aspect will be discussed subsequently (this thesis, p. 96). Habituation of the flexor reflex of the rat is also strongly controlled by supraspinal structures such as the frontal cortex (Griffin and Pearson, 1968b) and the dorsomedial thalamus (Griffin, 1970). While the flexor reflex may not be ideal for the study of synaptic plasticity it provides a model of relative complexity sufficient to determine the mechanisms by which habituation and sensitization are controlled. Furthermore, if multisynaptic mechanism contribute to habituation in more complex systems than the monosynaptic reflex, the flexor reflex will allow an examination of these processes.

The flexor withdrawal reflex (FWR) was defined as a reflexive withdrawal of either the hind or forelimb evoked by stimulation of the skin of the limb or its afferent nerves (Sherrington, 1910). The hindlimb displays a flexion of the hip, knee, and ankle; the forelimb a flexion of the elbow, shoulder, and wrist. The FWR may be elicited in the intact, decerebrate, or spinal animal, although the response differs somewhat in each of the preparations (Graham Brown, 1912). Muscles of the limbs which undergo contraction during the FWR are defined as flexor muscles (Sherrington, 1910), and regardless of the strength of stimulation only a fixed subset or group of muscles will contract. Spread of contraction to
other muscles will not occur. The flexor muscles are listed in Table I (Sherrington, 1910:31).

Reflexive relaxation of muscles also occurs during flexion and these muscles are defined as extensor muscles. This functional distinction between flexor and extensor muscles does not always correspond to classical anatomical nomenclature.

The receptive field of the FWR refers to that area of the skin of the limb wherein applied stimuli will evoke withdrawal. Noxious stimuli are by far the most effective stimuli in eliciting the reflex. Within the receptive field there exists a hierarchy with regard to the threshold of flexor elicitation, and the threshold is lowest in the skin of the foot and it increases proximally along the limb. It is greatest in the skin of the thigh. Evocation of the reflex from any point within the receptive field produces a stereotyped withdrawal. This flexion is composed of a "group of reflexes almost identical in form which when concurrent combine in harmonious action on the same common paths." (Sherrington, 1910).

Stimulation of the dorsal roots also evokes FWR of the muscles of the segment activated. In this regard, flexion of the ankle occurs most strongly with stimulation of caudal dorsal roots while hip flexion occurs most strongly with stimulation of more cephalic roots. However, stimulation of any dorsal root will evoke a full FWR but with the greatest strength of contraction prevalent in the muscles of the segment directly innervated by the stimulated root. This tendency extends to individual afferent nerves, and if a stimulus of increasing intensity is applied to
<table>
<thead>
<tr>
<th>Flexor Muscles</th>
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<tbody>
<tr>
<td>Ilio-psoas</td>
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<tr>
<td>Pectineus (slight)</td>
</tr>
<tr>
<td>Sartorius (part inserted into patella)</td>
</tr>
<tr>
<td>Tensor fasciae femoris</td>
</tr>
<tr>
<td>Rectus femoris</td>
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<tr>
<td>Gracilis</td>
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<td>Semitendinous</td>
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<td>Posterior biceps femoris</td>
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<td>Tenirissmus</td>
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<tr>
<td>Tibialis anticus</td>
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<tr>
<td>Peroneous longus</td>
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<tr>
<td>Extensor longus digitorum</td>
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to the skin of the foot (or to the afferent nerve supplying the foot) flexion will occur initially at the ankle, then at the knee, and finally at the hip.

Sherrington (1910) makes a distinction between two types of flexor reflexes. Withdrawal of the limb in response to a noxious (painful) stimulus results in a relatively long lasting response. This is the FWR as a protective or "nociceptive" reflex. This reflex is elicitable from a large surface area of the limb and also from the underlying muscles and fascia. The flexor reflex can also be evoked from subcutaneous structures of the limb in response to non-noxious stimuli. Thus, a distinction is made between a nociceptive flexion and a flexion evoked by weak stimuli. The latter flexion, it was suggested, is a component of reflex stepping while the former is a protective reflex allied to the response of the animal to painful stimulation. The "nociceptive" FWR was dominant as described by Sherrington (1910:73):

The peculiar character, in fact the "adequacy" of its stimulus lies in intensity. It is prepotent when pitted against the locomotor reflex: and this is a further mark of its nociceptive character, since nociceptive reflexes like painful sensations are habitually "dominant" in competition against others.

Lloyd (1943) classified a number of subcomponents of the FWR on the basis of the type of afferent activated to elicit the reflex. Activation of group I afferents (either by direct stimulation of the muscles nerves or by tendon tap) caused a contraction in flexor muscles if the nerve originated from that flexor muscle. This was not the FWR described by Sherrington (1910). Stretching of a flexor muscle caused contraction of that muscle due to activation of group II muscle nerves. Stimulation of
group II and III nerves from muscle, skin, and joints evoked a general flexor reflex. However, the activation of group III cutaneous afferents (Delta flexion) and group IV afferents leads to a FWR comparable to that observed by Sherrington (1910) that is, the "nociceptive" FWR.

Stimuli that activate high threshold afferents also maximally activate low threshold afferents unless an attempt is made to selectively block them. Mendell and Wall (1964) reported that selective stimulation of group IV afferents did not elicit any reflex discharge or the so called "C (group IV) reflex." It was suggested that group IV afferents merely facilitated the influence of low threshold afferents by a mechanism of PAH. In direct contradiction to these results many others have found that group IV afferents were capable of eliciting a discrete FWR and/or PAD (Franz and Iggo, 1968; Clark, et al., 1935; Franz, et al., 1966; Laporte and Bessou, 1958; Alderson and Dowman, 1960; Jänig and Zimmerman, 1971).

More recent evidence suggests that PAD and PAH can be evoked in the terminals of any particular group of afferents provided that group of afferents is itself stimulated (Mendell, 1972; Whitehorn and Burgess, 1973). The "C flexor reflex" was rapidly blocked by anaesthetic doses of ether, ethyl chloride, or sodium pentobarbital, but it was less likely to be reduced in animals anaesthetized with chloralose (Franz and Iggo, 1968). This may account for the failure of Mendell and Wall (1964) to record reflex discharge following stimulation of group IV afferents as their animals were anesthetized with sodium pentobarbital. It seems likely that a distinct "C flexor reflex" does exist even in the absence of low threshold afferent activity. This distinction may be somewhat arbitrary
as it has been shown that nociceptors are capable of evoking a powerful and independent discharge of dorsal horn interneurones (Iggo, 1974).

The FWR in the intact animal may include certain "long reflexes" such as the "spino-bulbo-spinal" flexion reflex. Apparently activation of afferents capable of eliciting the FWR also activates a pathway to the brain stem reticular formation that in turn can either excite or inhibit flexor motorneurones and the polysynaptic flexor pathway (Shimamura and Aoki, 1969). Spinal transection and injection of sodium pentobarital blocked "spino-bulbo-spinal" reflexes.

Groves and Thompson (1970) described two theoretical (inferred constructs) processes of behavioural and reflex decrement which occur as a consequence of regular stimulus repetition. The first is "habituation" to relatively weak stimuli and the other is "habituation of sensitization" which occurs with repetition of relatively intense stimuli. The many systems that demonstrate habituation as a consequence of "synaptic depression" tend to suggest that "habituation" occurs by a similar mechanism. It is not clear if "habituation of sensitization" is merely a superimposition of sensitization and "habituation" or whether it represents an altogether different process (Groves and Thompson, 1970).

Habituation of the FWR of the spinal cat has been employed as a model in which to determine whether or not inhibition might be responsible for this observed response decrement (Spencer, et al., 1966c). The decrement of the polysynaptic EPSP's evoked in flexor motorneurones was associated with habituation of the flexor reflex, but the injection of strychnine or picrotoxin was found to be ineffective in disrupting
habituation of the ventral root electrotonus. This was tested in both
decerebrate-spinal preparations and spinal animals anaesthetized with
pentobarbital. The "nociceptive" FWR described by Sherrington (1910)
is not likely to be that observed by Spencer et al., (1966 a, b, c). For
example, Spencer et al., (1966a) indicated that group II and some group
III afferents were involved, but it was never clear which afferents were
activated at any particular stage of the experiments. A failure by
various authors to indicate either the intensity of stimulation or
the afferents activated by that stimulation of the skin (Spencer et al.,
1966a, b, c; Wickelgren, 1967a; Groves and Thompson, 1973) have made it
impossible to determine the contribution of high threshold afferents to
habituation and sensitization of the FWR. The exclusive use of the
spinal preparation has also negated the possibility of determining if
any contribution is made by supraspinal structures and/or "spino-bulbo-
spinal" flexor reflexes.

The injection of strychnine or picrotoxin resulted in an elevation
of the response amplitude of the recorded ventral root electrotonus.
In compensation, Spencer et al. (1966c) lowered the stimulus intensity
for those animals which received an injection of strychnine or picrotoxin
but not for control animals. One of the parametric characteristics
of habituation (see this thesis, p. 14) is that a reduction in stimulus
intensity will result in a greater rate of habituation. This raises the
question of whether any impairment of habituation caused by the drugs
might have been counteracted by the opposite influence of reducing stimulus
intensity. The justification for such a manoeuver can also be questioned
on the basis that an artificially induced increase in response amplitude does not necessarily alter the rate of habituation of the FWR (Griffin and Pearson, 1968a; Pearson and Vickars, 1974) which negates the logic of reducing stimulus intensity for treated animals. While this study demonstrated that habituation can continue to take place following the injection of strychnine or picrotoxin the possibility remained that inhibitory mechanisms might contribute at least to some forms of response decrement.

Wall (1970) postulated that habituation of the FWR might be due to PTP of inhibitory synapses in the flexor reflex pathway. PTP of both pre-synaptic and post-synaptic inhibition has been reported in the mammalian spinal cord (Spencer and April, 1970; Schmidt, 1973). It does not seem likely, however, that inhibitory synapses should undergo PTP with stimulus parameters which do not produce PTP of excitatory synapses. This is certainly the case in invertebrates. Therefore it seems more probable that habituation to low intensity stimulation does not involve the build-up of inhibitory activity. Strong stimuli that cause sensitization might, on the other hand, provoke a build-up of inhibitory and excitatory activity. This proposed build-up of inhibition might limit sensitization and determine the rate of its decrement with repetition of the stimulation.

A process of inhibitory build-up might be found at a variety of levels of the central nervous system. These inhibitory mechanisms might be inherent to the spinal segments directly involved with the flexor reflex, however, intersegmental inhibition originating from outside these
segments might also contribute. Supraspinal inhibition might be manifest through modulation of "spino-bulbo-spinal" reflexes or perhaps more directly through an alteration in the tonic influence of inhibition originating from the bulbar reticular formation (originally described by Magoun and Rhines, 1946). The bulbar reticular formation (and for that matter the spinal cord) is also under the control of suprabulbar inhibitory systems (Pompeiano, 1973). In summary inhibition of the FWR could originate from various levels of the nervous system such as: 1) intrasegmental 2) intersegmental 3) supraspinal 4) suprabulbar.

This study was designed to explore the contribution of inhibitory mechanisms to habituation and sensitization of the FWR. This was accomplished by studying the reflex in the intact, decerebrate, and spinal rat in order to isolate the contribution of various levels of the central nervous system. Pharmacological isolation was achieved by eliminating post-synaptic inhibition (infusion of strychnine), pre-synaptic inhibition and some forms of post-synaptic inhibition (infusion of bicuculline), and bulbo-spinal inhibition mediated by serotonergic systems (pre-treatment with para-chlorophenylalanine (p-CPA) which is a selective depletor of brain serotonin, infusion of the drug methysergide which antagonizes serotonergic transmission, and by lesioning the nucleus raphe dorsalis (n.r.d.) one of the brain stem nuclei particularly high in content of serotonin and which when stimulated causes inhibition of the flexor reflex). Segmental mechanisms were also examined by comparing the effect of various levels of spinal transection and by subjecting the spinal cord to a temporary period of asphyxiation with the objective of causing
relatively selective destruction of inhibitory spinal interneurones.
CHAPTER II

METHODS: SECTION I

The Flexor Reflex

Animal Subjects

Albino rats (Wistar strain) were employed as experimental preparations in all cases. Male rats (200 to 700 g) were used exclusively in order to avoid any inhomogeneity in sample populations due to sexual differences. An attempt was made to avoid rats that showed any indication of respiratory disease. This was critical, as the use of ether as an anaesthetic greatly accentuates respiratory distress. Conscious rats had to be restrained following the implantation of chronic stimulating and recording electrodes and during testing of flexor reflexes. Restraint of animals during the period after electrode implantation, and prior to testing, required that some specific attention be paid to the care of the rats (p.57, this thesis).

Reflex Response

Electrical stimuli were delivered, subcutaneously, to the hindpaw of the rat in order to evoke a defensive withdrawal of the limb. The representative measure of flexor reflex response used in these experiments was the integrated electromyographic (EMG) discharge recorded from the posterior head of the biceps femoris muscle. The anterior head of this muscle is extensor in function, but the posterior head participates in functional flexion at the knee (Sherrington, 1910).

The integrated EMG may correspond either to the tension developed in the muscle or the activity in the motor nerve (electroneurogram) both of which are considered adequate measures of reflex response (Steiner,
The integrated EMG of the posterior head of the biceps femoris has previously been correlated, with a high degree of significance, to the isometric tension developed in this muscle. Habituation of the isometric tension and the integrated EMG, following repetitive stimulation, was also very similar (Griffin and Pearson, 1967). It can be concluded that the integrated EMG of the posterior head of the biceps femoris is an acceptable measure of the amplitude of the flexor reflex as described by Sherrington (1910).

Stimulus Delivery and Response Recording

Uniform, single pulse stimuli (5 msec. duration), were delivered by means of a Devices stimulator which was triggered at regular intervals by a Devices Digitimer. An integration unit was also appropriately triggered by the Digitimer. The electromyographic signals were amplified (Tektronix preamplifier type 122) and quantified by full-wave rectification followed by electronic integration. The integral values of the EMG were displayed on a digital voltmeter and were recorded by the experimenter. EMG activity was integrated for a period of 250 msec. (10 to 260 msec. after initiation of the stimulus pulse) subsequent to each stimulus pulse. Evoked activity was simultaneously monitored on a Tektronix 565 oscilloscope. Just prior to testing, and immediately following testing, a reading of background activity was recorded. The average background value was always subtracted from the evoked value of the EMG. The corrected values of the EMG were then used for an assessment of the amplitude of the flexor reflex.
Stimulus trains (0.5 msec. pulse duration, 50 pulses/sec., 0.5 sec. train duration) were given in a manner similar to the delivery of single pulses. However, a Devices Frequency Generator was added to the circuit in order to trigger uniform stimulus trains rather than single pulses. The Devices generator was triggered by the Digitimer which in turn triggered the Devices stimulator. The EMG activity was integrated for a 500 msec. period (10 to 510 msec.) after termination of the stimulus train. This integral is a measure of "after-discharge" as described by Sherrington (1906). It was not possible to record evoked activity during the stimulus train due to the presence of large stimulus artifacts. A schematic diagram is given in figure 4.

Stimulating Electrodes

Chronic stimulating electrodes consisted of two lengths of diamel coated silver wire (0.007 in. dia.). The ends of these were bared for about 0.5 cm. and surgically inserted into the skin of the hind-paw, always in the same location, i.e., one was placed between the third and fourth digit and the other was inserted on the lateral aspect of the fifth digit.

Recording Electrodes

Recording electrodes were constructed of diamel coated silver wire (0.010 in. dia.) bared for approximately 0.5 cm. at the ends. The bared ends were inserted, superficially, into the posterior head of the biceps femoris muscle (see figure 5). Care was taken to ensure that
Figure 4. Schematic diagram of the stimulating and recording equipment.
Figure 5. Anatomical location of the biceps femoris muscle of the rat in relationship to other muscles. (B.F. 1, anterior head of the biceps femoris; B.F. 2, posterior head of the biceps femoris; S.T., semitendinosis; Q.F., quadriceps femoris; G.M., gluteus maximus; G., gastrocnemius; T.A., tibialis anterior (antcus). The gluteus maximus had been completely removed to expose the underlying muscles. The location of EMG electrodes is indicated by the E.)
the electrodes did not penetrate through the biceps femoris into underlying extensor muscles. The ends of the wires were positioned about 3 mm. from each other. Recording (and stimulating) electrodes were always implanted one day prior to testing of reflexes.

Restraint and Care

Following the implantation of stimulating and recording electrodes it was necessary to restrain rats in order to prevent them from damaging or pulling out the electrodes. Rats were placed in Bollman restraining cages (see figure 6) with free access to food and water. The maximum period of restraint was two days. Prior to testing of the reflex rats were placed into a dark and soundproof box for an acclimatization period of 20 min. Testing of flexor reflexes took place with the rats inside this box. Special care was taken with spinal rats to ensure that the urinary bladder of each rat was emptied at least twice each day and immediately prior to testing of the reflex.

Anaesthetics

In all experiments it was necessary to anaesthetize rats with ether (Mallinckrodt Chemical Works). Ether anaesthesia was employed for all acute surgical procedures such as electrode implantation, decerebration, spinal ligation, and spinal asphyxiation. The depressant action of ether upon the centralnervous system is well known. Therefore, at least 24 hours were permitted to elapse following termination of the ether anaesthesia and the beginning of testing. The only exception was
Figure 6. A rat in a Bollman restraining cage. Flexor reflexes were tested while the rat was restrained in this manner.
in experiments in which a comparison was made between acute spinal rats and decerebrate rats when only 90 minutes separated the termination of the application of ether and testing of the reflex. An intact rat usually recovered from ether anaesthesia within this time period (90 min.) but residual anaesthesia may have been present during testing of the reflex. Special care had to be taken to give the ether for short and frequent periods during the induction of spinal asphyxiation. Often the rats had to remain anaesthetized for periods of up to 1 hour.

The only other anaesthetic employed was urethane (ethyl carbamate, 25% solution in 0.9% saline). Barbituates were avoided because of their antagonistic actions upon "C flexor reflexes", "after-discharge", and spontaneous activity within the spinal cord (Beecher, et al., 1939; Wall, 1967a; Franz and Iggo, 1968). As inhibition was examined in this study, chloralose was not used because of its tendency to prolong primary afferent depolarization (Schmidt, 1963, 1973). It must be admitted that little is known of the actions of urethane upon the central nervous system although it may have a considerable depressant action upon spinal activity (Schmidt, 1963). This anaesthetic was not given in the conventional manner (Intraperitoneally). Rats were initially anaesthetized with ether in order to permit the implantation of tracheal and jugular cannulae. Urethane was then given intravenously to a total dosage of 0.25 g/kg or less, and anaesthesia was not maintained for long periods after testing. This light level of anaesthesia was necessary so that motor responses (EMG) were not totally suppressed.
Surgical Procedures

Spinal Ligation ( Transection )

A laminectomy refers to the surgical exposure of the spinal cord by removal of a vertebral posterior arch. In the rat this is a relatively simple, although somewhat delicate procedure. An incision of the skin (5 to 10 cm.) was made over the appropriate region of the spinal cord. The muscles and fasciae of the back were then grossly separated from the vertebral column by blunt dissection. Little or no bleeding occurred. A pair of retractors was used to hold the large muscles away from the spinal column, while the muscle attachments to the column were scraped away using the back of a scalpel blade. At least 2 vertebrae were cleared of all soft tissue on their dorsal surfaces. The points of a pair of bone forceps were then inserted intervertebrally, and the dorsal (posterior arch) of the appropriate vertebra was gradually clipped away. A previously blunted surgical needle (1/2 in., half circle), with attached dental floss, was slipped under the cord without causing visible damage to the cord or dura. The dental floss was then drawn tight and knotted several times, constricting and effectively transecting the cord. The dura remained intact but the region of the cord under the ligation is forced caudal and cephalad to the ligation. No doubt can exist that the cord was transected. This method of transection reduced vascular disruption in comparison to actually cutting the cord. In addition, the cord caudal to the ligation was left in an enclosed dural sac. This was necessary in experiments
in which asphyxiation of the cord was to be induced. If the rat was to be kept chronically, a small amount of antibiotic was applied to the wound before the various layers of muscle and skin were sutured closed.

Asphyxiation

Both control and experimental rats had their cords ligated at the level of the second thoracic vertebra. A hypodermic needle (26 gauge) was inserted into the dural sac formed caudal to the ligation. Experimental rats were subjected to 22 min. of asphyxiation. With experimental rats the needle was connected, by means of polyethylene tubing, to a manometer bottle containing isotonic saline heated to 40°C. The pressure in the bottle was gradually increased, forcing saline into the dural sac, raising the intra-dural pressure, and causing occlusion of the spinal vasculature (Van Harreveld, 1943). The pressure was increased to 240 mm. Hg. over a period of 1 min. and was maintained at this level. Tremor and extension of the hindlimbs occurred in most rats when the pressure reached 90 to 120 mm. Hg. If this did not occur, the rat was not included in this study.

Decerebration

The skull of the anaesthetised rat was placed in a Student's stereotaxic holder (David Kopff) and an incision (2 to 4 cm.) was made across the skin of the dorsal surface of the head. The fasciae and underlying layers of tissue were cleared off the top of the skull in a region between the parasagittal ridges. Lambda and bregma were identified. At a point mid-way between lambda and bregma the muscles attached to the parasagittal ridges were cut free from the skull. Using
a high speed dental drill slits were cut into the bone extending from the base of the skull to its dorsal surface, from both sides. The bone was not cut in the region directly overlying the parasagittal sinus. The dura was exposed undamaged and a blunted surgical needle, with dental floss, was passed under the ventral surface of the brain. Decerebration was accomplished by tightly knotting the dental floss to the remaining dorsal piece of bone. Provided this procedure was carefully performed little blood loss resulted as the major vessels and sinuses were ligated rather than cut. Some rats failed to breathe spontaneously after decerebration and these rats were not used in this study. In decerebrate rats the carotid blood pressure was monitored using a Statham pressure transducer and a transducer convertor (S. E. Laboratories, Ltd.). Blood pressure was recorded on a Gilson pen recorder.

Experimental Procedures

Strychnine Sulphate

Strychnine sulphate (Sigma Chemical Co.) is soluble in physiological saline, and the drug was merely dissolved in the required volume of saline. The pH was adjusted to 7.4 if required.

Bicuculline

Bicuculline (K and K Laboratories, Inc.) was dissolved in saline by adding several drops of HCl (5N) to acidify the solution. The solution was then neutralized with NaOH (5N), prior to infusion.

Methysergide

Methysergide (Sandoz Pharmaceuticals) was acidified to facilitate
dissolution in saline. This drug was neutralized prior to infusion.

Infusion of Strychnine, bicuculline, and methysergide

Intact rats had jugular cannulae implanted one day prior to testing. Single pulse stimuli of 5 or 20 v stimulus strength were presented as series of 300 stimuli with an inter-stimulus interval of 5 sec. The infusion of a drug (or saline) was begun 20 min. prior to testing of the reflex (3 ml/hr.) and continued until the end of testing 45 min. later. Control rats received an infusion of saline but experimental rats received an infusion of:

- Strychnine sulphate (30 μg/kg/min.)
- Bicuculline (60 μg/kg/min.)
- Strychnine sulphate (15 μg/kg/min.) and bicuculline (30 μg/kg/min.)
- Methysergide (56 μg/kg/min.).

Pre-treatment with P-CPA

P-CPA (Pfizer, Inc.) suspended in saline (30 mg/ml) was injected intraperitoneally twice prior to testing. The initial injection was given 4 days prior to testing of the reflex and was followed by a second injection 2 days prior to testing. Each injection contained a dosage of 300 mg/kg. The procedure for preparation of this drug for injection was taken from Koe and Weissman (1966). Control rats received injections of saline. Rats received 300 stimulations with an inter-stimulus interval of 5 sec. (stimulus strength 5 or 20 v).

Rats with Lesions of the n.r.d. (nucleus raphe dorsalis)

The lesion electrode was lowered into the region of the n.r.d. Using a Students stereotaxic rat holder and attached electrode holder.
The position of the electrode tip was determined as follows: A (frontal plane), 160; L(sagittal plane), 0; H(horizontal plane), 800 (König and Klippel, 1963). Current was applied using a Wyss coagulator in the case of experimental rats but not for control rats. Three days following this procedure, recording and stimulating electrodes were implanted. Testing of the flexor reflex was carried out one day later. Rats were stimulated 300 times (inter-stimulus interval 5 sec.) with a stimulus strength of 5 v. The rats were perfused with fixative and sections of the midbrain were cut on a freezing microtome and stained with cresyl violet to verify the location of lesions.

Comparison between Decerebrate and Spinal Rats

Spinal ligation (T₂) was performed upon control rats and decerebration upon experimental rats as earlier described. Ninety min. later flexor reflexes were tested using stimulus trains (20 v or 60 v, inter-stimulus interval 10 sec.). Prior to decerebration, polyethylene cannulae (PE 60) were inserted into the right carotid artery of each rat. Arterial blood pressure was monitored and recorded during testing of flexor reflexes. The range of mean blood pressure was 80 to 150 mm. Hg. After testing rats were perfused with fixative and their brains were removed to determine the level and completeness of decerebration.

Spinal Rats and Asphyxiation of the Cord

Control rats and experimental rats had their cords ligated at the level of the second thoracic vertebra (T₂). Experimental rats were subjected to 22 min. of asphyxiation of the spinal cord. Two days after spinal ligation the flexor reflex was tested using stimulus trains
(20 v or 60 v, inter-stimulus interval 10 sec.).

Infusion of Strychnine

A group of spinal rats (T₅ ligation) was tested using stimulus trains. Control rats received an infusion of saline whereas experimental rats received an infusion of strychnine sulphate (30 µg/kg/min.) administered in the same manner as it was for intact rats. Two days after spinal ligation the flexor reflex was tested (20 or 60 v, inter-stimulus interval 10 sec.).

Infusion of Strychnine or Bicuculline

A group of spinal rats (T₅) was tested using single pulse stimulation. The day following spinal ligation jugular cannulae were implanted. Two days following spinal ligation the flexor reflex was tested (20 v, inter-stimulus interval 5 sec.). Each rat received two series of 300 stimuli separated by 2 hours. During the second series of stimuli control rats received an infusion of saline while experimental rats received an infusion of strychnine sulphate (30 µg/kg/min.) or bicuculline (60 µg/kg/min.). A further group of rats received saline during both the first and second series of stimuli.

Level of Spinal Transection

One group of rats had their cords ligated at the level of the seventh cervical vertebra whereas another group was ligated at the level of the tenth thoracic vertebra. The following day the flexor reflex was tested using stimulus trains (20 v or 60 v, inter-stimulus interval 10 sec.).
Injection of Strychnine in Anaesthetized Rats

Recording and stimulating electrodes were implanted as previously described. One day later, rats were anaesthetized with ether while jugular cannulae were inserted. Ether anaesthesia was discontinued and replaced by urethane anaesthesia (0.25 g/kg). Single pulse stimulation (60 v, inter-stimulus interval 1.5 sec.) was employed and EMG activity was integrated for 550 msec. after each stimulus. Two series of 120 stimuli, separated by 2 min., were applied to both control and experimental rats. One min. prior to the second series of stimuli, the control rats received an intravenous injection of saline (1 ml), whereas experimentals received an injection of strychnine sulphate (0.4 mg/kg, 1 ml.).
SECTION II

Spinal Interneurones

Flexor Reflex Interneurones

The flexor reflex is a polysynaptic reflex. However, there is virtually no evidence, either anatomical or physiological, that specifies which interneurones are directly involved in the flexor reflex (Wall, 1970, 1973). The response of spinal interneurones in the lumbar region of the cord has been correlated with the activity of the flexor reflex (Groves and Thompson, 1973). The defining characteristic of flexor reflex interneurones is not their capacity to evoke activity (or inhibition) in flexor motorneurones, but their property of response to flexor reflex afferents. Therefore, the spinal interneurones that respond to flexor reflex afferents may not project to motorneurones and may contribute to ascending afferent tracts, etc. Dorsal horn interneurones, in particular, have many collateral axons which branch extensively and which may terminate at the motor nuclei or at supraspinal structures (Matsushita, 1969). Flexor reflex interneurones may also project to various structures in the central nervous system.

Apparatus and Procedures

The use of microelectrodes to record spinal activity requires substantial restraint of the spinal column. This was accomplished using a spinal column holder designed specifically for the rat. It is composed
of a heavy metal base with two adjustable holders. One holder is used to fix the position of the rat's hips. Attached to the second holder is a towel clamp (Irex, Co. Ltd.) which can be used to grasp a dorsal vertebral spine or process. The towel clamp is connected to, and can be adjusted by a Narishige micromanipulator. Another such manipulator, also mounted on the second holder, serves to hold the microelectrode (Figure 7). The anterior end of the rat was suspended from a plexiglass ring which was sutured to the skin of the back in order to form an oil bath over the exposed cord. To this ring was attached a metal rod which was in turn clamped to a standard ring stand. The snout of the rat was also tied to this support rod.

Rats were anaesthetized with ether in order to permit the implantation of tracheal and jugular cannulae. Intravenous doses of 0.5 ml (25% solution) of urethane were then given repeatedly over a period of 30 min. The rats remained anaesthetized for the remainder of the experiment usually without subsequent injections of urethane. The total dosage was 0.5 g./kg. Rats were also paralysed with gallamine triethiodide (Flaxedil, 10 mg./ml) to a total dosage of 60 mg./kg. No reflex responses could be elicited from these animals even with very intense stimuli.

A laminectomy was performed to expose the lumbar enlargement. The rat was then placed in the spinal cord holder, the dural was removed, and the exposed cord was immediately covered with oil. The temperature of this oil bath and rectal temperature were monitored and kept at 37° C. Following paralysis with Flaxedil the rats were maintained on
Figure 7. Photograph showing the rat holder that was used to hold animals during the recording of interneurones in the spinal cord.
a rodent respirator (300 cc/min., positive pressure 0.5 to 1.0 cm. water). A pneumothorax was then performed in order to prevent respiratory movements from being transferred to the cord.

Stimulating electrodes (21 gauge, hypodermic needles) were inserted into the skin of the hind-paw on the same side as the recording site. Occasionally electrodes were also inserted into the contralateral hind-paw in a location homologous to that of the ipsilateral stimulating electrodes. A microelectrode was placed into the micromanipulator and connected to a cathode follower by means of silver wire. The cathode follower was grounded to the rat. The rat, spinal cord holder, lamp, and cathode follower were located inside a double-lined, copper wire cage fully surrounded on all sides except for an opening at the front of the cage which allowed the experimenter access to the preparation. The signal from the cathode follower was fed into a Tektronics 3A9 differential amplifier and a Tektronics 565A oscilloscope from which single unit responses could be monitored. The oscilloscope could be triggered simultaneously with the cutaneous stimulation. Single pulse stimuli or trains of stimuli were delivered as previously described. Spikes were counted using an EKG Ltd. rate meter which had the capacity to select the highest amplitude spikes. A Tennelec digital rate meter was triggered following the termination of the stimulus artifact and this rate meter could be set to count spikes for a pre-selected interval after the stimulus. The number of spikes/interval and the unit response itself were displayed on a Bell and Howell ultraviolet recorder. The power line was supplied with a voltage transformer in order to eliminate line interference.
The depth of penetration of the microelectrode was noted whenever a unit was recorded. Some recording locations were marked by iontophoresis of the dye pontamine sky blue (4 to 10 μA, 1 to 2 min.). Following termination of the experiment the rat was perfused with fixative and the cord was removed for histological verification of the recording sites.

Microelectrodes

Hard glass tubing (Pyrex Lab. glassware: tubing lab. std. wall - 2 mm.), previously cleaned in chromic acid, was pulled and broken back to tip diameters ranging from 1 to 2 μ with resistances ranging from 2 to 7 MΩ. These electrodes were then filled with NaCl (4M) or a 2% solution of pontamine sky blue in sodium acetate (0.5M). Filling was accomplished by injecting the desired solution into the barrel of the electrode. The tip was then broken to the required tip diameter and the electrode tip filled by capillary action. Small bubbles could often be teased out of the tip with lengths of very fine glass. Electrodes which were to be filled with pontamine sky blue often filled more readily if the tip was broken prior to injection of the solution into the barrel of the electrode. Saline electrodes tended to have lower tip resistances (2 - 5 MΩ) than electrodes filled with the dye solution (4 - 7 MΩ). Tip resistances were measured using a full wave generator.

Preparation of Pontamine Sky Blue Solution (from Hellon, 1971)

1) To a 0.5 M solution of sodium acetate a quantity of pontamine sky blue (ESBE Laboratories), sufficient to make a 2% solution,
was added. The dye is readily soluable.

2) This solution was then filtered (0.4 μ millipore filter) at least three times. It was then centrifuged (Beckman micro-sample centrifuge) for 5 min. The supernate was the final solution used for filling electrodes.

Cleaning of Glass Tubing

Tubing used to construct electrodes had to be absolutely clean in order to permit filling of the electrode. This was accomplished by completely immersing the glass in a solution of chromic acid (potassium dichromate in sulphuric acid) for a period of 2½ hr. The tubing was rinsed at least 3 times with distilled water and oven dried. The tubing was left in a sealed container until required.
SECTION III

Fixation of the Brain and Spinal Cord

Fixation of the central nervous system for verification of lesion and electrode tracts, level of decerebration, and location of pontamine sky blue markers was accomplished by infusing fixative into the heavily anaesthetized rat. A thoracostomy was quickly performed and the heart freed from surrounding tissue. A length of polyethylene tubing (PE 60) connected to a pressure bottle containing saline, was inserted through a slit made in the left ventricle and ultimately into the aorta. A small slit was made in the right ventricle to allow fluid to escape from the vascular system. A pressure of 200 to 300 mm. Hg. was applied to the pressure bottle for a period of 1 to 2 min. producing a saline flush of the vascular system. Without removing the polyethylene tubing it was reconnected to a second pressure bottle containing a solution of 10% formol-saline. Pressure was applied to the second bottle and the rat was perfused with fixative for 20 min. The desired section of the brain or cord was removed and placed in 10% formol-saline for 24 hrs. Sections were then cut on a freezing microtome and stained with cresyl violet (or safranin-0 for pontamine sky blue markers).
CURRENT RESEARCH ETHICS DEMAND THE USE OF CONVENTIONAL STATISTICAL TESTS. THE VALIDITY OF ANY STATISTICAL PROCEDURE IS DIRECTLY DEPENDENT UPON WHETHER OR NOT THEassumptions OF THAT STATISTICAL TEST ARE VALID FOR THE PRESCRIBED EXPERIMENTAL CONDITIONS. STATISTICS CAN BE A POWERFUL AID IN DETERMINING DIFFERENCES BETWEEN THE EXPERIMENTAL AND CONTROL POPULATIONS. HOWEVER, IF STATISTICAL TESTS ARE APPLIED WITHOUT A KNOWLEDGE OF THE TEST'S INHERENT ASSUMPTIONS A TOTALLY ERRONEOUS CONCLUSION MAY BE DERIVED. THE LEVEL OF MATHEMATICAL COMPREHENSION REQUIRED TO DETERMINE IF THE ASSUMPTIONS OF A TEST ARE UPHOLD IS OFTEN FORMIDABLE, ESPECIALLY WHEN THE TEST STATISTIC IS BASED UPON A COMPARISON OF A SAMPLE POPULATION TO A HYPOTHETICAL "NORMAL" DISTRIBUTION. FOR THIS REASON A SHORT DISCUSSION OF THE CRITERIA BY WHICH DECISIONS OF STATISTICAL APPROPRIATENESS ARE DERIVED IS APPROPRIATE.

STATISTICS IS NOT SIMPLY THE STUDY OF PROBABILITIES. STATISTICS IS THE MATHEMATICAL COMPARISON OF A CHARACTERIZED PROBABILITY MODEL WITH SAMPLED DATA IN ORDER TO MAKE INFERENCES ABOUT THE PROBABILITY OF OCCURRENCE OF ANY PARTICULAR EVENT. Seldom can the actual or "real" population distribution be described in detail, nor can any single probability model describe all possible population distributions. The best
known probability model is the "normal" or "Gaussian" distribution. It is not the only model nor is it necessarily the most appropriate model. Laplace and Gauss both described the normal distribution in relationship to the observed distribution of errors of astronomical measurement. These errors intuitively seemed to occur as frequently below the true value (a theoretical and by definition unmeasureable quantity approximated by the sample mean) as above and the larger the magnitude of any error the less frequently it would occur. Thus, the error appears to be distributed symmetrically and unimodally about the true value. The frequency of occurrence of these errors decreases monotonically as the error deviates in magnitude from this true value. If the magnitude of the errors is of the size (x) and if the relative frequency of occurrence of an error of magnitude (x) is (y) then such a distribution can be described as follows:

1) \( \frac{dy}{y} = -C \times dx \)
2) \( \ln y = -\frac{Cx^2}{2} + k \)
3) or \( y = e^{-C(x^2/2)} + k \)
4) or \( y = Ke^{-Cx^2/2} \)

Equation (4) gives the probability density of a normal distribution arrived at by the method used by Laplace and Gauss (C + k, constants). Astronomical observations were found to correspond with considerable regularity to this distribution. Quetelet, the Belgian astronomer, found that variations in anthropological measures of military personnel also corresponded to the normal distribution. For example, the variations in the heights of men were found to be distributed normally about a
hypothetical true population mean. From this correspondence of dis-
tributions with widely separated experimental measures there was a
tendency to elevate the normal distribution to the status of a law.
Seemingly chaotic events could be described by the order of the normal
distribution. Fisher described this attempt to dogmatize the normal
distribution as follows (1923:181):

All sorts of measurements were taken and the rapidly growing
collections of statistical data relating to economic and
social conditions as recorded by various government statis-
tical bureaus furnished material for further investigations.
But unfortunately in all these investigations the Gaussian
error law came to act as a veritable Procrustean bed to which
all possible measurements should be made to fit....Statist-
ticians could not conciliate themselves with the thought of the
possible presence of "skew" frequency curves, although
numerous data offered complete defiance to the Gaussian dogma
and exhibited a marked skew frequency distribution. Sup-
posedly great authorities argued naively that the reason the
data did not fit the curve of Gauss was that the observations
were not numerous enough to eliminate the presence of skew-
ness. In other words, skewness was regarded as a by-product
of sampling and was believed could be made to disappear com-
pletely if we would take an infinite number of observations.

Against this background of the universality of the normal dis-
tribution it became apparent that while agriculture, astronomy, and
economics often contained data fitting the normal distribution the
behavioural sciences were likely to deviate from the expected normality.
Intuitively it was also apparent that Laplacian error law contradicted
the objectives of experimental science. The error law implies that
as errors become infinite their distribution approaches that of the
normal distribution. Thus, as the experimenter strives for greater and
greater control of his experimental parameters his data will less
likely resemble an approximation of the normal distribution. Ironically, the power of a statistical test based upon normality is indirectly dependant upon the failure to attain experimental control.

When the objective is the comparison of two independent populations it was evident that by setting the population variances equal to each other the derivation of the resultant test statistic could be considerably simplified. As a consequence, parametric tests (based on normality) require an additional pre-condition of homoscedastic (equal) variances. A test of equal variances then becomes a test which specifies whether or not two populations are drawn from identical normal populations. If the normality of the population distributions and the equality of their variances can be assumed then the means of the populations and the difference between them can be specified. Seldom can these conditions be met absolutely; however, they are usually considered to be approximately normal. This has resulted in a belief in the quasi-universality of the normal distribution a conclusion which is attractive to the mathematician but which leaves an experimenter in a quandry.

Once making the assumptions, the mathematics is simple and exact and fascinatingly beautiful: and Mathematicians will frankly say that it is our concern as researchers, not theirs, whether the assumptions are legitimate in the particular research situations with which we work. It happens that in most of the research in our field (behaviour) the assumptions are so far fetched as to abort the results of careful work. (Peters, 1943)

In particular the behavioural scientist found that he was using a parametric test based upon infinite sample size, homoscedasticity, and normality of distribution when he had no idea of the shape of the
underlying distribution and when he regularly employed very small sample sizes. In answer to this problem statistics has been returned to the approach current in the time of Bernoulli. Probabilities were expressed as the ratio of the number of successful outcomes of an event to a finite number of possible outcomes in opposition to the infinite or asymptotic distribution where probabilities were obtained by integration over mathematical densities. Therefore, tests which were independent of distribution were developed so that non-normality would not obliterate test validity. "Distribution-free" tests possess this property. In other words, a "distribution-free" test is independent of the distribution of the sampled population. As a consequence, sample populations do not have to demonstrate either normality nor homoscedasticity in order to employ such tests. Tests which do require normality and homoscedasticity of the sample populations are called "parametric" tests because they depend upon these parameters of the sample populations. On the other hand, tests which are independent of these parameters are called "non-parametric" tests. Thus, they are also "distribution-free" tests.

This thesis makes use of parametric and non-parametric statistical tests. Both types of tests have inherent assumptions.

Non-parametric tests assume:

1) Sample observations are independent.

2) The variable under study has underlying continuity.

3) The variable under study can be measured at least on the ordinal scale (some tests valid for measurements made of the nominal scale).
Parametric tests assume all of the above except (3) and also assume:

4) Sample populations are normally distributed.
5) Sample variances are equal (homoscedastic).
6) Measurements are made at least on the interval scale.

Most of the above assumptions can only be assumed to be true or at least almost true (Siegel, 1956). However, the homoscedasticity of variances can be tested using Snedecor's F test (or Variance Ratio test).

Non-parametric tests have some advantages in certain situations, whereas parametric tests have advantages over non-parametric tests in other situations. The advantages of non-parametric tests are summarized as follows:

1) Non-parametric tests make few assumptions about the population distribution.
2) If small sample sizes are used (n = 6) then there is no alternative but to use a non-parametric test. In fact the "statistical efficiency" (Bradley, 1968) of non-parametric tests is greatest with small sample sizes and least with infinite sample sizes.
3) Non-parametric tests are derived with minimal mathematics.
4) They are easily applied and require little time in their application.
5) Even if all the assumptions of parametric tests are upheld non-parametric tests are only slightly less powerful than parametric tests.

The disadvantages of non-parametric tests are summarized as follows:

1) Provided all the assumptions of the parametric test are met the non-parametric test is wasteful of information. This can be overcome simply by increasing sample size, however.
2) Non-parametric tests cannot be adapted to study interactions in the analysis of variance model without making special assumptions about additivity. In this regard, the analysis of variance model requires one additional assumption. This assumption is that the means of normal and homoscedastic populations must be linear combinations of effects due to columns and/or rows.

The application of statistical tests involves several steps and these steps are listed as follows:

1) The null hypothesis ($H_0$) is stated.

2) The appropriate statistical test is chosen depending on whether or not the experimental data are valid for the assumptions underlying the chosen test.

3) A sample size is chosen ($N$) and a level of significance is specified.

4) The sampling distribution is determined or it is assumed.

5) A region of rejection of $H_0$ is chosen.

6) The value of the statistical test is calculated for the experimental data. If this value lies within the region of rejection the $H_0$ may be rejected.

The application of statistical tests and the required assumptions are summarized and presented as follows:

1) The analysis of the data from this thesis employs a common $H_0$. The flexor response of the control group is assumed to be identical to that of the experimental group. In other words, the mean ($\mu$) responses from each group are assumed to be equal ($H_0: \mu_{\text{control}} = \mu_{\text{experimental}}$).
The alternate hypothesis or the research hypothesis is that the experimental group has a response greater (or less than) that of the control group. Thus, there are two research hypotheses \( H_1: \mu(\text{experimental}) > \mu(\text{control}) \) and \( H_2: \mu(\text{experimental}) < \mu(\text{control}) \).

2) The level of significance \( \alpha \) is the probability of rejecting \( H_0 \) when it is in fact true (type I error). The lowest value of \( \alpha \) accepted in this thesis was 0.050, a value which is commonly accepted in the behavioural sciences (Siegel, 1956). Thus, if the value of a statistical test was found to be equal to or less than 0.050, the \( H_0 \) was rejected in favour of the research hypothesis (a significance level of 0.052 was accepted for the Mann Whitney U tests when \( n_2 \leq 7 \)).

3) The sample distribution was assumed to be normal.

4) The region of rejection was determined from \( H_1 \) or \( H_2 \) and therefore one-tailed regions of rejection were employed.

5) The flexor response is assumed to be measured on a ratio scale.

6) Individual observations (responses) are assumed to be independent.

7) Experimental and control groups were assumed to be independent. Therefore, the statistical tests employed were Student's t-test, two-way analysis of variance, Snedecor's F test, and Mann-Whitney U test (Bailey, 1959; Siegel, 1956; Snedecor, 1956). There were two exceptions to this independence of experimental and control groups. In experiments 2D and 3A control and experimental groups were related or dependent (animals serve as their own controls) and a Wilcoxon Matched-Pairs Signed-Ranks test was employed (Siegel, 1956). At no time can data from control and experimental groups be pooled unless the groups are dependent.
8) Prior to the application of any parametric test (Student's t-test, two-way analysis of variance) Snedecor's F test was applied in order to determine if the assumption of equal variances would be upheld. The only occasion when the variances were found to be significantly different was for experiments 2B and 2E. Cochran's modification of the t-test (Snedecor, 1956) was then applied because it corrects for unequal variances. However, non-parametric tests do not make this assumption and application of the Mann-Whitney U tests was more appropriate (and indeed more powerful).

9) The data was presented in many cases, graphically. Such a presentation usually includes a measure of dispersion and often standard errors are drawn on the graphs. Standard errors are not measures of dispersion for non-parametric tests and should not be included for data tested in this manner (interquartile ranges are the non-parametric counterpart but this measure is seldom included in a graphic presentation). When parametric tests were employed standard errors were not included in the graphic presentation for a number of reasons which are as follows:

a) The value of the statistical test gives the exact answer to the question of whether or not the $H_0$ should be rejected whereas standard errors give only a crude approximate.

b) The use of standard errors on the graphs of this thesis often confused rather than clarified the data presented.

10. The choice of statistical tests was based upon three factors:

1) the appropriateness of the test (for example do the data support the assumptions of the test) 2) the power of the test (The power of a test is defined as the probability of rejecting the $H_0$ when it is in fact false,
i.e., the power is equal to one minus the type II error. The type II error is the probability of accepting the $H_0$ when it is false.) 3) the power-efficiency of the test. The power-efficiency of a test is defined as the increase in sample size necessary to make one statistical test as powerful as another statistical test. For example the power-efficiency of the Mann-Whitney U test is approximately 95% for moderate sample sizes ($N = 9$ to 20) when compared to the most powerful parametric test (Student's $t$-test) (Siegel, 1956).
Habituation of behavioural responses cannot be assumed to be the result of the operation of a single mechanism and it is likely that at least two or more processes are responsible for determining the degree of response decrement (Hinde, 1970; Groves and Thompson, 1970; Peeke and Peeke, 1973). This complication of interaction of a number of processes is further aggravated by a lack of uniformity in the method used to measure the degree (or rate) of habituation. The degree of habituation is measured in three ways which are listed below:

1) The degree of habituation may be measured as the number of stimulations required to reach a specified response level. For example, the number of stimulations required to extinguish the response is often taken as the specified criterion. This may require as few as 20 stimulations in the case of the galvanic skin response (Jackson, 1974) or it may take many hundreds of stimulations as in the case of the flexor reflex (Griffin and Pearson, 1967). This is not a particularly useful measure when the flexor reflex is employed as the reflex may never reach zero response (dependent upon stimulus intensity). The response does tend to approach an asymptotic level but if this level is taken as the criterion level it must be recognized that it is only an approximate value. Furthermore, this measure is only a particular case of the absolute decrement of response (described below (2)) measure.
2) The degree of habituation may be measured as the absolute change in response during the habituation procedure. This absolute measure is often found to be directly related to the amplitude of the initial response (Peeke, 1969; Groves and Thompson, 1970).

3) The degree of habituation may be measured as the relative decrement of response during the habituation procedure. There are three methods of measuring relative habituation. One technique widely used when measuring habituation has been to give a small number of stimuli at widely spaced intervals prior to the habituation procedure (testing of habituation) Groves, et al., 1969; Thompson, et al., 1973). The average of these responses is then used as a reference to which test responses (recorded during the habituation procedure) are compared. A similar technique was used by Wickelgren (1967a) but a further series of widely spaced stimuli were given following the testing of habituation. The degree of habituation was then considered to be the percentage decrease in response of the post-test stimuli to the pre-test stimuli. These methods have the disadvantage that the pre- and post-test stimuli may themselves alter habituation (Farel, 1971; Davis, in press). This thesis avoids using pre- or post-test stimuli and uses the initial response of the flexor reflex as a reference. All other responses were then expressed as percentages of this reference. This method of data presentation was recommended by Figler (1970) and has been employed previously in order to study habituation and sensitization of the flexor reflex (Griffin and Pearson, 1968a; Pearson and Krajina, 1972; Pearson, 1974). The relative measure of habituation is independent of the amplitude of the flexor reflex.
To illustrate how these various measures of habituation can be used to arrive at conflicting conclusions three hypothetical response curves are presented. These curves are illustrated in Figure 8 and are labeled a, b, and c. From this figure it can be shown that the degree of habituation depends upon the type of measure employed. If the time (number of stimuli) to reach criterion is taken as a measure of habituation the greatest habituation occurred with curve c (c > b > a). If the absolute change in response is taken as a measure of the degree of habituation the greatest habituation occurred with curve a (a > c > b). On the other hand, the greatest relative decrement of response occurred with curve c (c > a > b).

This thesis employs a relative measure of habituation because relative measures of habituation are independent of the amplitude of the reflex and because a relative measure can be used to answer this important question: What is the relationship of the final response level to that of the initial response level? The responses of each animal were converted to the relative measure prior to averaging the data for a group of animals in order to maintain an independence from response amplitude. The importance of this technique can be illustrated by giving several simple (hypothetical) response curves. Figure 9 illustrates the response curves for a sample population of two animals (a and b). Curve c represents the average (absolute) response curve for the sample population and curve d is the average (relative) response curve derived by the method employed in this thesis. Curves a and b demonstrated identical degrees of relative habituation and the sample variance is equal to zero for curve d.
Figure 8. The degree of habituation is shown to be dependent upon the type of measure used to determine habituation. Three hypothetical response curves are presented (a, b, and c).
Figure 9. Demonstration of the independence of the relative measure of habituation from response amplitude. Two hypothetical response curves are presented (a and b above) and the average of these two curves is also shown (c). The relative response curve is shown below (d). The variance of the relative curve is not affected by differences in response amplitude but the average (absolute) response curve has a variance dependent upon response amplitude.
absolute measure of response decrement was 3 units for curve a and 1.5 units for curve b. The sample variance (maximum) for curve c is 2.25 units. This serves to illustrate that the absolute measure of habituation is dependent upon response amplitude while the relative measure is independent. Furthermore, if we compare two sample populations it would not be surprising to find that the absolute measure is insensitive to differences in habituation (because of dependence upon response amplitude) while the relative measure is more sensitive to group differences in the degree of habituation. However, it can not be empirically assumed that an alteration in response amplitude will not modify the degree of habituation. To be more specific, an experimental manipulation may alter the amplitude of the reflex and also alter the relative measure of habituation. For example, it has been suggested that glutamate may be the transmitter released from primary afferent terminals in the spinal cord. The action of glutamate and of primary afferent terminals upon spinal interneurones is blocked or reduced by the glutamate antagonist glutamic acid diethyl ester (Haldeman and McLennan, 1972). Infusion of this drug might therefore reduce the sensory input to the spinal cord and reduce the amplitude (contribution of polysynaptic pathways) of the flexor reflex. Figure 10 illustrates the habituation curves for rats receiving an infusion of saline (controls) or glutamic acid diethyl ester. This drug caused a significant reduction in the initial response amplitude of the flexor reflex (p ≤ 0.010, Mann-Whitney U test), and the relative degree of habituation was significantly less in these rats compared to those receiving saline (p ≤ 0.010, Mann-Whitney U test).
Figure 10. The effect of glutamate acid diethyl ester (GDEE) on habituation of the flexor reflex (intact rats). Infusion of saline (controls, 8 rats) or GDEE (8 rats) was begun 1 min. prior to testing of the reflex and was continued until its termination. GDEE reduced the relative degree of habituation \( (p \leq 0.010, \text{Mann-Whitney U test}) \) by reducing the initial response amplitude \( (p \leq 0.010) \).
It is unlikely, however, that this alteration in relative habituation is due to any factor other than a reduction in the number of spinal interneurones participating in the reflex.

Often the relative measure of habituation has been presented without any indication of the initial response amplitude. This is an important question as the amplitude of the flexor reflex is directly related to stimulus intensity and because the parametric characteristics of habituation are dependent upon the type of measure employed. In fact, Groves and Thompson (1970) have proposed that the relative degree of habituation is inversely related to stimulus intensity but that the absolute degree of habituation is directly related to stimulus intensity. Therefore, in this thesis the average absolute response curves for each sample population are presented as well as the relative measures of habituation.

The usual method of presenting the absolute response is to average the absolute responses of the animals in each group. While this allows a determination of whether or not the amplitude of the flexor reflex is greater in one group than another it is a relatively poor measure of the degree of habituation. For example curve c in Figure 9 is an example of this method of presenting the data. Although this curve indicates a 50% decrement of response the variance (maximum) of this curve is equal to 2.25 units. In comparison the average relative response curve (d) has zero variance. Admittedly this example is an extreme case but it serves to illustrate that the variance of the absolute curve increases with dispersion of values of reflex amplitude whereas the variance of
the relative response curve does not. However, the relative response curve is particularly sensitive to sample population heterogeneity with regard to habituation and sensitization. Figure 11 gives an extreme example of this type of population heterogeneity. Curve a demonstrates habituation (absolute or relative). The average absolute response curve (c) shows neither habituation nor sensitization but the maximum variance of the curve is still 2.25 units. The average relative response curve (d) indicates a slight sensitization but the maximum variance of this curve is enormous (variance = 5,625%). Thus, it is absolutely critical to retain some form of absolute response measure to guard against the effects of population heterogeneity.

Another factor in the presentation of data, also of some importance is the representation of data points (response values) in blocks (averages of small numbers of responses). If data is presented in this manner an early sensitization of the flexor reflex (spinal animal) is overlooked (Groves, et al., 1969). In the intact animal an initial response increment may also be overlooked. For example Figure 12 shows flexor responses of two groups of intact rats tested with a stimulus intensity of 5 or 20 v. Rats tested with a 5 v. stimulus showed an initial increment of response while rats tested with a 20 v. stimulus showed a relatively rapid and greater degree (absolute and relative) habituation. If the data is now presented as blocks of responses (averages of 10 responses) the initial increment of response (rats tested with 5 v. stimuli) is not apparent (Figure 13). Furthermore, over the course of 300 stimulus presentations the relative degree of habituation was significantly
Figure 11. Demonstration of the sensitivity of the relative measure of habituation to sample population heterogeneity. Two hypothetical response curves are presented (a and b, above) and the average of the two curves is shown (c). The relative response curve is shown below (d). The variance of the average response curve (c) is relatively small compared to that of the relative response curve (d). This type of population heterogeneity results in very large dispersion of the relative measures.
Figure 12. Response of the flexor reflex to repeated stimulation with a stimulus strength of 5 v. or 20 v. The greatest degree of habituation (absolute or relative) occurred with the group tested with 20 v. stimuli. The data are presented as averages of individual responses. These data were taken from control rats used in the comparison with rats which received an infusion of strychnine.
Figure 13. Response of the flexor reflex to repeated stimulation with a stimulus strength of 5 v. or 20 v. The greatest relative decrement of response occurred with the group tested with 5 v. stimuli. The responses are blocked (averaged in groups of 10) and presented as percentages of the first block of responses. This is the same data that were shown in Figure 12.
greater for rats tested at 5 v. compared to those tested at 20 v. (Figure 13, p ≤ 0.010; Mann-Whitney U test). Thompson, et al. (1973) have recently suggested that stimulus intensity has only a weak effect upon the relative degree of habituation. This parametric characteristic of habituation is open to interpretation.

On the basis of this result two decremental components of the flexor reflex may be defined as follows: 1) a decrement of response apparent early in the test series provided the intensity of stimulation is relatively great 2) a decrement of response apparent late in the test series which is only weakly related to stimulus intensity.

A number of the experimental manipulations employed in this thesis are dependent upon or are performed upon the intact, decerebrate, or spinal rat. However, decerebration and spinal transection can cause a substantial modification of the flexor reflex. For example, the flexor reflex is tonically inhibited following decerebration (Holmqvist and Lundberg, 1961). In addition, spinal transection can cause a temporary suppression of the flexor reflex due to the phenomenon of spinal shock, however, spinal shock is either very short lived or non-existent in the spinal rat. There is some indication that even in primates spinal shock may be due to vascular disruption. Intra-dural section of the cord may prevent spinal shock in the monkey for example (Denny-Brown, et al., 1973).

The application of repeated stimuli characteristically elicits a smaller amplitude flexor reflex (biceps femoris) in the spinal compared with the intact rat (Figure 14). This difference in response
Figure 14. EMG discharge evoked in response to stimulation of the ipsilateral hind paw. In the left column are shown the responses obtained from a rat with an intact spinal cord. In the right column are shown the responses from a spinal rat. Habituation in the intact rat and sensitization in the spinal rat are illustrated. This figure is taken from Pearson and Wenkstern (1972:110).
amplitude is diminished with many stimulus repetitions. As a consequence the intact rat shows an initial decrement of response to a level corresponding to that attained in an incremental manner by the spinal rat (Figure 15). This distinction in response amplitude may be related to the depression of reflex "after-discharge" that occurs following spinal transection (Forbes, et al., 1923).

In order to elicit an "after-discharge" of the flexor reflex in the spinal animal Sherrington (1906) used a stimulus train as opposed to a single pulse stimulation. Sherrington (1906) also found that the size of the "after-discharge" was directly related to stimulus intensity and that the maximum "after-discharge" occurred following cessation of the stimulus train. Therefore, in order to evoke a discharge of sufficient size to demonstrate habituation of the flexor reflex in the spinal and decerebrate rat it was necessary to use high intensity stimulus trains.
Figure 15. Mean flexor reflex responses to stimuli applied at 10 sec. intervals (top) and 1 sec. intervals (bottom). Habituation in intact rats is shown on the left, and sensitization in spinal rats is shown on the right. This figure is taken from Pearson and Wenkstern (1972:113).
CHAPTER IV

RESULTS: SECTION I

The Flexor Reflex

Experiments on Unanaesthetized Rats with Intact Spinal Cords

1A. Infusion of Strychnine

The effect of strychnine on flexor reflex responses to 300 stimuli, at an intensity of 5 v., is illustrated in Figure 16 (above). The responses to each successive group of 10 stimuli were averaged and expressed in absolute units (volts per integration period: 250 msec.) or as percentages of the average of the first 10 responses (relative measure). The amplitudes of responses were consistently and significantly greater in those rats to which strychnine had been administered than they were in control rats. The relative degree (and rate) of habituation was unaffected by strychnine infusion (Figure 16, below).

For experiments in which a stimulus strength of 20 v. was used administration of strychnine resulted in an impairment of relative habituation (Figure 17, below), but did not cause a significant elevation of the level of reflex response to the initial stimuli (Figure 17, above). The 5 v. stimulus has been found to be just suprathreshold for reflex activation of the biceps femoris muscle (unpublished observation) and the 20 v. stimulus has been found to be just suprathreshold for group IV afferents in the sural nerve (unpublished observation).

1B. Infusion of Bicuculline

The effect of bicuculline on habituation of the reflex (20 v. stimuli)
Figure 16. The effect of strychnine on habituation of the flexor reflex. The control group received an infusion of saline (15 rats) and experimental rats received an infusion of strychnine (10 rats). The stimulus intensity was 5 V. The absolute response of experimental rats was greater than that of the controls (p < 0.010, Student's t-test). The relative degree of habituation was the same in both groups (below).
Figure 17. The effect of strychnine on habituation of the flexor reflex. The control group received an infusion of saline (15 rats) and experimental rats received an infusion of strychnine (12 rats). The stimulus intensity was 20 v. The absolute response of experimental rats was significantly greater than that of controls ($p \leq 0.010$, Student's t-test). The relative degree of habituation was greater in control animals ($p \leq 0.010$, Student's t-test).
is shown in Figure 18. As was the case with strychnine, this drug also reduced the relative degree of habituation (Figure 18, below). However, bicuculline had no significant action on the absolute response amplitude of the reflex. This is an example where the relative measure of habituation is sensitive to an alteration in habituation that is obscured in the absolute response curve due to its dependence upon response amplitude (see p. 91, this thesis).

IC. Infusion of both Strychnine and Bicuculline

The responses from experiments in which a combination of strychnine and bicuculline were given (20 v. stimuli) were compared to those in which strychnine or bicuculline were given alone (Figure 19). Whereas the drugs given together did not result in an impairment of the relative degree of habituation after 300 stimuli, there was a greater impairment of decrement (in fact an increment occurred) during the early stages of the experiment than was apparent for either strychnine or bicuculline alone. The combined drug infusion also reduced the relative degree of habituation with regard to animals receiving an infusion of saline.

ID. Pre-treatment with p-CPA

Pre-treatment of rats with p-CPA caused an alteration in excitability of the flexor reflex in rats tested at either 5 or 20 v. Figures 20 and 21 (above) illustrate the changes that occurred in absolute response. Regardless of the intensity of stimulation responses to the initial stimuli in the p-CPA treated animals were greater than those in their respective controls. Following the presentation of 300 stimuli this relationship was reversed and therefore the absolute degree of
Figure 18. The effect of bicuculline on habituation of the flexor reflex. The control group received an infusion of saline (10 rats) and the experimental groups received an infusion of bicuculline (10 rats). The absolute measure indicated no significant differences between control and experimental rats (above). The relative degree of habituation was greatest in control animals (p ≤ 0.010, Student's t-test).
Figure 19. The effect of a combination of strychnine and bicuculline (9 rats) on habituation of the flexor reflex is compared to that of strychnine and bicuculline alone. The asterisk (*) indicates a significant difference (Student's t-test, $p < 0.050$). Further control groups (saline infusion) are shown in the two previous figures.
Figure 20. The effect of pre-treatment with p-CPA on habituation of the flexor reflex using a stimulus strength of 5 v. Control rats (11 rats) received sham implants whereas experimental rats (10 rats) received p-CPA implants. The relative degree of habituation was significantly greater for experimental rats (below). Furthermore, the initial responses for experimental rats were significantly greater than those for controls, and final responses were significantly lower for experimental rats (above). See the text for discussion of significance levels.
Figure 21. The effect of pre-treatment with p-CPA on habituation of the flexor reflex using a stimulus strength of 20 v. Each group consisted of 9 rats. Pre-treatment with p-CPA had the same effect upon absolute and relative response curves as it did when the rats were tested with a stimulus strength of 5 v. See the text for discussion of significance levels.
habituation was greater in p-CPA treated animals. A two-way analysis of variance (Bailey, 1959) was used to assess the initial and final differences in response amplitude. The initial responses in treated rats were significantly greater \( (p \leq 0.010) \) than those in the control group and the final responses in treated rats were significantly lower \( (p \leq 0.010) \) than those of their appropriate controls. The probability levels refer only to the initial and final 20 responses.

Treated rats demonstrated a significantly greater degree of relative habituation \( (p \leq 0.025 \) for rats tested at 20 v. and \( p \leq 0.001 \) for rats tested at 5 v) as assessed using the Mann-Whitney U test. In accordance with the parametric characteristics of habituation the greatest degree of relative habituation occurred with the group of control rats tested with the 5 v. stimuli in comparison with the control group tested with 20 v. stimuli (Mann-Whitney U test, \( p \leq 0.050 \)).

IE. Rats with lesions of the n.r.d. (nucleus raphe dorsalis)

The effect of lesions of the n.r.d. on flexor reflex amplitude is shown in Figure 22 (above). The stimulus strength was 5 v. Confirmation of the locations of these lesions of the n.r.d. was demonstrated in all rats included in the experimental group and the extent of these lesions is illustrated in Figure 23. Two-way analysis of variance was applied to the initial and final 20 responses. In lesioned rats the initial amplitude of the reflex was significantly greater than that of controls and the final response amplitude was significantly lower \( (p \leq 0.001) \). The relative degree of habituation was significantly greater in lesioned rats than in controls \( (p \leq 0.001; \) Mann-Whitney U test).
Figure 22. The effect of a lesion in the n.r.d. on habituation of the flexor reflex using a stimulus strength of $5\%$ v. Control rats were sham lesioned whereas experimental rats were lesioned in the n.r.d. The greatest relative degree of habituation occurred with experimental rats (below). The initial response amplitude was greater in experimental rats and the final response amplitude was greater in the control rats (above). See the text for discussion of significance levels.
Figure 23. The extent of the lesions of the n.r.d. is indicated by the shaded area. This diagram was been redrawn from the atlas of König and Klippel (1963).
Two-way analysis of variance was performed on the initial and final 20 responses of the treated (and lesioned) rats and their appropriate controls. Such an analysis indicated a significant effect of trials ($p \leq 0.010$) for all comparisons. This simply means that there was a significantly different effect of repeating the stimulus on the experimental as opposed to the control group. There was no significant effect of groups which is not surprising as the experimental and control response curves invariably crossed over each other (the group analysis simply asks if the experimental group tended to be greater or less than the control group without regard to differences occurring at different places on the curves). A significant trials versus group interaction was demonstrated with animals pre-treated with p-CPA and tested with 5 v. stimuli ($p \leq 0.050$) and for lesioned rats ($p \leq 0.010$), therefore demonstrating a difference in the rate of habituation between experimental and control animals.

IF. Infusion of Methysergide

This dosage of methysergide did not have a significant effect upon the habituation of the flexor reflex. However, the initial few responses were significantly larger than comparable control responses (Student's t-test, $p \leq 0.050$) (Figure 24, above). This resulted in a slight increase in the initial relative sensitization of the reflex (not statistically significant) (Figure 24, below).
Figure 24. The effect of methysergide on habituation of the flexor reflex using a stimulus intensity of 20 v. Each group consisted of 9 rats. The asterisks(*) indicate that the response during infusion of the drug was significantly greater than the corresponding control responses (above). The relative degree of sensitization is shown below.
Experiments on Unanaesthetized Rats with Transected Spinal Cords (or Decerebration)

2A. Comparison between Decerebrate and Spinal Rats

Rats which had undergone spinal transection exhibited flaccid paralysis of the hind limbs, but the muscular tone of decerebrate rats was not obviously different from that of intact rats. These decerebrate rats displayed exaggerated startle response to strong stimuli, and the application of a strong pinch to a hind-paw resulted in a powerful reflex withdrawal of the limb. The magnitude of this response quickly diminished with repeated stimulation. Similarly, "pseudoadective reflexes" such as vocalization and orientation of the head towards the site of stimulation, when present, also gradually diminished. These findings are similar to those described by Sherrington (1906). Dis-habituation of the FWR could be brought about by pinching the contralateral hind-paw.

For decerebrate rats the relative degree of habituation of the flexor reflex was not significantly different to that of spinal rats provided the intensity of the stimulus trains was 20 v. (Figure 25, below). The amplitude of the flexor reflex was not significantly different in decerebrate rats when they were compared to equivalent responses in spinal rats (p < 0.052), Mann-Whitney U test). When a stimulus strength of 60 v. was employed the flexor reflex responses of decerebrate rats differed from those of spinal rats both in terms of the amplitude of the responses and with regard the the relative degree of habituation.
Figure 25. Habituation of the flexor reflex to repeatedly applied stimuli (20 v.) in spinal and decerebrate rats. There were 8 rats in each group. Decerebration had no significant effect on the absolute (above) or relative degree of habituation (below).
The responses to the initial 5 stimuli were significantly greater ($p < 0.003$, Mann-Whitney U test) in decerebrate rats than the corresponding responses in spinal rats (Figure 26, above). In the case of the decerebrate rats the reflex magnitude decreased rapidly, whereas the opposite occurred in spinal rats, so that after 50 stimulations the responses from decerebrate rats were significantly lower ($p < 0.005$, Mann-Whitney U test) than those from rats which had undergone spinal transection. Thus, decerebrate rats demonstrated relative habituation while spinal rats showed a long term sensitization (Figure 26, below); $p = 0.000$, Mann-Whitney U test).

In order to emphasize the rapidity of response habituation in decerebrate rats Figure 27 is included. In this figure individual responses for decerebrate rats (tested with 60 v. trains) and for spinal rats (60 v.) with various levels of transection are shown. Regardless of the level of spinal transection spinal rats demonstrated an initial increment of response while decerebrate rats invariably showed decrement ($p < 0.000$, Mann-Whitney U test).

2B. Spinal Rats and Asphyxiation of the Cord

Flaccid paralysis, the normal immediate consequence of spinal transection, was seen in both control rats and rats which had undergone asphyxiation for 22 min. The control rats had strong withdrawal reflexes in response to a pinch of the hind-paw within 30 min. after ligation of the cord but no reflex could be elicited in the rats which had undergone asphyxiation until 4 to 5 hours after the cords were ligated.

Groves, et al. (1969) described both an early and a late sensitization
Figure 26. Changes in flexor reflex response to repeated application of intense stimuli (60 v.) in spinal and decerebrate rats. The initial response amplitude was greater in decerebrate rats but this response fell until it was significantly less in decerebrate rats (above). Decerebrate rats showed relative habituation while spinal rats showed a long term sensitization (below). There were 8 rats in each group.
Figure 27. A comparison of habituation of the flexor reflex between spinal and decerebrate rats all tested with a stimulus intensity of 60 v. Each response is expressed as a change in amplitude from the first response (volts). Decerebrate rats showed only decrement of response while spinal rats invariably showed an increment of response.
of the flexor reflex in the spinal cat. A similar observation was made in this study but the early component tended to be obscured by presenting the data in blocks (see p. 92, this thesis). For this reason the initial 20 responses are occasionally plotted as individual data points. Figures 28 and 29 (above) illustrate this early response sensitization.

Using 20 v. stimulus trains the early sensitization and the later habituation were not significantly different in asphyxiated rats compared to controls (Figure 28). However, rats tested with 60 v. stimulus trains demonstrated an alteration in the early phase of sensitization and in the later relative habituation (Figure 29). Statistical significance was indicated using Snedecor's F test (p ≤ 0.050). This indicates a significant difference between groups with regard to variances. The Student's t-test cannot therefore be employed without a correction for unequal variances. Such a correction was accomplished using Cochran's modification of the t-test (see p. 82, this thesis). This test indicated a significant difference between experimental and control groups (p ≤ 0.050). The Mann-Whitney U test was also employed (p ≤ 0.010) and it is in fact superior to Cochran's modification as it makes no assumptions about equal variances.

The large variances that were calculated for the group of rats that underwent asphyxiation (demonstrated by a significant F test) suggest sample population heterogeneity with regard to habituation and sensitization (see p. 91', this thesis). Therefore, it is necessary to carefully consider the absolute response data. Figure 30 illustrates these
Figure 28. The effect of asphyxiation on habituation of the flexor reflex in the spinal rat. Stimulus trains (20 v.) were applied at 10 sec. intervals. The relative degree of sensitization (above) and habituation (below) were similar for control (14 rats) and asphyxiated rats (14 rats).
Figure 29. The effect of asphyxiation on habituation of the flexor reflex in the spinal rat. Stimulus trains (60 v.) were applied at 10 sec. intervals. The relative degree of sensitization was significantly greater in asphyxiated rats (above) while the degree of habituation was significantly less that that of controls (7 rats, 11 rats asphyxiated).
Figure 30. Flexor responses, in absolute units (volts), of control and asphyxiated rats tested with a stimulus intensity of 60 v.
results for control and asphyxiated rats tested at 60 v., in absolute response units. No statistical difference was apparent between these two curves using the Mann-Whitney U test (at the beginning and end of curves, p > 0.050). The groups did show a significant difference in terms of variances (F test) during the initial and final stimulations which negates the use of a parametric test, but this significance can itself be used to indicate that the rats were drawn from two different and independent populations. Regardless of statistical significance, two aspects are apparent from this graph and they are as follows: 1) asphyxiation reduced the initial response amplitude and 2) asphyxiation increased the final response amplitude, in relationship to control responses. Expressing the data as percentages indicated an initial increase in early sensitization (relative) but this relationship holds only because of a reduction in reflex amplitude. There was a tendency, however, for asphyxiation to reduce a later reflex decrement.

Figure 31 illustrates a comparison between controls (spinal rats) tested with 20 or 60 v. trains in which the data are expressed as relative measures. The relative degree of habituation was significantly greater in rats tested with 60 v. trains (p ≤ 0.010, Mann-Whitney U test) and the degree of sensitization was significantly greater in rats tested with 20 v. trains (p ≤ 0.010, Mann-Whitney U test). These data contradict the parametric characteristic of habituation that states the relative degree of habituation is inversely related to stimulus intensity.
Figure 31. The effect of varying stimulus intensity on habituation of the flexor reflex in the spinal rat. A greater relative degree of habituation occurred with high intensity stimulation than with low and a greater degree of sensitization occurred with low rather than high intensity stimulation.
2C. Infusion of strychnine

For spinal rats transected at the level of the fifth thoracic vertebra, and tested with a stimulus strength of 20 v. (trains), strychnine consistently elevated the amplitude of response (Figure 32, above). However there was no alteration in the relative rate and degree of habituation (Figure 32, below). Stimulation with 60 v. trains resulted in a sensitization of control responses, but the infusion of strychnine prevented this characteristic response pattern (Figure 33, below). Rats which received an infusion of strychnine demonstrated a significantly greater response amplitude (p ≤ 0.001, Mann-Whitney U test) but following 25 stimulus presentations both groups responded at the same level of response. Therefore, it can be concluded that strychnine prevented a long term sensitization and revealed habituation.

2D. Infusion of strychnine and bicuculline (single pulse stimuli)

It was difficult to demonstrate habituation of the flexor reflex in the spinal rat using single pulse stimuli (see p. 98, this thesis) and as a consequence it was decided to take the largest amplitude block of 10 responses as a reference and to express all subsequent responses as a percentage of this reference, provided this block occurred within the first 5 response blocks. It was also necessary to increase the stimulus frequency to 1 per second in order to accentuate habituation.

The experimental procedure used for spinal rats (tested with single pulse stimuli) was different than that used in other experiments in this thesis. Two series of 300 stimuli were given to each rat. During the
Figure 32. The effect of strychnine on habituation of the flexor reflex in the spinal rat. The stimuli were trains (20 v.). Intravenous infusion of saline (9 rats) or strychnine (12 rats) was commenced 20 min. prior to the first stimulus and continued until the end of the experiment. Strychnine significantly elevated response amplitude but had no effect upon the relative degree of habituation.
Figure 33. The effect of strychnine on habituation of the flexor reflex in the spinal rat. The stimuli were trains (60 v). Strychnine significantly elevated initial response amplitude and the controls showed a long term sensitization whereas rats receiving strychnine demonstrated habituation (15 control rats and 10 experimentals).
first series saline was administered. This was followed two hours later by the second series during which an infusion of strychnine or bicuculline was given. Habituation to the first series of stimuli thus served as a control to which the effect of a drug on habituation, during the second series, could be compared. A separate control group of rats was tested using this regime but saline was given during both series. The purpose of this group was to ensure that the degree of habituation during the second series of stimuli was the same as that of the first and this proved to be the case.

In Figure 34 the relative degree of habituation during the first series of stimuli (saline) and during a second series (strychnine) is shown. Strychnine caused a significant reduction in the final degree of habituation. Bicuculline had no significant effect upon habituation of the FWR. Because experimental and control groups are dependent (each rat serves as its own control) statistical significance was assessed using the Wilcoxon Matched-Pairs Sign-Ranks test (Siegel, 1956).

2E. Level of Spinal Transection

In order to determine if the level of transection might have some influence upon habituation and sensitization of the FWR a series of rats had their cords transected at the level of the last cervical vertebra (C7) and were compared to a group of rats with transection at the tenth thoracic vertebra (T10). The most rostral section was chosen rather than the C1 section used by Wickelgren (1967a) so that the supraspinal drive to the motor neurones of the diaphragm remained intact whereas the most caudal section was chosen rather than the T12
Figure 34. The effect of strychnine on habituation of the flexor reflex using single pulse stimuli of 20 v. stimulus strength. Experimental rats served as their own controls (8 rats). Strychnine caused a significant reduction in the relative degree of habituation (p \leq 0.010, Wilcoxon-Matched Pairs Signed-Ranks test).
section used by Groves, et al. (1969) to ensure that the direct FWR pathway was not damaged. Rats with either level of transection were tested with 20 v. and 60 v. stimulus trains.

Rats with the caudal section demonstrated no significant difference in response amplitude when tested with 20 or 60 v. stimulus trains ($p > 0.052$, Mann-Whitney U test) (Figure 35, above). The greatest relative degree of habituation occurred with the 20 v. stimulus trains ($p \leq 0.025$, Mann-Whitney U test) (Figure 35, below).

In contrast, rats with the rostral transection demonstrated significantly greater response amplitude when tested with 60 v. trains in comparison to similar rats tested with 20 v. trains (Figure 36, $p \leq 0.025$, Mann-Whitney U test). Tested at a stimulus strength of 20 v., rats with the rostral transection were also significantly lower in response amplitude than with the caudal transection, regardless of the stimulus intensity ($p \leq 0.052$, Mann-Whitney U test).

The group of rats, with rostral transection, tested with 60 v. trains demonstrated sample population heterogeneity with regard to relative degree of habituation and sensitization (see p. 91, this thesis). To be specific 2 rats showed low response amplitude with sensitization while 6 rats showed high amplitude responses and marked habituation. As a consequence, the relative measure of habituation had a variance of thousands of percent and it failed to be a reasonable measure. To avoid this particular distortion the data was presented as differences in absolute response from the initial block of responses (Figure 36, below). The greatest absolute degree of habituation occurred with the 60 v. stimulus
Figure 35. The effect of stimulus intensity on habituation of the flexor reflex in the spinal rat. There was no significant difference between rats tested with 20 v. (8 rats) and those tested with 60 v. (8 rats) in absolute response; however, the relative degree of habituation was greatest for rats tested with the lower intensity. These rats were transected at the level of the tenth thoracic vertebra.
Figure 36. The effect of stimulus intensity on habituation of the flexor reflex in the spinal rat. Rats tested with 20 v. trains (6 rats) had responses significantly lower than those rats tested with 60 v. trains (8 rats). As a consequence, rats tested with the highest intensity had a greater absolute decrement of responses. These rats were transected at the level of the seventh cervical vertebra.
trains primarily because of the higher initial response amplitude.

**Flexor Reflex in Anaesthetized Rats**

3A. Injection of Strychnine

The use of urethane anaesthesia greatly changed the response pattern of the FWR in the intact rat. The response of an unaesthetized rat (EMG) is shown in Figure 14 (p. 97). Following injection of urethane the reflex was manifest as an early and late reflex (Figure 37). Repetition of the stimulus produced a fusion of these components until the duration of the response outlasted the inter-stimulus interval. Continued stimulation resulted in a decrement of the response usually due to a dropping out of individual units and a reduction in the amplitude and duration of the EMG discharge. The integrated EMG recorded during two consecutive series of stimulation is shown in Figure 38. There was no significant difference between the first and second series of stimuli. A second group of rats was tested in the same manner with the exception that rats received an injection of strychnine 1 min. prior to the second series of stimuli. These results are illustrated in Figure 39 in absolute response (volts). The response amplitude at the end of the second run was significantly greater than at the end of the first series of stimuli (p < 0.050, Wilcoxon Matched-Pairs Signed-Ranks test). The first run for controls and those rats that received strychnine were not significantly different (p ≥ 0.050, Wilcoxon Matched-Pairs Signed-Ranks test).

In order to determine the influence which strychnine had upon the
Figure 37. Responses of the flexor reflex (EMG, biceps femoris) to repeated stimulation (60 v., 22.5 mA, inter-stimulus interval 1.5 sec.) in the anaesthetized, intact rat. The EMG is shown above and the integrated EMG (550 msec.) is representer below. The initial stimulus evoked a short latency response (10 to 20 msec.) followed by a long latency (500 msec.) response. On presentation of the second stimulus the early and late responses fused.
Figure 38. Sensitization and "habituation of sensitization" of the flexor reflex in the anaesthetized, intact rat (60 v. 22.5 mA, inter-stimulus interval 1.5 sec.). The graph illustrates the mean responses of rats (7 rats) receiving two series of stimuli separated by 2 min. One min. prior to the second series an injection of saline was given. The first series had no significant effect on the second series.
Figure 39. The effect of strychnine on "habituation of sensitization" of the flexor reflex in the intact rat. Control and strychnine series were separated by two min. One min. prior to the second series of stimuli an injection of strychnine was given. Strychnine significantly impaired the absolute degree of "habituation of sensitization." (8 rats, 60 v., 22.5 mA, inter-stimulus interval 1.5 sec.).
decrement of the response, a special measure was synthesized. Responses
to the 110th to 120th stimuli were averaged and expressed as a percentage
of the means of the 6th to 15th responses, for both the first and second
series of stimuli. This percentage decrement was then expressed as a
difference between the first and second runs for both controls and ex-
perimentals. The percentage decrement of the second run was always sub-
tracted from that of the first run which meant that this difference
could be either positive or negative. A negative difference would in-
dicate that less decrement had occurred in the second run as compared
to the first run. This measure gives an indication of the influence
of strychnine on response decrement which is largely independent of the
influence of the first run on the second. Comparison of experimentals
with controls, using the Mann-Whitney U test, demonstrated a significant
impairment of response decrement as a consequence of strychnine (p <
0.005, control + 8.8%, strychnine + 65.2%)
SECTION II

Spinal Interneurones

The microelectrode was lowered vertically through the dorsal surface of the cord until either spontaneous or evoked activity was encountered. The distribution of spontaneously active units was not uniform, and the more ventral the location of the electrode tip the more likely that spontaneous activity would be detected. These unit discharges fulfilled criteria that would define them as neuronal discharge (Dafny and Gilman, 1973).

Spontaneously active interneurones had resting discharge rates which ranged from about 5 to 200 spikes/sec. These units usually had a relatively stable discharge rates. Often in preparations that had been used for long periods the discharge was oscillatory. Such units were not studied.

Orthodromic stimulation evokes a number of characteristic discharge patterns:

1) A short duration high frequency burst (100 to 1000/sec.) with a relatively short latency (2 to 5 msec.). Increasing the intensity of stimulation decreases the latency of the response and increases the number of spikes in the burst (5 to 20 spikes).

2) A long duration and moderate frequency train (10 to 200/sec.) of discharges. These neurones have greater latencies (5 to 500 msec.). This type of response has been given several names including late discharge, sustained discharge, repetitive discharge, and after-discharge.

This distinction in response patterns is not absolute and many
intermediate patterns are encountered. This classification of excitatory response patterns has been established in the spinal cord by numerous authors (Frank and Fuortes, 1956; Wall, 1959; Price and Wagman, 1970; Groves and Thompson, 1973). The term after-discharge is adopted in this thesis because of the similarity of this neuronal response pattern and "after-discharge" of the flexor reflex. Figure 40 illustrates an example of each of the response patterns. These interneurones were not spontaneously active which served to facilitate observation of the response patterns.

Repetitive stimulation has different effects upon each of the discharge patterns. Frank and Fuortes (1956) originally reported that the after-discharge built up with stimulus repetition (increased in duration and frequency). This build-up was confirmed by a number of authors including Groves and Thompson (1973) who equated it with sensitization of the FWR and labeled these interneurones "S" cells. Certain of these interneurones demonstrated a later decrement of discharge following the initial build-up and these were called "S-H" cells. On the other hand, Groves and Thompson (1973) found that interneurones which responded with a high frequency burst underwent only decrement (reduction in number of evoked spikes) following stimulus repetition ("H" cells). In these respects, the results of the present study did not differ from previous studies.

Interneurones that responded with a high frequency burst often had relatively low thresholds to cutaneous stimulation. Habituation of such an interneurone is illustrated in Figure 41. The threshold for excitation
Figure 40. Interneuronal responses typical of a high frequency burst (above) and after-discharge (below).
Figure 41. An example of habituation of a high frequency burst illustrated graphically. The response to 500 uniform stimuli (1 mA) was a gradual decrement of response. The response to the 1st and 500th stimuli are also shown.
(0-6mA) of this interneurone was less than that of the FWR. Increasing the stimulus strength greatly reduced the rate of decrement of this type of response and at stimulus intensities ranging from 10 v. (3.8 mA) to 60 v. (22.5 mA) these units showed little in the way of decrement (see Figure 45).

Interneurones which demonstrated after-discharge were examined in considerably more detail than those showing a high frequency burst. As the intensity of the stimulation was increased, the degree of sensitization of after-discharge increased (Figure 42), and increasing stimulus frequency had a similar effect. Other interneurones demonstrated a later phase of response decrement (Figures 43 and 44).

Groves and Thompson (1973:187) found that certain interneurones responded with both a high frequency burst and a later after-discharge. These particular interneurones demonstrated decrement of the high frequency burst and build-up of the after-discharge. In this study a number of interneurones was encountered that responded with both patterns of discharge but it often happened that the high frequency burst had a lower threshold to cutaneous stimulation. Interneurones which responded with a high frequency burst were often found to possess a later after-discharge provided the intensity of stimulation was increased sufficiently. Records obtained from such an interneurone is shown in Figure 45. The high frequency burst had a threshold well below 1 mA but the after-discharge was not evoked until the stimulus strength was raised to 8 mA. The upper part of the figure demonstrates the response of the high frequency burst to repetitive stimulation (20 mA, 58 v.).
Figure 42. Sensitization of after-discharge to various intensities of stimulation illustrated graphically. Increasing the intensity increased the degree of sensitization.
Figure 43. Sensitization of after-discharge and a later decrement of response following repetitive stimulation (20 mA). This interneuron was not spontaneously active. The decrement of sensitization was gradual and was similar to that shown in Figure 44.
Figure 44. Sensitization of after-discharge and a later decrement of response following repetitive stimulation. This interneurone was spontaneously active. The decrement of sensitization was gradual.
Figure 45. Response of an interneurone demonstrating both a high frequency burst and a later after-discharge. The high frequency burst did not change with repeated stimulation (above) whereas the after-discharge showed a marked build-up of response followed by a later decrement (below). This interneurone was not spontaneously active.
Little or no change occurred in evoked response. However, when the period of counting was increased to include the after-discharge (the high frequency burst was not counted) there was a considerable build-up of response followed by a decrement. It is only an assumption that this represents the activity of a single interneurone.

Interneurones were both excited and inhibited by the stimulation, but both the inhibitory and excitatory phases were capable of undergoing progressive changes following repetitive stimulation. The factor which determined whether excitatory or inhibitory influences would dominate appeared to be the frequency of stimulation. This factor was originally described by Frank and Fuortes (1956:429): "Apparently the afferent volley produced short periods of inhibition which fused and predominated over more enduring phases of excitation when the frequency of stimulation was sufficiently high." Figure 46 illustrates the response of an interneurone that was inhibited by the stimulus. This inhibition was reduced with repeated stimulation due to build-up of excitation. The duration of this inhibition was greater with higher stimulus intensities and a particularly strong stimulus was used to accentuate the inhibition.

One of the objectives of this thesis was the demonstration of the build-up of inhibitory activity. A number (28) of spontaneously active interneurones was progressively inhibited by the repetition of cutaneous stimulation, and this occurred with single pulse stimulation (Figure 47) and with application of stimulus trains (Figure 48). Maximal build-up of inhibition was demonstrated with stimulus trains. The cessation of
Figure 46. Inhibition of a spontaneously active interneurone. A typical record of pre-stimulus activity is shown in (A). Repeated stimulation (30 mA) every 0.5 sec. initially inhibited this activity (B) but the inhibition gradually wore off and was replaced by a build-up of activity (C) reaching a level greater than its pre-stimulus condition. The decrement of inhibition may therefore be the consequence of a competing build-up of excitation.
Figure 47. Inhibitory build-up of a spontaneously active interneurone in response to single pulse stimulation (20 mA, inter-stimulus interval 1.5 sec.). Repeated stimulation was associated with a gradual dropping out of spontaneous activity until no activity was present. No recovery occurred until stimulation was recommenced 20 sec. after the last stimulus (40). An increase in the background activity occurred during the stimulation and this might represent the activity of nearby interneurones, perhaps the inhibitory interneurone driving the spontaneously active interneurone.
Figure 48. Inhibitory build-up of a spontaneously active interneurone in response to stimulus trains (10 mA, 0.5 msec. pulses, 0.5 sec. train duration, inter-stimulus interval 10.0 sec.). Each stimulus train is shown as an open box and the responses to the first (A), fifth (B), and fifteenth (C) are shown. Full recovery is illustrated in (E). The time bar indicates 1.5 sec. with regard to (A,B,C and E), however, it indicates 4.5 sec. for (D). (D) illustrates the gradual recovery of activity. No activity was present until 3.5 min. following the last stimulus (25 trains) and a second series of trains given after full recovery inhibited the interneurone for a period of 7.0 min. following the cessation of the stimuli. Single pulse stimuli also produced inhibitory build-up similar to that seen in Figure 47.
spontaneous activity outlasted the period of stimulation (after-inhibition) from periods of 0.5 sec. to 7 min.

An interneurone was considered to exhibit inhibitory build-up only if it fulfilled certain criteria: 1) the pre-stimulus activity had to be relatively constant 2) the stimulus had to clearly evoke inhibition of the spontaneous activity 3) the period of inhibition (duration of halted activity) had to increase progressively with repeated stimulation 4) the interneurone had to clearly demonstrate recovery after cessation of the stimulation 5) the build-up of inhibition had to be demonstrated at least twice for each interneurone.

This build-up of inhibition demonstrated a number of characteristics as follows:

1) During stimulus repetition the period of inhibition developed gradually (Figure 49). This inhibition often appeared to reach asymptotic level. A total of 28 interneurones demonstrated inhibitory build-up but another 13 showed a build-up of inhibition followed by a decay of this inhibition during the stimulation period. The maximal duration of inhibition in the latter cells was usually relatively short (< 500 msec.) in comparison to interneurones showing inhibitory build-up, and the decay of inhibition was associated with a simultaneous build-up of excitation (Figure 46).

2) The rate of build-up and the duration of the inhibition were directly related to the intensity of stimulation (confirmed in 16 interneurones). In this respect, most inhibitory build-up was observed at intensities that activated high threshold afferents (groups III and IV),
Figure 49. The effect of stimulus intensity on inhibitory build-up. Above is shown the progressive increase in the duration of inhibition for two relatively strong stimulus strengths (7.5 and 20 mA). With a lower stimulus strength (1.9 mA) the frequency of spontaneous activity was gradually reduced (below). The rate and duration of inhibitory build-up is proportional to the intensity of stimulation.
although inhibitory build-up was observed at intensities well below that of group IV afferents (> 6 mA). Figure 49 illustrates the influence of three different stimulus strengths on inhibition of the same interneurone. Although a clear period of inhibition was not apparent with relatively low stimulus intensity (5 v., 1.9 mA), repetition of the stimulus caused a gradual reduction in spontaneous activity. There was a relationship between the frequency of the spontaneous activity and the intensity of stimulation capable of halting the activity. The higher the frequency of the spontaneous activity the greater was the intensity of stimulation required to halt the activity. Several interneurones with relatively low spontaneous rates were strongly inhibited by weak stimuli (5 v., 1.9 mA).

3) The rate of inhibitory build-up was directly related to the frequency of stimulation (confirmed in 9 interneurones) (Figure 50). A build-up of inhibition was observed in some interneurones with frequencies as low as 0.2/sec. when using single pulse stimulation and at frequencies as low as 0.1/sec. when stimulus trains were employed.

4) After cessation of the stimulation, the inhibition appeared to decay spontaneously and the spontaneous activity was often depressed for periods after the interneurones re-commenced discharging.

5) Repeated series of stimuli often resulted in a more rapid development of inhibition and longer periods of inhibition after the cessation of stimulation (Figure 51). Confirmed in 4 cells.
Figure 50. The effect of stimulus frequency on inhibitory build-up. The build-up was proportional to the frequency of stimulation.
Figure 51. The effect of repeated series of stimuli on inhibitory build-up. Two series of stimuli (10 mA, inter-stimulus interval 1.5 sec.) were separated by 1 min. The build-up of inhibition was greatest in the second series. This may indicate a residual inhibition was carried over from the first to second series of stimuli, although the inter-neurone recovered its spontaneous discharge rate between the first and second series.
6) In several interneurones an interaction between the build-up of excitatory activity by repeated (ipsilateral) stimulation and the build-up of inhibition by repeated (contralateral) stimulation was observed (Figure 52). Repetition of the ipsilateral stimulus evoked an after-discharge which gradually doubled (the number of spikes evoked by each stimulus doubled) until a plateau of response was reached. The ipsilateral stimulation was continued, but a simultaneous stimulation of the contralateral hind paw was begun. This resulted in a gradual reduction of the evoked activity. Following cessation of the contralateral but not the ipsilateral, stimulation there was a gradual build-up of response until the plateau level was again reached.

7) The interneurone illustrated in Figure 47 was stimulated during the period of inhibition that occurred following the initial series of stimuli. The interneurone was dis-inhibited by re-implementation of the stimuli, presumably due to an excitation originally masked by the build-up of inhibition (Figure 53). Continued repetition of the dis-inhibiting stimuli lead, again, to a build-up of inhibition. This effect was confirmed in 3 other interneurones.

8) In 6 rats, when an interneurone was encountered that demonstrated inhibitory build-up, an injection of strychnine was given (0.1 mg/Kg). In half of these rats the build-up of inhibition was blocked by strychnine (Figure 54) and without elevating the pre-stimulus discharge of the interneurone (Figure 55), or at least by no great extent. Strychnine blocked this inhibition in 3 of the rats (3 interneurones), however, in 3 others it either had little effect on the inhibition or it reduced the build-up in association with a large increase in the spontaneous discharge
Figure 52. The interaction of excitatory and inhibitory build-up. This interneurone showed a build-up of response to ipsilateral stimulation (20 mA, inter-stimulus interval 1.5 sec.). Contralateral stimulation (same parameters) progressively inhibited the ipsilateral evoked response. On cessation of the contralateral stimulation the response built-up to its original response level.
Figure 53. Dis-inhibition of inhibitory build-up. Inhibitory build-up of this interneurone is shown in Figure 47. Twenty sec. after cessation of the stimulation the stimulus is recommenced (1) and then turned off after a second stimulus (2). The cell was fully recovered by these two stimuli. If the stimulation was begun again and continued inhibitory build-up also occurred again.
Figure 54. The effect of strychnine on inhibitory build-up. Inhibitory build-up was demonstrated before injection of strychnine (above), however, after strychnine the inhibition was almost eliminated and no build-up occurred.
Figure 55. The effect of strychnine on inhibitory build-up illustrated graphically. This is the same interneurone shown in Figure 54. Pre-stimulus discharge rates are indicated and strychnine did not elevate spontaneous activity. Although some inhibition remained after injection of strychnine repeated stimulation did not result in a build-up of inhibition.
rate. This suggests that inhibition, other than strychnine sensitive inhibition, may be partially responsible for inhibitory build-up.

A number of interneurones was anatomically located in the cord by iontophoresis of the dye pontamine sky blue. These interneurones (recording sites) were then placed in the appropriate Rexed laminae (Figure 56) according to Steiner and Turner (1972) who examined lamination in the cord of the rat. Depths of recorded interneurones were correlated with the depths of marked interneurones in order to determine the approximate location of non-marked interneurones. Interneurones demonstrating inhibitory build-up were located in laminae V to VII and two were recorded in the vicinity of lamina I.

A total of 154 interneurones were recorded in the spinal cords of intact rats (total of 41 rats). Of these interneurones 101 were exclusively excited by the stimulus, 13 demonstrated both excitation and inhibition, 12 were inhibited but repeated stimulation did not alter the duration of the inhibition, and 28 demonstrated inhibitory build-up. The majority of the interneurones excited by the stimulus demonstrated after-discharge, although many interneurones responded with a high frequency burst and an after-discharge.
Figure 56. The laminary location of various interneurones. Recording sites were marked with pontamine sky blue and lamination was determined as indicated by Steiner and Turner (1972). The location of 9 inhibitory build-up responses is indicated above.
SECTION III

Interneurones Recorded in the Cord of Spinal Rats

An attempt was made to locate interneurones in the spinal rat (6 rats, transection T5) that demonstrated inhibitory build-up. Characteristically interneurones that demonstrated relatively long periods of inhibition to a single stimulus ( > 100 msec.) also demonstrated inhibitory build-up, in the intact rat. It has not been possible to locate any interneurones demonstrating inhibitory build-up in the spinal rat; however, the sample of interneurones is small (10 interneurones). On the contrary, the relatively long duration inhibition (after-inhibition) which was observed in the spinal rat, decayed with stimulus repetition regardless of the stimulus strength or frequency. This decrement of the after-inhibition occurred even though these interneurones did not appear to be excited by the stimulus. Figure 57 illustrates the typical inhibitory response to single pulse stimulation and to stimulus trains.
Figure 57. The decrement (habituation?) of inhibition found in interneurones in the spinal rat. Single pulse stimuli evoked an inhibition of decreasing duration (20 mA, inter-stimulus interval 1.5 sec.). Only one inter-neurone is shown in this figure.


CHAPTER V

DISCUSSION: SECTION I

Inhibition and the Flexor Reflex (FWR)

This study has been based upon the supposition that inhibitory processes might contribute significantly to the establishment and integration of behavioural events which manifest themselves as habituation and sensitization of the flexor reflex and, resisting a current trend towards reductionism, it was thought important to consider the cybernetic role of inhibition. Therefore, it was not the objective of this study to propose a general theory of habituation, but rather it was designed to examine the possible role that inhibition might play in modifying flexor responses to repeated stimulation. Isolation of various components of the reflex has been accomplished both surgically and pharmacologically. A number of preparations were studied (decerebrate, spinal, and intact) instead of relying solely upon the use of the spinal preparation, and the justification for this approach becomes apparent from an understanding of the composition of the flexor reflex. In order for some form of inhibitory process to contribute to habituation of the flexor reflex activation of flexor reflex afferents must cause either inhibition or a dis-facilitation of the flexor reflex.

Definition of Flexor Reflex Afferents (FRA's)

A single shock to the skin or nerve of a limb evokes an indirect (multisynaptic) facilitation of flexor motorneurones and likewise
reciprocal inhibition of extensor motor neurones, located ipsilateral to the site of stimulation. This is the flexor reflex defined by Sherrington (1910). Recent electrophysiological experiments have described certain afferents as flexor reflex afferents (FRA's) by virtue of their actions upon flexor and extensor motor neurones.

Stimulation of group II and III muscle afferents (Lloyd, 1943, 1946; Brock et al., 1951; Laporte and Lloyd, 1952; Laporte and Bessou, 1959; Eccles and Lundberg, 1959 a,b; Paintal, 1961) and group II and III cutaneous afferents (Lloyd, 1943; Hagbarth, 1952; Laporte and Bessou, 1958; Eccles and Lundberg, 1959 a,b) evoke excitation of ipsilateral flexor motor neurones and inhibition of ipsilateral extensor motor neurones. Cutaneous group IV afferents may also be considered FRA's (Franz and Iggo, 1968).

This definition of FRA's was extended by Wall (1970:180-182) in relationship to their action on flexor motor neurones:

Motor neurones which supply axons to flexor muscles are driven into activity by a variety of peripheral stimuli. A class of these stimuli sets off the flexor reflex, which seems designed to move the limb away from the stimulus point. Which muscles contract or which motor neurones fire depends therefore not only on the type of afferent fibres stimulated but also on their spatial origin. In other words, each motor neurone can be said to have a receptive field with respect to the flexor reflex stimulus... As a stimulus at one point is increased in strength, more and more muscles take part in the response. In terms of the single motor neurone, this means that threshold varies within its receptive field. Increase in stimulus strength not only recruits more neurones but also produces repetitive firing of active motor neurones. Repetitive stimulation produces repetitive response. The motor neurones are therefore subject to both spatial and temporal summation... The threshold for the reflex is affected by the existence of other peripheral stimuli and central activity.
However, flexor and extensor motorneurones are not the exclusive targets of FRA's and Oscarsson (1967, 1970) defined ascending tracts, carrying information which was not rightly classified as either proprioceptive or exteroceptive, as FRA tracts. These tracts project to the cerebellum and with many components in the medial lemniscal system, to the cerebral sensorimotor cortex (Grampp and Oscarsson, 1968). The ascending FRA pathways have the following features in common (Evarts, 1971:100):

1) The information from the periphery is without modality specificity, because excitation and/or inhibition is evoked by all components of the FRA.

2) The receptive fields are large and may include one or several limbs. They sometimes consist of excitatory and inhibitory areas, but they permit only crude spatial discrimination.

3) The FRA effects to ascending pathways are mediated by pools of interneurones in the spinal cord and/or brainstem.

4) These interneurones are strongly excited and inhibited by descending tracts.

The actions of FRA's are mediated by spinal and brain stem interneurones which can be denoted "flexor reflex interneurones" (FRI's).

This conundrum is presented as follows: Can these FRI's be regarded primarily as flexor reflex centres, under the control of descending influences, or do they represent a focus by which descending motor activity is modulated by peripheral input? However, such a distinction may be meaningless as considerable evidence suggests that motor performance depends, at least in part, on the initiation of excitation and inhibition
of reflex arcs by higher centres (Lundberg, 1966; Hongo, et al., 1966a, b). Oscarsson (1970) has suggested that ascending FRA's function to provide a continuous feedback as to the state of the FRI's in relationship to motor commands from higher centers.

Exceptions to Reciprocal Inhibition

Exceptions to the rule of ipsilateral excitation of flexor motor-neurones and reciprocal inhibition of extensor motor neurones have long been recognized. For example, Sherrington (1906) described "extensor thrust" whereby pressing the skin underneath the toe pads of the hindfoot (dogs) elicited extension of that limb. Similarly it was found that stimulation of the appropriate peripheral nerve trunks could provoke ipsilateral extension (Sherrington and Sowton, 1911). Thus, it was concluded that ipsilateral stimulation sometimes yields reflex contraction of extensor muscles (Graham Brown and Sherrington, 1912).

Contradictions to reciprocal excitation and inhibition were further emphasized in the experiments of Graham Brown (1912). Flexion of the ankle was elicited by electrical stimulation of a cutaneous nerve of the limb. The variability of response led to the suggestion that ipsilateral stimulation not only activates flexor and inhibits extensor muscles but also inhibits flexor and excites extensor muscles. (Ranson and Hinsey, 1930). A similar contradiction was found with stimulation of the forelimbs (Denny-Brown and Liddell, 1928). These reflex actions did not correspond to "extensor thrust", however, because activation of this reflex is restricted to the plantar nerves (Sherrington, 1906) whereas the afferent sources of flexor inhibition and extensor
excitation were widely distributed to stimulation of various nerves.

Hagbarth (1952) described inhibition of flexor monosynaptic responses (myographic and ventral root electrotonus) and excitation of extensor responses with ipsilateral cutaneous stimulation. Pinching the skin of the limb could either facilitate or inhibit flexor responses depending upon the location of the stimulus on the surface of the skin. It was recognized that for any flexor or extensor muscle both excitatory and inhibitory fields could be mapped out on the skin of the limb.

Inhibition of Flexor Motorneurones by FRA's

Eccles and Lundberg (1959a) first reported that FRA's not only evoked EPSP's in flexor motorneurones but also but also IPSP's. It was suggested that FRA's might activate two pathways to FMN one excitatory and one inhibitory. Both of these pathways are themselves subject to supraspinal inhibitory control. That is FRI's are inhibited by supraspinal centres, a distinction which became apparent when the decerebrate preparation was compared to the spinal animal (Job, 1953; Eccles and Lundberg, 1959b).

Holmqvist and Lundberg (1961) described the transmission from FRA's to flexor motorneurones in a variety of preparations. In the spinal cat activation of FRA's evoked, overwhelmingly, excitation of flexor motorneurones and inhibition of extensor motorneurones. The only exception has been shown to occur with stimulation of the skin overlying an extensor muscle which excites that extensor muscle and inhibits flexors (Hagbarth, 1952). In the decerebrate cat both the excitatory and inhibitory pathways to flexor motorneurones were tonically inhibited.
A "low pontine lesion" selectively released, from descending inhibition, the inhibitory pathways from FRA's to flexor and extensor motorneurones. A "caudal medullary lesion" selectively released the excitatory pathways from FRA's to flexor and extensor motorneurones. In the intact (anaesthetized) animal both pathways were patent although the excitatory pathway to flexor motorneurones was predominant.

The preceding description serves to illustrate that FRA's evoke inhibition of ipsilateral motorneurones, in very different degrees, depending upon the preparation examined. This inhibition of flexor motorneurones may be present in the direct flexor reflex pathway or it may be manifest indirectly as a descending inhibition of FRI's. Inhibition of flexor motorneurones was shown to occur in a number of motor nuclei including that of the biceps femoris (Holmqvist and Lundberg, 1961). It was not clear if group IV afferents were also capable of inhibiting flexor motorneurones but pinching the skin and thermal stimulation of these afferents produced inhibition of flexor motor responses in the spinal cat (Hagbarth, 1952).

Strychnine Sensitive Inhibition, Habituation, and Inhibitory Build-up

Eccles (1964) and others have demonstrated a close relationship between strychnine sensitive inhibition and the intracellularly recorded IPSP's of motorneurones. The short duration of the individual IPSP's makes it unlikely that they represent a basic mechanism of habituation. However, IPSP's elicited by stimulation of joint and cutaneous afferents can be prolonged due to "...temporal dispersion of the activation of the inhibitory synapses rather than by the slower action of individual synapses" (Eccles, 1964;160). In other words, prolonged IPSP's can be
evoked in motoneurones as a consequence of high intensity stimulation of cutaneous nerves and, in addition, repetitive interneuronal discharge of an inhibitory pathway has been suggested as a reasonable explanation for this phenomenon (Eccles, 1964).

A. Spinal Animal

The complex IPSP evoked in flexor motoneurones by activation of FRA's undergoes a progressive decrement (as does the EPSP) during habituation of the flexor reflex in the spinal cat (Spencer, et al., 1966c). Furthermore, Renshaw cell inhibition does not cause habituation of the flexor reflex in the spinal cat (Spencer, et al., 1966a). Similarly, Buchwald, et al. (1965) found that the inhibition of tonically active units in the ventral root of the spinal cat underwent a progressive decrement during habituation of phasic (excited) units. These results would seem to agree with the finding of this thesis wherein the inhibition of tonically active interneurones in the spinal cord of the spinal rat also underwent a progressive decrement during repeated stimulation.

Administration of strychnine failed to prevent habituation of the ventral root electrotonus in the spinal cat (Spencer, et al., 1966c). In contrast, this thesis did demonstrate an impairment of the relative degree of habituation of the flexor reflex (see p. 128) in the spinal rat (when single pulse stimulation was employed). This impairment of habituation was probably not due to an action on the underlying mechanism of habituation. For example, it was difficult to demonstrate habituation in the spinal rat using single pulse stimulation and there was always an initial sensitization of response. Furthermore, Pearson and Richardson
(personal communication) have demonstrated that the infusion of strychnine (spinal rat, single pulse stimuli) caused a gradual potentiation of sensitization. The impairment of decrement observed in the spinal rat is likely due to a potentiation of sensitization and not due to an action on habituation. When stimulus trains were employed, strychnine not only failed to retard habituation but actually facilitated decrement (or at least prevented a long term sensitization) (see p. 126, this thesis). Spencer, et al. (1966c) also found that strychnine occasionally facilitated decrement of the ventral root electrotonus. The progressive decrement of inhibition of tonically active interneurones may account for long term sensitization of the flexor reflex in the spinal rat. Therefore, it is unlikely that strychnine sensitive inhibition contributed to habituation of the flexor reflex in the spinal rat.

B. Intact Animal

This thesis has demonstrated an impairment of the relative and absolute degree of habituation of the flexor reflex in the intact rat. The intensity of the stimulation had to be relatively great (20 v). This impairment of the relative degree of habituation, unlike the results for the spinal rat, was greatest during the initial period of the test (see p. 102, this thesis); however, a reduced habituation during the infusion of strychnine may have occurred for several reasons:

1) Strychnine may have eliminated a tonic inhibition of interneurones so that new interneurones now participate in the flexor reflex. These interneurones may have little or no tendency to demonstrate habituation with a resultant reduction in the degree of response: (motor) habituation.
Thus, the impairment by strychnine would simply be the consequence of an alteration in the number of interneurones participating in the reflex.

2) Strychnine may have changed the frequency of activity within the reflex arc. If it is assumed that habituation is a result of synaptic depression then an increase in activity should reduce the degree of habituation. Furthermore, strychnine may have had an effect upon the process underlying habituation that is independent of strychnine's actions upon post-synaptic inhibition.

3) The impairment of habituation might have been due to a blockage of a build-up of inhibition either in the direct (spinal) pathway from FRA's to flexor motorneurones or indirectly as a blockage of a build-up of inhibition originating from supraspinal structures. The former possibility is unlikely but the latter possibility seems to be supported by the demonstration of inhibitory build-up of spinal interneurones in the intact rat. It is unlikely that this build-up of inhibition occurs in the inhibitory pathway from FRA's to extensor motorneurones because there is no such build-up in the spinal rat even though this pathway is predominant in the spinal animal (Holmqvist and Lundberg, 1961). Therefore, the results of this thesis suggest that a build-up of supraspinal inhibition may contribute to habituation of the flexor reflex in the intact rat.

The flexor reflex is inhibited tonically by the reticular formation. If this inhibition were to represent a build-up of supraspinal inhibition the decerebrate rat should demonstrate a more pronounced habituation than the spinal rat. This thesis has shown that the decerebrate rat
demonstrated a rapid decrement of response to repeated stimulation (stimulus trains 60 v., see p. 116, this thesis).

This rapid decrement was best illustrated in Figure 27 (p. 117, this thesis). No group of spinal rats, regardless of the level of spinal transection, ever failed to show some degree of sensitization during the initial 10 stimuli, but decerebrate rats uniformly demonstrated a rapid decrement.

The threshold for the elicitation of the FWR is greater in the decerebrate than in the spinal animal (Sherrington and Sowton, 1915) and this difference in threshold may be attributable to the tonic inhibition of FRA transmission by the brain stem. It was found in this thesis that the response of the FWR was initially greater than that of spinal rats but the amplitude of the reflex was rapidly reduced to levels below that observed in spinal rats provided the stimulus was repeated (see p. 116, this thesis). Even tested at a strength of 20 v. decerebrate rats demonstrated a marginally greater amplitude of response for the first 5 responses. Perhaps tonic brain stem inhibition of FRA transmission requires stimulus repetition before it develops fully. Thus, there would appear to be an association of rapid response decrement with tonic brain stem inhibition of the FWR. This seems to be a re-statement of an observation made by Graham Brown (1912:262) with regard to the flexor reflex in the decerebrate as compared to the spinal animal: "...the greater excitability of the factor of inhibition [refers to decerebrate preparation]; the greater the liability of the factor of contraction to "fatigue" [habituation]...."
Bicuculline Sensitive Inhibition, Habituation and Inhibitory Build-up

The actions of bicuculline are highly complex. This drug blocks the action of gamma-aminobutyric acid (GABA) whether it is the post-synaptic hyperpolarization of interneurones (Curtis, et al., 1970; Curtis, et al., 1971b) in the brain and spinal cord or if it is the depolarization of primary afferent terminals in the spinal cord (Levy, 1974). Therefore, the administration of bicuculline is associated with the antagonism of some forms of post- and pre-synaptic inhibition (Curtis, et al., 1971a, b, c; Schmidt, 1973; Levy, 1974; Levy and Anderson, 1974).

The complexity of the actions of bicuculline are demonstrated by its actions upon dorsal root potentials (DRP's). This drug eliminates DRP's (both negative and positive) and pre-synaptic inhibition induced by stimulation of spinal afferents (segmental DRP's) (Levy and Anderson, 1974), but DRP's and presumably pre-synaptic inhibition of primary afferents (spinal cord) induced by activation of supraspinal structures (supraspinal DRP's) are facilitated by intravenous injection of bicuculline (Benoist, et al., 1972). However, application of bicuculline directly to the surface of the spinal cord eliminates both segmental and supraspinal DRP's. On the other hand, application of bicuculline to the surface of the cortex facilitated supraspinal DRP's induced by stimulation of the cortex. This suggests that the facilitation of supraspinal DRP's is the result of dis-inhibition at the cortical level (Benoist, et al., 1974).

The Flexor Reflex

It has been demonstrated that alterations in the excitability (PAD)
of cutaneous afferent terminals (located in the region of lamina IV) were not related to habituation of the flexor reflex in the spinal cat (Groves, et al., 1970). Furthermore, picrotoxin failed to prevent habituation of the ventral root electrotonus in the spinal cat (Spencer, et al., 1966c). Also, bicuculline failed to impair habituation of the flexor reflex in the spinal rat (this thesis). However, these results do not eliminate the possibility that pre-synaptic inhibition or bicuculline-sensitive inhibition might contribute to habituation of the flexor reflex in the intact animal. This thesis has demonstrated that bicuculline impairs the relative degree of habituation of the flexor reflex in the intact rat (see p. 104, this thesis). Because bicuculline had no effect in the spinal rat it might be suggested that its actions in the intact animal are primarily at the supraspinal level. For example, the frontal cortex exerts an inhibitory action upon the reticular formation and lesions of the frontal cortex remove this inhibition and also impair habituation of the flexor reflex (Griffith and Pearson, 1968b). Bicuculline might have had a similar action.

Recent evidence has stressed the importance of descending brain stem inhibition in the control of spinal reflexes. Afferent input to the spinal cord can be inhibited by repetitive stimulation of various brain stem structures (Hagbarth and Kerr, 1954) and long term (hrs.) inhibition of spinal reflexes may occur (Abrahams, 1974). Furthermore, brain stem stimulation may cause either post- or pre-synaptic inhibition of FRA transmission (Pomeiano, 1973). This inhibition is exerted at the
level of the FRI's and it is carried to the cord as part of the dorsal reticulo-spinal system (Pompeiano, 1973), and it originates from regions located in the medial part of the lower brain stem (Holmqvist and Lundberg, 1961). In the decerebrate animal this inhibition is tonically active. This system is believed to be independent of another system of descending inhibition which originates in the medullary raphe nuclei and which sends axons to the spinal cord within the dorso-lateral funiculus (Brodal, et al., 1960). Destruction of the medullary raphe nuclei or infusion of serotonergic antagonists was shown to give a partial release from the tonic descending inhibition of transmission of the FRA's (Engberg, et al., 1968).
SECTION II

Segmental Inhibition

The hindlimbs are subject to inhibition during activation of the forelimbs (Schiff-Sherrington effect), and repeated stimulation of afferent nerves has been reported to depress spinal reflexes by increasing "general inhibition" (Beritoff, 1965). This "general inhibition" is not restricted to those pathways directly involved with the activating stimulus and cannot be accounted for entirely on the basis of PTD or a similar phenomenon. Furthermore "general inhibition" can also be induced by repeated stimulation of supraspinal structures. It has been postulated (Beritoff, 1965) that "general inhibition" is mediated by the substantia gelatinosa and its segmentally arranged connections carried in the tract of Lissauer. Until recently little experimental evidence was available to support such an hypothesis; however, a recent study of the cutaneous receptive fields of the Macaque monkey has provided substantial evidence for the existence of segmentally mediated inhibition (and to a lesser extent excitation) that is tonically active and mediated by the substantia gelatinosa and the tract of Lissauer (Denny-Brown, et al., 1973). It was found (spinal animal) that the receptive field for an isolated dorsal root preparation was under a tonic inhibitory influence of the cells of the substantia gelatinosa located both caudal and rostral to the entry zone of the isolated root by as many as four segments. This inhibition was carried in the tract of Lissauer and it was eliminated following injection of strychnine.
Stimulation of the substantia gelatinosa inhibits flexor responses (Isolliani, 1958) to cutaneous stimulation and this suggests that the level of flexor reflex excitability might be under the control of the substantia gelatinosa.

In the present study a caudal transection (T\textsubscript{10}) resulted in a flexor response amplitude that was significantly greater than that of the rats with rostral transection (C\textsubscript{7}). This is based upon a comparison of rats tested at 20 v. (C\textsubscript{7}) with rats tested at 20 v. and 60 v. (T\textsubscript{10}) (see pp. 130-132, this thesis). This depression of reflex excitability obviously could not have been due to a damaging of the direct flexor reflex pathways and, as it appeared to be overcome in rats tested at the higher intensity (C\textsubscript{7}, 60 v.), a tonic depression of reflex excitability is implied. One possible explanation is that the reduced reflex excitability is the result of a tonic inhibition by the substantia gelatinosa which extends from the region of the caudal transection to the rostral transection.

During the infusion of strychnine (20 v., trains) there was no impairment of flexor response decrement. With higher intensity trains (60 v.) strychnine appeared to favour response decrement by preventing a long term sensitization of the reflex. This sensitization was characteristic of rats with spinal transection at T\textsubscript{5}. From Figure 30 (p. 126) it can be seen that strychnine released an initial period of high reflex excitability, and therefore it might be suggested that, in the spinal rat (T\textsubscript{5}) without strychnine, flexor inhibition is gradually reduced in effectiveness resulting in a long term sensitization. To a
certain extent such an implication seems to correlate with the decrement of inhibition of spinal interneurones in the spinal rat.

Temporary asphyxiation of the spinal cord leads to the selective destruction of small interneurones (\(< 20 \mu\) dia.) particularly within the dorsal horn (Davidoff, et al., 1967). The onset of asphyxiation is associated with a reduction in the amplitude of dorsal root potentials (DRP's). One particular component of the DRP (DRP nomenclature of Lloyd and McIntrye, 1949), DRPV was susceptible to asphyxiation. Periods of asphyxiation, sufficient to cause destruction of small spinal interneurones, resulted in a permanent loss of DRPV but not other components of DRP's (Van Harreveld and Niechají, 1970). Lloyd (1971) postulated that DRPV most likely represents the activity of interneurones and Wall (1962, 1964) has postulated that the origin of this activity is the substantia gelatinosa.

The close structural relationship of substantia gelatinosa neurones with glial elements (Ralston, 1968; Scheibel and Scheibel, 1969), coupled with their uniquely small size (5 to 20 \(\mu\) dia.), has lead to the suggestion that they have a particularly high metabolic rate and requirement for oxygen. It would therefore seem likely that the cells of the substantia gelatinosa are susceptible to asphyxiation.

Davidoff, et al. (1967) reported that temporary ischaemia of the spinal cord caused a selective decrease in the concentrations of glycine, aspartate, and glutamate, and there was a correlation between the decrease in glycine and aspartate and the destruction of small
interneurones. The concentrations of GABA and glutamine were unaltered by ischaemia of the cord. It was assumed that decreases in the concentrations of glycine and aspartate were the consequence of the destruction of spinal interneurones. This was further supported by a loss of inhibition and polysynaptic reflexes. The loss of glutamate, however, was not directly related to the loss of interneurones. The authors suggested that a decrease in the glutamate concentration of dorsal root terminals may have occurred as a consequence of destruction of the interneurones upon which they normally synapse.

Pearson and Krajina (1972) reported that spinal ischaemia (22 min.) resulted in an impairment of habituation of the FWR in the intact rat. Originally, it was decided to use asphyxiation to cause selective destruction of spinal interneurones in the spinal rat. The effect of destruction of FRI's (flexor reflex interneurones) would be two fold: 1) impairment of FRA transmission of excitation to FMN 2) impairment of FRA transmission of inhibition to FMN. It was the objective of this experiment to cause a selective disruption of the latter pathway and therefore asphyxiation would present an indirect means to impair a hypothetical build-up of inhibition. However, this hypothesis is no longer tenable. Asphyxiation did impair flexor response decrement in the spinal rat. However, it must also be recognized that it did so by a decrease in the initial level of reflex excitability (this thesis, p. 121). At the end of the stimulus series the reflex excitability was actually greater in asphyxiated than control rats. This was perhaps the consequence of removal of segmental inhibition.

Davidoff, et al., (1967) postulated that depletion of glutamate was responsible for the loss of polysynaptic reflexes following long
periods of spinal ischaemia, possibly due to depletion of primary afferent terminals. Infusion of the glutamate antagonist glutamic acid diethyl ester (Haldeman and McLennan, 1972) can also impair habituation of the FWR but primarily because of a reduction in the initial level of reflex excitability (this thesis, p 90). The initial reduction in reflex excitability which occurred following asphyxiation may have been the result of the loss of glutamate (or destruction of interneurones) and therefore the loss of excitatory pathways to flexor motorneurones. On the other hand, the later increased reflex excitability may have been the consequence of a loss of inhibitory interneurones.
SECTION III

Afferent Inflow to the Spinal Cord

The afferent nerves and their related sensory apparatus have never been conclusively eliminated as contributors to habituation and/or sensitization of the FWR, although habituation of the reflex can continue if direct stimulation of afferent nerves is substituted for cutaneous stimulation (Wickelgren, 1976b). Repeated cutaneous stimulation at frequencies below 1/sec. can lead to "fatigue" of group IV afferent volley (Törerbjörk and Hallin, 1974) whereas slightly higher frequencies of stimulation can lead to "fatigue" of lower threshold afferents (Campbell and Taub, 1973; Törerbjörk and Hallin, 1974). There is also evidence that long duration hyperpolarizations (sec.) occur in isolated peripheral nerves (particularly non-myelinated afferents) following repetitive stimulation of the nerves (Ritchie and Straub, 1956; 1957). This hyperpolarization served to increase the magnitude and duration of the pre-synaptic spike and thus an increase in the amount of transmitter released by the primary afferent terminals might be expected. "Wind-up" (sensitization) might be a consequence of such an effect as it has been reported to be a property of repeated activation of group IV afferents. However, hyperpolarization of non-myelinated nerves did not occur at frequencies of stimulation below 6/sec. whereas "wind-up" occurs at frequencies as low as 0.3/sec.

Spencer, Thompson, and Neilson (1966a) concluded that habituation
of the flexor reflex does not depend upon the type of afferent activated but rather upon the number of afferents activated. Clearly, there is a relationship between high threshold afferents (group III and IV) and sensitization. There is some evidence that group III afferents are themselves capable of eliciting sensitization of the FWR in the spinal rat (Richardson and Pearson, personal communication); however, it should always be remembered that stimuli sufficient to activate group IV afferents are also supramaximal for lower threshold afferents. In addition, the electrical stimuli employed in this study may have lead to activation of deep afferent nerves (muscle, joint, etc.).

Stimulus electrodes form an epicentre of current intensity with a current field of decreasing density radiating away from this centre. Even with low intensity stimulation high threshold afferents may be activated at the electrodes. Similarly with high intensity stimulation only low threshold afferents may be activated at the edge of the current field and the number of low threshold afferents activated may be much larger than that activated by low intensity stimuli. The stimulus strength, and therefore the size of the current field, will also determine the size of the sensory field activated for any individual FRI or flexor motorneurone. As a consequence, the stimulus strength may also determine whether or not the field activated is excitatory, inhibitory or both. These factors make it difficult to determine if inhibitory build-up is dependent (or for that matter independent) of the type of afferent activated.

Nociceptors are especially influenced by local chemical changes
where various substances such as histamine, serotonin, bradykinin, and prostaglandins are implicated. Although nociceptors (mechanical and thermal) respond with latencies too short to be accounted for by chemical intermediates repetitive stimulation may lead to a significant accumulation of such substances. These chemical factors might have significant actions in terms of receptor sensitivity and reflex sensitization. Furthermore, vasomotor responses to noxious stimulation of the skin (galvanic skin response, GSR) are subject to habituation and might alter receptor sensitivity and sensitization of the flexor reflex.

Le Blanc and Gapiton (1974) have reported inhibitory build-up in response to strong electrical stimuli and also to strong mechanical stimulation of the skin which suggests that inhibitory build-up is not dependent upon some characteristic of the electrical stimulation. The role of sensory mechanisms in behavioural plasticity of the FWR is beyond the scope of this thesis but it is recognized that these mechanisms may have played a significant role. The failure to demonstrate inhibitory build-up in the spinal rat does seem to indicate that the build-up of inhibition does not depend upon peripheral mechanisms. Furthermore, preliminary experiments have shown inhibitory build-up in response to repetitive stimulation of the sural nerve.

Stimulation of the skin will cause either excitation or inhibition (or both) of the FRI's depending upon: 1) the location of the stimulus with respect to the receptive field (excitatory or inhibitory) of the FRI. 2) the type of afferent activated 3) the number of afferents activated 4) the integrative control of access to the FRI exerted by higher centres.
of the central nervous system.

Schmidt (1973) has suggested that the afferent input to the spinal cord is for the most part "surplus", that is any stimulus evokes more afferent activity than the spinal cord can reasonably process. As a consequence, inhibition is required to reduce the afferent input. This process of inhibition may occur at the receptor level (this mechanism is found in intertebrates), the level of the primary afferent terminal (presynaptic inhibition) or at the level of the second order sensory neurones. Furthermore, Schmidt proposed that the greater the afferent inflow to the cord the greater would be the resultant inhibition. This would serve two purposes: 1) to adjust the sensitivity (intensity) of the inflow 2) to focus afferent signals into specific integrative components of the nervous system (in a sense to "focus attention" on to specific elements of the massed afferent input. This would in fact increase the information content of the afferent inflow). This type of inhibitory control would have spatial and temporal aspects as well (Melzack, 1973;82):

It is apparent, then, that a central cell normally has large skin area that can drive it (the receptive fields of many fibres that project on to a central cell), but that only a portion of the fibres is capable of doing so at any time. Because receptive fields may vary in size and sensitivity from moment to moment, any transient input—such as a stimulus—produces activity that must be selected by the brain from a continually changing background.

The results of this thesis suggest that both the inclusion (if the stimulus lies within an excitatory field) and exclusion (if the stimulus lies with an inhibitory field) of afferent activity are capable of undergoing progressive changes in response to repeated stimulation. For example, the more intense the stimulus (and thus the greater the afferent
inflow) the greater will be the resultant inhibition (inhibitory build-up). This inhibition is dependent upon centrifugal pathways.

In this regard, Haber and Wagman (1974) have reported inhibitory build-up of spinal interneurones in response to repetitive stimulation of the nucleus reticularis gigantocellularis (n.r.g.). The n.r.g. demonstrated both excitatory and inhibitory build-up to repeated cutaneous stimulation of the limbs (Le Blanc and Gapiton, 1974). On the other hand, repetitive stimulation of this nucleus lead to inhibitory build-up of spontaneously active spinal interneurones distributed in laminae I and IV to VII. Inhibitory build-up of evoked activity elicited by intense peripheral stimulation was also demonstrated (Haber and Wagman, 1974). The n.r.g. therefore seems to be a possible source of the inhibitory build-up observed in the experiments of this thesis.

Inhibitory build-up may be secondary to excitatory build-up of interneurones driving inhibitory interneurones; however, inhibitory synapses are also capable of plastic changes. For example, the characteristics of inhibitory build-up, with regard to stimulus intensity, are similar to the frequency facilitation of inhibitory synapses observed in Aplysia (Waziri, et al., 1969) and the mammalian spinal cord (Kuno and Weakly, 1972b). Most likely, inhibitory build-up is the result of a recruitment of the influence of interneurones demonstrating some form of frequency facilitation or PTP but requiring the participation of supraspinal structures. Complex interactions such as progressive dis-inhibition and dis-facilitation may also be involved.

Brief periods (sec.) of noxious stimulation of the skin have also
been shown to alter activity at various levels of the nervous system such as the spinal cord, the reticular formation and the thalamus, either inhibiting or facilitating activity for 5 to 10 min. Longer periods of stimulation resulted in longer periods of altered activity. These effects were highly dependent upon the depth of anaesthesia and were observed only during moderate and not light or heavy anaesthesia (Melzack, et al., 1968, 1969).

The inhibition of activity recorded in this study is assumed to be due to excitation of inhibitory interneurones that exert their effects upon spontaneously active interneurones. The blockage of inhibitory build-up following an injection of strychnine tends to support this assumption. It is difficult to determine the longest period of inhibition which might be induced. This is difficult to do because after-inhibition is measured in terms of the absence of activity. If the spontaneously active interneurone is "lost" during the period of inhibition the experimenter may be left in the ludicrous situation of recording a lack of activity where no activity exists. Therefore, only limited periods of inhibition are safely recorded (due to movement of the electrode) and the longest possible duration of after-inhibition may be much greater than that reported in this thesis. In this regard, the inhibition of the plantar reflex described by Abrahams (1974) developed progressively with repeated stimulation of the brain, lasted a number of hours after termination of the stimulation, and remained even after subsequent transection of the spinal cord.
The results of this thesis indicated that inhibitory build-up and habituation of the flexor reflex to repeated noxious (painful) stimuli bear some relationship to the perception of pain because:

1) Strychnine impaired habituation of the flexor reflex only if the intensity of stimulation was sufficiently strong to activate group IV afferents. This stimulus (20 v., 7.5 mA) was sufficient to cause pain when tested on this experimenter (electrodes inserted into the skin).

2) Inhibitory build-up was directly related to the intensity of stimulation. This build-up of inhibition was particularly apparent with stimuli of an intensity sufficient to cause pain.

3) The flexor reflex has been closely associated with the perception of pain by a number of authors. Sherrington (1910), in fact, defined the flexor reflex as a "nociceptive reflex".

The sudden application of a noxious (painful) stimulus is followed by a number of stereotyped responses or "psuedaffective reflexes" as named by Sherrington (1906). Melzack and Wall (1965:976) described these "psuedaffective reflexes" (now shortened to "affective reflexes"):

Sudden, unexpected damage to the skin is followed by 1) a startle response; 2) a flexion reflex; 3) postural readjustment; 4) vocalization; 5) orientation of the head and eyes to examine the damaged area; 6) autonomic responses; 7) evocation of past experience in a similar situation and prediction of the consequence of the stimulation; 8) many other patterns of behavior aimed at diminishing the sensory and affective components of the whole experience, such as rubbing the damaged area, avoidance behavior, and so forth.
The decerebrate dog displays such "pseudffective reflexes," in response to noxious stimulation, which are presumably divorced from any higher perception of pain. Sherrington (1906) observed that in the decerebrate dog these responses underwent a rapid decrement of response amplitude following repeated elicitation: "The movement, even when most vigorous and prompt, dies away rapidly, to be succeeded in some cases by a few weaker repetitions, each successively weaker and more transient than the last." Sherrington did not indicate if this rapid response decrement extended to the flexor reflex. The results of this thesis have confirmed Sherrington's results and have extended them to show that the flexor reflex also undergoes a rapid decrement in the decerebrate animal.

The FWR shares a number of characteristics with the perception of pain. The sudden application of a painful stimulus to the skin is perceived (in man), almost immediately as a sharp pain or "first pain" which is then followed, approximately 0.5 to 1.0 sec later, by a more diffuse, burning, or "second pain". This perceptual separation of pain into an early and a late component has been attributed to differing conduction velocities of cutaneous afferents: "first pain" with group III (A) afferents and "second pain" with group IV (C) afferents (Lewis and Pochin, 1938 a,b; Noordenbos, 1959; Sinclair and Stokes, 1964; Sinclair, 1967). Phylogenetically, "first pain" is believed to correspond to a relatively new and specific discriminatory or "epicritic system", and the "second pain" is believed to be representative of an old and diffuse or "protopathic system" (Head, 1920; Landau and Bishop, 1953; Collins, et al., 1960). In a similar if not identical manner,
the flexor reflex is also composed of an early and a late reflex component (Kugelburg, 1948; Dimitrijvec and Nathan, 1970; Price, 1972; this thesis, p. 133). The early flexor reflex has been associated with "first pain" and the late flexor reflex is associated with "second pain" (Price, 1972).

The threshold for the perception of pain and that of the FWR, in man, are identical (Hardy, 1953). Price (1972) has suggested that FRI's also function as the interneurones transmitting pain. Furthermore, the perception of pain may habituate (Glaser and Griffin, 1962; Le Blanc and Potvin, 1966; Campbell and Taub, 1973; Torebjork and Hallin, 1973; Pearson, personal communication) or sensitize (Noordenbos, 1959; Price, 1972) following repetitive stimulation.

The discharge of spinal interneurones may also be correlated with the early flexor reflex (high frequency burst) and the late flexor reflex (after-discharge). Repetitive stimulation results in a sensitization of the after discharge or "wind-up" (Mendell and Wall, 1965; Mendell, 1966; Wagman and Price, 1969; Price and Wagman, 1970, 1971; this thesis p. 143). The inhibition of neuronal after-discharge may be related to the induction of analgesia and the decrement of after-discharge may result in a reduction in the perception of pain. Therefore, interaction might be expected between the sensitization of after-discharge and the build-up of inhibition.

Intense stimulation of the skin of a limb is known to induce analgesia of the contralateral limb by "counter-irritation" (Mendell, 1973). Strong stimulation of the skin of a limb is also associated with inhibition of contralateral spinal interneurones believed to transmit
noxious information (Taub, 1964; Mendell, 1966; Brown and Franz, 1969). Therefore, repetitive stimulation of the skin might be expected to cause a progressive inhibition of after-discharge of contralateral spinal interneurones. This hypothesis was supported by the results of this thesis where such an interaction was demonstrated (p.156, this thesis).

Stimulation of the central grey of the brain stem in the vicinity of the n.r.d. induces a profound inhibition of the FWR, the interneurones of lamina V of the spinal cord, and produces a strong analgesia in the skin of the hindlimbs (Reynolds, 1969; Liebeskind, et al., 1974; Mayer and Liebeskind, 1974; Melzack and Melnickoff, 1974). Wall (1970) proposed that lamina V interneurones were likely to be FRI's; however, no anatomical connexion has been established between lamina V interneurones and flexor motorneurones. There is evidence which suggests that lamina VI interneurones send collaterals to the motorneuronal pools (Matsushita, 1969).

Considerable attention has been focused upon the role of lamina V interneurones in the transmission of pain. These interneurones respond to noxious cutaneous stimulation either electrical, chemical, or natural (pinch) (Liebeskind, et al., 1974). Some lamina V interneurones contribute to the spinocervical and spinothalamic tracts, and they are also the site for convergence of visceral and somatic group IV afferent input to the spinal cord (Pomeranz, et al., 1968). In response to peripheral stimuli extensive excitatory and inhibitory fields can be mapped for any particular lamina V interneurone. In addition stimuli applied to the inhibitory field can inhibit the response of such a neurone to a noxious

The inhibitory fields of lamina V interneurones are under strong inhibitory control by supraspinal mechanisms and the size of the receptive fields are tonically reduced in the decerebrate animal (Wall, 1967). The inhibition of lamina V interneurones, induced by stimulation of the n.r.d., was blocked by the injection of lysergic acid diethyl amide (LSD) (Liebeskind, et al., 1974), and the inhibition of spinal reflexes that occurs following stimulation of the ventro-medial region of the medulla (bulbospinal inhibition) was also blocked by infusion of LSD and methysergide (Clineschmidt and Anderson, 1970). The administration of LSD caused a specific inhibition of the activity of the neurones in the raphe nuclei (Haigler and Aghajanian, 1974). Furthermore, the cutaneous analgesia induced by stimulation of the n.r.d. is blocked by prior administration of p-CPA (Akil and Mayer, 1972) presumably due to the specific depletion of serotonin (Koe and Weissman, 1966; Sheard, 1973). In addition, lesions of the raphe nuclei (dorsal and median), pre-treatment with p-CPA, and infusion of serotonin antagonists such as LSD are associated with a potentiation of sensitization of various behavioural responses (Carlton and Advokat, 1973; Davis and Sheard, 1974; Davis and Sheard, in press); or increases in dishabituatory responses (Aghajanian and Sheard, 1968; Conner, et al., 1970); or decreased thresholds
to noxious stimuli (Ténen, 1967; Harvey and Lints, 1971).

In derivation from these various results, blockage of descending serotonergic inhibition would be expected to produce a greater degree of flexor response sensitization and perhaps hyperalgesia. In this study the initial level of reflex excitability (absolute sensitization) was increased by pre-treatment with p-CPA, by lesions of the n.r.d., and to a lesser extent by infusion of methysergide. These results imply that the removal of bulbospinal inhibition and/or the influence of the n.r.d. eliminates a tonic level of inhibition of the FWR. Continued repetition of the stimuli eventually lead to a FWR of an amplitude below that of controls, in p-CPA treated and lesioned (n.r.d.) rats. This particular finding is difficult to reconcile in terms of the removal of a tonic inhibition. It appears, however, that the raphe nuclei exert an inhibitory influence upon behavioural arousal. The arousal mechanism seems to exert both excitatory and inhibitory influences upon spinal reflexes (this thesis, p. 200) which suggests that both components may be released by removal of the influence of the n.r.d. and the serotonergic system. Thus a greater expression of inhibitory build-up might be predicted with a resultant increase in response decrement. These findings further support a correlation between a potentiation of sensitization of the FWR and cutaneous hyperalgesia.

Cutaneous analgesia induced by repeated stimulation of the brain stem does not commence immediately following the initiation of the stimulus (Melzack and Melinkoff, 1974). Furthermore, the period of analgesia may outlast the stimulation by periods of up to 5 min. (Reynolds, 1969).
Repetitive peripheral stimulation can also induce an analgesia which develops gradually and which outlasts the period of stimulation (Wall and Sweet, 1967; Meyer and Fields, 1972; Andersson, et al., 1973; Campbell and Taub, 1973). Electro-analgesia induced in this way can occur if the intensity of stimulation is non-noxious, although noxious stimulation may be required to evoke percutaneous analgesia. It has been postulated that some forms of analgesia may occur as the result of inhibition of the sensory transmission of pain, and the gradual induction of electro-analgesia has been attributed to a progressive build-up or recruitment of inhibition within the central nervous system. It has also been suggested that the balance between excitation and inhibition of spinal interneurones might determine the effectiveness of pain transmission (Melzack, 1973). Weak stimuli, below group IV afferent thresholds, have been shown to inhibit the response of dorsal horn interneurones to noxious stimuli (Wall, 1967). The stimuli employed in this thesis were usually of an intensity above the threshold of group IV afferents (6 mA) in order to produce a marked inhibitory build-up of spinal interneurones; however, some interneurones demonstrated a reduction in spontaneous activity with repetition of relatively weak stimuli. The induction of acupuncture analgesia usually involves repetition of stimuli at much greater frequencies and for much longer periods than used in this study. It is possible that increasing the period of stimulation and the frequency of stimulation would produce a significant build-up of inhibition of the neurones subserving the transmission of pain.

Considerable interest has developed as to the possible relationship
between the transmission of pain and central inhibition (Melzack, 1973). Dusser de Barenne (1910) presented evidence that the topical application of strychnine to the dorsal surface of the spinal cord produced hyper-algesia and hyper-reflexia in the dermatomes supplied by that region of the cord. Of course hyper-reflexia was to be expected; however, Dusser de Barenne also reports behaviour that must be associated with spontaneous pain and hyper-algesia:

A few seconds after the repeated contact of the poison [strychnine] with the spinal cord at this spot, the dog that meanwhile has nearly awakened from the narcosis, begins to lick the skin of the right half of the trunk over a region, extending like a band of moderate breadth from the mid-dorsal to the mid-ventral line, passing over the most caudal ribs....The hyper-reflectory symptoms are: 1) wrinkling of the skin, 2) curving of the vertebral column, the concavity turned to the right, 3) with intervals scratching movements of the right hind-limb, resembling closely those of the well known Sherrington's "scratch reflex"....Continually repeating this gentle, mechanical irritation, I gradually approached that region, and as soon as I have passed its boundary, the hyper-reflectory symptoms described above are aroused or become much more intense, whilst in most cases, the animal shows at the same time by howling and biting that the subjective symptoms are likewise aroused or their intensity increased.

These results indicated a tonic inhibition of pain transmission at the spinal level. Inhibitory build-up, decrement of the FWR to painful stimuli, and acupuncture analgesia (McLennan and Gilfillan, personal communication) are antagonized by the infusion of strychnine which implies a common mechanism for inhibitory build-up and some forms of analgesia. Furthermore, acupuncture analgesia is also blocked or reduced following the injection of bicuculline and methysergide (McLennan and Gilfillan, personal communication) and acupuncture analgesia has been associated with an increase in the brain content of serotonin (Acupuncture Anaesthesia Research Group, 1973).
Inhibition and the Theories of Habituation

The major theories of habituation are as follows: 1) Dual-Process theory (Groves and Thompson, 1970) 2) Conditioned Inhibition (Sokolov, 1965; Stein, 1966) 3) Afferent Neuronal Habituation (Hernandez-Peon, 1961) and each theory recognizes two separate sensory systems. One, a specific sensory system, which refers to the classical paucisynaptic afferent pathways that carry modally, temporally, and spatially specific information from the receptors to the corresponding sensory-motor cortical projection areas or to the motorneurones. This is the "S-R" pathway described by Groves and Thompson (1970). The other system is the non-specific sensory system ("state" system) which is associated with phenomena such as arousal, attention, and "affective" responses. The information carried by this system is not temporally or spatially coded nor is it modality specific. In other words, it has characteristics similar to the information transmitted by FRA's. The non-specific system is characterized by sensitization of reflex responses whereas the specific sensory system demonstrates habituation (Webster, 1971; 1974). Response decrements do occur in the non-specific sensory system (Sharpless and Jasper, 1956; Hernandez-Peon, 1960; Knispel and Siegel, 1973; Deutsch and Dennis, 1975), however.

The "state" system may be synonymous with the brain stem reticular mechanism that controls the level of behavioural excitability or arousal. This central excitatory state is conceptually identical to the "central arousal state" (Lindsley, 1960; Duffy, 1962). The reticular formation is
usually accepted as the major component of the arousal system (Moruzzi, 1964) and stimulation of the reticular formation will cause EEG arousal and dishabituation (or sensitization) of habituation in the specific sensory system (Mancia, et al., 1959). Hernandez-Peon's (1960) hypothesis that "afferent neuronal habituation" results from a "reticular centrifugal inhibition" upon the specific sensory pathways has not been confirmed (Buchwald and Humphrey, 1973).

The Dual-Process theory is based almost entirely on habituation of the flexor reflex in the spinal cat. As a consequence, the flexor reflex was isolated from the reticular formation and other central nervous system structures critical to the expression of behavioural arousal. In essence the Dual-Process theory presents two inferred constructs, one a process of response decrement in the S-R pathway and the other a process of response increment in the "state" system (see this thesis, p.13), which when summated, are responsible for the final motor response of the flexor reflex. Confirmation of this hypothesis was found at the interneuronal level. The process of inferred decrement was represented by habituation of the high frequency burst discharge and sensitization of the after-discharge was representative of the inferred incremental process. Weak (touch, hair movement, etc.) stimulation of the skin tends to evoke a high frequency burst in spinal interneurones (Price and Browe, 1973; this thesis) which undergoes a decrement of response (Groves and Thompson, 1973; this thesis) when the stimulus is repeated. The after-discharge of spinal interneurones is evoked, for the most part, by high intensity (pinch) stimulation of the skin (Price and Browe, 1973; this thesis)
and this response builds-up with repeated stimulation. This sensitization of after-discharge is often, but not always, followed by a decrement of response in both the spinal (Groves and Thompson, 1973) and intact animal (this thesis). It is assumed that the decrement of the high frequency burst is likely to be a form of "synaptic depression" and that the sensitization of after-discharge is perhaps a consequence of "frequency facilitation". Even the laminar location of interneurones displaying the high frequency burst is similar to those associated with the transmission of relatively specific sensory information (Price and Browe, 1973). In contrast, spinal interneurones displaying after-discharge are located in laminae which contain interneurones contributing to spino-reticular tracts and which receive a large convergence of activity from spinal and supraspinal structures (Matsushita, 1969; Bowsher, 1972). Thus, after-discharge of spinal interneurones may be itself considered a property of the "state" system whereas the high frequency burst response is likely characteristic of the specific sensory system.

The results of this thesis and the results presented by Spencer, et al. (1966c) demonstrate that drugs such as strychnine, picrotoxin, and bicuculline do not stop habituation of the flexor reflex in the spinal and intact animal. Neither does the inhibition of spinal interneurones in the spinal rat indicate any significant potentiation of inhibition (Groves and Thompson, 1973; this thesis) during repeated cutaneous stimulation. In the spinal rat inhibitory mechanisms, possibly short chained intersegmental systems, may modify FWR amplitude but do not appear to play a causitive role in response decrements. In fact they may prevent response decrements under certain circumstances (this thesis, p.178).
Thus, there is no evidence to indicate that habituation is primarily a result of "conditioned inhibition." If chemical synapses characteristically display various forms of synaptic plasticity it is not unlikely that habituation and sensitization are the consequence of the properties of the synapses of the central nervous system.

Habituation of the flexor reflex in the intact rat is not identical to habituation of this reflex in the spinal rat (p. 97, this thesis). Habituation is readily apparent in the intact animal with an intensity of stimulation which produces almost no habituation in the spinal animal. This difference is probably related to the elimination of spino-bulbo-spinal reflexes (Shimamura and Aoki, 1969) and the reduction of flexor reflex "after-discharge" (Forbes, et al., 1923). Therefore, supraspinal structures contribute a component of excitation to the spinal flexor reflex, and intact rats may show a maximal response to strong stimuli without a prior build-up of activity (p. 97, this thesis). Habituation of this supraspinal component is greatest with relatively strong stimuli and least with weak stimuli (p. 94). It is impaired by the infusion of strychnine and bicuculline and it is facilitated by pre-treatment with p-CPA and lesions of the n.r.d.

The reticular formation has been proposed as the site of generation of behaviour that is associated with reactions to environmental stimuli of novel, aversive, and painful perceptual characteristics. Stimuli of these types produce arousal, increased vigilance, escape behaviour, and affective responses. Some of these reactions such as behavioural arousal, EEG arousal, and the orienting reflex are assumed to be mediated by the
the reticular formation. Somatic and autonomic reflexes may also be the vehicles of such behaviours. For example, the flexor reflex has a nociceptive component and a low threshold component associated with reflex stepping (Sherrington, 1910). Presumably the nociceptive component is associated with reticular activation, arousal, etc. In other words, the flexor reflex is both an affective reflex and a simple spinal reflex.

It is proposed that cutaneous stimuli are capable of activating reticular interneurons which in turn have the capacity to inhibit tonic activity in the flexor reflex pathway. Repeated stimulation then results in a build-up of this inhibition. This inhibition is partially blocked by strychnine which may indicate the involvement of glycine and glycine-like transmitters. However, other forms of inhibition are also involved (perhaps GABA mediated inhibition). The decrement of the FWR related to a build-up of inhibition is only apparent when the stimulus is of an intensity which is noxious (possibly painful) and it may represent a mechanism for adaptation to painful stimuli repeated at regular intervals. Serotonergic systems are known to inhibit reticular activity (Simon, et al., 1973) and behavioural arousal (Fibiger and Campbell, 1971; Mabry and Campbell, 1972) presumably by an action upon the "limbic-forebrain" system which itself exerts an inhibitory action upon the reticular formation (Morgane and Stern, 1973). Thus, lesions of the nucleus raphe dorsalis and pre-treatment with p-CPA may eliminate an inhibition of the reticular neurones responsible for the build-up of inhibition observed
in the spinal cord. This proposed mechanism of habituation is similar to a "reticular centrifugal inhibition" of the non-specific sensory system suggested by Hernandez-Peon (1960).

Voronin and Sokolov (1960) came to the conclusion that habituation of the orienting reflex was the result of "conditioned inhibition". This concept was based in part upon a study of the plastic responses evoked by repeated stimulation (light) of neurones in the visual system (Sokolov, 1965). In this situation, repeated stimulation evoked sensitization of neurones in the retina, superior colliculus, and the geniculate body but interneurones of the visual cortex (pyramidal neurones) and especially the interneurones of the hippocampus, underwent rapid response decrements. Inhibitory build-up of spontaneously active interneurones was also demonstrated (Sokolov, 1969:699) in the visual cortex. Such results prompted Sokolov (1965) to suggest that PTP of the visual pathways resulted in sensitization of activity in some interneurones and an "elaboration of inhibition" in others. Furthermore Sokolov (1965:342) stated that: "The observation of complete inhibition of spike discharges during habituation leads to the conclusion that interneurones are involved not as negative feed-back loops of pyramidal neurones, but as "parallel inhibition chains," summing parallel inputs of excitatory and inhibitory pathways to pyramidal neurones." Sokolov (1965) inferred that the mechanism of this "elaboration of inhibition" might be PTP of inhibitory synapses. There is an obvious similarity between Sokolov's results and the results of this thesis.

Arousal induced by stimulation of the reticular formation appears
to be under the inhibitory control of the frontal cortex (Clemente and Sterman, 1967; Lynch, et al., 1969; Lynch, 1970) in the rat. Therefore, the observation that lesions of the frontal cortex prevent habituation of the FWR (Griffin and Pearson, 1968b) is probably related to an increase in behavioural arousal. A stimulus which originally did not activate the arousal system might now demonstrate a continuous dishabitation (or sensitization) of the flexor reflex. Decerebration should release reticular arousal from the inhibitory influence of the frontal cortex and thus cause an impairment of flexor response habituation. However, in comparison to the flexor reflex isolated from the reticular formation by spinal transection, decerebration seems capable of facilitating initial response decrements (this thesis, p.117). Thus, with high intensity stimulation the reticular formation contributes to habituation of the arousal component of the flexor reflex. Lesions of the n.r.d. and pre-treatment with p-CPA also facilitated flexor habituation in comparison to the intact animal which suggests such manipulations increase arousal and habituation of arousal.

Thompson, et al. (1973:214) state that: "Under certain circumstances (strong stimuli presented regularly at relatively slow rate) temporal conditioning of sensitization of state may occur." This thesis presents no substantial evidence for temporal conditioning of inhibition, but if the excitatory component is capable of such conditioning it is not unreasonable to expect a similar conditioning of the inhibitory component of the "state" system.

According to Sokolov (1960, 1969) the orienting reflex is a complex
of responses to novel stimuli which serves to increase sensitivity to peripheral stimuli. As such, Sokolov would predict a resistance to habituation with stimuli of a strength close to threshold for the reflex and less resistance with strong stimuli. This hypothesis contradicts the Dual-Process theory which would predict that low intensity stimulation will produce the greatest habituation. A number of studies, examining a variety of reflex and behavioural responses, has indicated a contradiction to this aspect of the Dual-Process theory. Two of these studies (Wickelgren, 1967a; Pearson and MacDonald, 1973) may have produced contradictory results simply due to a secondary alteration in FWR excitability associated with the level of spinal transection. On the other hand, experiments performed on the intact animal have also produced contradictory results (Harper, 1968; Peeke, 1969; Davis and Wagman, 1968; Jackson, 1974; this thesis, p. 94). An example is illustrated in Figure 58 taken from Peeke (1969). These results refer to the biting behaviour (territorial) of male three-spined sticklebacks in response to stimuli of two differing strengths. One curve (RM) represents the response to another and live male stickleback whereas the other (MM) represents the response to a crude model of a male stickleback. Assuming that the natural stimulus (live stickleback) is perceived as being of greater significance or intensity these results are surprisingly similar to those shown in Figure 11 (this thesis, p. 94). Similar relationships have been shown for the galvanic skin response (Harper, 1968; Jackson, 1974) and for the startle response of the rat (Davis and Wagman, 1968). In these various studies the absolute and relative habituation were greatest with
Figure 58. Mean number of bites per minute delivered at the stimulus by a male three-spined stickleback Gasterosteus aculeatus, for a group presented with a real, male conspecific confined to a glass tube (RM) and a group presented with a crude wood model male (MM) moved in a circular path in the territory of the subject fish. Each point represents the average of 3 successive minutes (Peeke, 1969).
the highest intensity of stimulation. In the case of the FWR this relationship held only for the intact rat and, furthermore, if the stimulation was repeated a sufficient number of times it was obvious that the relative degree of habituation was greatest with the lower intensity of stimulation.

Thompson, et al., (1973:245) account for the contradiction in the results presented by Peeke (1969) as follows: "....the periods of response measurement are critical -- too large time blocks [analogous to response blocks used in this thesis] wash out initial response sensitization" (see p. 92, this thesis). Thus, the response decrement is classified as "habituation of sensitization" by Groves and Thompson, (1970). Unfortunately, "habituation of sensitization" is not strictly distinguished from that occurring in the spinal animal as opposed to that in the intact animal. The terminology "habituation of sensitization" also implies the necessity for a build-up or increment of response and as a consequence I propose the term "arousal habituation" which refers to a decrement of that component of reflex response attributable to activation of the "state" system. Specifically, "arousal habituation" is the gradual withdrawal of the "state" system from the reflex level. Both the size of the arousal component and the degree of arousal habituation are directly related to stimulus intensity. The contribution of the "state" system is both excitatory and inhibitory provided the reticular formation is not isolated from the reflex arc under study. With this preface an addendum to the Dual-Process theory is offered.

Presentation of a strong stimulus will activate the "state" system
with a resultant reticular activation (this results in the interpolation of excitation into the reflex arc). Repeated stimulation may cause sensitization of the arousal (excitation) but a maximal response may be triggered by the first stimulus presentation. The withdrawal of arousal ("arousal habituation") from the reflex arc is due to a build-up of inhibition of the more tonic components of the reflex. This inhibition is probably manifest at all levels of the "state" system and it may be analogous to the "general inhibition" of the spinal reflexes described by Beritoff (1965).

The build-up of inhibition demonstrates these characteristics:

a) The process of inhibitory build-up occurs in the "state" system (requires the reticular formation) but not in the S-R pathway. It is manifest as a progressive reduction in the tonic activity of the reflex ("after-discharge").

b) During repeated stimulation the build-up of inhibition is progressive and therefore arousal decays or habituates. The inhibition may later decay but other decremental mechanisms (synaptic depression?) will have become predominant.

c) The rate of inhibitory build-up and the duration of inhibition following cessation of the stimulation (after-inhibition) are directly related to the intensity of stimulation. At low intensities little or no activation of the arousal system occurs.

d) The rate of inhibitory build-up is directly related to stimulus frequency.

e) The inhibition outlasts the duration of stimulation (after-inhibition) and then decays spontaneously.
f) Repeated series of strong stimuli result in progressively longer periods of after-inhibition.

g) Inhibitory build-up may demonstrate generalization to the extent that the generalized stimulus lies within an inhibitory field.

h) Dis-inhibition of after-inhibition may occur if the dis-inhibitory stimulus lies within an excitatory field.

i) There is a possibility that temporal conditioning of after-inhibition will occur with repetition of strong stimuli at relatively low rates.

The results of this thesis have presented evidence for one further inferred construct which might underlie habituation of a particular component of the flexor reflex. This process is one of a sensitization of centrifugal inhibition of the more tonic elements of the FWR. To this extent this thesis differs from the Dual-Process theory and supports Sokolov's theory of Conditioned Inhibition. However, this thesis adds to and does not contradict the Dual-Process theory. Furthermore, it supports the concept that stimulation of sensory afferents elicits both significant inhibition and excitation which co-exist within the central nervous system. The interaction of this developing excitation and inhibition presents a unique way in which the central nervous system can control sensory inflow to the spinal cord and reflex behaviour.

Pavlovian inhibition does not draw a distinction between the phenomena of "synaptic depression" and inhibitory build-up (inhibition is used in the Sherringtonian sense) (This thesis, p. 2). Either mechanism might be classified as being a manifestation of inhibition
by Beritoff; however, a mechanism such as "synaptic depression" seems at least superficially, to be analogous to Sherrington's concept of central "fatigue". Sherrington's concept of inhibition fails to account for the long term inhibition of the plantar reflex (Abrahams, 1974) and the long term analgesia which is related to inhibition (Liebeskind, et al., 1974), both of which are induced by repeated stimulation of the brain and which have the capacity to last for periods of hours after cessation of the stimulus. This type of inhibition is a confirmation of the Russians' hypothesis of "general inhibition", but is this a form of Sherringtonian inhibition? It is readily admitted that the inhibition of spontaneously active spinal interneurones (after-inhibition) is of a duration well below that described for the inhibition of the plantar reflex; however, facilitation of the action of excitatory synapses can last for many hours in vertebrates and it is not unreasonable to expect similar phenomena to occur for inhibitory synapses.


Hardy, J. D. "Thresholds of pain and reflex contraction as related to noxious stimulation" J. Appl. Physiol., 5:725-739, 1953.


