An Investigation of the Neurophysiological Correspondents of Learning and Memory in Two Forebrain Regions of the Day-Old Chick

Thesis

AN INVESTIGATION OF THE NEUROPHYSIOLOGICAL CORRESPONDENTS OF LEARNING AND MEMORY IN TWO FOREBRAIN REGIONS OF THE DAY-OLD CHICK.

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This Thesis is dedicated to the memory of my father Francis John Edwin. Rest in peace.

ABSTRACT

Spontaneous bursting (5 or more spikes of 200-450mV amplitude at 400Hz) occurs in many areas of chick forebrain. Day-old chicks trained on a one-trial passive avoidance task show a bilateral increase of up to 350% in bursting following training in one of these areas: the intermediate medial hyperstriatum ventrale, or IMHV (Mason & Rose, 1987; 1988).

An investigation was carried out into the time course and lateralization of this change in bursting activity following the training of day-old chicks on a passive avoidance task. Chicks were trained to either avoid a bead coated with the bitter-tasting substance methylanthranilate (M-birds) or were trained to peck a water coated bead (W-birds). Bursting was recorded sequentially from the IMHV of both hemispheres at 8 time points over the period 1 to 9 hours post-test. The results indicate that there are significant differences in bursting activity recorded from M-birds only during the period 3-7hr posttest, when compared to W-birds. Between 6-7hr posttest there are significant differences in the burst firing patterns of the right IMHV of M-birds compared to the left. At other time points tested there are no significant differences between hemispheres. No between hemisphere differences are evident in W-birds.

Multi-unit recordings were made from the lobus parolfactorius (LPO), another forebrain structure to show changes in biochemistry and morphology following passive avoidance training. M-birds showed a higher incidence of bursting when compared to W-birds over the period 1-10hr posttest. No lateralization of bursting was seen in either group at any time posttest.

In a further experiment, chicks trained to avoid the methylanthranilate coated bead were subjected to subconvulsive electroshock 5min posttraining. This procedure was used to test whether the training-induced increase in bursting in the LPO was a direct

correlate of memory formation for the task. This electroshock treatment produced two groups of birds: one group that avoided the bead (remembered the task) and another that pecked the bead (forgot the task). Multi-unit recordings from the LPO of these two groups revealed that the group that avoided the bead had a significantly higher mean burst-frequency when compared to the group that pecked the bead, indicating that increased bursting in the LPO following training is directly associated with recall for the task. These results are similar to those of Mason and Rose (1988) who showed that amnesia abolished a training-induced enhancement of bursting in the IMHV.

The effects of pretraining bilateral LPO lesions on IMHV bursting activity were examined. The IMHV of four groups of birds was recorded from following training: two groups of M-birds, one with LPO lesions the other with sham LPO lesions and two similarly treated groups of W-birds. A significant increase in overall IMHV bursting activity was observed in sham-lesioned M-birds when compared to sham-lesioned W-birds. However, no significant difference in bursting activity was seen between lesioned M-birds and lesioned W-birds. There was a trend towards a higher overall level of bursting in lesioned W-birds, when compared to sham-lesioned W-birds.

These results are discussed with reference to previous electrophysiological studies concerning the role of burst-firing patterns in models of learning and memory.

CHAPTER 1. General Introduction.

The reception, storage and retrieval of information are general properties of neuronal systems that serve to adapt the behaviour of an organism to its environment. Without the ability to learn items and retain them in memory for subsequent retrieval, it would be impossible to either repeat successes according to experience or to avoid failure. But what underlies the storage of such items of information? Perhaps the most popular proposal has been that learning and memory mechanisms affect the encoding of information via some change in the 'wiring' of the brain, typically with the production of new synaptic connections. Evidence for this type of neuronal plasticity has come from studies of embryonic development, regeneration of peripheral tissue, injury and environmental pressures (Cotman & Nieto-Sampedro, 1984; Kwak & Matus, 1988). The fundamental unit that appears to be of importance in this growth is the synapse. One of the major problems in neurobiology is to explain the biophysical and biochemical processes occurring in the nervous system that are responsible for the synaptic changes in the neural pathways and circuits that underlie learning.

The importance of the electrical activity of neurons in shaping patterns of connections is becoming increasingly clear (Brewer & Cotman, 1989; Changeux & Danchin, 1976; Fields et al., 1991; Jones, 1990; Wiesel & Hubel, 1965). From theoretical considerations alone, some aspect of neuronal activity must influence the synaptic patterns that exist amongst nerve cells, since it is hard to imagine how an individual could learn or remember otherwise. There is a tremendous amount of literature indicating that electrical activity in the developing nervous system plays a crucial role in the patterning of neuronal geometry and synaptic connections. With regard to studies of learning and memory, there is widespread agreement that the processing of information in the cortex is associated with complex spatio-temporal patterns of activity. Yet the vast majority of experimental work is either based upon single neuron recordings (e.g., Rolls, 1987) or upon recordings made with gross electrodes to

which tens of thousands of neurons contribute in an unknown fashion (e.g., Landfield et al., 1972). Although these experiments have provided some information concerning the organization and function of the cortex, they have not enabled any detailed examination of the spatio-temporal organization of neuronal activity.

It appears, therefore, that some form of compromise is required between two of the 'camps' of neurophysiology, on the one hand single cell recording and on the other recording of focal electroencephalogram potentials. One such 'compromise' is the use of electrodes with relatively low impedance, when compared to those used to record intracellularly, to allow the recording of what is termed 'multi-unit activity'. This multi-unit technique allows the simultaneous recording of small groups of cells. This has an obvious advantage over both single cell and focal EEG recordings when searching for any neurophysiological changes produced by learning: whilst the level of analysis is quite specific (the firing of individual cells), the ability to sample a population of cells allows the analysis to operate at a 'network' level. Looking for such learning-induced changes can be referred to as looking for the proverbial needle in a haystack. Whilst this is also true for studies concentrating upon either biochemical or morphological changes, the time course over which the respective changes will occur is vastly different: the electrophysiologist speaks in terms of periods lasting fractions of one second, the time domain of the biochemist or the morphologist is of the order of minutes to tens of minutes to hours. This is, perhaps, the reason why the proponents of the majority of learning paradigms have looked for electrophysiological changes at either the time of training or the time of testing. However, as is becoming increasingly clear, changes underlying memory formation do not occur exclusively around the time of training. Not only is this true for animal models of learning but also for the 'reduced' neurophysiological learning models (Doyle et al., 1990; Gibbs & Ng, 1977; Mason & Rose, 1987; Matthies, 1989; Zamani & Rose, 1990).

For the reasons described above the experimental work described in this thesis was undertaken using a mutli-unit recording approach to study the neurophysiological consequences of a single-trial passive avoidance task on relatively small groups of neurons. This recording was extended over several hours posttraining in order to examine any long-term effects of training upon neuronal firing. In writing a General Introduction for the use of prolonged posttraining recording of spontaneous multi-unit activity, one is handicapped by the lack of its historical use as an electrophysiological approach to the study of learning and memory. It is not simply the case of "X and Y procedures were carried out in species Z so I did the same thing in species A". The popular approach used to study the electrophysiology of learning and memory has become rather 'bottom up' in recent years. Initial experiments examined electrophysiological changes occurring during learning at the level of the electroencephalogram (EEG), in particular at the theta EEG band (e.g., Landfield et al., 1972; see pages 29-32 of this Thesis for the section on theta rhythm). The experimental emphasis then shifted towards first identifying a lasting change in the enhancement of synaptic transmission and then attempting to 'back associate' it with the phenomena of learning and memory. It appears that many researchers have made the *a priori* decision that spontaneous, memory-specific neurophysiological changes occurring at the cellular level during learning and also memory consolidation would be impossible to (a) localize and (b) interpret. Whilst these conclusions are understandable they do not provide excuses for 'not looking in the first place'.

The General Introduction presented below is designed around the points introduced above. The initial section is a short review of firing patterns, a logical first step in a thesis which examines changes in neuronal firing following training. This is followed by a brief overview of previous and current neurophysiological models of memory formation. The final part of the General Introduction examines the applicability of neuronal firing patterns and neurophysiological models to animal models of learning

and memory.

Brief overview of neuronal firing patterns.

This brief outline of neuronal firing patterns is by no means meant to be exhaustive and will concentrate upon neurons that express bursting activity, a firing pattern proposed to underlie a form of long-term synaptic plasticity: long-term potentiation (Buzsaki, 1989) and also to be involved in the acquisition of a passive avoidance task in chicks (Mason & Rose, 1987; 1988).

Neocortical neurons have in the past been classified according to various criteria such as morphology, location, synaptic relationships (both locally and distally) and biochemical properties, in particular neurotransmitters and their associated enzymes (Peters & Jones, 1984; White, 1989). However, to understand a neuron's functional role within any circuit it is necessary to know not only the characteristics outlined above but also its electrical 'fingerprint', as determined by its intrinsic membrane properties.

It has been known for some time that neuronal membranes do not all behave similarly (Hodgkin, 1948). Neurons differ in terms of the types and distribution of specific ion channels on their soma and dendrites. These intrinsic differences are evident in the shapes of individual action potentials. They also produce distinctive temporal patterns of repetitive firing, thus determining to a large extent the way individual neurons transform synaptic input into spike (axonal) output. As these 'fingerprints' can be extremely uniform between cells within a particular neuronal class (e.g., amongst cerebellar Purkinje cells, thalamic relay cells) these intrinsic physiological membrane properties constitute a reasonable basis for neuronal classification (Connors & Gutnick, 1990; Llinas, 1988).

Neurons of the neocortex do not appear to be physiologically homogenous in terms of

their firing repertoires. Three basic types of intrinsic physiology have been recognized: regular spiking, fast spiking and intrinsically bursting neurons (Connors & Gurnick, 1990; Llinas, 1988). For each type, classification is based upon three general variables: (1) the characteristics of individual action potential afterhyperpolarizing potentials; (2) the response to a just threshold intracellular current pulse; and (3) the repetitive response to prolonged intracellularly applied stimuli. Intracellular staining experiments suggest that these neuron classes also exhibit distinct morphological correlates (Peters & Jones, 1984; White, 1989). These classes are not meant to be exclusive. Sub-categories are likely to exist and there may be some as yet unidentified cell types.

(1) Regular spiking neurons.

This cell type is the most commonly encountered class in electrophysiological studies, described initially by Mountcastle and colleagues (Mountcastle et al., 1969) as firing 'regular' action potentials. Most published *in vivo* recordings from neocortex are from this cell class (e.g. Calvin & Sypert, 1976). Individual regularly spiking action potentials are relatively long-lasting, possibly due to a slow rate of repolarization. Each spike is usually followed by a complex series of intrinsically generated afterhyperpolarizations (AHPs) and afterdepolarizations (ADPs). Threshold stimulation of a regularly spiking neuron generates only a single spike and, in contrast to intrinsically bursting cells, as the stimulus amplitude increases, the first interspike interval decreases as a function of current intensity. Regularly spiking neurons also display marked adaptation of spike frequency with prolonged, constant amplitude stimuli. (Figure 1.1a).

(2) Fast-spiking neurons.

Fast spiking neurons have only been rarely described (Mountcastle et al., 1969) and seem to be elusive when using extracellular recording. Fast spiking cells have a 'fast'

Figure 1.1; Differences in firing patterns between regular-spiking, fast-spiking and intrinsically bursting neurons.

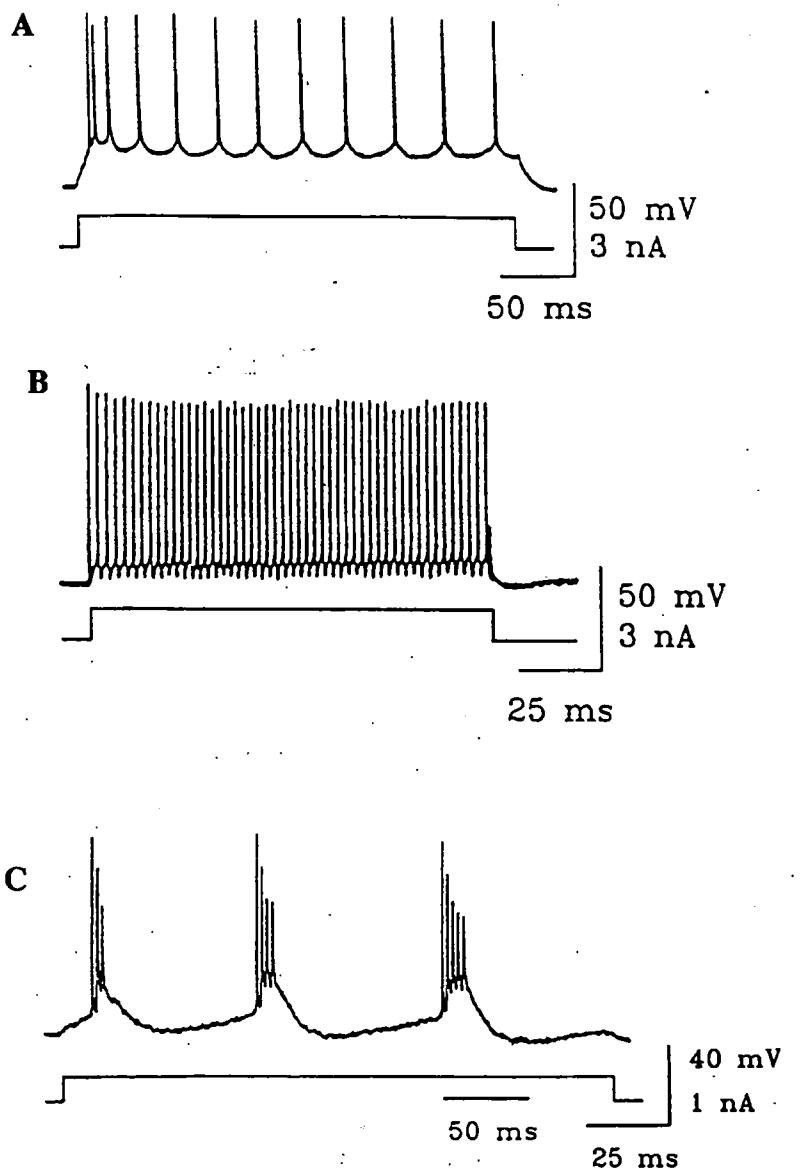


Figure 1.1; (A) Regular-spiking neuron stimulated with suprathreshold intracellular current. Initial response is high frequency spike output that rapidly declines to a lower, sustained frequency. (B) Fast-spiking neuron recorded under similar conditions to A. High-frequency spikes are generated and sustained for the duration of the stimulus. (C) Intrinsically bursting neuron under similar conditions, firing repeated bursts of spikes to a prolonged stimulus (taken from Connors & Gutnick, 1990).

action potential of less than 0.5ms duration. Repolarization is very quick, each spike being curtailed by a deep, relatively brief AHP (Figure 1.1b). Fast spiking cells have no pronounced hyperpolarizing or depolarizing potentials (unlike intrinsically bursting and regularly spiking cells) and undergo little or no adaptation. When strongly stimulated fast spiking cells can sustain spike frequencies of at least 500-600Hz for hundreds of milliseconds. Fast spiking cells are, therefore, able to 'follow' stimulation very closely, producing a faithful conversion of synaptic input to output over a wide dynamic range (in strong contrast to intrinsically bursting and regularly spiking cells).

(3) Intrinsically bursting neurons.

Intrinsically bursting neurons are distinguished by the tendency for their spikes to appear in a stereotyped, clustered pattern, called a burst (Agmon & Connors, 1989; Connors et al., 1982). Bursts are often the minimal response to a just threshold intracellular stimulus. Individual spikes of intrinsically bursting cells are quite similar to those of regularly spiking cells, although they are often followed by more prominent ADPs which may summate to form a slow, low-amplitude depolarizing wave during the burst. Within a burst, each successive spike usually declines in amplitude. This is presumed to occur because of inactivation of sodium conductances through sustained depolarization (Figure 1.1c).

A prolonged intracellular stimulus to an intrinsically bursting cell can produce a complex, often periodic pattern of bursts and single spikes. Fast spiking or regularly spiking cells will respond with monotonic frequency patterns to the same stimulation.

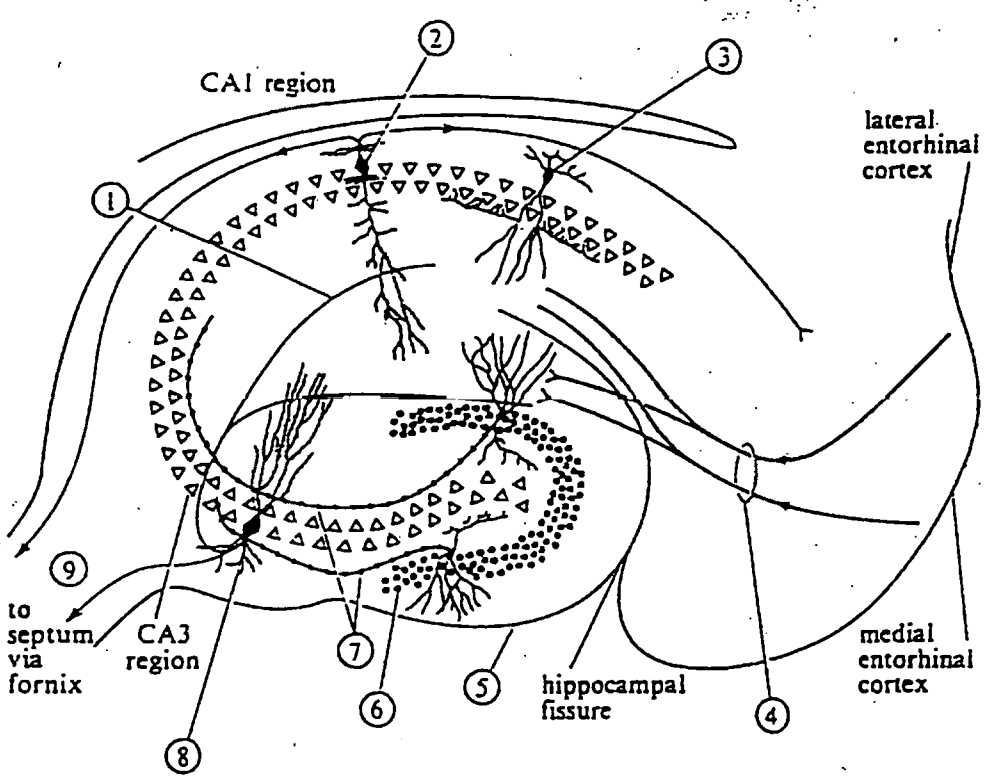
The term 'burst' is commonly used in the neurophysiological literature to describe a neuron's firing behaviour. However, the designation 'bursting cell' is ambiguous unless modified to indicate whether the firing pattern represents an inherent property of the cell, or a direct response to its afferent drive. The term 'intrinsically bursting' refers to a cell's tendency to generate clusters of high-frequency spikes solely as a

manifestation of its intrinsic membrane properties - independent of its synaptic input. Almost any neuron could produce clusters of spikes in response to phasic synaptic input. However, this response pattern does not itself justify classification of an intrinsically bursting neuron (Connors & Gutnick, 1990). Extracellular recording cannot distinguish between dendritic and axonal/somatic bursting and, therefore, intracellular recordings are the only means of classification, providing the cell is isolated from its extrinsic connections.

Much of the research into receptor-activated neuronal bursting has been performed in the mammalian hippocampus (for a schematic diagram of the rat hippocampus see Figure 1.2). Because of this, the following brief review of the physiology of bursting neurons will concentrate upon hippocampal studies. Bursts recorded from hippocampal pyramidal neurons typically consist of trains of two to six action potentials 'riding' upon a depolarizing envelope (Wong & Prince, 1978), the event lasting about 30msec. Intracellular recordings show intrinsic rhythmicity of bursting responses at frequencies of 0.2 to 5Hz, the inter-burst interval being determined by the development and subsequent delay of a post-burst membrane hyperpolarization.

Bursting in the hippocampus can reliably be produced with the application of an agonist to one of the glutamate receptors: N-methyl-D-aspartate (NMDA). Both NMDA and magnesium free artificial cerebrospinal fluid can induce population and endogenous bursts in CA3 neurons of the hippocampal slice preparation. Single CA3 pyramidal cell stimulation in the presence of NMDA or magnesium free artificial cerebrospinal fluid can partially synchronize a population of neurons (Neuman et al., 1989). This synchronization is similar to that produced in the disinhibited slice (Miles & Wong, 1983). Bursting in CA1 pyramidal cells is also sensitive to NMDA. Microiontophoretic application of NMDA to CA1 in the hippocampal slice produced bursting that was sensitive to 2-amino-5-phosphonovalerate (AP5) and cobalt (Peet et

Figure 1.2; Diagram showing the major divisions of the Hippocampus



1. Schaffer collateral	**4. Perforant path**	**7. Mossy fibres**
2. CA1 pyramidal cell	**5. Dentate gyrus**	**8. CA3 Pyramidal cell**
3. Basket cell	**6. Granule cells**	**9. Fimbria**

The major input to the hippocampus is via the entorhinal cortex, the entorhinal cortex projects via the perforant path to the dentate gyrus. The dentate gyrus projects via mossy fibres to CA3 pyramidal area. CA3 projects to CA1 via Schaffer collaterals. The major outputs of the hippocampus are through the fimbria and entorhinal cortex.

al., 1986). Application of quisqualate evoked tetrodotoxin-sensitive spikes (TTX), those produced by NMDA consisted of bursts of TTX-sensitive action potentials superimposed upon an underlying depolarizing shift of membrane potential (the DAP). The action of AP5 and cobalt on CA1 pyramids was to selectively (and reversibly) antagonize the NMDA-induced depolarization and DAP (Peet et al., 1986). The ionic conductances underlying burst generation appear to be as follows (Wong et al., 1986): membrane depolarization above threshold activates a fast sodium current causing the cell to fire an action potential; this depolarization causes an increase in calcium conductance, which is a long lasting increase, decaying only slowly to sustain a long secondary depolarization following the initial sodium spike (the DAP). The amplitude of the DAP reaches threshold in such a way that it generates a sequence of action potentials, seen as a burst. As the intracellular concentration of calcium increases during the burst, it 'turns on' an accumulative, repolarizing potassium conductance. This potassium conductance gradually increases and eventually becomes sufficient to terminate the burst, sustaining the slowly decaying afterhyperpolarization.

Burst firing, at least in hippocampal pyramidal neurons, appears to be an apical dendritic phenomenon. The usual model of the neuron depicts the dendrites as passive 'cables' (Rall, 1977). Their integrative function merely consists of conveying the synaptic signal from the synapse to the soma/initial segment region of the cell. However, a number of supraspinal neurons do not appear to conform to this generalization: their dendrites are excitable. Direct intradendritic recordings from neurons in mammalian neocortex (Purpura et al., 1965), cerebellar Purkinje cells (Llinas, 1988; Llinas & Hess, 1976; Llinas & Nicholson, 1971) and hippocampal pyramidal cells (Wong et al., 1979) have revealed that action potentials can be generated locally. The pattern of these action potentials is predominantly burst firing. Some spike components of the burst are TTX-resistant, that is, they do not reflect sodium spikes. Evidence to suggest that these dendritic spikes are not simply electronically propagated soma activities comes from pyramidal cell recordings (Wong

et al., 1979). Firstly, the sizes of the recorded dendritic action potentials were much larger than would be predicted purely by passive electrotonic spread. Secondly, simultaneous intracellular recordings from electrotonically coupled soma and dendritic elements revealed that action potentials in the two regions were not correlated in a 1:1 fashion. Figure 1.3a shows two traces, one from distal dendrite, the other from soma, that probably come from the same cell, as hyperpolarizing pulses applied to the dendrite produced electrotonic hyperpolarization of the soma. As can be seen from this Figure, depolarization of the dendrite produces burst firing, causing only solitary cell body spikes at the soma. Depolarization of the soma elicited only single spikes and did not produce bursting in the dendrite. This suggests that dendritic bursting may facilitate communication between those dendrites and the cell body. In the CA1 region of the hippocampus powerful excitatory input impinges upon the dendritic fields of pyramidal cells. If this input produces dendritic bursting, then a significant amplification of the input will occur in the post-synaptic cell. Bursting can, therefore, be envisaged as a process for securing the transmission of signals between cells in a synaptically connected network. Such bursting properties provide the basic step in the generation of synchronized discharge in the hippocampus -- a simple epileptiform event.

Since burst firing in the soma or dendrites of pyramidal cells can be elicited by short duration depolarizations, it is easy to imagine that phasic excitatory synaptic potentials should also trigger postsynaptic bursts. However, orthodromic activation of pyramidal cells does not lead to burst generation (Wong, 1982). The reason for this is that suprathreshold orthodromic stimulation elicits an excitation-inhibition sequence in the postsynaptic neuron. Inhibition in the hippocampus is, at least in part, generated by an intrinsic group of GABAergic neurons (Andersen et al., 1963; Ribak et al., 1978).

Synchronization of the neuronal population can be elicited when substances such as

Figure 1.3; Intracellular recordings from (a) two synaptically coupled neurons and (b) electrotonically coupled dendrite and cell body of the same neuron.

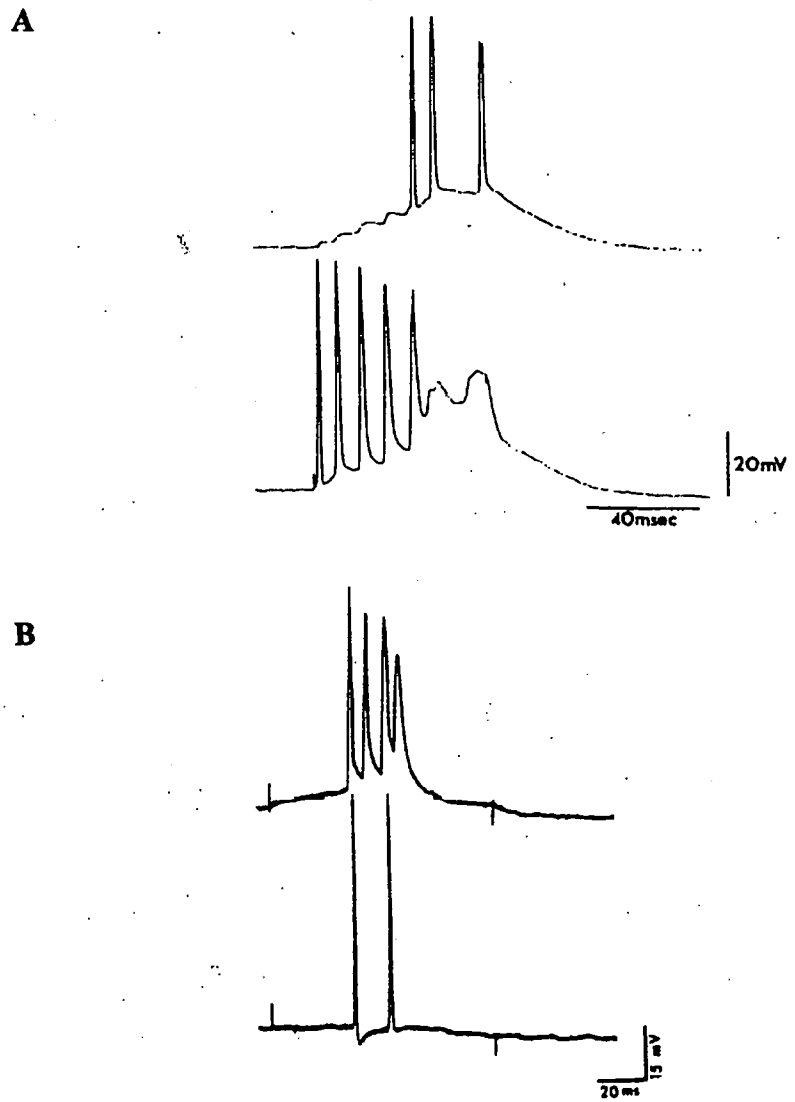


Figure 1.3; (A) Recurrent excitatory connection between pyramidal cells. Each action potential in the presynaptic cell triggered an EPSP in the follower cell, eventually leading to a burst of action potentials. (B) Differential firing patterns recorded intracellularly from the dendrite (top trace) and cell body (lower trace) of hippocampal CA1 pyranidal cell. Note that depolarizing current injected into the dendritic recording site elicits a burst of dendritic action potentials, but only solitary cell body spikes (taken from Wong et al., 1986).

penicillin are added to the hippocampus (Miles et al., 1984). This synchronization is produced by a reduction in inhibition. Bursting can also be initiated via application of morphine, presumably acting to inhibit inhibitory GABAergic interneurons (Zieglgansberger et al., 1979). In models of hippocampal neuronal networks, the strength of inhibition can determine the number and pattern of bursting excitatory cells that will fire in synchrony (Traub et al., 1987). A similar mechanism may apply in neocortex, with the added feature that synchronous activation of the intrinsically bursting cell minority leads to either net inhibition or excitation of the non-bursting cell majority, depending upon the efficacy of GABA-mediated IPSPs (Chagnac-Amitai & Connors, 1990).

Burst firing in a CA3 neuron can trigger a burst in the post-synaptic cell via a monosynaptic connection (Figure 1.3b). This may allow a single neuron to initiate and reset the rhythm of synchronized discharge of an extremely large cell population. Such discharges can be envisaged as cascading excitation: a few pyramidal cells near to threshold fire bursts of action potentials which allow recruitment of additional pyramidal cells through sequential activation of excitatory connections. Eventually, a critical proportion of pyramidal cells become simultaneous active, recruiting virtually the whole population. This is thought to occur upon blockage of normal inhibition, via actions described above. Tetanic stimulation of hippocampal slices also causes cascading excitation (Miles & Wong, 1987), presumably via physiological GABA inhibition. As will be discussed in the next section of the General Introduction, tetanic stimulation causes increases in the strengths of excitatory connections, and the tetanic stimulation noted above may be similar to that of LTP. It can be inferred from this evidence that the phenomenon of bursting relies upon a reduction of inhibitory 'tone' in the neural network. However, this need not necessarily be the case. Stelzer et al. (1987) showed that repetitive stimulation of the hippocampal slice reduces the GABA response via the activation of NMDA receptors. Application of a selective NMDA antagonist, for example AP5, maintains GABA activity following stimulation.

Increasing the activity of NMDA receptors causes seizure-like discharges, and blocking of NMDA receptors (for example by AP5 or any divalent cation, obviously with the exception of calcium) prevents the onset of such discharges (Anderson et al., 1986; Peet et al., 1986). Such antagonism has also been shown to impair spatial learning (Morris et al., 1986) and also to reversibly prevent LTP induction of the population spike recorded from both the Schaffer/commissural pathway and the synaptic region of CA1. From this it seems that a reduction of inhibition is not necessarily required for seizure-like events to occur, provided that excitatory connections are sufficiently strong and each neuron is sufficiently close to threshold.

The relevance of neuronal firing patterns, in particular that of burst-firing, to neurophysiological models of learning and memory will now be discussed.

Neurophysiological Models of Learning and Memory.

Although several aspects of synaptic structure appear to change through experience (Purves et al., 1986), the most consistent potential correspondent of memory storage during learning is an alteration in the number and/or pattern of synaptic connections. This change in connectivity could be accomplished through changes in the 'strengths' of previously existent synapses, allowing the incorporation of new patterns into the organization of the brain (Hebb, 1949). Hebb's neurophysiological postulate for learning proposes that the strength of plastic synapses can be enhanced if the use of those synapses is associated with the nearly simultaneous occurrence of postsynaptic electrical activity. The Hebb model has, therefore, been referred to as a 'successful use' model (Kupfermann & Pinsker, 1969) because the modification depends upon the use or activity of the presynaptic cell and activity in the postsynaptic cell. Another form of plasticity, complementary to that of Hebb, has been proposed by Stent (1973). A Stent synapse between two neurons, A and B, will be formed under the following conditions: the presynaptic cell A must repeatedly and persistently fail to

excite the postsynaptic cell B, whilst B is firing under the influence of other inputs; this will produce changes in one or both cells such that A's efficiency, as one of the cells firing B, will be decreased.

An alternative cellular model to that of either Hebb or Stent is that proposed by Eccles in which the use of a synapse itself, independent of any postsynaptic effects, produces an alteration in synaptic efficacy (Eccles, 1953). The model is derived from early observations that changes in spike activity in presynaptic neurons leads to changes in the magnitude of postsynaptic potentials.

A simple conception concerning the neuronal basis of learning is that an item of information could be first stored as a 'dynamic engram' in the form of reverberating excitation in a spatio-temporally arranged pattern. Such reverberatory activity has been seen in the red nucleus of the cat (Tsukahara, 1981) and the rodent hippocampus (Buszaki, 1989). This circulating activity could bring about structural changes at the synapses involved and thus produce consolidation into a 'structural engram'. Conceivably, the memory could then be retrieved by an activation of such synapses. This concept of reverberatory circuits, although lacking in much experimental evidence, is consistent with the subjective experience that we must practice material in order for it to be learned (i.e., let it pass repeatedly through our consciousness).

Changes in synaptic efficacy during and after stimulation have come under increasing investigation recently as they may represent the mechanisms which underlie learning and memory. Mechanisms of synaptic modulation are often referred to as either short-term or long-term, according to their time course of development and decay. Four major types of short-term, use-dependent modulation have been defined; facilitation, augmentation, posttetanic potentiation and synaptic depression (Zucker, 1989). These types of modulation appear to be consistent with the Eccles postulate, that is, they

involve presynaptic changes only.

Synaptic facilitation is an enhancement of transmitter release that results in enlargement of the postsynaptic potential. It develops gradually over one second during repetitive stimulation and then decays over a similar timespan. If it lasts for several seconds, it is termed augmentation. Both phenomena are apparently caused by a buildup of calcium in the presynaptic terminal, causing increased transmitter release (Katz & Miledi, 1968). Most synapses display facilitation or augmentation, but the magnitude appears to vary, perhaps due to differences in the calcium clearing mechanisms amongst synapses.

Posttetanic potentiation refers to an enhancement of transmitter release that follows a brief, high frequency stimulation train and lasts 2-5 minutes (Magleby & Zengel, 1975). It is thought that this form of modulation also relies, at least in part, upon residual calcium on the presynaptic terminal, the slow decay possibly reflecting a slow phase of calcium removal.

Short-term depression is a gradual decrease in synaptic strength following repetitive stimulation. It is often caused by progressive depletion of releasable neurotransmitter stores. Its time course is variable, but seldom lasts longer than a minute (Zucker, 1989).

In terms of function, synaptic potentiation amounts to making a central nervous system process occur more readily as a result of repeated use (practice). It can be argued that this constitutes a learning process. Perhaps the most significant form of potentiation is that of long-term potentiation (LTP, also known as long-term enhancement or long-lasting potentiation). It was originally described in the rabbit hippocampus (Bliss & Lomo, 1973). In addition to LTP there is a form of synaptic potentiation known as long-term depression, the 'inverse' of LTP thought to be a

model of 'neuronal' forgetfulness. Long-term depression is a relatively rare phenomenon and its mechanisms are still largely unknown. It has been observed in only three areas of the vertebrate brain: the cerebellum (Ito, 1989), the hippocampus (Levy & Steward, 1983); and the the visual cortex (Artola et al., 1990).

The next three sections of this chapter deal with brief reviews of LTP, LTD and theta rhythm, One feature of all of these models is the involvement, to a lesser or greater extent of burst-firing patterns, suggesting that this ubiquitous type of neuronal response may have a special involvement in mechanisms of learning and memory.

(1) Long-Term Potentiation.

Long-term potentiation (LTP) can be defined as a stable, relatively long lasting increase in the magnitude of the post-synaptic response to a constant afferent volley following brief tetanic stimulation of the same afferents (see Figure 1.4). This potentiation is generally described as an enduring increase of the population excitatory postsynaptic potential (EPSP) and/or population spike. However, whilst the population spike may reflect changes in the EPSP, it is to a large extent controlled by a parallel disynaptic feed-forward inhibitory postsynaptic potential that follows activation of the same afferents that cause the monosynaptic EPSP (Abraham et al., 1987; Wigstrom & Gustaffson, 1985), as well as by general cellular excitability. One consequence of LTP induction is that when the slope of the potentiated field EPSP is returned to its pre-tetanization level by lowering the stimulus strength, the EPSP is then associated with a larger population spike than before. This enhanced EPSP-spike relationship has been termed E-S potentiation (Bliss & Lomo, 1973; Andersen et al., 1980). Wilson et al. (1981) suggested that E-S potentiation could be accounted for by a greater effect of tetanization on LTP at monosynaptic excitatory pathways when compared to disynaptic inhibitory pathways. This suggestion was supported by experiments conducted by Abraham et al. (1987). The population spike may,

Figure1.4; Induction of LTP in dentate gyrus following tetanic stimulation of the perforant path.

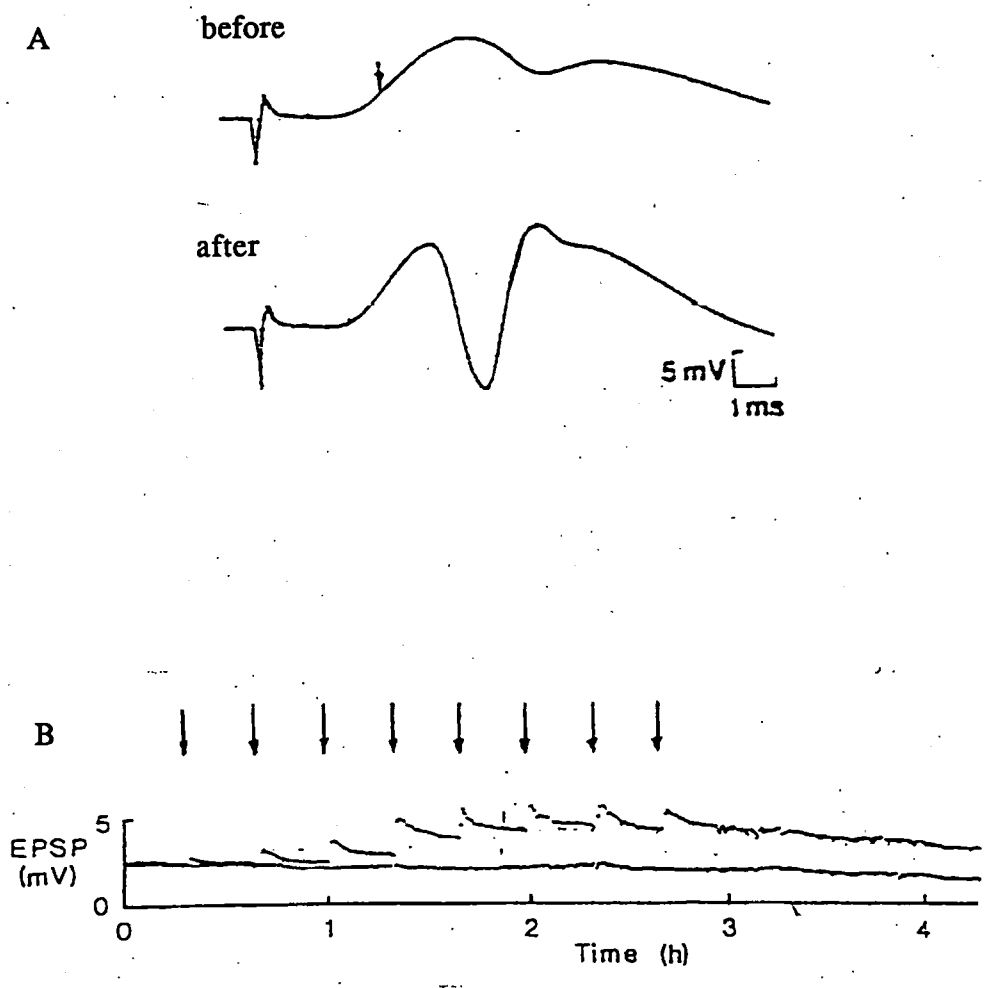


Figure 1.4; (A) Dentate population response to single test stimulation of the perforant path before and after a series of high-frequency trains of stimulation to the same input (tetani shown in B)

(B) Test responses following tetanic stimuli (arrows) of the perorant path. The slope of the EPSP is measured during the early rising phase of the response (arrow in A). Taken from Bliss, Goddard and Riives (1983).

therefore, be a less reliable measure of potentiation of excitatory synaptic transmission when compared to the EPSP. Because of this, Gustaffson and Wigstrom (1988) suggest that the term 'LTP' should refer exclusively to changes in the EPSP.

LTP was first demonstrated by Bliss and Lomo (1973) in an anaesthetized preparation, and by Bliss and Gardner-Medwin (1973) in an unanaesthetized preparation (rabbit hippocampus in both cases). In these experiments tetanic stimuli were applied to the perforant path and responses recorded in the dentate gyrus. This marked the first demonstration of an alteration in the neurophysiological activity of the mammalian brain that was capable of possessing a considerable time course: LTP could be seen as an increase of up to 50% in the amplitude of the post-synaptic response which lasted for at least ten hours in the anaesthetized and up to sixteen weeks in the unanaesthetized preparation, respectively. But do these neuronal changes really underlie some form of memory storage? This critical question, which is still not completely answered, was recognized by Bliss and Lomo at the time:

"Whether or not the intact animal makes use in real life of a property which has been revealed by synchronous, repetitive volleys to a population of fibres, the normal pattern of activity along which are unknown, is another matter."

Bliss and Lomo, 1973

Two stages of LTP can be differentiated: its induction and its maintenance. The vast majority of published work has dealt almost exclusively with the former.

The induction of LTP begins with the delivery of tetanic stimulation to afferent fibres. Depending upon the intensity, frequency and pattern of afferent activation, LTP can be induced in a gradual or an all or none fashion. Potentiation does not develop immediately following a tetanus (McNaughton, 1983). In the CA1 hippocampal region it is delayed by about 3 seconds with an additional delay of 15-20s before the

maximum potentiation is observed. At higher stimulus intensities and higher tetanic frequencies, LTP reaches an asymptotic level after only one, or perhaps a few tetanisations. Tetanus frequencies used to elicit LTP range from 0.2 to 400 Hz (Douglas 1977; Skelton et al., 1983), the number of pulses delivered usually being between one to two hundred. At stimulation rates lower than about 50 Hz, a heterosynaptic depression can be seen, probably due to the activation of inhibitory interneurons projecting onto their target cells (Alger et al., 1978). At slightly higher frequencies, between 100 and 400 Hz, such depression is rarely observed. Initial studies in the dentate gyrus (Bliss & Gardner-Medwin, 1983; McNaughton et al., 1978) and later in CA1 (Lee, 1983) showed that the induction of LTP requires the magnitude of stimulation to be great enough to activate a sufficient number of synapses. This cooperativity can be seen as an associative interaction between separate excitatory inputs in which a weak input (that does not itself induce LTP) becomes potentiated only after tetanization in combination with a strong one. This occurs in synapses from the perforant path onto dentate granule cells in the hippocampus (Levy & Steward, 1979) and also from the Schaffer collaterals onto CA1 cells (Barrionuevo & Brown, 1983; Levy & Steward, 1979). This suggests that stimulation of a set of synapses increases the probability that other synapses on the same neuron will be strengthened if they are activated at the same time or a few milliseconds later. This type of potentiation has been termed 'associative LTP' and provides a possible neural mechanism for encoding associations between different afferent inputs that occur concurrently.

The importance of postsynaptic activation in LTP induction was first demonstrated via the facilitation of LTP induction through blockade of postsynaptic inhibition (Wigstrom & Gustaffson, 1985). It has also been shown that EPSPs evoked by low frequency single volleys can induce LTP when paired with depolarizing current pulses (Gustaffson et al., 1987; Sastry et al., 1986; Wigstrom et al., 1986b). Neither the

presynaptic volley, nor the postsynaptic depolarization is sufficient, by itself, to induce LTP. These studies indicate that LTP obeys Hebb's rule, at least in the CA1 and CA3 regions of the hippocampus, in that synaptic modification only occurs when the synapse is active in close temporal contiguity with postsynaptic activity (Jaffe & Johnston, 1990; Kelso et al., 1986). There is some evidence that postsynaptic firing, when present, also plays a role in LTP induction. When spikes evoked by brief depolarizing current pulses are paired with a weak afferent stimulus, not producing LTP by itself, substantial LTP can be produced (Wigstrom et al., 1988). However, the induction of LTP does not require the firing of the postsynaptic cell, only the depolarization of the postsynaptic membrane, since LTP can still be induced after blockade of action potentials by intracellularly injected local anaesthetics (Gustaffson et al., 1987; Kelso et al., 1986).

Although NMDA receptors do not appear to be important for normal low-frequency synaptic transmission, they have been intimately associated with LTP induction. The NMDA receptor is one subtype of the glutamate class of receptors. Glutamate, the transmitter released from excitatory afferents in the CA1 region of the hippocampus, was originally shown to activate three receptor subtypes (Watkins & Evans, 1981). These subtypes were classified by the then selective exogenous analogues of glutamate; N-methyl-D-aspartate (NMDA), kainate (K) and quisqualate (Q). Subsequent research has shown that the Q receptor has two subtypes; Qi, an ionotropic receptor subtype and Qp, a metabotropic receptor subtype (for review see Sladeczek et al., 1988). The Qp subtype has been shown to activate (and be regulated by) protein kinase C (Manzoni et al., 1990). If the membrane potentials of CA1 pyramidal cells are more negative than -70mV, the application of AP5 has little effect upon the EPSP evoked by single stimuli (Collingridge et al., 1983). This suggests that with low frequency stimulation, synaptically released glutamate appears to act preferentially upon non-NMDA glutamate receptors. However, blockade of NMDA

receptors with AP5 entirely blocks the induction of LTP (Collingridge et al., 1983). If NMDA receptors are not involved with normal synaptic transmission, how are they involved in LTP induction?

In the CA1 field of the hippocampus synaptically released glutamate normally acts upon both NMDA and non-NMDA glutamate receptors. However, NMDA receptors are normally blocked by extracellular magnesium at resting membrane potentials (Wigstrom et al., 1986a; Coan & Collingridge, 1987). To observe the NMDA-mediated response it is necessary either to sufficiently depolarize the membrane to relieve the magnesium block, or simply remove magnesium from the bathing medium. Although this explains why NMDA responses are seen during strong depolarization produced by tetanic stimulation, it does not explain why the NMDA receptor is essential for the induction of LTP.

The NMDA receptor may be essential because its activation allows the influx of calcium, that is, NMDA receptors are coupled to calcium channels. The postsynaptic role played by calcium in LTP induction was indicated by Lynch et al. (1983) by loading the postsynaptic cell with EGTA, a calcium chelator. This blocked the induction of LTP, providing clear evidence that both the postsynaptic cell and a postsynaptic rise in calcium are necessary for LTP induction. However, the postsynaptic entry of calcium through voltage sensitive calcium channels is, by itself, incapable of inducing LTP (Malenka et al., 1989). The rise in postsynaptic calcium presumably comes from another source. It has been found that the ion channel associated with the NMDA receptor, unlike those associated with Q and K receptors, is highly permeable to calcium ions (Jahr & Stevens, 1987). Calcium influx through NMDA receptor-associated ion channels has also been shown to be enhanced during LTP induction in the CA1 region of the rat hippocampus (Stanton et al., 1989). Although this suggests that calcium entry through NMDA receptor ion channels is essential for the induction of LTP, it does not rule out a role for calcium release from

postsynaptic stores. These results suggest that the postsynaptic requirements for LTP induction are the activation of NMDA receptors coupled with sufficient depolarization to cause a brief increase in the postsynaptic calcium concentration. If these are indeed the necessary conditions for LTP induction, what are the necessary conditions for LTP maintenance?

Before a postsynaptic locus for the induction of LTP had been identified, evidence had been presented suggesting that LTP in the dentate gyrus was associated with an enduring increase in the release of glutamate (Dolphin et al., 1982; Bliss et al., 1986; Errington et al., 1987). However, Aniksztejn et al. (1989) used a similar method to that of Bliss et al. (1986) and found that LTP induction was not followed by a sustained increase in glutamate release. Since the induction of LTP had been shown clearly to be postsynaptic, it was hypothesized that LTP induction caused the release of some 'plasticity factor' postsynaptically which then diffused back to the presynaptic terminal (Williams & Bliss, 1988) and that this caused the enhanced presynaptic release of glutamate. This sustained release was suggested to be the mechanism responsible for the maintenance of LTP. Although there is no known mechanism for such retrograde synaptic communication, a potential class of 'retrograde messengers' has been suggested: the eicosanoids, metabolites of arachidonic acid (Williams et al., 1989). Activation of NMDA receptors can stimulate the calcium-dependent enzyme phospholipase A_2, causing release of free arachidonic acid into the surrounding medium (Dumius et al., 1988). If this occurs at hippocampal synapses, arachidonic acid, or its metabolites, which can pass easily across cell membranes, could pass from a postsynaptic site into the presynaptic terminal to initiate the potentiation of transmitter release (Linden et al., 1987; Williams & Bliss, 1988).

Another possible mechanism for LTP maintenance is an increase in the sensitivity of postsynaptic receptors. If LTP maintenance were due to a sustained increase in the

presynaptic release of glutamate, then one would expect to observe a parallel increase in the receptor potentials of both the Q and NMDA components of the monosynaptic EPSP after LTP induction. Kauer et al. (1988) performed an experiment in which they examined the NMDA component of the monosynaptic EPSP in isolation from the Q component. This was accomplished by: (1) adding CNQX to the bathing medium; and (2) partially relieving the magnesium block of the NMDA receptor-coupled ion channel by holding the postsynaptic membrane at -55mV. A tetanus that would normally have produced LTP was then delivered to the Schaffer collateral inputs. This tetanus produced an increase in the NMDA component of the EPSP that persisted for only 1-3min. Although there was no persistent increase in the NMDA component in the presence of CNQX after tetanic stimulation, the Q component was considerably potentiated over control values upon removal of the CNQX block. This indicated that LTP induction occurred in the presence of CNQX and that LTP induction was independent of of the activation of CNQX-sensitive Q receptors. The results of this experiment have been supported by Muller et al. (1988) using the Q receptor antagonist DNQX. Malenka et al. (1989) have suggested on the basis of these findings that the long-term change underlying LTP is most easily explained by an increase in the sensitivity of the postsynaptic membrane to glutamate acting upon Q, rather than NMDA, receptors. Although the biochemical events thought to occur following an LTP-producing tetanus are beyond the scope of this introduction the mechanism underlying 'increased sensitivity' LTP maintenance may be outlined as follows. During tetanisation of Schaffer collaterals Q/K receptors on CA1 pyramidal cells will be activated sufficiently to produce a large depolarization. This results in a relief of the magnesium block of the NMDA channel. As a consequence of this calcium enters the postsynaptic spine (although there is no direct experimental proof for the existence of NMDA receptors that are co-localized with Q/K receptors on the same dendritic spine, this situation accounts for all the properties of LTP induction, as first proposed by Wigstrom & Gustaffson, 1985a). The consequence of this may be two-fold. For

calcium to exert its effect it must bind to calcium/calmodulin dependent protein kinase (CaM), presumably within the spine (CaM is a major constituent of postsynaptic densities, specializations of the cytoskeleton that lie directly beneath postsynaptic membranes, Kennedy et al., 1984).

The activation of postsynaptic glutamate receptors may also cause the activation of phospholipase C, via the action of a G protein, which in turn may break down phosphoinositol biphosphate to form two intracellular messengers: diacylglycerol and inositol trisphosphate. Diacylglycerol causes the translocation of PKC to the membrane from the cytosol. Although there is no evidence that NMDA receptors are coupled to this second messenger pathway, the activation of protein kinase C could occur via the stimulation of postsynaptic Qp receptors (Manzoni et al., 1990). Inositol trisphosphate causes calcium to be released from intracellular stores. CaM kinase causes PKC to become attached to the membrane, presumably via a calmodulin-dependent phosphorylation of either PKC or its membrane anchoring proteins (Wolf & Sayhoun, 1986). This is assumed to protect PKC from processes that normally detach it from the membrane such as proteolysis (Melloni et al., 1985). The long-term association of PKC with the membrane can phosphorylate ion channels or their constituents, particularly in response to transient calcium concentration elevation, to increase cellular excitability by decreasing potassium conductances or modulating synaptic conductances (Bank et al., 1989). The persistent change responsible for LTP maintenance in this model is postsynaptic, the increased sensitivity of the Q/K receptor. Malenka et al (1989) have proposed that a phosphorylation step, possibly of the Q/K receptor, underlies this change, resulting in a larger synaptic response. Recent evidence has, however, suggested that NMDA receptor-mediated transmission may also undergo long-term potentiation, at least in the CA1 region of the hippocampus (Bashir et al., 1991). In terms of LTP, this means that a tetanus, in addition to increasing the efficiency of synaptic transmission, could directly alter the plasticity in the pathway for long periods. Subsequent tetani could then result in correspondingly

greater enhancements of synaptic transmission as LTP of the NMDA component will provide increased entry of calcium, producing further LTP. This has implications both for the genesis of epileptiform activity and also the generation of kindling, an animal model of epilepsy. The limited scope of this General Introduction does not allow a more detailed discussion of these topics.

These observations suggest that (at least) two interacting mechanisms, increased presynaptic neurotransmitter release together with enhanced postsynaptic sensitivity of Q/K receptors, may in some way converge to potentiate synaptic transmission during LTP establishment.

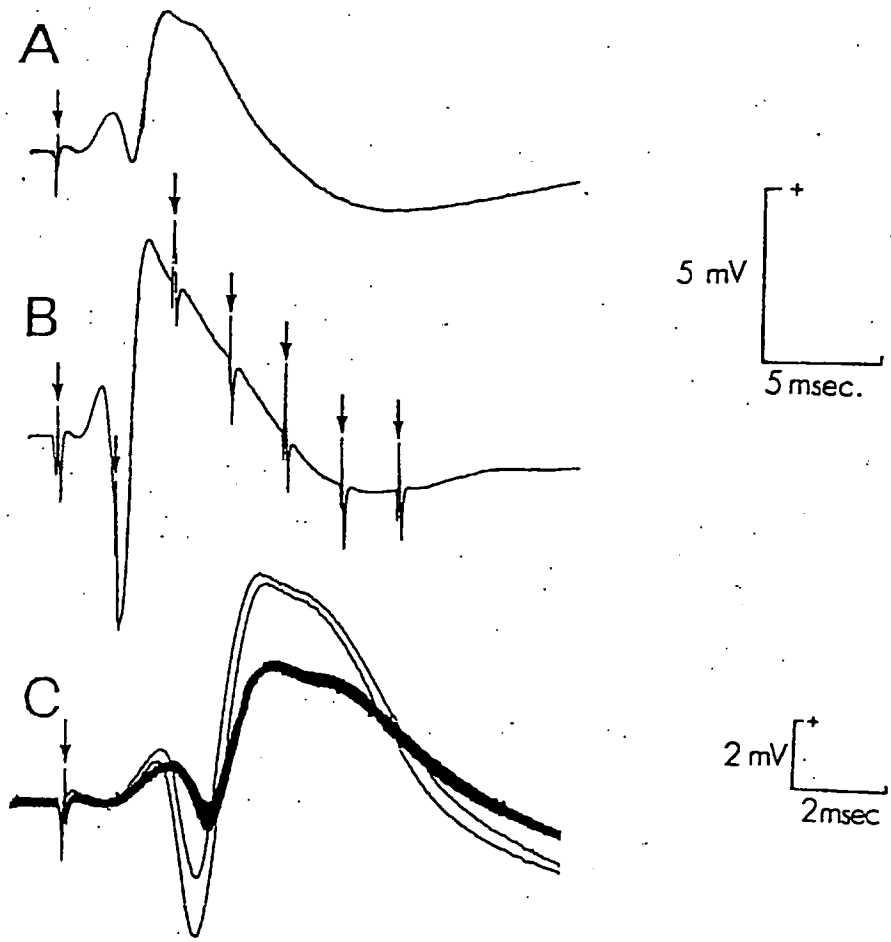
Bliss and Lomo (1973) originally made it quite clear that the stimulation parameters they were using to induce LTP were decidedly 'unphysiological'. This outlines one of the major conceptual difficulties in relating LTP to an endogenous mechanism for memory formation: the parameters commonly used for LTP induction are well beyond the normal physiological firing rate of hippocampal neurons. To produce LTP usually 50-400 stimuli are delivered at frequencies of 100-400Hz (Teyler & DiScenna, 1984). Although hippocampal pyramidal cells are known to fire in bursts or 'complex spikes' at frequencies well above 100Hz, groups of only 3 or 4 action potentials seem to be the usual output of these cells (see previous section and Suzuki & Smith, 1985). Continuous high frequency discharge is only seen under experimentally-induced or pathological conditions. Because of this disparity between normal patterns of cell firing and those stimuli patterns commonly used to induce LTP, experimenters have designed their patterns of stimulation to mimic the natural firing properties of pyramidal burst-firing neurons. In one of the earliest studies to use such patterned stimulation, Douglas (1977) delivered the tetanus as a package of short bursts (6 to 10 stimuli) of 400 Hz stimulation every few seconds or minutes. This technique reliably produced LTP, avoided the problem of after-discharges seen with continuous high

frequency stimulation, and provided a more 'physiological' stimulation. Douglas compared this type of patterned LTP stimulation to the normal tetanic (see Figure 1.5) and found that the response to a burst (stimulation in perforant path, recording in dentate gyrus) was very similar to that evoked by a single test pulse. After-discharge was never observed. Figure 1.5 shows both the typical potential evoked by a series of 10 test pulses and also the response to a burst after 9 previous bursts. The LTP induction from patterned stimulation was as large and as reliable as that reported in previous studies, although no post-tetanic depression was observed.

Changes in the inter-burst interval had no effect upon LTP induction in the Douglas (1977) study - bursts could be repeated at rates from only one per second to one per minute without any gross effect upon the potentiation observed. However, the number of bursts seemed to be critical, with less than 8 bursts producing a small, often unreliable potentiation. This lack of stimulus specificity of LTP in this study is in stark contrast to later studies using 'theta patterned' stimulation.

The possible influence of theta rhythm upon LTP induction has recently been investigated. Theta rhythm is a sinusoidal EEG potential of 5-9Hz found in the dentate gyrus and CA1 fields of the hippocampus (see section 3). Staubli and Lynch (1987) showed that maximal LTP to short bursts of stimuli (10 bursts of stimuli, 4 pulses per burst at 100Hz) was obtained in the CA1 field of a hippocampal slice when the bursts were delivered at theta rhythm periodicity, in this case at 5Hz. The amount of potentiation was significantly less when shorter or longer inter-burst intervals were chosen. This strong relationship between LTP induction and theta rhythm differs from that found in the study by Douglas (1977), in which no such relationship was found: the induction of LTP was independent of changes in the interburst interval. Reasons for the difference between the Staubli and Lynch result and that of Douglas may be due to : (a) the use of different hippocampal areas, i.e., CA1 vs. dentate and (b)

Figure 1.5; LTP as induced by "physiological" stimulation



A. Field potential evoked by a single pulse (arrow) applied to the perforant path. The positive wave is due to EPSPs in the granule cells, the postsynaptic elements. Superimposed upon this is a negative going population spike, produced by the almost synchronous firing of the granule cells.

B. Response to a burst of 7 pulses, same animal as above. The potential is larger than A because of the growth in response over the series of 20 bursts.

C. Potentiation induced by B. The width of the line pairs are the 95% confidence limits for two averages. The pre-tetanus average is shown filled. The average 48hrs later is shown unfilled. Note that both the EPSP and population spike components are larger. The onset to population spike is also shorter.

Taken from Douglas (1977)

chronic (Staubli & Lynch) vs. acute (Douglas) stimulation. Pavlides et al. (1988) used a similar preparation to that of Staubli and Lynch (1987) and were able to demonstrate that LTP induction in the dentate gyrus of rats is preferentially induced on the positive phase of theta rhythm. Theta was induced via stimulation of the midbrain and bursting stimulation was applied either at the peak or at the trough of the evoked theta rhythm (10 bursts of stimuli, 5 pulses per burst at 400Hz). The presence of theta in the dentate gyrus, and in particular its phase, appears to affect the ability of perforant path neurons to induce LTP, via repetitive firing, in the dentate granule cells. Perforant path stimuli appear to enhance granule cell synaptic efficacy when presented at the peak of theta rhythm, while there is either no synaptic enhancement, or even a depression of synaptic efficacy, when stimulation is coincident with the theta rhythm trough. This seems to be in agreement with the finding that bursting cells fire upon the positive phase of theta rhythm during rat locomotion (Fox et al., 1986) and that inhibitory interneurons fire (and inhibit bursting pyramidal cells) during the negative phase of theta (Buzsaki & Eidelberg, 1983).

A slightly different stimulus patterning to induce LTP has been employed by Diamond et al. (1988), a pattern they call 'primed burst potentiation'. This pattern consists of a single priming pulse followed 170ms later (i.e. at a theta frequency of 6Hz) by a high-frequency burst of pulses. This pattern can reliably produce LTP in the *in vitro* CA1 field with the application of only 5 patterned pulses (Rose & Dunwiddie, 1986). This form of LTP also appears to have dendritic activation, rather than somal spiking, as a necessity for induction. Further experiments have shown that the threshold for primed burst potentiation is extremely low, with LTP being produced after only one priming pulse followed by a burst of 3 spikes. The involvement of NMDA receptors was also found to be necessary for this potentiation, with AP5 reliably inhibiting its expression (Diamond et al., 1988). It appears from these results that primed burst potentiation has a greater per pulse efficacy, when compared to 'nonpatterned' continuous trains of

pulses, in producing LTP.

It can be suggested from these lines of evidence that two naturally occurring phenomena (theta rhythm and neuronal burst firing) could be associated with what is an unnatural, candidate mnemonic device, namely LTP and that LTP, as induced by bursting stimulation, may occur as a normal process in the hippocampus. Further evidence to support this contention comes from experiments that have investigated 'behavioural LTP', that is, experiments that have attempted to discover whether LTP is in fact the mechanism that underlies learning and memory. The proposal that LTP is a 'mnemonic device' (Teyler & Discenna, 1984) has received support from diverse experimental paradigms. Laroche et al. (1987) discovered an increase in the release of glutamate in the dentate gyrus following classical conditioning in the rat, similar to that found previously following LTP induction. In a further experiment, Laroche et al. (1989) showed that the acquisition of a classical conditioning task in rats was associated with the level of LTP induction. In this experiment high frequency stimulation of the perforant path (the conditioned stimulus) was paired with footshock (the unconditioned stimulus). The conditioned suppression of a previously learnt, food-motivated lever-pressing task served as the behavioural measure of conditioning. Animals learned to associate the perforant path stimulus (which produced LTP in the dentate gyrus) with footshock. A linear relationship was found between the magnitude of LTP induced in the dentate gyrus and the level of associative learning. Chronic administration of the NMDA antagonist AP5 blocked both the induction of LTP and acquisition of the association.

In another conditioning task, the rabbit nictitating membrane response, the induction of LTP in the hippocampus is greatly facilitated the acquisition of conditioned responses (Berger, 1984). The activation of protein kinase C, which has been implicated in the development of LTP (Akers et al., 1986; Routtenberg et al., 1985),

has also been shown to be important for the expression of the nictitating membrane response (Bank et al., 1989).

LTP-like effects are also thought to underlie at least the initial stages of the storage of spatial information. When rats are placed in and explore a novel environment, a robust increase in the initial slope of the population EPSP and a corresponding decrease in both the area and latency of the population spike can be seen in the dentate gyrus (Green et al., 1990; Sharp et al., 1989). One inhibitor of the behavioural acquisition of a spatial task has proved to be 2APV; Morris et al. (1986) showed that infusion of this NMDA receptor antagonist prevents place-learning in rats.

These observations of behaviourally correlated LTP-like effects suggests that LTP may underlie memory storage. However, as indicated by Teyler and DiScenna (1984), these observations raise questions regarding the underlying phenomenon of LTP. For example, it is unclear why participation in a variety of behavioural tasks should be reflected in alterations of hippocampal efficiency at electrode positions that are apparently chosen at random. However, the evidence presented has led Teyler and DiScenna (1984; 1987) to the conclusion that LTP is a candidate mnemonic device underlying memory storage in the brain.

(2) Long-term depression.

Long-term depression (LTD) has been most intensely studied in the cerebellum. LTD occurs when impulses arrive at a Purkinje cell almost simultaneously through two distinct afferent pathways; one from cerebellar mossy afferents through granule cells and their axons (the parallel fibres), the other from olivocerebellar afferents through their climbing fibre terminals. LTD, in contrast to LTP, is manifest as a long-lasting reduction of synaptic efficacy (Ito et al., 1982).

An essential feature of LTD is that it is associative, i.e., it requires a combination of

parallel-fibre and climbing fibre stimulation (1-4Hz stimulation for 0.5-8 mins). Stimulation of either pathway alone does not result in LTD. Repetitive stimulation of parallel fibres alone will actually elicit a slight potentiation of synaptic efficacy in parallel fibre-Purkinje cell transmission that lasts 20-50 mins. Stimulation of climbing fibres alone neither increases, nor decreases parallel fibre to Purkinje cell efficacy (Ito et al., 1982; Sakurai, 1987). The timecourse of LTD appears to be as follows: an initial phase of 10 mins followed by a later phase lasting 1-3 hours (Ito, 1989).

The role of glutamate in LTD was indicated by experiments in which glutamate was iontophoretically applied to Purkinje cells whilst simultaneously stimulating climbing fibres (Ito et al., 1982). This produces a depression of the glutamate sensitivity of Purkinje cells that closely followed the timecourse of evoked LTD. The subclass of glutamate receptor involved in LTD was identified as quisqualate, as application of quisqualate, but not aspartate or kainate, produced LTD when in conjunction with climbing fibre stimulation (Dupont et al., 1987; Kano et al., 1988). The pairing of glutamate application with stimulation to produce LTD can be prevented by the blockade of glutamate receptors with kynurenic acid (Kano & Kato, 1988). These observations suggest that LTD is produced by a desensitization of quisqualate receptors, the exact opposite of what is thought to be one of the mechanisms underlying the induction/maintenance of LTP.

The postsynaptic influx of calcium has been associated with the development of LTD, as the injection of EGTA into the dendrites of Purkinje cells abolishes LTD (Ito, 1986). The working hypothesis for calcium's postsynaptic action is that calcium influx causes the accumulation of cyclic GMP postsynaptically and that cyclic GMP, by process(es), as yet unknown, causes desensitization of quisqualate receptors (Ito, 1986).

When LTD is compared to other types of synaptic plasticity, such as LTP in the hippocampus and sensitization in *Aplysia*, calcium ions commonly seem to play a key role, although the mechanisms by which it plays its role are different: via NMDA receptor-associated calcium channels in the CA1 region in the hippocampus; via voltage-sensitive channels in *Aplysia* neurons and cerebellar Purkinje cells.

Whilst enhancement of transmitter release from presynaptic terminals accounts for both sensitization in *Aplysia* and, at least partly, for LTP in the hippocampus, there is no evidence to suggest such a scheme for LTD. Parallel fibre-Purkinje cell synapses have a fairly complex pharmacology. Purkinje cells express $GABA_B$ receptors (Wilkin et al., 1981) and adenosine receptors (Goodman et al., 1983) and the sensitivity of glutamate receptors on Purkinje cells is reduced by serotonin (Lee et al., 1986). The relationship of LTD with these chemical regulatory mechanisms could prove very complex.

The behavioural significance of LTD is unclear. Studies of the flocculus have implicated LTD in the learning of the vestibular-ocular reflex (Ito et al., 1974). The flocculus is a phylogenetically old part of the cerebellum. Purkinje cells of the flocculus receive mossy fibre input from the semicircular canals and visual information from climbing fibre input (Lisberger, 1988). Climbing fibres appear to convey 'retinal error signals' as to the effectiveness of vestibular-ocular reflex eye movements compensating for head movements. During head turns, the vestibular-ocular reflex normally generates smooth eye movements that are equal to and opposite rotatory head movement. If a monkey, for example, undergoes passive head rotation in darkness, the 'gain' of the vestibular-ocular reflex, defined as compensatory eye velocity divided by head velocity, is 0.95 to 1.0. The vestibular-ocular reflex undergoes motor learning whenever errors in the in the vestibular-ocular reflex are signalled by the temporal coincidence of retinal slip and head turns (Lisberger, 1988). The gain of the

vestibular-ocular reflex will decrease if retinal slip and head turns are in the same direction and will increase whenever these two inputs are in opposite directions. LTD has been put forward as a mechanism underlying vestibular-ocular reflex that will 'disconnect wrong wiring' in the flocculus and so eliminate any such errors in performance (Ito, 1984).

LTD has also been studied in the hippocampus. From their studies using a hippocampal slice preparation, Stanton and Sejnowski (1989) have proposed that LTD can be seen as a model of 'forgetting'. LTP has become widely accepted as a neuronal 'substrate' for learning and memory. Because forgetting is such a conspicuous complement to learning, it is possible that the cellular analogue of forgetting is the inverse of LTP: such 'neuronal' forgetfulness might be an enduring decrease in synaptic strength following the heavy use of a synapse. This hypothesis came from an experiment in which Stanton and Sejnowski induced LTD in the CA1 region of the hippocampus. Stimuli were applied to two different inputs to this region. Burst patterned stimuli at theta periodicity (a similar pattern of stimulation to that used to induce LTP) were presented to the Schaffer and commissural collateral inputs to CA1 (the 'conditioning input'). The other stimulus consisted of single pulses applied to the subicular input to CA1 (the 'test input'). When these separate inputs to CA1 were stimulated 'in phase' (i.e. synaptic activity was positively correlated) LTP was induced. This increase in synaptic strength could be reversed when activation of the two inputs was applied 'out of phase' (i.e. synaptic activity was negatively correlated), producing LTD. The induction of LTD was shown to AP5-insensitive indicating that the activation of NMDA receptors was not required for LTD expression. If test pulses were applied that contained an equal number of 'in phase' and 'out of phase', randomly mixed stimuli no net change in synaptic strength occurred. This zero net change is in accord with the covariance model (Sejnowski, 1977a; 1977b) which predicts that stimuli with no covariance should produce no synaptic change. The presence of LTD was also reflected in the size of EPSPs

recorded intracellularly from CA1 pyramidal cells. The EPSP was markedly larger when 'in phase' stimulation was presented, whereas 'out of phase' stimulation produced the reverse i.e. a marked reduction in the EPSP and reduced ability of the test pulse to elicit the firing of action potentials.

Further experiments indicated that hyperpolarization of CA1 pyramidal neurons, if coupled with simultaneous presynaptic activity, was sufficient to induce LTD - an 'inverse-hebbian' mechanism. The authors suggested that this form of plasticity may be involved in learning processes that lead to the weakening of stimulus-response relationships such as habituation and reversal learning.

The important factor underlying LTD in the Stanton and Sejnowski (1989) study was the voltage of the neuron: LTD was only found when the neuron was prevented from depolarizing during stimulation of its excitatory afferents. A much more specific theory for the necessary conditions for LTD expression has been suggested from studies using a slice preparation of the visual cortex (Artola et al., 1990). In this preparation LTD also appeared to be the inverse of LTP. LTD in the visual cortex appeared to occur when a neuron was depolarized, providing that this depolarization was insufficient to reach the threshold for calcium entry through NMDA receptor channels. It appeared that neurons in visual cortex have a narrow 'window' of voltages at which LTD will be produced when the synapse is used. If their potential is near or below resting levels then the strength of synapses will not alter with their use. Above a depolarization of around 20mV LTP developed but between resting potential and the threshold for LTP induction LTD developed. The membrane potential was not regulated by intracellular current injection in this experiment. This was accomplished by altering the dose of the $GABA_A$ antagonist bicuculline to permit the neuron to be more or less depolarized (according to the dose) by antagonizing the inhibitory activity that accompanied the tetanizing stimulus. With no bicuculline, neither LTP nor LTD

was observed. With small doses LTD was found after tetanization. With doses that blocked most of the inhibitory synaptic activity LTP followed tetanization.

(3) Theta Rhythm.

Theta rhythm was first described by Jung and Kornmuller in 1938. It is an approximately sinusoidal, extracellularly recordable EEG potential of 5-9 Hz found in dentate gyrus and CA1 fields of the hippocampus. It can be seen in a number of sub-primate species during species specific behaviours (Winson 1972). In the rat, it occurs during voluntary movements, e.g., exploration, as well as during REM sleep (rapid eye movement, or paradoxical sleep), observable as a synchronization in the EEG record assumed to reflect periods of dreaming in man. The source of this rhythmicity is the 'pacemaker' cells of the medial septum and the diagonal band of Broca (Petsche et al., 1962). Neurons in and near the medial nucleus of the septum emit rhythmic signals along a pathway to the hippocampus (Apostol & Creutzfeldt, 1974; Bragin & Vinogradora, 1983; Green & Ardini, 1954). The output of these septal cells is thought to be controlled by ascending inputs from the brainstem, which are themselves non-rhythmic but are translated into rhythmic activity by neurons in the septal region (Petsche et al., 1965; Vertes, 1981). There appear to be two spatially separated theta rhythm dipoles in both pyramidal and granule cells, one on the cell body and the other on apical dendrites (Buzsaki et al., 1983). These appear to be septally driven, the former via a cholinergically driven inhibitory interneuron, the latter via direct cholinergic innervation.

As outlined above, recent reports have indicated that LTP can be induced maximally by applying burst-patterned stimuli at a theta frequency. However, the association of theta rhythm with memory formation has a much longer history.

Early literature emphasized the correlation between theta rhythm and learning. It was

observed in a number of learning tasks that theta activity is weak at the start of learning but becomes more pronounced (or higher in frequency) when the level of performance is improving most rapidly. Such a correlation has been found in tasks which involve classical conditioning (Buzsaki et al., 1979; 1981), appetitive conditioning (Adey et al., 1960; Bennett, 1970; Grastyan et al., 1959; 1966), active avoidance learning (Graliewicz, 1981; Lissak & Grastyan, 1960) and in discrimination tasks (Pickenhain & Klingberg, 1967). Morrell (1961) postulated that theta activity might be correlated with "the inscribing of an experience into neural structure" as EEG in the theta range (4-9Hz) had been associated in many experiments with the early stages of conditioning.

Landfield et al. (1972) examined theta rhythm in cortical EEG 30 min after one-trial footshock conditioning in rats. They found that the amount of theta seen posttraining was positively correlated with the amount of subsequent retention for the task. In a further experiment, one group of rats received electroconvulsive shock (ECS) 10sec after training. These rats showed a range of retention. The amount of retention was directly related to the amount of theta, as measured two days post-training: animals receiving ECS and exhibiting good recovery of theta exhibited good retention two days post-trial, whereas animals that had no ECS following footshock, but showed low theta two days post-trial, exhibited low retention scores. Such changes were also evident during a 30min post-training recording. In a later study rats with implanted electrodes were subjected to two avoidance tasks, one active and one passive, three weeks apart (Landfield, 1977). Following training, experimental animals received 20 min of either low frequency (LF) or high frequency (HF) septal stimulation, which 'drove' or 'blocked', respectively, the endogenous hippocampal theta rhythm (Gray, 1972; Gray & Ball, 1970; Yokota & Fujimori, 1964). Animals receiving theta-driving LF stimulation exhibited improved retention for the active avoidance task in comparison to HF or implanted controls. In the passive avoidance task, LF animals performed significantly better than HF animals, although not significantly better than

implanted controls (there was, however, a trend towards improvement). Perhaps the most essential result from this experiment was the improvement in performance, in both tasks, after post-trial theta stimulation (when compared to post-trial theta blocking). This post-trial stimulation neatly circumvents the criticisms of stimulation effects upon arousal, perception or motivation since the animals were stimulated after being removed from relevant environmental cues. Thus, it can be inferred that stimulation had some effect upon the processing of already acquired information, through an enhancement of storage and/or retrieval mechanisms. These studies are further supported by results showing that drugs that facilitate memory in a time-dependent manner, when given post-trial, increase slow wave theta (McGaugh & Dawson, 1971).

More recent experiments have shifted the emphasis of theta research toward correlations between theta and aspects of behaviour unrelated to learning. Important between-species differences in the behavioural correlates of theta activity have come to light. In rats and other small-brained mammals, theta activity appears to be particularly associated with certain types of body movements (Kramis et al., 1975; Vanderwolf, 1969). The frequency of theta rhythm is lower for small movements from a fixed base than for whole-body movements such as locomotion. Immobile rats do not show theta activity except when paralyzed with curare (Macader et al., 1970), during REM sleep (Vanderwolf et al., 1978) or in the immobile moments before jumping (Vanderwolf, 1975). Theta rhythm exhibited by rabbits is similar to that seen in rats, although rabbits will exhibit theta whilst immobile in response to sudden stimuli (Kramis et al., 1975). Cats also show theta rhythm whilst immobile, in association with orienting responses and during locomotion (Brown, 1968; Coleman & Lindsley, 1975; Whishaw & Vanderwolf, 1973).

However, the correlations between theta and behaviour may not be as strong as recent

publications have suggested. In some experiments, locomotor activity has been shown to occur in the absence of theta rhythm. Routtenberg (1968) was able to show this after placing rats in a novel environment. Once the rats had habituated to the environment very little theta activity could be recorded from them. Pickenhain and Klingberg (1967) observed that rats which received random electric shocks initially made chaotic escape attempts, unaccompanied by hippocampal theta rhythm. But when rats made a successful avoidance, theta rhythm could be seen in the EEG record. If a flashing light was presented to rats shortly before shock they quickly learned the association. The signal presented on each trial evoked theta rhythm before any avoidance response and in the absence of any other motor behaviour, suggesting that theta can occur during learning in the absence of locomotor or other whole-body movements. Although the correlations between various types of behaviour not directly related to learning and theta activity are well established, Miller (1989) has suggested that they should only be regarded as correlations and not explanations of theta activity. Because of this, examples of learning-related changes in theta rhythm can coexist with apparently contradictory examples of movement-related theta activity.

Animal Models of Learning and Memory

This section is aimed toward briefly describing some of the basic training procedures used in behavioural and cellular studies of learning and memory. Much of the research into the cellular analysis of learning has utilized both nonassociative and also associative learning paradigms. Nonassociative learning is a form of learning that is independent of paired events. Examples of this form of learning are habituation and sensitization. Associative learning, however, involves the temporal pairing of two events and underlies a wide range of behavioural modifications. Two of the most extensively studied of these modifications are classical and operant conditioning. Nonassociative learning is of some importance as its underlying cellular mechanisms may also form a part of associative learning.

The animal models outlined below utilize habituation/sensitization (*Aplysia*) and classical conditioning (red nucleus of the cat and the rabbit nictitating membrane response). Habituation is possibly the simplest and most ubiquitous form of nonassociative learning. During habituation an animal learns that the consequences of a weak stimulus are neither noxious nor rewarding through a series of exposures to that stimulus. With repeated stimulation the animal learns to 'ignore' (at least behaviourally) the stimulus and exhibit progressively smaller reflex responses. It can be distinguished from fatigue as responsiveness can be rapidly restored by the presentation of a novel stimulus to the animal (dishabituation). Sensitization is also a form of nonassociative learning and refers to the enhancement of a behavioural response as a result of applying a novel stimulus to the animal. The stimulus may be of the same modality and applied at the same site as a test stimulus used to elicit the response, or it may be of a different modality, applied to a different locus. Dishabituation and sensitization are similar and may utilize identical underlying cellular mechanisms (Carew et al., 1979).

Classical conditioning is an example of associative learning in which the presentation of a reinforcing stimulus or unconditioned stimulus is made contingent upon that of a preceding, conditioned stimulus. An example of classical conditioning is that used by Pavlov (1927) to condition salivation in dogs. Before training began it was noticed that the presentation of meat powder, the unconditioned stimulus, reliably elicited salivation, the unconditioned response. During training meat powder was made contingent upon the conditioned stimulus, in this case a bell, via repeatedly pairing the sound of the bell with meat presentation. After training, presentation of the conditioned stimulus alone (ringing the bell) could elicit salivation (the conditioned response).

(1) Aplysia Defensive Behaviour (Gill/Siphon withdrawal).

Aplysia californica is a marine opisthobranch mollusc that can grow to 1Kg in weight and up to 1m in length. Its nervous system contains some 20,000 neurons, grouped into ten clusters or ganglia of roughly equal size. Not only are the neurons few in number, they are also extremely large. Because of their large size (up to 1mm diameter) and their invariance from animal to animal, neurons can be given specific names e.g. R2 and L7. These characteristics make them ideal for electrophysiological, biochemical and morphological studies.

Aplysia, like other molluscs, has a gill that is enclosed in a respiratory chamber called the mantle cavity. This cavity is covered by a protective sheet, the mantle shelf, that terminates in a fleshy spout, the siphon. When the mantle shelf or siphon is stimulated, the gill, mantle shelf and siphon all contract vigorously and withdraw into the mantle cavity. This is a defensive withdrawal behaviour which is sensitive to modification through experience. In *Aplysia*, the defensive reflex can be modified by three different forms of learning: habituation, sensitization and classical conditioning. Each of these three types of learning exhibits both a short and a long-term phase of memory.

In habituation, a weak tactile stimulation of the *Aplysia* siphon will initially cause brisk withdrawal of the gill and siphon. However, during a session of 10 stimuli, the response gradually diminishes. The habituation from such training will last up to a few hours (Pinsker et al., 1970).

To define the specific locus of memory and the nature of the mechanism(s) of storage, the complete "wiring diagram" of the gill-withdrawal reflex was investigated. The circuit proved to be relatively simple. The gill is controlled by six motor cells, which receive information from the siphon via 24 sensory and numerous interneurons. The

constituent cells and connections appear to be invariant (Kandel, 1976). Skin stimulation will activate sensory neurons which connect to interneurons. Interneurons then synapse with the motor cells which connect directly to the muscle, causing contraction.

The locus for short-term habituation is the presynaptic connection of sensory neurons to the motor neurons and interneurons (Castellucci et al., 1970). This identification allowed direct measurements to be made to investigate the electrophysiological correlates of short term habituation in these cells. The EPSPs of motor cells were recorded intracellularly in response to intracellular stimulation of sensory cells. During a training session of 10 stimuli, repeated firing of the sensory neuron (at a rate that produces habituation in the intact animal) depressed the synaptic connection i.e. a reduction EPSP amplitude and rise-time (Castellucci & Kandel, 1974). This reduced EPSP was caused by a reduction in transmitter release. The amount of transmitter release largely depends upon the concentration of free calcium in the presynaptic terminal. Calcium flows across the presynaptic membrane upon depolarization from action potentials. This allows the fusion of synaptic vesicles with the presynaptic membrane and exocytosis of the neurotransmitter. Klein and Kandel (1980) found that during habituation the calcium influx was depressed. The retention of short-term memory for habituation, therefore, appears to reside (at least in part) in the duration of inactivation of the presynaptic calcium current.

Further research has indicated that both the locus and the mechanism underlying long-term habituation are identical to those observed during short-term habituation i.e. a decrease in postsynaptic EPSP of motor neurons (Castellucci et al., 1978). However, there is a striking difference in the degree of reduction in synaptic depression. Whereas a transient depression in synaptic efficacy underlies short-term habituation, long-term habituation is accompanied by a more dramatic and prolonged alteration: a

functional inactivation of the previously existing connection between sensory and motor neurons.

It is important to note at this point that alterations in neuronal efficacy have been found at multiple sites within the nervous system of *Aplysia* following conditioning of the gill withdrawal reflex. Lukowiak (1986) discovered that changes in synaptic efficacy that occur at the sensory to motor neuron synapse are only present during the initial stages of conditioning. In further studies, Colebrook and Lukowiak (1988) discovered that changes in the synaptic efficacy between siphon sensory and gill motor neurons were neither necessary nor sufficient for changes in gill behaviour following conditioning of the gill-withdrawal response. This suggested that neural changes were also occurring at other sites within the *Aplysia* nervous system during conditioning. Colebrook and Lukowiak (1988) were able to show such a change in the ability of the motor neuron to induce gill withdrawal after unpaired (i.e., control) presentations of conditioned and unconditioned stimuli. Lukowiak and Colebrook (1987) further analyzed this change in gill withdrawal and were able to show that following conditioning, the ability of the gill motor neuron to elicit gill withdrawal was potentiated. These results indicate that important changes in the nervous system of *Aplysia* that underlie learning occur distal to the sensory-motor neuron synapse.

The changes in electrophysiology induced by long-term habituation appear to be accompanied by morphological changes. In animals exhibiting long-term habituation, sensory neurons have fewer and smaller active zones (active zones are thought to be specialized areas of presynaptic membrane responsible for the positioning and subsequent exocytosis of synaptic vesicles). The number of synaptic vesicles at each release site also appeared to be lower in long-term habituated animals when compared to control animals (Bailey & Chen, 1983).

The second form of non-associative learning displayed by *Aplysia* is sensitization. A

single strong stimulus applied to the tail (or head) of the animal enhances the gill and siphon withdrawal reflexes produced by tactile stimulation of the siphon. This enhancement lasts for several hours. Repeated stimulation of the tail or head enhances the reflex from several days to weeks (Pinsker et al., 1973). Whereas habituation requires a reduction in response to a repeated stimulus, sensitization requires the enhancement of a behavioural response to a stimulus because the stimulus is potentially accompanied by (in this case) painful or dangerous consequences.

At the cellular level, sensitization involves an enhancement of synaptic transmission at the same site as habituation (the sensory neuron to motor neuron synapse). Thus, the same synaptic locus can be regulated in two opposing ways: it can be depressed by habituation and it can be facilitated by sensitization.

Several lines of evidence suggest that short-term facilitation of sensory neuron synapses is mediated by the activation of heterosynaptic pathways and the release of the facilitatory neurotransmitter 5HT onto the presynaptic terminals of the sensory neuron to enhance transmitter release. 5HT may also have an important role in the long-term enhancement of the response. Repeated applications of 5HT evoke a long lasting enhancement of sensorimotor synapses in a cell culture preparation through a long-term enhancement of the EPSP (Dale et al., 1988; Montarolo et al., 1986). This culture preparation also exhibits the structural changes in sensory neurons seen in the *in vivo* preparation of long-term sensitized animals (Bailey & Chen, 1983; Glanzman et al.; in press in Schacher et al., 1990). These structural changes were the opposite of those found after long-term habituation, the size and number of active zones was increased as were the number of synaptic vesicles. The number of synapses at the sensory neuron to L7 motor neuron connection is also increased following long-term sensitization (Bailey & Chen, 1988). The morphological changes seen *in vitro* (Glanzman et al., 1990), in particular the increase in the number of synapses, depends

upon the presence of the post synaptic motor cell, suggesting that the post synaptic motor cell has a role in these structural changes.

The use of the *Aplysia* cell culture preparation can be criticized as being too reductionist: do two neurons grown in culture really reflect the mechanisms of learning and memory seen in the intact animal? Colebrook and Lukowiak (1988) have shown that conditioning of the gill withdrawal reflex results in changes in synaptic efficacy at loci other than the sensory/motor neuron synapse. The isolated preparation is being promoted as a molecular model for behavioural learning. This may be premature as the preparation does not appear to include every modifiable synapse that underlies the response. Rather than describing the nervous system of *Aplysia* as 'simpler' than that of a vertebrate perhaps a more valid adjective is 'smaller'. It is this small size that ought to make the loci of change easier to locate in an invertebrate such as *Aplysia* when compared to the nervous system of a vertebrate. When and if this search is completed the major problem then becomes the relevance (if any) of the learning and memory mechanisms of *Aplysia* to those of vertebrates.

(2) Classical conditioning mediated by the red nucleus of the cat.

Conditioning mediated by the cat red nucleus (RN) is an example of a conditioning procedure that has been applied to reduced experimental preparations. As seen above in the case of *Aplysia*, these preparations can be criticized for being neural analogues and not behavioural models. However, the advantage of these 'reduced' preparations is that it has been possible to mimic the natural input to produce a preparation that is more amenable to electrophysiological analyses.

Conditioning of the forelimb flexion response was originally demonstrated by Smith (1970) who showed that pairing a tone (conditioned stimulus) with electric shock to the forelimb (unconditioned stimulus) caused the tone to produce limb flexion.

Tsukahara et al. (1979; 1981) simplified this procedure by substituting the tone with direct stimulation of the fibres in the cerebral peduncle which project from the cortex to the RN (Figure 1.6). The unconditioned stimulus remained as electric shock to the forelimb. To produce conditioning, paired shocks (with an interval of 100ms) were delivered once every 30s for a total of 120 trials per day over a 10 day period. The effects of the conditioned stimulus were restricted to the corticorubrospinal pathway by sectioning the cerebral peduncle caudal to the RN. With paired conditioned-unconditioned stimulus presentations, initially ineffective conditioned stimuli gave rise to forelimb flexion that reached a plateau after about 7 days. The minimum current needed for 100 correct performances (100% performance current) decreased in parallel with the increase in performance (Tsukahara et al., 1981). Random conditioned stimulus or unconditioned stimulus presentations, backward pairing or presentation of conditioned stimulus or unconditioned stimulus alone failed to produce the same degree of conditioning. Therefore, the result of conditioned stimulus-unconditioned stimulus pairings was to enhance the efficacy of transmission from neurons of the corticofugal pathway to forearm flexor neurons. Supporting evidence for this facilitation of transmission has come from recording the activity of RN cells in the awake cat. Induction of conditioning increases the probability of firing of some RN cells to the conditioned stimulus.

The RN receives another excitatory input from the nucleus interpositus (IP) of the cerebellum. To test the possibility that the primary site of neuronal change following conditioning is below RN, the IP was stimulated. If the site of change was below RN, then the stimulation of the IP should produce a similar increase in performance with a concomitant decrease in the current required for 100% performance. However, no appreciable decrease of current intensity was observed with IP stimulation, even though the conditioned response had already been established by cerebral peduncle stimulation. The most likely sites of neuronal change, therefore, are the corticorubral synapses.

Figure 1.6; Diagram to show the experimental design of the cat red nucleus conditioning paradigm.

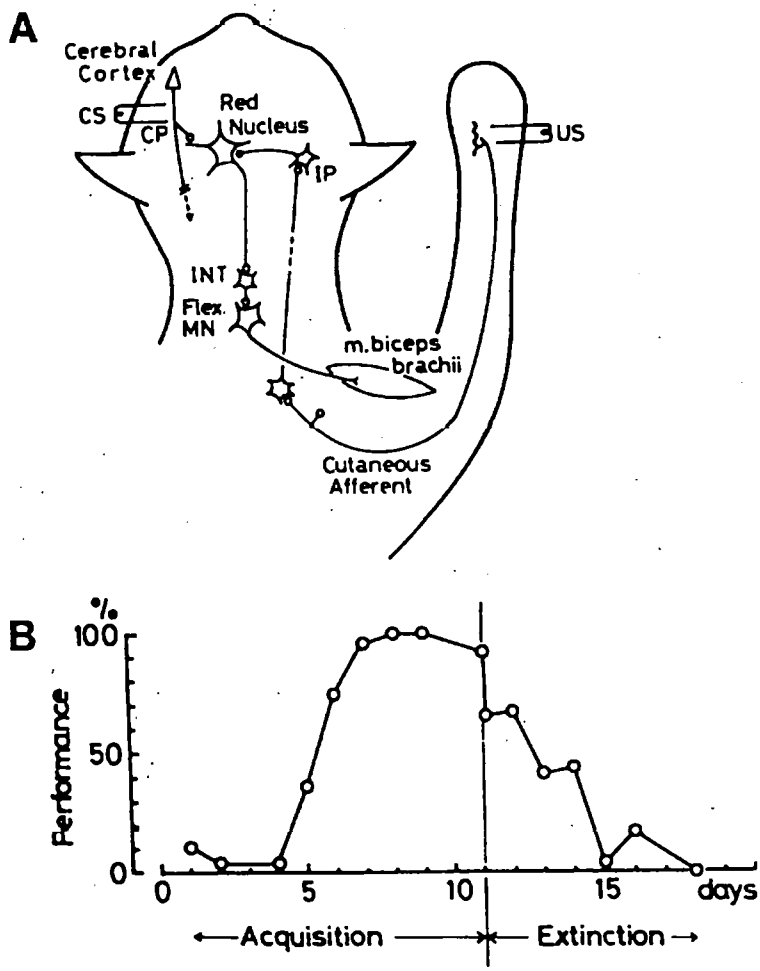


Figure 1.6; Classical conditioning mediated by the red nucleus in the cat. (A) Experimental design. Conditioning stimuli (a train of five stimuli with 2 msec intervals, CS) are applied to the cerebral peduncle (CP), followed 100 msec later by an unconditioned stimulus (electric shock, US) to the skin of the forelimb. (B) Change in performance. Delivery of the CS alone initially produces no forelimb flexion (day 1). After 7 days of training (pairing CS-US 120 times each day) presentation of CS alone produces forelimb flexion (aquisiton phase). After establishing the conditioned response by CS-US pairing, backward pairing was used to extinguish the conditioned response (extinction phase). Modified from Tsukahara et al., 1981.

To discover whether changes consequent upon conditioning were indeed occurring in RN cells, intracellular recording from RN cells were made. It was found that after establishment of the conditioned reflex, a new fast-rising component appeared in the corticorubral dendritic EPSPs in response to the conditioned stimulus.

In previous studies, Tsukahara and his colleagues had observed the development of similar fast-rising EPSPs following IP lesions and also after cross-innervation of the peripheral flexor and extensor nerves (Fuyito et al., 1982; Murakami et al., 1977; Tsukahara et al., 1975). The new fast EPSP was attributed to neuronal sprouting at corticorubral synapses. Tsukahara and Oda (1981) therefore proposed that the fast EPSP observed after classical conditioning is due to the formation of new synaptic connections (although the possibility of other mechanisms, e.g., increased neurotransmitter release, have yet to be investigated). These results may, therefore, represent the first description of a morphological change produced by a classical conditioning procedure.

(3) Rabbit nictitating membrane response.

The nictitating membrane response (NMR) has been extensively examined at both behavioural and cellular levels, providing an excellent experimental preparation for the investigation of the cellular mechanisms of associative learning.

The nictitating membrane of the rabbit consists of a curved piece of tissue that can be drawn across the cornea. Weak air puffs delivered to the cornea reliably elicit an extension of the nictitating membrane, presumably in an effort to protect the sensitive cornea from a potentially harmful stimulus.

Gormezano et al. (1962) discovered that the NMR could be classically conditioned. The unconditioned stimulus was a 100ms air puff to the cornea, the unconditioned

response being extension of the nictitating membrane. The conditioning stimulus was an 800Hz tone presented for 600 ms. The interval between presentations of conditioned and unconditioned stimuli was 500 ms. Seventy conditioned-unconditioned stimulus pairings were presented each day for 8 days. With repeated pairings the conditioned stimulus produced extension of the nictitating membrane.

Initial attempts to define the neural systems involved in NMR conditioning investigated the activity of the motor system controlling the response. Recordings of neural activity from one of the critical motor nuclei for the response (the Adbucens) simultaneously with measurement of NM extension, or eyelid closure, showed that the pattern of increased neural unit response preceded and closely paralleled the amplitude-time course of behavioural NM response (Cegarske et al., 1976)

The search then turned to the neuronal system(s) responsible for generation of the NMR (Figure 1.7). A series of lesioning experiments was undertaken to test the effects of removal of certain brain areas upon the acquisition of the response. In the standard conditioning paradigm animals with total lesions of the cerebral neocortex or hippocampus were still able to learn (Oakley & Russell, 1972; Solomon & Moore, 1975), as were animals following removal of all brain tissue above the level of the thalamus (Norman et al., 1977). That a decerebrate animal could learn the eyelid response does not necessarily mean that the tissue above the thalamus is not involved in the learning of or memory for the response, only that the remaining tissue is capable of supporting learning. It seems very likely that memory trace systems develop in higher brain regions during classical conditioning. The hippocampus has been strongly implicated in the memory trace system for NMR conditioning (Berger & Thompson, 1978). However, the hippocampus is not required for learning or memory of the conditioned response.

As a first step in the identification of the neural system(s) involved in learning and

Fig 1.7; Diagram to show the hypothetical memory trace circuit for the rabbit nictitating membrane conditioning paradigm.

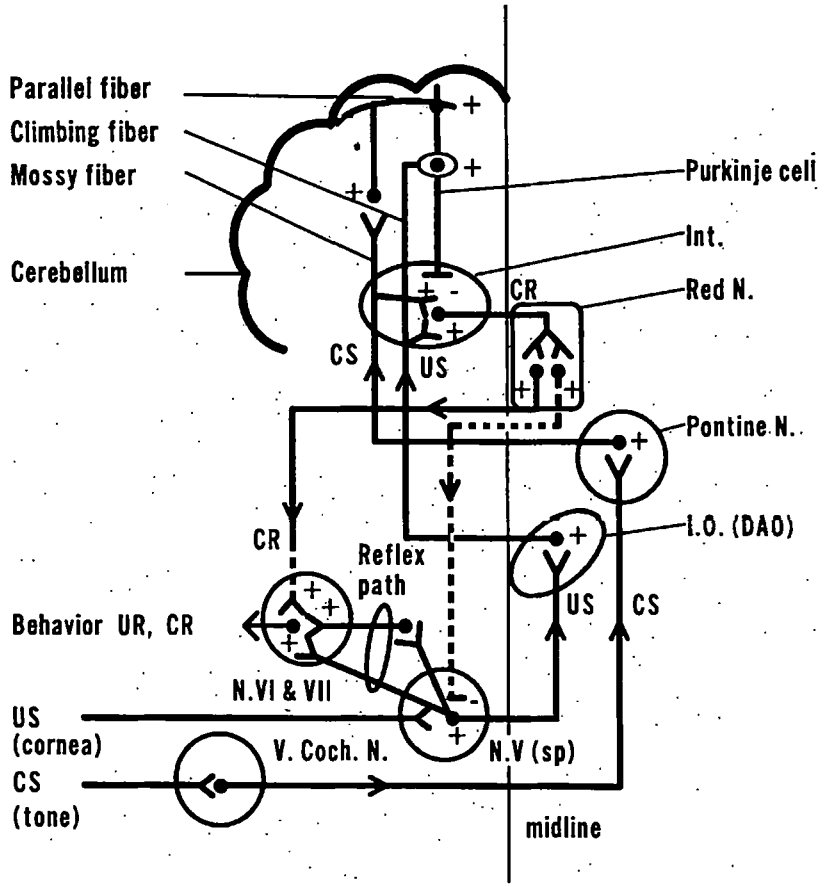


Figure 1.7; Classical conditioning mediated by rabbit nictitating membrane. The unconditioned stimulus (corneal airpuff, US) consists of somatosensory projections to the dorsal accessory portion of the inferior olive (DAO) and its climbing fibre projections to the cerebellum. The tone conditioned stimulus (CS) pathway consists of an auditory projection to the pontine nuclei (Pontine N.) and their mossy fibre projections to the cerebellum. The efferent (eyelid closure) conditioned response (CR) pathway from the interpositus nuclei (Int) of the cerebellum to the red nucleus (Red N.) and via the descending rubral pathway is thought to ultimately act on motor neurons. The Red N. may also exert inhibitory control over the transmission of somatic sensory information about the US to the inferior olive (IO) to 'dampen' US activation of climbing fibres. Other abbreviations: NV(sp), spinal 5th cranial nucleus; NVI & NVII, 6th & 7th cranial nuclei; V Coch N, ventral cochlear nucleus. Taken from Thompson, 1986.

memory for the NMR, the nuclei involved with transmission of auditory information concerning the conditioned stimulus were examined. Using multiple and single-cell recording from the cochlear nucleus, central nucleus of the inferior colliculus and the ventral division of the medial geniculate body, no evidence was found to suggest that training induced changes in neuronal activity occurred in the conditioned stimulus sensory channel (Kittner & Thompson, 1982).

However, changes induced by NMR training were found to occur in the hippocampus. Multiple unit recordings from the hippocampus during NM conditioning revealed a profound training-induced alteration in neural activity (Berger & Thompson, 1978). During paired conditioned-unconditioned stimulus conditioning trials the neuronal activity in the CA1 pyramidal layer rapidly developed an excitatory response which increased in amplitude over conditioning and which formed a 'temporal model' of the amplitude and time-course of the behavioural NM response. The initial response from the CA1 region was to the unconditioned stimulus, but the response gradually shifted over successive trials to the conditioned stimulus at the time when conditioned NM responses occurred to the conditioned stimulus. The neuronal response preceded the NM response by about 40ms and did not develop in control animals receiving unpaired conditioned-unconditioned stimulus presentations. The neuronal response could also predict the occurrence of the conditioned response over a variety of conditions. For example, changes in procedure which produced no behavioural conditioned responses (e.g., a decrease in the conditioned-unconditioned stimulus interval) produced no associative hippocampal neuronal response (Thompson et al., 1980). Intraperitoneal morphine injections, which abolished the occurrence of behavioural conditioned responses in trained animals, also abolished the conditioned neuronal response, whilst leaving the unconditioned behavioural and neuronal responses unaffected (Mauk et al., 1982).

As mentioned previously, the hippocampus is unnecessary for the learning of retention of the conditioned response. However, the hippocampus does appear to be important if the conditioned-unconditioned stimulus association is made 'more difficult' for the animal, i.e., if the conditioned-unconditioned stimulus interval is increased to 0.5s. Animals with hippocampal lesions appeared severely deficient in this form of association when compared to control animals (Solomon et al., 1983). This procedure required the animal to retain the memory of the conditioned stimulus during the interval between conditioned stimulus offset and unconditioned stimulus onset and also required more conditioning trials for normal, non-lesioned animals than in the standard task where there was no interval between offset and onset. It appears, therefore, that whilst the hippocampus is unnecessary for acquisition of a simple delay Pavlovian procedure, it becomes necessary for optimal acquisition as the training situation places increasing demands upon the animal.

Recent evidence has suggested that long-lasting neuronal plasticity is established in the hippocampus with NMR conditioning (Mamounas et al., 1984; Weisz et al., 1984). Although the cerebellum appears to be the essential site of memory storage for NMR conditioning, a second 'memory trace', temporally associated with the changes in cerebellum, appears in the CA1 region of the hippocampus. This hippocampal neuronal response appears to develop within the hippocampus itself (Berger et al., 1980), as recordings made from the entorhinal cortex and medial septum, the two regions from which the hippocampus receives most of its afferent innervation, show no long-lasting changes over the course of conditioning. The response also shows a decrease of firing frequency in correlation with behavioural extinction during the unconditioned stimulus period, but in advance of behavioural extinction during the conditioned stimulus period (Berger & Thompson, 1978).

During the course of training, the dentate population spike shows a marked and persistent increase in amplitude in response to test pulses delivered to the perforant

path during inter-trial intervals. There is also a translocation of PKC from cytosol to membrane of CA1 cells after conditioning (Bank et al., 1988). This translocation appears to be largely in the dendritic regions of the cells. Once PKC has been translocated to the plasma membrane it becomes activated in the presence of diacylglycerol and low calcium concentration (Berridge, 1984). The activation of PKC is thought to alter the excitability of CA1 pyramidal cells by reducing the afterhyperpolarizing potential and enhancing the summation of postsynaptic potentials, possibly via the reduction of a calcium-dependent potassium current (Bank et al., 1989). This is consistent with the possibility that a long-term potentiation-like mechanism may underlie the training-induced within-trial increase in pyramidal neuronal conditioned response as a similar translocation of PKC occurs after the induction of LTP in the same area (Berger, 1983; Bank et al., 1988). It can be inferred from this that 'higher-order memory traces' are formed in the hippocampus during learning, but that these traces are neither necessary nor sufficient for the acquisition of the basic association between tone conditioned stimulus and the precisely timed, adaptive behavioural response. The role of the hippocampus may be to modulate the acquisition and expression of conditioned responses (Bank et al., 1989).

Total deficits in NMR conditioning were found after lesions of the cerebellum (McCormick et al., 1982). Ablation of the lateral cerebellum ipsilateral to the trained NM completely and permanently abolished the conditioned response in a previously trained animal, whilst producing no effect in the unconditioned response. Subsequent training using the untrained NM contralateral to the lesion showed a conditioned response that developed rapidly. Similar effects can be produced if the lesions are localized to the dentate and interpositus nuclei (and surrounding fibres) or the superior cerebellar peduncle, the major efferent cerebellar pathway (Clark et al., 1982).

The effects of lesions of the cerebellum are consistent with recorded multiple unit

activity from the interpositus and cerebellar dentate nuclei following training. Both the conditioned stimulus and the unconditioned stimulus produce evoked responses, but a temporal model of the unconditioned response, as seen from hippocampal recordings, was not evident in cerebellar recordings. What was found in cerebellum was an emergence of a temporal neuronal model of the conditioned response, which preceded the conditioned response by about 50ms. Hence, in contrast to the hippocampus, the cerebellar unit activity from discrete nuclei was restricted to a model of the conditioned response and preceded both the behavioural response and the hippocampal neuronal response, suggesting that the hippocampal response may be dependent upon the development of the cerebellar response. Evidence to suggest this hippocampal dependence upon the cerebellum has come from demonstrations that unilateral lesions of the dentate and lateral interpositus nuclei abolish not only the ipsilateral conditioned NM response to the conditioned stimulus, but also the ipsilateral hippocampal neuronal response to the conditioned stimulus (Clark et al., 1982).

Thompson and Donegan (1986) produced a hypothetical model to show how the association for NMR conditioning might be acquired. An implicit assumption of their model is that the site of the memory trace resides in the cerebellum at the principle neurons (Purkinje cells) and/or their associated interneurons and/or in the interpositus nucleus. A conditioned stimulus is assumed to activate a subset of cerebellar granule cells which then weakly activate all principle cells via their parallel fibres. The unconditioned stimulus pathway is assumed to be the climbing fibres from the dorsal accessory olive, projecting via the inferior cerebellar peduncle. Any unconditioned stimulus is assumed to only activate a limited number of principle cells coding the motor program for the defensive response that is specific for the unconditioned stimulus (e.g., eyelid closure, leg flexion). When parallel fibre activation occurs just before climbing fibre activation, the connections of the parallel fibres to the principle cells activated by the particular unconditioned stimulus are strengthened. This may

involve processes similar to those of LTD and/or LTP, both of which have been shown in the cerebellum. LTD-like processes may serve to 'disconnect wrong wiring' in order to eliminate erroneous performance, as has been proposed for learning of the vestibular ocular reflex (Ito, 1984). The strengthening of synapses may be produced by LTP-like processes.

Each aspect and assumption of this hypothesis is testable. With regard to the unconditioned stimulus pathway, lesions of the appropriate region of the dorsal accessory olive prevent acquisition and produce normal extinction of the behavioural conditioned response with continued paired training in previously trained animals (McCormick et al., 1985). Electrical microstimulation of this same region will elicit the appropriate behavioural response and serves as an effective unconditioned stimulus for the normal learning of conditioned stimuli; the exact behavioural response elicited by dorsal accessory olive stimulation is learned as a normal conditioned response to a conditioned stimulus (Mauk et al., 1986). Lesion and microstimulation data suggest that the essential conditioned stimulus pathway includes the above mossy fibre projections to the cerebellum via the pontine nuclei. Sufficiently large lesions of the medial cerebellar peduncle prevent acquisition and immediately abolish retention of the eyelid conditioned response to all modalites of conditioned stimulus (Soloman et al., 1986), whereas lesions in the pontine nuclear region can selectively abolish the eyelid conditioned response to an acoustic conditioned stimulus (Steinmetz et al., 1985). Electrical stimulation of the mossy fibre system serves as a very effective conditioned stimulus producing rapid learning (Steinmetz et al., 1985).

Finally, appropriate forward pairing of mossy fibre stimulation (conditioned stimulus) with climbing fibre stimulation (unconditioned stimulus) yields normal behavioural learning of the response elicited by climbing fibre stimulation (Thompson, 1986). Lesion of the interpositus abolished both the conditioned response and the unconditioned response in this preparation. The results from all of these experiments

promote a strong case for localization of the essential memory traces to the cerebellum, especially in the 'reduced' preparation, with mossy fibre conditioned stimulus and climbing fibre unconditioned stimulus.

Concluding remarks.

In conclusion, the electrophysiological contribution to the study of learning and memory can be broken down into two areas: the electrophysiological changes that underlie models of memory formation, such as LTP ('how the world should be') and those electrophysiological changes that underlie animal models of memory formation ('how the world is'). The extent to which electrophysiological models can adequately explain the observed neurophysiological changes seen with learning is still far from satisfactory. However, the evidence available to support models such as LTP as the fundamental mechanism(s) of learning and memory is increasing in weight.

The following Chapter describes the background and methods used to record spontaneous multi-unit activity from the forebrain of the day-old chick following passive avoidance training. The results of the associated Experiments will be discussed with reference to both previous experimental work using the same paradigm and also to putative electrophysiological models of memory formation.

CHAPTER 2. Background and Methods for the Recording of Multi-Unit Activity from the Forebrain of the day-old Chick Following Training on a Passive Avoidance Task.

Introduction.

Day-old chicks (*Gallus domesticus*) are excellent subjects for behavioural experiments, mainly because they are a precocial species, i.e. they are capable of a wide variety of behaviours almost immediately posthatch. For some days after hatching chicks show a predisposition for pecking small, visually conspicuous objects such as chrome beads or faecal pellets. This behaviour allows the animal to test its environment, especially for the presence of food and drink, as the difference between food and non-food items is obviously of prime importance to the animal at this early post-hatch stage. This behaviour has been introduced into learning and memory research in the form of a one-trial passive avoidance task (Lee-Teng & Sherman, 1966; Cherkin, 1969). If a chrome bead covered with a bitter tasting substance, such as methyl anthranilate, is offered to chicks, they will peck and show a characteristic disgust response. The chick will consequently avoid similar dry beads. Birds that have pecked an identical but water coated bead do not show disgust responses and consequently continue to peck upon re-presentation of the bead.

The major attraction of the passive avoidance task is that it provides a paradigm free from the confounding affects of rehearsal and reinforcement, which are intrinsic characteristics of multiple-trial and long-term exposure tasks. The appropriate learned response is an adaptive behaviour and does not require the acquisition of any unnatural movements. Also, since the time of training is discrete, requiring just one trial, the paradigm has proved most useful for studying the biological changes following learning. In particular, time courses for specific changes can be accurately constructed

relative to the time of training. This is obviously not the case for the majority of learning tasks in which multiple trials are used. The chick has also proved an excellent model system for biochemical and pharmacological studies of learning and memory: the chick has only a poorly functioning blood-brain barrier, allowing peripherally injected precursors or pharmacological agents to rapidly diffuse into the brain. Another advantage to using the chick as a model is that the skull of the young chick is very thin. This means that surgical procedures prior to either electrophysiological recording or the placement of lesions are very simple and it also allows the easy placement of localized intracerebral injections. However, as with many model systems, the passive avoidance task does have disadvantages. Although the task is advantageous because it involves discrete learning, this can be a problem. By definition, chicks trained to avoid the methylanthranilate coated bead effectively 'do nothing' at test, that is, if they remember pecking the methylanthranilate coated bead, they should avoid pecking it subsequently at test. Therefore, it is difficult to distinguish between those chicks that remember and those that, for whatever reason, don't 'feel like pecking'. Also, chicks tend to perform with a high level of retention (about 80%), that is, they have effectively reached a ceiling of performance. This precludes the use of agents which have been shown to enhance memory in more 'traditional' learning and memory tasks.

The training of day-old chicks on a passive avoidance task has become well established as an animal model of learning and memory. Many experiments have been designed to reveal both the loci and the nature of changes within the chick brain that are crucial to the learning and memory of the task. Initial biochemical investigations using this model focussed upon the forebrain of the chick, dissecting it rather crudely into two fractions: forebrain roof and forebrain base. Mileusnic et al. (1980) trained chicks on a passive avoidance task to produce two groups of chicks: methylanthranilate-trained chicks (M chicks) and water-trained chicks (W chicks). Mileusnic et al. (1980) were then able to demonstrate a persistent increase in the incorporation of radioactively labelled leucine into soluble protein of the forebrain roof

of M, compared to W, chicks for up to 24hr posttraining. Such an increase was also seen in tissue slices cut from the forebrain roof of M chicks incubated with labelled leucine, when compared to similarly treated slices from W chicks (Schliebs et al., 1985).

Increased binding of receptor ligands in chick forebrain following passive avoidance training has also been reported. Muscarinic cholinergic receptor binding, using the ligand QNB (quinuclinidinyl benzilate), is significantly increased following passive avoidance training by 22% in the forebrain roof of day-old M-birds (Rose et al., 1980). This increase was present at 30min posttraining but was not evident at either 10min or 3hr after training. Untrained birds that merely tasted methylanthranilate showed no such increased QNB binding, suggesting that increased binding was not simply a concomitant of the aversive taste of methylanthranilate. Bilateral intracerebral injection of either ouabain or cycloheximide, substances that render chicks amnesic, is also correlated with an absence of QNB binding, suggesting that increased binding is a correlate of memory formation.

Changes in the phosphorylation of specific proteins have also been observed in the forebrain of day-old chicks following passive avoidance training (Ali et al., 1988a). In this experiment chicks were trained as normal, producing M and W groups. They were then tested at either 10, 30 or 360min posttraining. Following testing their brains were removed and synaptic plasma membrane fractions were prepared from whole forebrain samples. The phosphorylation patterns of synaptic plasma membrane proteins showed a significant decrease in phosphorylation of a 52KDa band in M trained chicks, as compared to W controls. This decrease was only significant at the 30min posttest time point, suggesting that there were changes in protein phosphorylation at the synapse soon after training. In a further experiment protein kinases were also implicated in memory for passive avoidance training (Ali et al.,

1988a). In this study day-old chicks received bilateral intracranial injections of the protein kinase inhibitors polymixin B or mellitin into the forebrain 5min before training. Both of these treatments were amnestic. Injections of mellitin 5min posttraining were also amnestic, suggesting that the injection of mellitin was not simply interfering with the acquisition of the task. However, injection of mellitin 1hr posttraining was without effect. The authors suggested that this indicated that the effect of mellitin was to directly interfere with processes underlying long-term memory formation, rather than with those of recall or recognition (Ali et al., 1988b). This implicated protein kinases in the formation of long term as opposed to short term memory.

Another biochemical change has been seen in the forebrain of M chicks following passive avoidance training: an increase in the synthesis of glycoproteins. Rose and Harding (1984) trained day-old chicks on a passive avoidance task and then measured the incorporation of radioactively labelled fucose into homogenates of whole forebrain base or anterior forebrain roof. This analysis showed that there was an increased uptake of fucose following passive avoidance training in day-old M chicks compared to water controls. This increased uptake was abolished when M chicks were rendered amnesic through the use of immediate posttraining subconvulsive electroshock. If the shock was delayed until 10min posttraining no amnesia developed. M chicks that received this delayed shock treatment exhibited increased fucose incorporation. This suggested that increased fucose incorporation was associated with memory for the task.

Chicks can also be rendered amnesic if injected with 2-Deoxy-galactose (2-D-gal), a substance that interferes with the fucosylation of glycoproteins. Bilateral injections of 2-D-gal into the forebrain of day-old chicks at any time between 3.5hr before and 3.5hr after training on a passive avoidance task renders M chicks amnesic at a 24hr

test (Rose & Jork, 1987). In a later experiment the amnestic action of 2-D-gal was investigated at times later than 3.5hr posttraining (Zamani & Rose, 1990). In this experiment it was shown that M chicks show significant amnesia at a 24hr test if injections of 2-D-gal are given 6hr posttraining. This suggests that there are (at least) two distinct periods during which the fucosylation of glycoprotein is necessary for the retention of passive avoidance training. From these three experiments it can be proposed that: (1) memory for the task is associated with an incorporation of fucose into glycoprotein (Rose & Harding, 1984) and (2) that this increased fucosylation is required for memory formation, as its inhibition produces amnesia (Rose & Jork, 1987; Zamani & Rose, 1990).

Because the experiments outlined above have examined very large samples of forebrain, each containing many different brain regions, recent experiments have attempted to define more precisely the forebrain regions involved in learning and long-term retention of passive avoidance training. Kossut and Rose (1984) were able to show that anatomically distinct structures within the brain of the day-old chick exhibit increased metabolic activity following passive avoidance training. In this autoradiographic study, 2-deoxyglucose (2-DG) was injected immediately before training. The relative amount of 2-DG uptake into various regions of the brain was taken as an index of both metabolism and blood flow/O_2 usage. The basis for the use of the 2-DG technique is as follows. It can be assumed that the immediate consequence of an experiential event is an increased neuronal firing rate within the population(s) of cells involved with the perception and central processing of the incoming information concerned with that event. This increased neuronal activity raises the metabolic rate of the cells involved. As glucose is the only source of metabolic energy utilized by neurons, increases in neuronal firing should, therefore, increase glucose uptake. Sokoloff et al. (1977) showed that neurons take up 2-DG in an identical manner to that of normal glucose. However, once present intracellularly, 2-DG is converted to 2-deoxyglucose-6-phosphate, which cannot be further

metabolized. The level of neuronal activity can be 'assayed' by measuring the accumulation of labelled ^{14}C-2-DG-6-phosphate, using serial autoradiography of thin brain sections following the injection of labelled 2-DG.

As mentioned earlier, the chicken has only a weakly established blood-brain barrier which allows substances injected peripherally to have central effects. Kossut and Rose (1984) gave chicks pericardial injections of ^{14}C labelled 2-DG immediately after pretraining. Half the chicks were then trained on a water coated bead (W chicks), the other half on a bead coated with methylanthranilate (M chicks). The birds were then killed 30 min posttraining, their brains removed and prepared for autoradiograms. Densitometric analyses of these autoradiograms revealed that only 3 of 13 identified structures exhibited significantly enhanced labelling when M birds were compared to W birds (see Figure 2.1). These three regions were: the intermediate medial hyperstriatum ventrale (IMHV); the lobus parolfactorius (LPO); and the paleostriatum augmentatum (PA). This result suggested that these three regions were involved in the acquisition of passive avoidance training. However, because the design of the experiment used a pretraining injection of 2-DG, the observed differences in 2-DG accumulation reflected not only the metabolic sequelae of the 30min posttraining consolidation period but also those of the training experience itself, including the tasting of methylanthranilate and the motor behaviour of the associated disgust response.

In an attempt to 'dissect' the effects of training from those of consolidation a second experiment was undertaken (Rose & Csillag, 1985). In this experiment day-old chicks were injected with 2-DG at one of three times: either 5min before training or 10 or 30min after training. Birds were then killed 30 min postinjection. In contrast to the earlier experiment of Kossut and Rose (1984), the three areas previously shown to exhibit enhanced labelling were on this occasion dissected out individually using a

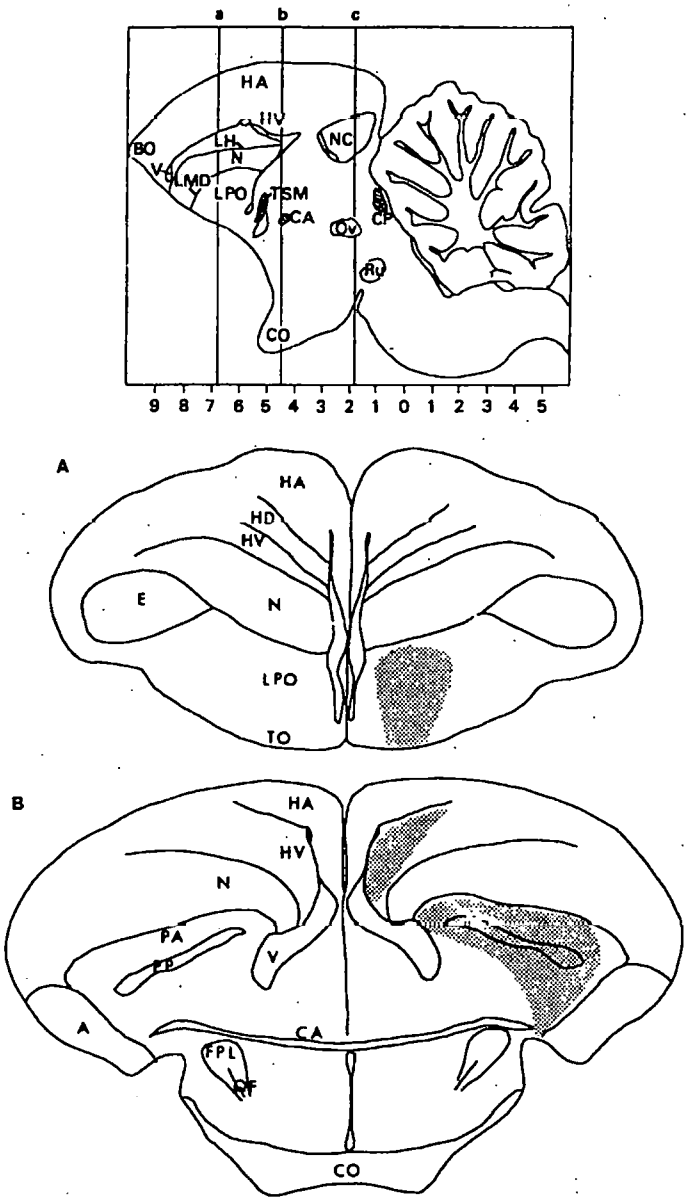
micropunch. The uptake of label was then measured as specific radioactivity of either left or right IMHV or LPO and standardized to the mean PA specific radioactivity, as no differences were found in the PA either between groups (M vs W) or hemispheres. Analysis of samples of the LPO indicated that only the LPO of the left hemisphere exhibited an increased uptake of label and then only when injections of 2-DG were given either 5min pre-training or 10min post-training. No significant increase was seen after a 30min post-training injection. Significant increases in 2-DG uptake into IMHV were also seen in the left hemisphere, in this instance at all injection times, with a significantly increased uptake into the right hemisphere only after the 10min injection - increased uptake into the right IMHV was not evident following the 30min injection.

The localized biochemical changes observed in chicks following passive avoidance training formed the basis for subsequent ultrastructural investigations. These investigations were carried out at both the light and electron microscope (EM) level.

Stewart et al. (1984) examined under EM the postsynaptic thickening of IMHV neurons from day-old chicks following passive avoidance training. A hemispheric asymmetry in the length of the postsynaptic thickening was found in W birds (12% greater in the left IMHV). This asymmetry was absent in M birds. The left IMHV has also been shown to exhibit other morphological changes in day-old M-chicks examined 25hr after training. These changes include: (a) a 23% increase in the number of synapses per unit volume of neuropil, compared to right IMHV; (b) a 61% increase in the number of vesicles per synapse in left IMHV compared to right IMHV; and (c) a 72% higher vesicle count in left IMHV. No changes in PA morphological characteristics have been reported following passive avoidance training.

In a second EM study the LPO of chicks was studied 24hr following passive avoidance training (Stewart et al., 1987). A significant increase of up to 59% in the

Fig 2.1; Diagram to show the relative positions of IMHV and LPO in the chick forebrain.



Schematic drawings of coronal slices of day-old chick brains prepared using a brain mould to show the locations of IMHV and LPO. The position and angle of cuts is shown in the sagittal scheme (stereotaxic coordinates after Youngren and Phillips 1978). The first slice represented by face A (taken at level a) was used by Rose and Csillag (1985) for dissecting LPO, and the second one, represented by face B (taken at level b) was used by them for dissecting PA and IMHV. The dissected regions are indicated by stippling. The nomenclature of frontal sections was based on the atlas by Karten and Hodos (1967): A=archistriatum, BO=bulbus olfactorius, CA=commissura anterior, CP=commissura posterior, CO=chiasma opticum, E=ectostriatum, FPL=fasciculus

Taken from Rose and Csillag (1985).

numerical density of synapses was observed in the LPO of both hemispheres in M-chicks compared to W-chicks. The density of both synaptic vesicles and synaptic vesicles per presynaptic bouton were 50% greater in the left LPO of M chicks compared to those of W chicks.

These changes observed at the EM level have been accompanied by changes seen at the light microscope level. Day-old M chicks examined 25hr posttraining had significantly increased spine densities in the IMHV of both hemispheres, with an asymmetry favouring the left hemisphere (Patel & Stewart, 1988). Other changes seen in this study were an increase in spine head diameter and a decrease in spine shaft length. In a follow-up study (Patel et al., 1988) day-old M chicks that received subconvulsive electroshock 5min posttraining and exhibited retention had a greater spine density at 25hr posttest than those that were amnesic from the shock. This second experiment directly implicated the spine increase in memory formation for the task. The other two spine parameters found to change in the previous study, spine head diameter and spine neck length, were no longer significantly different, suggesting that they may be associated with parts of the training experience that were non-specific to memory formation, e.g., the taste of methylanthranilate .

Recent studies involving the use of restricted electrolytic lesions of the IMHV and LPO have also implicated these regions in the acquisition and consolidation of passive avoidance training. Day-old chicks given bilateral lesions of the IMHV at 15 to 20hr pretraining on a passive avoidance task were amnesic for the task when tested 3hr posttraining (Davies et al, 1988; Patterson et al, 1990a). This indicates that the IMHV has an important role in the learning and/or retention of memory for passive avoidance training. In further studies to examine any lateralization of the effect, the left or right IMHV of day-old chicks was lesioned 24hr pretraining (Patterson et al., 1990a).When tested 3hr posttraining, chicks that had received a pretraining lesion to the left IMHV

were amnesic. Chicks with lesions to the right IMHV showed good recall. This suggests that the left IMHV (and not the right) is a necessary structure for the acquisition and/or retention of the task. Posttraining lesions of the IMHV were then undertaken to discover whether the IMHV does indeed have a role in memory storage. The results of this study demonstrated that the IMHV, although necessary for acquisition, is unnecessary for long term memory storage of memory for passive avoidance training. This was shown by placing bilateral IMHV lesions either 1 or 6hr posttraining with a test 24hr after lesioning (Patterson et al., 1990a). Lesions placed at either posttraining interval were not amnestic. It appears, therefore, that although neither hemisphere of the IMHV is necessary for long-term memory storage of the avoidance response the left IMHV is necessary for its acquisition. This suggests that areas other than the IMHV may maintain the memory trace following training.

In an attempt to define areas involved in long term storage of the task, lesion studies were extended to the LPO, the other area that 'lit up' in the 2-DG study. Day-old chicks given bilateral LPO lesions 24hr pretraining were not amnesic (Gilbert et al., in press). However, bilateral LPO lesions placed 1hr posttraining produced significant amnesia when chicks were tested 24hr after training. These experiments indicate that the LPO is not necessary for the acquisition of memory for the task, but is required for longer-term stages of memory formation/storage.

Gibbs and Ng (1977) have proposed a three-stage model of memory formation for passive avoidance training in day-old chicks. This model was based upon the temporal characteristics of amnesia produced by the intracerebral injection of different classes of drugs. Formation of short-term memory (STM) was disrupted by depolarizing agents such as lithium or potassium chloride or glutamate. The second stage, intermediate term memory (ITM) was disrupted by sodium/potassium ATPase inhibitors such as ouabain and ethacrynic acid. Long-term memory (LTM) was inhibited by the injection

of protein synthesis inhibitors such as anisomycin and cycloheximide. These stages of memory formation were sequentially dependent. Patterson et al. (1986; 1988) performed similar experiments to those of Gibbs and Ng (1977) and found similar results, supporting a three-phase model of memory formation. In addition, amnestic drugs were found to have lateralized effects. The left IMHV was susceptible to amnesia, if injections were placed in the right IMHV no amnesia developed. Chicks were amnesic if localized injections were placed in the right lateral neostriatum (Patterson et al., 1986; 1988). Injections placed in the left neostriatum were without effect. The amnesia that developed from injections to right neostriatum followed a similar time course to that produced in the left IMHV. From this it appears that the right neostriatum is also involved in memory storage for passive avoidance training and that this memory follows in a three-stage fashion.

Both the neurophysiological and animal models of learning presented in Chapter 1 demonstrate that behavioural learning requires changes in neuronal connectivity. In most cases these changes in connectivity were first observed at the electrophysiological level. Training a day-old chick on a passive avoidance task has been shown to produce memory-specific biochemical and morphological changes in identified regions of forebrain. If these alterations underlie the neural representation of memory for passive avoidance training, then they should be associated with and possibly produced by changes in the electrical activity of neurons in the identified loci of change. Until quite recently there were no investigations of the neurophysiological correlates of training day-old chicks on a passive avoidance task. The initial investigation, therefore, sought to record electrophysiological activity from as many different forebrain regions of the day-old chick as possible after training on the passive avoidance task (Mason & Rose, 1987. The sampled forebrain regions were as follows: the hyperstriatum accessorium; the hyperstriatum dorsale/intercalatum supremum; the IMHV; the neostriatum; and the medial PA. Two of the regions, the IMHV and PA, had been shown previously (using the 2-DG technique) to exhibit

increased neuronal activity following training on the task (Kossut & Rose, 1984). Multi-unit activity was recorded from both M and W birds at all of the above sites over 1-13hr posttest. The sites were sampled from each hemisphere during a vertical electrode penetration from the surface of the brain, 1mm lateral to the midline. Each recording site was 200μm apart and 2min of recording was taken at each point. The multi-unit activity that was recorded could be broken down into four major components (Mason & Rose, 1987): tonic, small amplitude spikes (100-175μV peak-to-peak); occasional large amplitude spikes (200-450μV peak-to-peak); 'bursting' episodes, 15-20ms in duration, consisting of high amplitude, high frequency spikes (200-450μV peak-to-peak, 400-450Hz); and noise (36μV peak-to-peak). When the first three of these components were expressed as mean firing rate per region sampled, significant differences could be seen between treatment groups. M birds exhibited a significantly higher mean firing rate, when compared to W birds, in three structures of the right hemisphere: the hyperstriatum accessorium; the IMHV; and the medial PA. No significant differences in mean firing rate were observed between groups for left hemisphere structures. However, the most remarkable between-group difference was a massive increase in the occurrence of the second of the four multi-unit components listed above: burst-firing episodes. The IMHV of both hemispheres of M birds showed a significant increase in burst-firing episodes when compared to W birds. Although bursting was observed in all of the forebrain structures examined, this was the only structure to exhibit a significant difference in bursting.

Previous experiments to examine training-induced biological changes in the day-old chick have attempted to directly associate these changes with memory formation by the use of amnestic treatments, e.g., subconvulsive electroshock. Such an experimental design was used in the follow-up experiment to Mason and Rose (1987) in order to distinguish which neuronal firing pattern (if any) was directly associated with memory for the task. In this follow-up experiment Mason and Rose (1988) recorded multi-unit

activity from the IMHV of M-trained day-old chicks in an identical manner to that reported in their previous paper. However, in this instance chicks were given subconvulsive electroshock that was delivered either immediately after training (approximately 10sec posttraining) or delayed until 10min posttraining. This procedure produced two groups of M-trained chicks: a group that received immediate electroshock and was amnesic and a second group that received delayed electroshock and showed recall. In a second experiment, W-trained birds were also included in the analysis. Immediately after training these W birds were either shocked or held identically and "sham-shocked" (i.e., had electrodes pressed against the cranium with the current turned off). Two other groups of birds were also included. The first of these consisted of M-trained chicks that had been given immediate electroshock and yet still remembered the task, the other group was made up from M-trained chicks that had received delayed electroshock but were amnesic.

The results of this experiment showed that when levels of bursting in M-trained chicks that remembered the task were compared to W-trained chicks that also remembered, there was a significant bilateral increase in burst-firing in the IMHV, similar in magnitude to that seen previously (Mason & Rose, 1987). M birds that were amnesic displayed a similar level of bursting to W-trained chicks. These results strongly suggest that the enhancement of phasic bursting activity in the IMHV of day-old M chicks is a direct concomitant of the mnemonic processes underlying memory formation/consolidation. The increase in mean firing rate of the right IMHV of M trained chicks reported by Mason & Rose (1987) was not seen in the later, electroshock study (Mason & Rose, 1988). This result implies that the increase in the mean firing rate of the right IMHV of M trained chicks is produced by aspects of the training experience not specific to memory formation: M chicks that receive electroshock, but display recall at test, show no increase in the mean firing rate of left or right IMHV above those of either W chicks or amnesic M chicks.

These two studies (Mason & Rose, 1987; 1988) form the basis for the work presented in the following chapters of this thesis. Mason and Rose (1987) demonstrated an electrophysiological effect produced by training day-old chicks on a one-trial passive avoidance task: a bilateral increase in bursting in the IMHV of M-trained chicks during 1-13hr posttest. This effect was then directly related to memory formation (Mason & Rose, 1988). That there is an effect is an exciting result in itself. However, as mentioned in the General Introduction, researchers in the field of Learning and Memory are becoming increasingly concerned with neuronal events whose expression is both (a) associated with memory and (b) time-locked to phases at some interval from the point of test, e.g., phases of protein synthesis that may occur many hours following training. With this in mind the Experiment described in Chapter 3 describes an extended timecourse of bursting in both M and W-trained chicks. Chapters 4 and 5 are concerned with recording multi-unit activity from the LPO, a region that displays increased neuronal activity following training, as measured by 2-DG uptake. This region was not recorded from by Mason and Rose in either of their neurophysiological experiments (Mason & Rose, 1987; 1988). Chapter 6, the final experimental chapter, examines the effects of lesions of the LPO on burst-firing activity in the IMHV.

The final part of this chapter will review the general housing, training, anaesthetic, surgical and recording procedures used in the following experiments. Any deviations from these procedures will be outlined at the appropriate time.

Methods for Recording Multi-Unit Activity after Passive Avoidance Training.

The behavioural training, surgical and anaesthetic procedures are similar for all of the experiments in this thesis. They will be dealt with in detail here and referred back to in other chapters with details of any changes as appropriate.

(1) Subjects.

Ross-1 eggs from a commercial hatchery were incubated and hatched in communal brooders on a 12hr light/dark cycle and chicks of both sexes were then maintained at 38.5-40.5°C until approximately 24hr old. On the day of training, pairs of chicks were placed in small pens (20x25x20cm) illuminated from above by 25W red lights. Once housed, a small amount of commercial chick-crumb was scattered onto the floor of each pen. Chicks had no access to water in these pens.

(2) Training and testing procedures.

Chicks were allowed to equilibrate for 1hr. They were then pre-trained (to initiate pecking) by three presentations of a white bead (2.5mm diam.) over a five minute period. After a further 10min, the chicks were then trained using a chrome bead (4.0mm diam.) coated with either methylanthranilate (M-chicks) or water (W-chicks). The chicks' pecking behaviour was recorded. The chicks were tested 1hr after training by a 30sec presentation of a dry chrome bead and peck or avoid responses were recorded. Criteria for selection of experimental animals were: (1) that all chicks should peck upon training; and (2) that W-chicks pecked at test whilst M-birds avoided the test bead. Animals were trained and coded by either Prof. S.P.R. Rose or Dr. T.A. Patterson. All subsequent procedures were carried out blind by J. Gigg. Animals were selected on a random basis, the first bird being made ready for recording immediately after testing, the others being taken at successive intervals. Usually a total of four birds per session were used. No animals were taken for recording after 10hr posttest. Data were decoded only when all experimental and analytical procedure had been carried out.

(3) Anaesthetic and surgical procedures prior to recording.

Chicks were anaesthetized with an intraperitoneal injection of a 40% w/vol solution of

Urethane (Sigma) at a dose of 0.65ml/100g body weight. After injection chicks were placed in an insulated box that contained a mirror to help reduce isolation stress. When the chick was under deep surgical anaesthesia (judged by lack of 'withdrawal reflex' upon pinching interdigital skin) it was transferred to a Baltimore Universal Stereotaxic Frame (Model L) where its core temperature was maintained at 40-41^{o}C by a Palmer Homeothermic Underblanket thermostatically controlled via a cloacal thermistor probe. Additional thermal insulation was provided by a thick covering of cotton wool.

The cranium of the chick was then secured in a modified small animal holder. The cranium was placed at the desired angle by locking the beak bar 5mm below and 11.5mm anterior to the central axis of the ear bars - an orientation similar to that of the resin brain mould of Rose and Csillag (1985) and to that of Youngren and Phillips' (1978) atlas of the 3-day old chick. Care was taken to ensure that the minimum amount of pressure was used during insertion of the ear bars, as the chick skull is easily distorted. Prior smearing of the ear bars with electrode gel aided their subsequent insertion (Neptic electrode gel, Sandev Ltd.). This gel had the added advantage of ensuring an efficient electrical contact between the tissues of the external ear and the metal of the ear bars. This was important as it ensured a good connection to ground via the low-resistance electrode placed on the ear bars. The beak was then securely taped to the beak bar to provide an extra degree of stability. Surgical procedures were carried out under overhead and side illumination.

A stereo binocular microscope, mounted on a tripod, was used during the more delicate surgery. The chick's scalp and underlying muscle were incised. The coordinates used for recording from the IMHV were 1.15mm anterior to the ear-bars and 1mm lateral to the skull midline. For LPO recordings the coordinates were 4.8mm anterior to the ear-bars and 1.2mm lateral to the midline. An electrode was mounted in a vertical, stereotaxic-mounted electrode holder and lowered carefully to mark the skull

microelectrode would pass to record from either the IMHV or the LPO. At these coordinates square craniotomies roughly 1mm in length were performed using a scalpel blade. The underlying dura was then carefully incised. Warm silicon fluid (~40°C) was placed into these incisions to prevent excessive cooling of the brain and to reduce the seepage of cerebrospinal fluid.

(4) Lesioning procedures.

Chicks were taken when they were 18hr old and were given intraperitoneal injections of Equithesin at a dose of 0.28 ml/100g body weight. When narcosis became apparent, the chick was transferred to a stereotaxic instrument. The chick's temperature was maintained by an electric underblanket. When the bird was fully anaesthetized, the cranium of the chick was secured in a modified small animal headholder, with the beak bar locked at an identical angle to that described above. The scalp and underlying muscle insertions were deflected and small craniotomies were made in the skull directly over the electrode placement sites. After incision of the dura, the temperature-sensitive electrode was lowered under stereotaxic control. The coordinates used for the LPO were 4.0mm anterior to the ear bars, 1.1mm lateral to the midline and 4.5mm ventral to the surface of the brain. The lesions were induced by radiofrequency using an RGF 4 Lesion Maker (Radionics Inc., Burlington, Mass. USA). The temperature at the electrode tip was maintained at 60°C for 90 sec. The electrode was removed when the temperature of the electrode probe returned to body temperature, the skull flap was closed and secured with warm bone wax. The scalp was sutured and the chick was left to recover in a warm recovery box for several hours. The chicks were then given water and allowed to recover in these boxes until several hours before training or testing. Sham control chicks underwent identical procedures to those of the lesioned animals; however, no current was passed through the electrode tip.

(5) Manufacture of microelectrodes.

Microelectrodes used to record from the IMHV were manufactured from borosilicate glass capillary tubing ("Kwik-Fill", Clark Electromedical Ltd., 1.2mm outside diameter and 0.65mm inner diameter). Electrodes were pulled on a vertical electrode puller (Scientific Research Instruments Ltd. Model No. 2001) and filled under vacuum with 0.5M sodium acetate solution containing 2% Pontamine Sky Blue for the marking of electrode positions. The electrode tips were broken back using a small pair of scissors. Electrodes were monitored for diameter and resistance (10μm o.d., 3-5μm i.d., 0.5 MΩ, respectively).

Glass-insulated tungsten microelectrodes were chosen for LPO recording because of their consistent, thin longitudinal profile when compared to the glass microelectrodes used for IMHV recording. It was felt that such a small profile would minimize the tissue damage caused to higher structures when recording from the LPO, which is a structure situated in the forebrain base. These microelectrodes were fabricated using a method similar to that of Merrill and Ainsworth (1972). Pieces of pre-straightened tungsten wire (127μm diameter, 15cm length. Clark Electromedical) were submitted to a 2-stage etching procedure. The first stage involved immersing one end of the wire into a solution of KNO_2 (150g per 100ml distilled water) whilst passing A.C. current (12V, 1.75A) across the tip of the wire from a large carbon rod also immersed in the solution. This first etch consisted of three immersion stages: (1) the first 2cm of the wire was immersed for 20s; (2) the very tip of the wire was then allowed to form a meniscus with the solution very briefly a total of five times; (3) finally, the first 2mm of the wire was immersed very briefly a total of seventy times. The wire was then inserted non-etched end first into a borosilicate glass capillary (0.38mm outer diameter, 15cm length, Plowden and Thompson Ltd) until the etched end passed 2cm in from the end of the capillary. The wire was then superglued in place at the non-etched end.

The electrode was then inserted into the vertical electrode puller, the top chuck holding the wire and the bottom chuck holding the bare glass end of the capillary. The electrode was then pulled (ensuring a good coating of glass insulation to the tip of the electrode) and mounted in a horizontal micromanipulator. The tip was then advanced under low-power magnification into a small (2mm) molten bead of glazing flux (Pyrotenax Ltd.) until any protruding glass and the very tip of the electrode passed into the bead. The current used to melt the bead was then switched off and the bead allowed to harden. The electrode was then firmly pulled away from the bead, leaving the very tip of the wire exposed from the insulating glass. More than one insertion into the bead was occasionally found to be necessary. The tip was then subjected to a second etch in KNO_2 (12V, 1.2A current A.C.), this time under low-power magnification, until the tip assumed a 'bullet' shape. The impedance of the electrode was then measured, with impedances of 1MΩ being being favoured.

(6) Multi-unit recording procedures.

(a) Recording from the IMHV.

Multi-unit signals were monitored using a digital storage oscilloscope (Gould OS4000) and the audio output of a spike processor (Digitimer 130). All recordings were made relative to a distant reference electrode at the skull base. This reference electrode consisted of a hook of chlorided silver wire. The chloride helped to reduce the effect of junction potentials. The preparation was grounded via a low-resistance junction at the ear-bars (crocodile clip). The active electrode consisted of a 3cm piece of chlorided silver wire, carefully soldered to a short length of input lead. This wire was inserted into the glass electrode prior to recording. To shield the preparation from extraneous electrical noise a small aluminium shield was placed over the active electrode during recording and grounded to the stereotaxic frame. All electrodes were connected to the input sockets of a headstage (NL100, Digitimer Ltd.) which was connected via a shielded cable to an AC preamplifier. Signals were amplified 1000 times in two stages (Neurolog AC preamp NL 104, Neurolog AC DC amp NL 106,

both Digitimer Ltd) and band-pass filtered (300Hz-5KHz, Neurolog filter NL125) to remove both low frequency "slow-wave" and very high frequency components. This amplified signal was then fed to a spike processor (Digitimer D130) to ascertain the average spike firing rate (above a pre-determined discrimination voltage, set for removal of electrical noise). Slow-wave signals were high frequency filtered (100Hz, 50Hz notch filtered, Neurolog NL 115) and fed directly, together with multi-unit signals, to a digitizing unit (Neurocorder DR484, Neurodata Instruments Corp.) and from here to a video tape recorder (Panasonic AG 6010) for subsequent storage. After recording from each hemisphere, the electrode was withdrawn and Pontamine Sky Blue was iontophoresed to mark each recording site (3min at -10nA, Neurophore BH2/IP2, Medical Systems Corp.). Following this the brain was removed, immediately frozen in isopentane and stored at -70°C. Sections were cut (20μm thick) on a cryostat at a later date to determine the electrode track.

Vertical measurements of electrode tip position were taken from the brain surface. Penetrations were made from this 'zero' point as follows. For recording from the IMHV the electrode was initially stepped down 1000μm (TrentWells Microstepper Mk. 3) in two steps of 500μm; the electrode was then lowered in 200μm steps until the upper border of the IMHV was reached. This was determined by a large increase in the amplitude of damage potentials as the recording electrode approached the IMHV. A series of eight sequential steps of 200μm were then made, 2min of recording being made at each step. A 30sec pause for the termination of damage potentials was allowed at each penetration point before recording. This process normally produced six 2min recordings from the IMHV of each hemisphere. The order in which hemispheres were recorded from was randomized.

(b) Recording from the LPO.

The methods used to record multi-unit activity from the LPO were very similar to

those reported above. Spontaneous multi-unit activity signals were amplified 1000 times in two stages and high band-pass filtered (300Hz-5KHz) for subsequent storage on video tape. The preparation of tungsten-in-glass electrodes was as described above. Once the electrode was mounted in the electrode holder the free end was trimmed back to reduce the pickup of extraneous noise. The connection of electrodes to the headstage was via a short length of shielded cable. This cable was attached to the trimmed, free end of the electrode with a mini crocodile clip. The electrode was surrounded by a grounded, aluminium shield during recording.

Vertical measurements of electrode tip position were again taken from the surface of the brain. The electrode was initially stepped down 3500μm in four steps of 750μm and one step of 500μm. A series of eight sequential steps of 200μm were then made with 2min of recording at each step. A 30sec pause for the termination of damage potentials was allowed before recording. This process normally produced five 2min recordings from the LPO of each hemisphere. The order in which hemispheres was recorded from was randomized. At the termination of recording the free metal end of the electrode was heated with a soldering iron for 90sec, producing a small lesion at the electrode tip to mark the site of recording. The brain was then removed, immediately frozen in isopentane and stored at -70°C. Sections were then cut 20μm thick on a cryostat to determine the position of the electrode.

(7) Data analysis.

Data analysis was similar for all experiments and was performed off-line. Recorded multi-unit activity was replayed from video tape, through the digitizing unit to a monitoring oscilloscope (Gould OS4000) and from here to a spike-processor (Digitimer D130). Spikes were discriminated as follows: the lower (in this case negative) discriminator was adjusted until only spikes of sufficient amplitude to be included within bursts were registered, i.e., spikes greater than or equal to 100μV negative. The resulting discriminated spikes were output as 75μs T.T.L. pulses on the

'Lower' terminals of the D130. These pulses were then fed to the digital 'in' port of a CED1401 (Cambridge Electronic Design) computer interface unit and sampled at 500Hz for storage to hard disk (Zenith Z200 PC).

The computer program used for analysis was modified from one of the CED 'Spike2' software programs (see Appendix i). The modifications allowed the user to input directly the parameters which would be used by the program to perform burst analysis and also allowed the analysis of burst start times via the use of interburst interval histograms and autocorellelograms. The input parameters used to perform burst analysis were: (1) the number of spikes per burst; (2) the maximum interval between spikes to constitute a burst, i.e. the intraburst inter spike interval (intraburst I.S.I.); and (3) the minimum interval between spikes to constitute the end of a burst i.e., the interburst I.S.I. To produce bursts with the required spike number and frequency (5 or more spikes with a mean frequency. of 400Hz or above), the optimum values for these parameters were derived from a series of pilot runs of the program, using real data. The final parameters chosen were an intraburst I.S.I. of 4.9ms and an interburst I.S.I. of 5.0ms. These values were used in all data analysis. However, it is important to realize that these values represent a compromise. Although during a burst some intervals between spikes may be longer than 2.5ms (a frequency less than 400Hz), the mean I.S.I. of the burst may actually be 2.5ms (or less). Therefore, if the cut-off level for the intraburst I.S.I. were set to 2.5ms, then bursts meeting criterion with a mean I.S.I. of 400Hz would be (incorrectly) rejected. Although the upper I.S.I. limit of 4.9ms seems high, it was chosen because higher or lower values than this allowed into the analysis either a smaller number of faster bursts or a higher number of slower (<400Hz) bursts, respectively. In fact, the 4.9ms value produces a majority of bursts with I.S.I.s of >500Hz.

The statistics returned by the program included: (a) mean number of spikes per burst;

(b) mean intraburst I.S.I.; (c) the percentage of discriminated spikes to fall within bursts; and (d) the number of bursts per 2min. These data are presented in the results section.

The modifications to the program also allowed computer-discriminated burst start times to be saved to disk for later analyses designed to obtain quantitative data concerning burst firing patterns. Burst start times were used to plot both interburst interval histograms and also autocorrelelograms of bursting activity

The frequency data for burst firing patterns were normalized using the square root transform (Sokal & Rohlf, 1981) and subsequently analyzed using two-tailed unpaired Student's t-tests and analysis of variance tests to assess any differences between groups. For clarity, all data are presented graphically in their original form, i.e., non-transformed.

CHAPTER 3. Recording Multi-Unit Activity from the IMHV of the Day-Old Chick Following Passive Avoidance Training.

Introduction.

Electrophysiological recordings have shown that training on a passive avoidance task increases the level of neuronal burst-firing in the IMHV over the period 1-13hr posttraining (Mason & Rose, 1987): a change that has been directly associated with recall for the task, as amnesia abolishes the increased bursting (Mason & Rose, 1988). The experiments described here were undertaken in an attempt to extend these electrophysiological observations to include more detailed analyses of the timecourse, lateralization and patterning of the bursting phenomenon in the IMHV of the day-old chick. Because of the number of graphs to be presented, this experiment has been divided into three sections. The first of these sections serves as a replication of the original effect described by Mason and Rose (1987). The second describes the timecourse and lateralization of bursting in the IMHV. The third and final section describes in more detail the pattern of bursting.

Methods.

The behavioural, anaesthetic, surgical and recording procedures were carried out as described in Chapter 2 for IMHV recordings. This method can be summarized as follows: pairs of day-old chicks were housed in pens and then trained by Prof. S.P.R. Rose. This training produced two treatment groups, M chicks and W chicks. M chicks were presented at training with a methylanthranilate coated bead: only birds that pecked the bead and showed a disgust response were included in later parts of the experiment, birds that either did not peck the bead or did not show a disgust response were rejected. W chicks were presented with a water coated bead at training: only those chicks that pecked the bead were included in the experiment. Chicks that trained successfully were tested one hour posttraining with a dry bead. M chicks that pecked

the dry bead and W chicks that avoided the dry bead were rejected on the grounds that this behaviour indicated that they had, for whatever reason, failed to remember their training. Chicks were then assigned codes and these codes were passed to J. Gigg. Chicks were then taken at regular intervals for IMHV multi-unit recording. The intervals at which chicks were recorded from were; 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8 and 8-9hr posttraining. At least four chicks from each treatment group were recorded from at each of these intervals. Hemispheres were recorded from in a random order. Figure 3.1 shows a typical histological reconstruction of recording sites.

3.1 Effects of passive avoidance training on bursting recorded over 1-9hr posttest.

Results.

A typical burst recorded from the IMHV is shown in Figure 3.2. The lower trace represents the associated focal EEG. Mean burst frequencies (i.e., the mean of left and right hemispheres) for both methyl (M) and water (W) trained animals are presented in Figure 3.3. There is a significant difference between groups ($p<0.01$; Anova F-ratio=12.23). The mean bursting frequency for each hemisphere is presented in Figure 3.4. This shows that both hemispheres of M birds exhibit significantly higher burst frequencies than the respective hemispheres from W birds ($p<0.02$ for both comparisons).

Discussion

The overall burst frequencies recorded from the IMHV of M birds are significantly higher when compared to those for W birds in data pooled over the 1-9hr posttest period. Both the right and left hemispheres of M birds show increased bursting over the same period when compared to the respective hemispheres of W birds. These results are similar to the electrophysiological effects found by Mason and Rose (1987) after training day-old chicks on the same passive avoidance task. Therefore, the

Figure 3.1; Schematic representation of recording sites in IMHV.

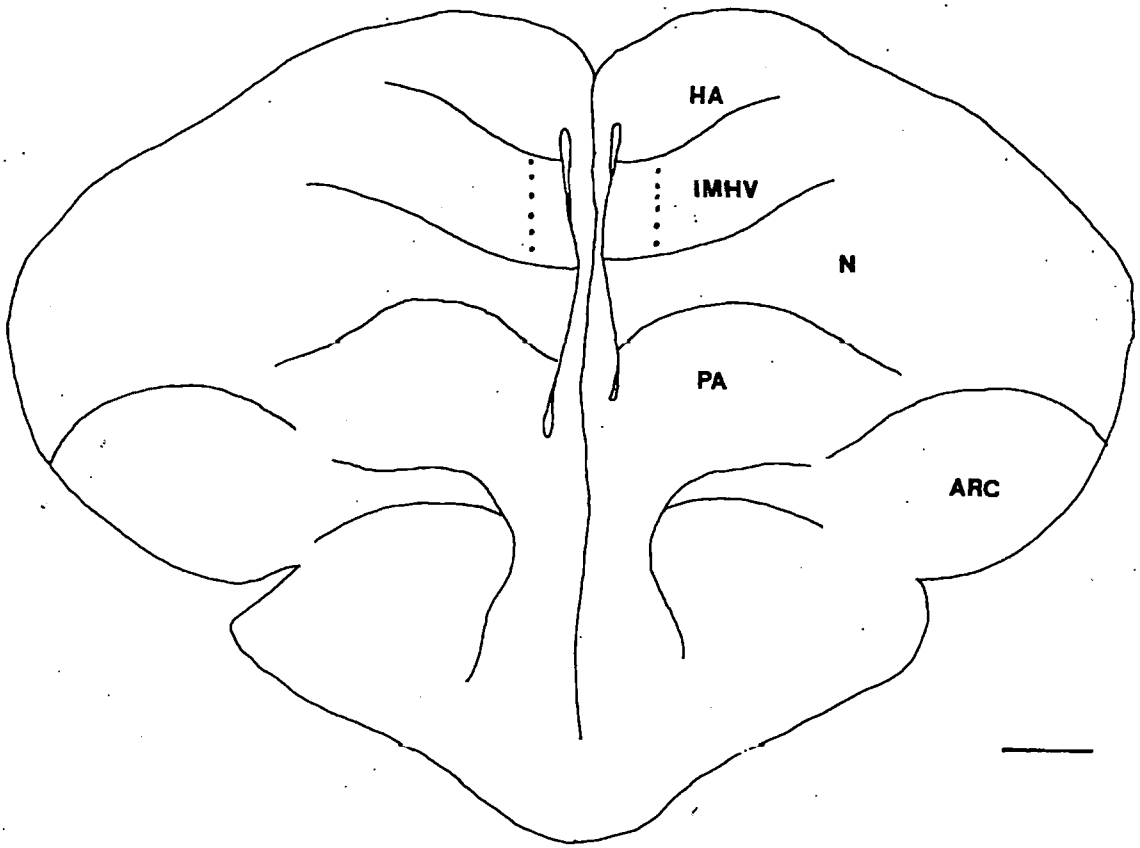


Figure 3.1. Schematic representation of IMHV recording sites. Filled circles indicate recording sites. The section corresponds to the anterior 3.5 reference in the Youngren and Phillips (1988) atlas of the 3 day-old chick. This section indicates that recordings were made from an anterior portion of the IMHV. Abbreviations: HA, Hyperstriatum accessorium; IMHV, intermediate medial hyperstriatum ventrale; N, neostriatum; PA, paleostriatum augmentatum; ARC, archistriatum. Scale bar = 1mm.

Figure 3.2(A); Multi-unit Activity Recorded from the IMHV

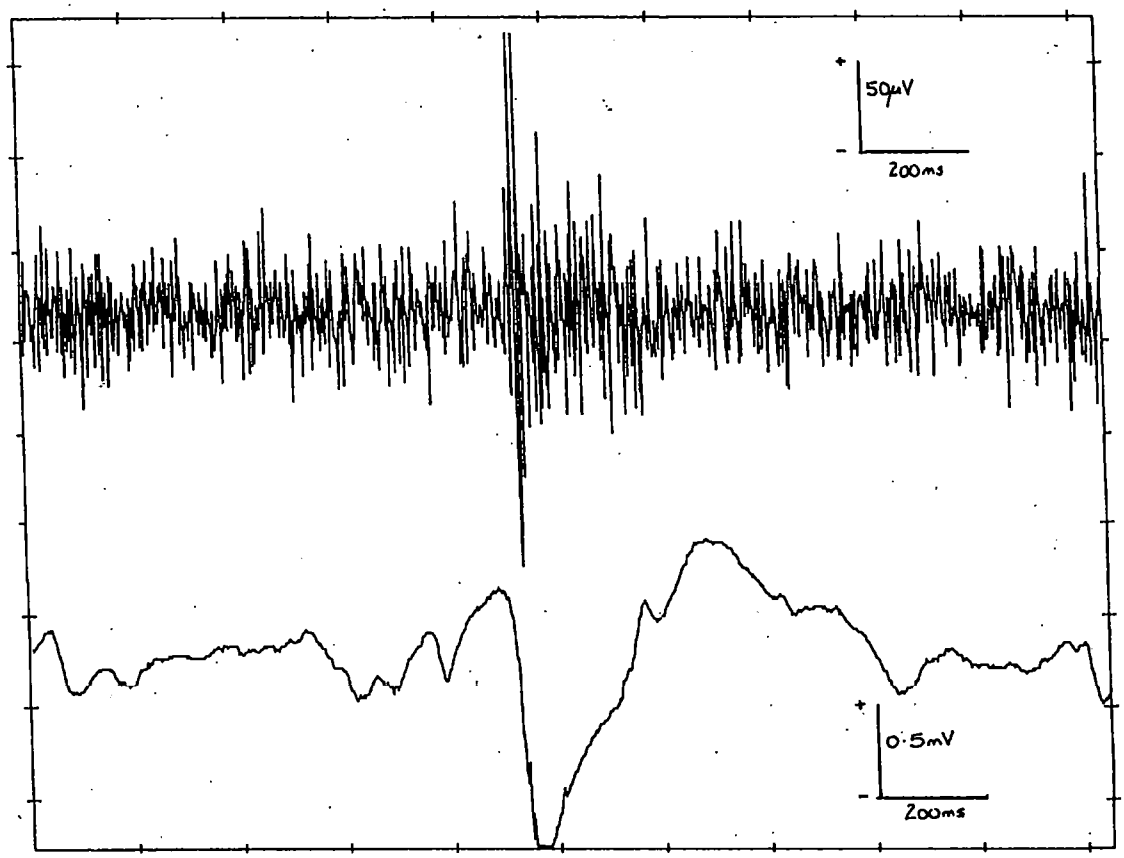


Figure 3.2 (A); IMHV multi-unit activity. Top trace includes a bursting epoch, lower trace represents simultaneously recorded local field potential. Note that the negative shift in field potential is coincident with the occurrence of bursting.

Figure 3.2(B); IMHV Multi-unit Activity to Show: (1) Occurrence of "Theta" Rhythm in IMHV; and (2) a more Detailed Picture of a Burst, Using a Faster Oscilloscope Sweep Time to That Used in 3.2(A).

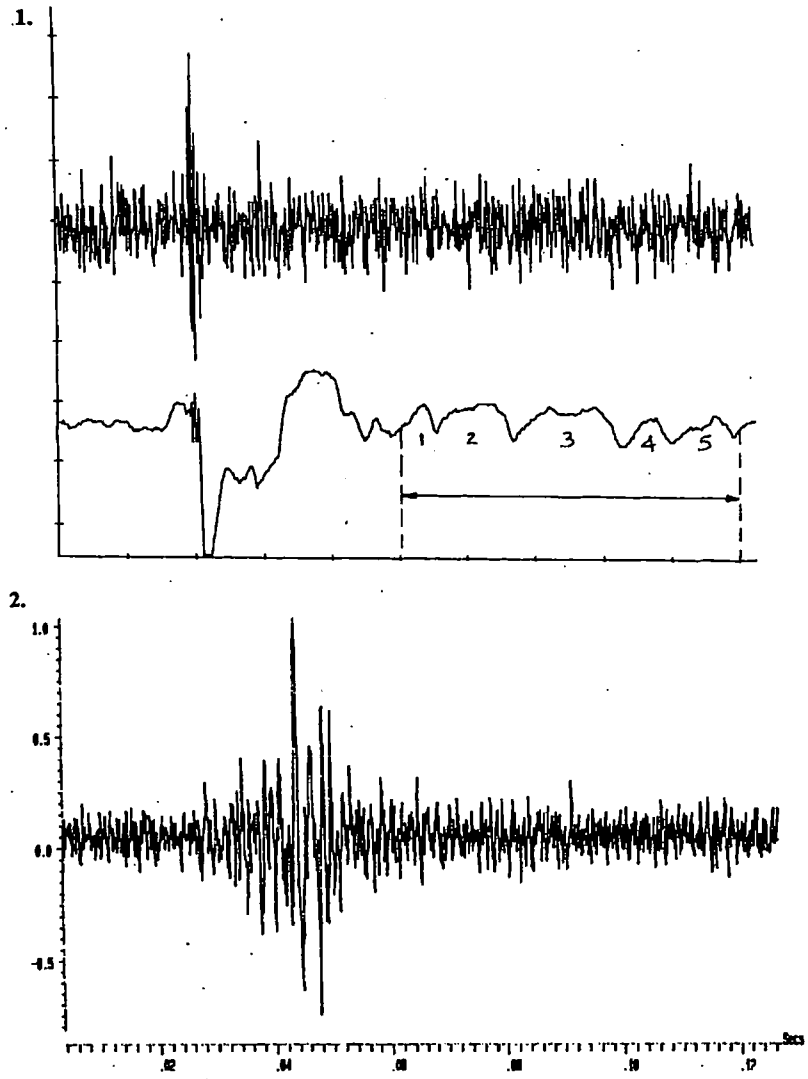


Figure 3.2 (B); IMHV multi-unit activity. (1) The occurrence of "theta" rhythm in the IMHV: following the burst the field potential (lower trace) has a sinusoidal frequency of approximately 5hz (arrow). This frequency is within the theta range. Calibration as per Figure 3.2A. (2) Burst at higher resolution, 0.12sec trace, to show individual spikes. Calibration: mV (vertical scale); seconds (horizontal scale).

Figure 3.3; Mean IMHV Burst Frequencies for Methyl and Water Trained Day-old Chicks over 1-9hr Posttest.

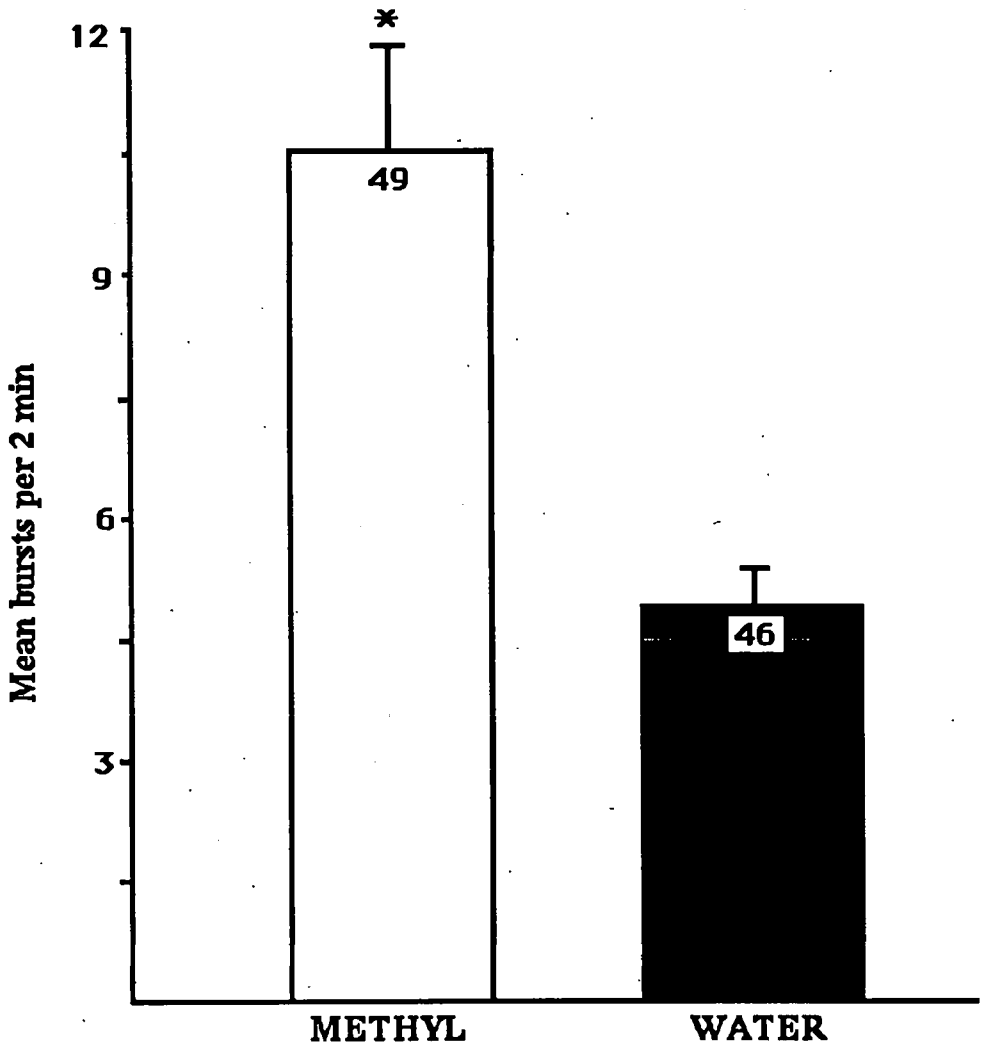


Figure 3.3; Mean bursting per 2min for the IMHV of methyl and water-trained, day-old chicks over 1-9hr posttest. The level of bursting represents the mean value for left and right hemispheres. The numbers in bars represent sample size. Chicks trained on a methylanthranilate coated bead exhibit a significantly higher level of bursting when compared to chicks trained on a water coated bead ($p<0.01$). Error bars are standard error of the mean.

Figure 3.4; Hemispheric Differences in Mean IMHV Burst Firing for Methyl and Water-trained Day-old Chicks over the period 1-9hr Posttest.

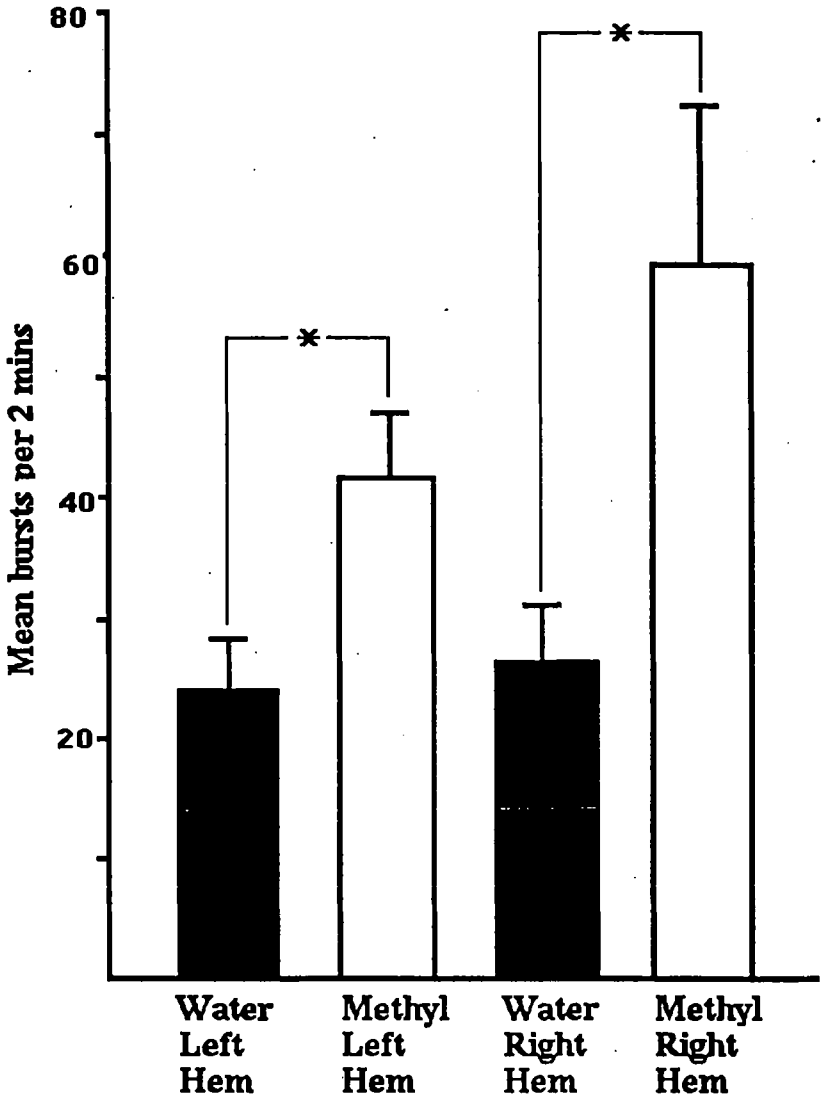


Figure 3.4; Hemispheric differences in bursting per 2min between methyl- and water-trained day-old chicks over 1-9hr posttest. The sample sizes for each group are as per Figure 4.3, that is, 49 methyl-trained chicks and 46 water-trained chicks. Chicks trained on a methylanthranilate-coated bead exhibit a significantly higher level of bursting in both left and right hemispheres when compared to the respective hemispheres of water-trained animals ($*p<0.02$). There is no significant difference in burst-firing rates between hemispheres within either group.

present results replicate the effect they originally described. The general level of bursting for both M and W chicks reported here is somewhat lower than that described by Mason and Rose (1987; 1988). The mean burst rates quoted in this Experiment for both groups of chicks are approximately an order of magnitude lower. There may be several reasons for this. Firstly, as will be seen in later sections of this Chapter, the level of burst-firing in the IMHV of M chicks follows a distinct timecourse following training. The exact posttest time at which recordings are made from the IMHV may, therefore, introduce a sampling-bias into overall levels of bursting, especially if large numbers of M chicks are recorded from during any time-locked peak of bursting. This raises the possibility, therefore, that a significant number of M birds in the studies of Mason and Rose (1987; 1988) were recorded from during periods of highly elevated bursting, producing a much higher overall burst-firing frequency than that reported in this Experiment. Secondly, the analysis method in this Experiment uses a computer program to discriminate burst-firing, a more quantitative method to that used by Mason and Rose (1987; 1988) who employed a rather qualitative 'by eye' method of analysis. Mason and Rose may have overestimated levels of burst-firing, that is, they may have discriminated bursts that would have been rejected by the program used here. Evidence to support this contention comes from pilot studies in which the intraburst interval was raised to 8msec during computer-aided analysis. This produced a level of bursting similar to that reported by Mason and Rose (1987). However, the bursts generated had interspike intervals higher than 2.5ms and as such were too 'slow' to be included as bursts, that is, their interspike frequency was lower than the *a priori* burst definition of 400Hz. This raises the possibility that not all of the bursts discriminated by Mason and Rose met their own *a priori* conditions. A third suggested reason for the disparity in bursting levels is the difference in the choice of anaesthesia: instead of Urethane, as used here, Mason and Rose (1987; 1988) chose the barbiturate-based Equithesin as an anaesthetic. This was a poor choice for three reasons: (1) barbiturates depress the very type of neuronal activity under study, that

is, burst-firing; (2) the safety margin between levels of barbiturate required for surgical anaesthesia and those that produce medullary paralysis is very small in bird species when compared to other laboratory animals (Wright, J.G. 'Veterinary Anaesthesia', Williams & Wilkins Co., Baltimore, 1957); and (3) Equithesin is a short-acting, recovery anaesthetic that produces narcosis very quickly after injection and allows a fairly rapid return to consciousness. Urethane, however, has a relatively non-depressant action on neuronal firing, a higher safety margin for paralysis and maintains a stable level of anaesthesia for some hours. Chicks anaesthetized with Equithesin may have been less deeply anaesthetized when compared to Urethane anaesthetized birds in order to minimize the risk of respiratory failure. The initial dose of Equithesin may have been barely sufficient to induce anaesthesia and, once anaesthetized, the chick would be quickly recovering consciousness. This may explain why levels of bursting from chicks injected with Urethane are different to those from chicks injected with Equithesin.

Although the replication of Mason and Rose (1987) reported here adds further credence to the training effect upon bursting activity, it provides little information regarding the dynamics of the effect. These temporal characteristics can be seen when the data presented in Figure 3.3 are plotted against time as well as treatment group. This is presented in the next section.

3.2. The Timecourse and Lateralization of Bursting following Training.

Results.

The burst frequency for each group is plotted against time in Figure 3.5. This shows the timecourse of bursting over 1-9 hours posttest for both W and M-trained day-old chicks. Subjecting these data to an analysis of variance test demonstrated that there was a significant effect of time posttest upon bursting (F-ratio 3.29, p=0.004).

Figure 3.5; The Timecourse of IMHV Bursting Measured Over 1-9hr Posttest for Methyl and Water-trained Chicks.

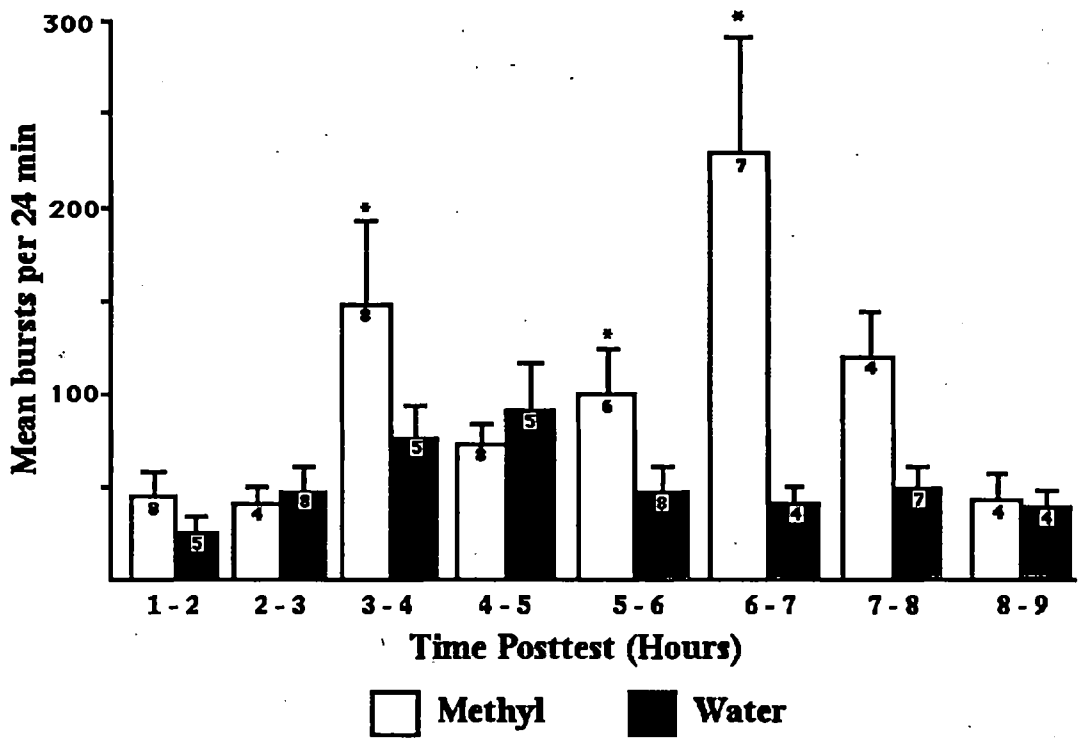


Figure 3.5; The timecourse of bursting for methyl and water-trained chicks over the 1-9hr posttest period. The sample sizes for each group are indicated in bars. Methyl-trained chicks exhibit significantly higher burst-frequencies between 3-4, 5-6 and 6-7hr posttest when compared to water-trained chicks ($p<0.05$). Error bars are standard error of the mean.

Figure 3.6; The Lateralization of IMHV Bursting Measured Over 1-9hr Posttest for Methyl-trained Chicks.

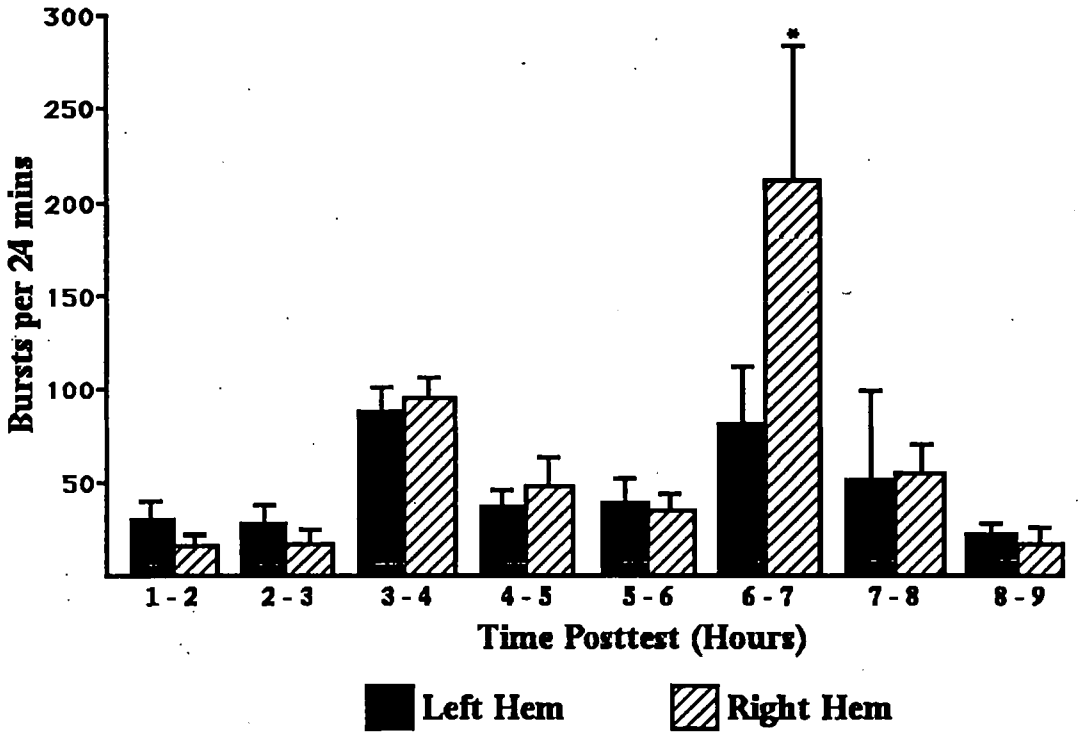


Figure 3.6; The lateralization of bursting in the IMHV for methyl-trained chicks over the 1-9hr posttest period. The sample sizes for each methyl-trained group are as per Figure 3.5. Bursting in the right IMHV is significantly higher than that measured from the left hemisphere of the IMHV between 6-7hr posttest ($p<0.05$). There are no significant differences between hemispheres at any other time. Error bars are standard error of the mean.

Figure 3.7; The Lateralization of IMHV Bursting Measured Over 1-9hr Posttest for Water-trained Chicks.

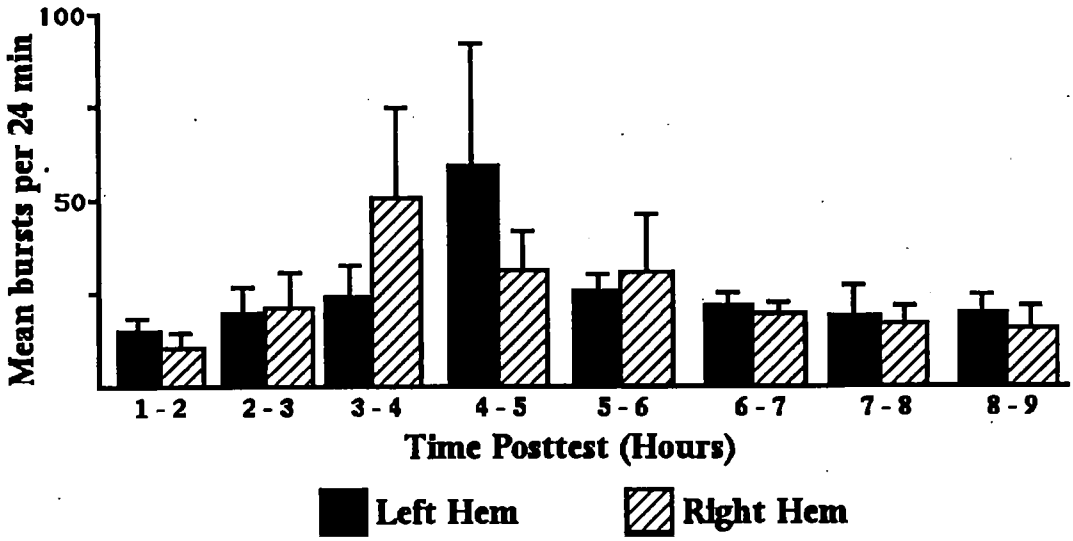


Figure 3.7; The lateralization of bursting in the IMHV for water-trained chicks over the 1-9hr posttest period. The sample sizes for each water-trained group are as per Figure 3.5. There are no significant differences between bursting measures at any time. Error bars are standard error of the mean.

Figure 3.8; The Percentage of Discriminated Spikes to Fall Within Bursts for both Methyl and Water-trained Chicks as a Function of Time Posttest.

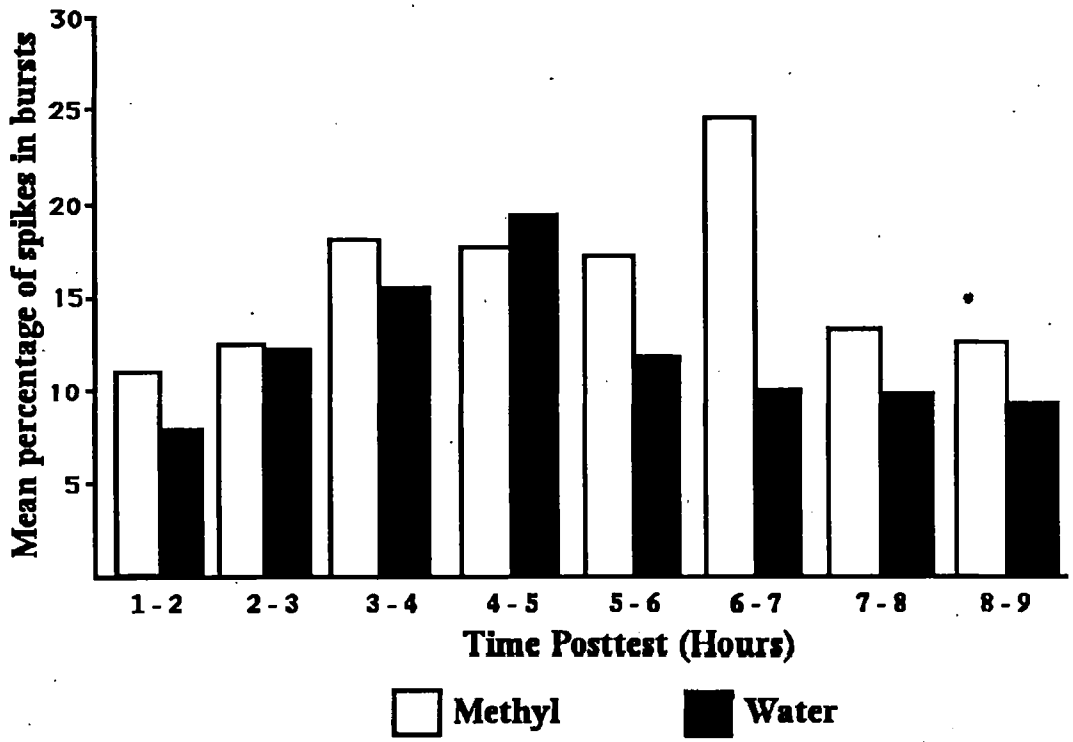


Figure 3.8; The percentage of discriminated spikes included within bursts for both methyl and water-trained chicks over the 1-9hr posttest period. The sample sizes for each group are as per Figure 3.5.

Figure 3.9; The Mean Number of Spikes per Burst for both Methyl and Water-trained Chicks as a Function of Time Posttest.

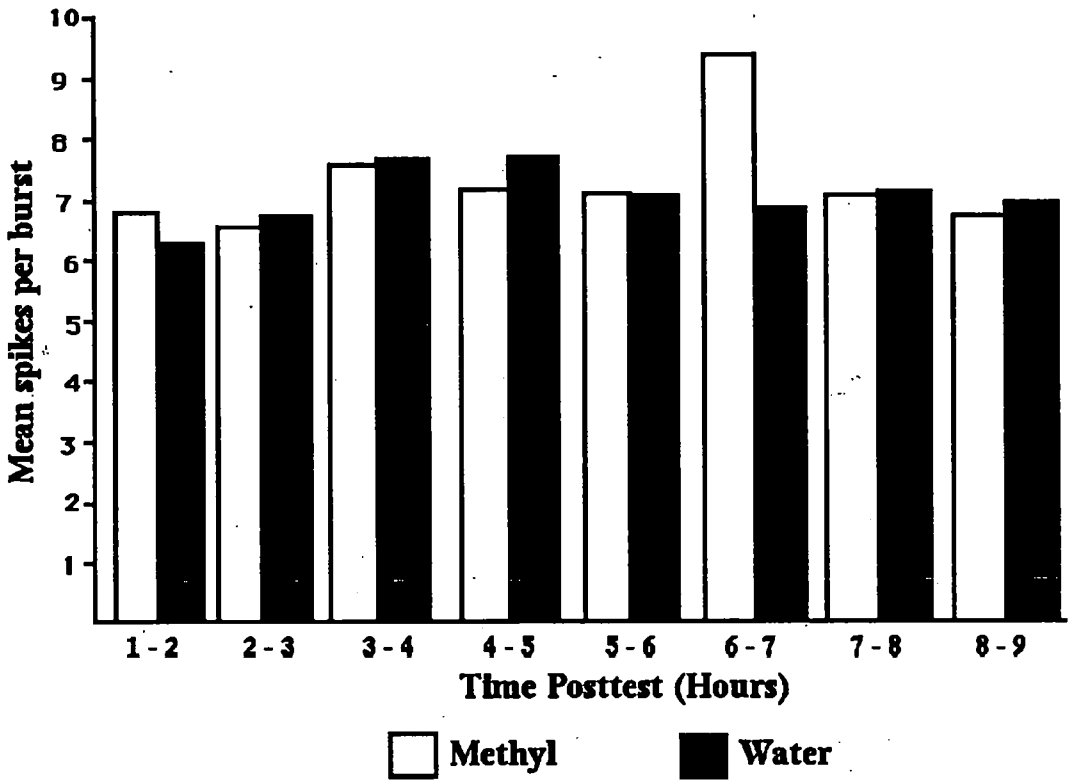


Figure 3.9; The mean number of spikes per burst for both methyl- and water-trained chicks over the 1-9hr posttest period. The sample sizes for each group are as per Figure 3.5. No significant difference was seen between groups at any time. Error bars are standard error of the mean.

Subsequent *post hoc* Student's t-tests revealed that there is a significant increase in bursting in M trained animals compared to W animals at the 3-4, 5-6 and 6-7hr timepoints ($p<0.05$ in all comparisons). The maximum level of bursting in M birds is evident between 6-7 hours posttest.

Figure 3.6 shows the same data described in Figure 3.5, broken down into bursting in each hemisphere for M birds across different timepoints. Figure 3.7 is the counterpart for W trained animals. M birds display a hemispheric asymmetry in burst frequency at the 6-7 timepoint, with the right hemisphere displaying a significantly higher level of bursting compared to the left ($p<0.05$). No asymmetry of burst firing was found in W birds (Figure 3.7).

The data presented in Figure 3.8 represents the mean percentage of discriminated spikes that fall within bursts for both M and W birds, displayed once again as a timecourse. As can be seen M birds display a greater percentage of spikes within bursts at almost all time points, with the greatest difference between groups 6-7 hours posttest.

The data in Figure 3.9 shows the mean number of spikes per burst for M and W birds, again as a timecourse. There is no significant difference between groups at any timepoint. The mean number of spikes/burst for M birds shows a 35% increase over that for W birds between 6-7 hours posttest.

Discussion.

As can be seen, the increased bursting seen in M chicks does not exhibit a generalized increase with time, but rather shows a distinct timecourse with definite peaks. Bursting in M birds is significantly higher between 3-4, 5-6 and 6-7 hours posttest. Bursting is similar between groups during the period 4-5hr posttest. The maximum

level of bursting appears in the last of these periods, 6-7 hours posttest. The percentage of spikes that occur in bursts and the number of spikes per burst are also maximal at this posttest time. This suggests that the firing-pattern of large amplitude neuronal activity becomes predominantly burst-firing during this posttest period.

An asymmetry of bursting can also be seen in M birds during 6-7hr posttest: the right hemisphere of M birds has a significantly higher frequency of bursting when compared to the left. No such asymmetry is found for bursting in W birds. This asymmetry of bursting in M chicks that favours the right hemisphere is surprising in light of the weight of evidence to indicate the importance of the left hemisphere in passive avoidance training. Morphological studies and the 2-DG studies have shown training-induced, memory-related changes that occur predominantly in the left IMHV of day-old chicks (Rose & Csillag, 1985; Stewart et al., 1984; Patel et al., 1988).

The next section describes an investigation into the patterning of burst firing in the IMHV after training.

3.3. The Characteristics of Bursting Patterns.

Introduction

During playback of recorded multi-unit activity, a definite pattern of bursting in the IMHV of M-trained chicks could be discerned: bursts tended to occur in small clusters, especially when the burst frequency was highest. To investigate this patterning the program used for burst analysis was modified to allow the registration of burst start times as single 'events' in a separate event channel. This allowed the processing of burst start times as both inter-burst interval histograms and also burst autocorrelelograms.

Results.

The pattern of bursting can be seen in the inter-burst interval histograms and burst

Figure 3.10; Bursting from the IMHV displayed in the form of inter-burst interval histograms and burst autocorrelelograms.

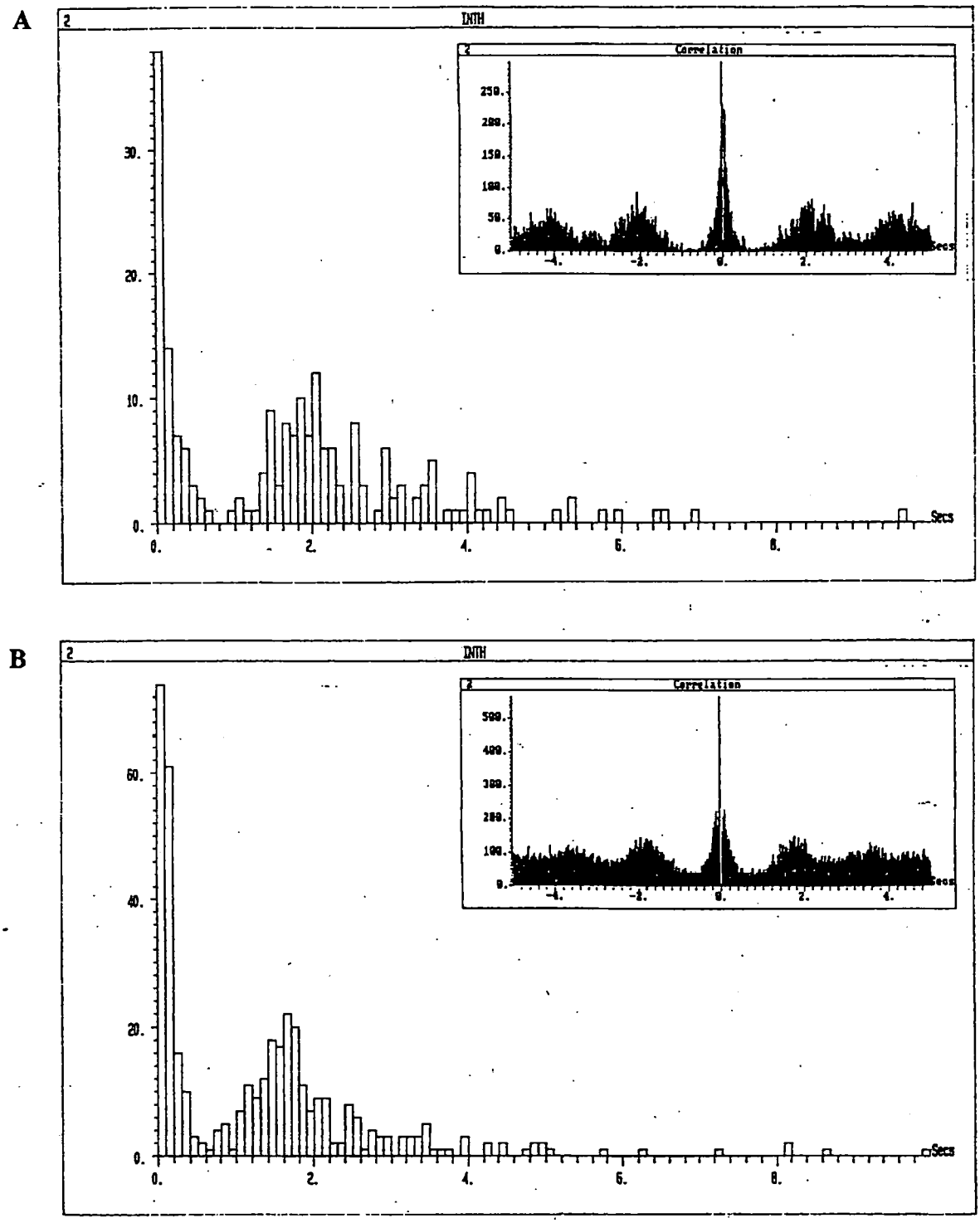


Figure 3.10; A, B. Bursting recorded from the right IMHV of two M chicks recorded between 6-7hr posttest. Each graph represents 6min of data.

Figure 3.10; Bursting from the IMHV displayed in the form of inter-burst interval histograms and burst autocorrelelograms.

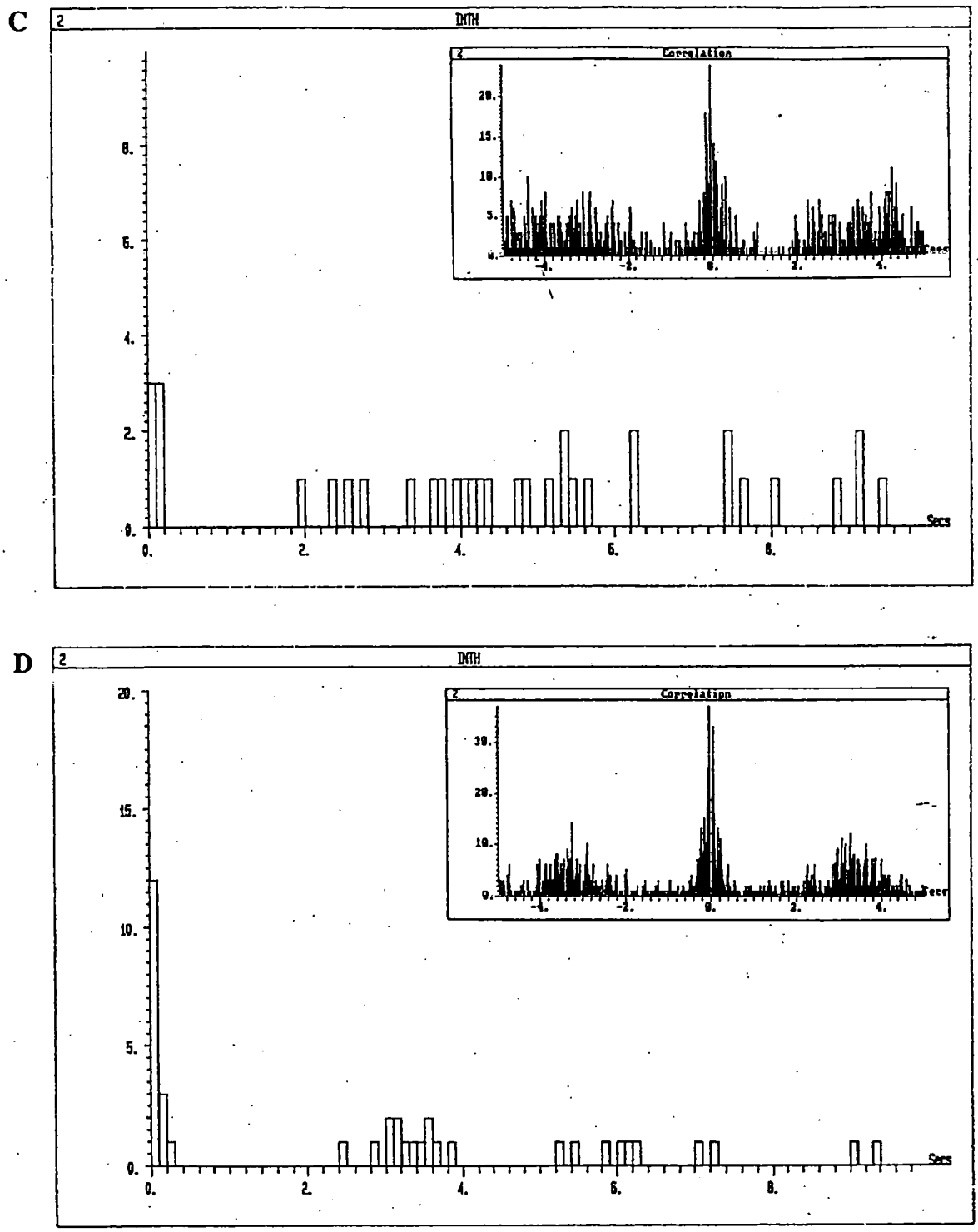


Figure 3.10; C, D. Bursting recorded from the right (C) and left (D) IMHV of water-trained chick recorded 4-5hr posttest. Each graph represents 12min of data.

Figure 3.10; Bursting from the IMHV displayed in the form of inter-burst interval histograms and burst autocorrelelograms.

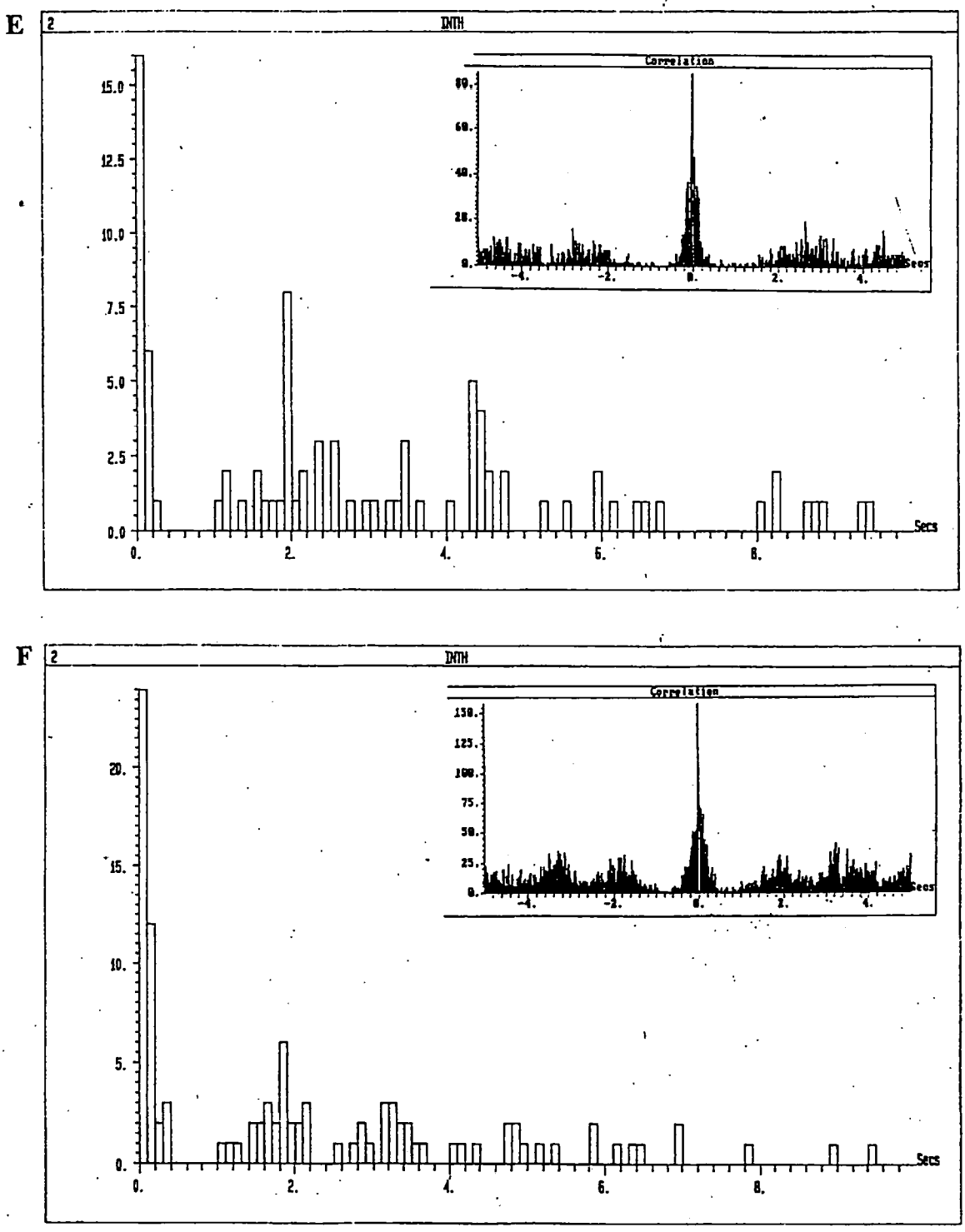


Figure 3.10; E, F. Bursting recorded from right (E) and left (F) IMHV of methyl-trained chick recorded 10-11hr posttest. Each graph represents 12min of data.

autocorrelelograms presented in Figure 3.10. These histograms represent the distribution of burst start times. The autocorellelograms are generated from the burst start times used in the histogram. Each graph represents the results from individual birds, recorded at the times indicated.

To test whether bursts were indeed occurring in groups ('bursts of bursts') burst start times were passed through the burst analysis program. For this analysis the maximum intra-burst (in reality an inter-burst value) was set to 0.4sec. The 'bursts' produced by this analysis are actually composed of the start times of individual, 'real' bursts. This produces a simple type of cluster analysis, results of which are presented in Table 3.11.

Table 3.11. Analysis of bursting recorded from the right hemisphere of four methyl-trained chicks between 6-7hr posttest.

	Average bursts/group	Average interburst time (sec)
1	2.28	0.132
2.	2.32	0.120
3.	2.68	0.080
4.	2.45	0.127
Average	**2.43**	**0.115**

Discussion

As can be seen from both the histograms and autocorrelations of Figure 3.10, the pattern of burst-firing in the IMHV is distinctly non-random. Between 6-7hr posttest, bursts tend to fire in small groups of 2-3 bursts (Table 3.11), with the interval between groups being roughly 2 seconds ('bursts of bursts'). This patterning can be

seen from the interburst interval histograms. The short latency peaks in these histograms represent the 'within group' inter-burst intervals whilst the longer latency interval represents the intervals between groups of bursts. This biphasic pattern is most evident when the burst frequency is maximal, that is, in the right hemisphere of M birds between 6-7 hours posttest.

General conclusions

In section 3.1 the mean burst frequency of the IMHV of M chicks was shown to be significantly higher than that of W chicks. What is the possible significance of this training-induced, memory-specific increase in bursting? In mammals, activation of the septohippocampal pathway has been implicated in processes of memory consolidation (See Chapter 1 and Jaffard et al., 1977; 1979). Electrical stimulation of the cholinergic septohippocampal pathway enhances retention by inducing theta rhythm, which produces an increase in the synchronization and frequency of burst-firing (Galey et al., 1983; Landfield, 1977; Wetzel et al., 1977). EEG activity within the theta frequency range has been recorded from the hyperstriatum of freely behaving chicks (Spooner, 1964). These chronic recordings indicated that chicks displayed brief bouts of 6-12Hz EEG that were particularly associated with orienting responses, in particular with shifts in visual attention. EEG within this frequency band can also be seen in the anaesthetized chick. The spike-trains presented in Figure 3.2 show focal EEG in the theta range recorded from the IMHV of the anaesthetised chick. The means of production of this 6-12Hz rhythm may be similar to that for mammalian, septohippocampally driven theta: the IMHV of the chick receives putative cholinergic input from the medial septal nuclei (Davies & Horn, 1983). Also, the IMHV appears to be rich in cholinergic receptors and enzymes (Coulter, 1982; McCabe et al., 1982). Bursting in IMHV may increase following training as a result of a theta 'priming' effect. In rats, theta activity occurs during exploratory behaviour (Vanderwolf, 1969). At the cessation of such exploratory behaviour, groups of pyramidal cells in CA1,

CA3 and subiculum fire in synchronous bursts (Buzsaki, 1989). This sequence of events may also occur in the day-old chick. As theta activity in the chick appears to be particularly associated with orienting responses (Spooner, 1964), theta rhythm should be evident in the hyperstriatum as the chick visually orients to the bead during passive avoidance training. In most instances, if the chick pecks at a methylanthranilate bead it subsequently displays an associated disgust response. During this effectively non-orienting behaviour theta rhythm may diminish, allowing the emergence of neuronal bursting in groups of cells in the IMHV. Bursting activity has been described as the most favourable neuronal activity for the enhancement of synaptic plasticity (Buzsaki, 1989). If there is an increase in theta activity during training, then this may weakly potentiate certain synapses between groups of cells in the IMHV to form a transient 'structural engram'. The potentiated synapses between these groups of cells would then be subject to long-term modification, brought about by an increase in the occurrence of bursting between the constituent cells. A similar sequence of events has been put forward by Buzsaki (1989) as a possible means of memory formation in the rat. It has been suggested that bursting activity is associated with integrative or mnemonic processes by producing: (1) an amplification of incoming afferent signals when bursts are generated at multiple sites on the soma and dendrites (Wong et al., 1986; Abeles 1982; 1988); and/or (2) an enhanced synthesis of neuronal structural proteins, via the stimulation of calcium/calmodulin-dependent protein kinases, through an associated increase in calcium influx (for review see Krnjevic, 1986). It is possible, therefore, that the observed increase in bursting in the IMHV represents some encoding of memory for the task via (structural?) synaptic changes between interconnected bursting cells. This encoding may exist as some potentiated connectivity between a distinct group of cells. An alternative to this encoding process is that the increased level of bursting represents some 'readout' of previously formed associations to different areas of the brain to perhaps provide more complicated associations or even to facilitate some 'transfer' of memory to other neuronal regions. These important issues will be fully discussed in the General Discussion Chapter.

Day-old chicks require a normally functional cholinergic system for the acquisition of behavioural tasks. Administration of the cholinergic antagonist scopolamine disrupts key-peck avoidance learning and also one-trial passive avoidance training in the day-old chick (Zolman et al., 1978; Patterson et al., 1990b). In the latter of these studies, pretraining scopolamine administration produced amnesia that became evident between 15 and 30min after training. This susceptibility to amnesia falls within the posttraining period ascribed to the formation of intermediate-term memory (Patterson et al., 1990b). This proposed involvement of cholinergic mechanisms in the formation of intermediate-term memory is supported by data showing that QNB binding is increased in day-old chicks 30min after passive avoidance training (Rose et al., 1980). Whether the cholinergic system shows increased activity during any later stages of consolidation remains to be seen. However, the data presented indicate that the increased frequency of bursting in the IMHV may be mediated/modulated by cholinergic input from the septal nuclei. This septal input to the IMHV is also of importance in the development of an *in vitro* LTP-like effect in the IMHV (Bradley, pers. comm.): slices that exhibit LTP most reliably are those that contain an intact septal projection to the IMHV, suggesting that the cholinergic system may also be important in the production of 'LTP' in this structure .

Figure 3.2 shows a recording of burst-firing from the IMHV. IMHV bursts are very similar to bursts recorded by Buzsaki (1986) from the dendritic region of the CA1 area of the hippocampus. Bursting in the two structures is accompanied by large negative deflections in field potential. These deflections in the hippocampus have been termed sharp-waves and possibly represent the synchronous activity of large numbers of bursting pyramidal neurons in the CA1, CA3, subicular and dentate fields of the hippocampus (Buzsaki, 1986). Buzsaki (1988) has proposed that sharp wave-associated bursting is the best candidate for a physiological basis for LTP, again

suggesting that an LTP-like mechanism may operate in the IMHV as part of the process that underlies memory formation for passive avoidance training.

The area(s) of neuronal membrane responsible for the generation of burst-firing in IMHV is impossible to say from extracellular, multi-unit recordings. Bursting may be produced by: (a) areas of active dendritic membrane; (b) individual axons/cell bodies; or (c) the near synchronous firing of axons from different cells. The available evidence seems to indicate the former, that is, that bursting in IMHV is of dendritic origin. As can be seen in Figure 3.4, the heights of the individual spikes that compose each burst are variable: if bursting were of axonal origin then the resulting spikes would all be of similar height as the axon would be acting as a point source. Bursting very similar to that recorded intracellularly from bursting mammalian pyramidal cells has also been recorded from single cells of the IMHV in a chick brain slice preparation (A. Webb, pers. comm.), implicating single cells as the source of bursting in the IMHV.

In section 3.2, bursting in the IMHV of M chicks was shown to exhibit a definite timecourse: bursting was significantly higher during the periods 3-4, 5-6 and 6-7hr posttest. A lateralization of bursting to the right IMHV was also seen during the period 6-7hr posttest. Some evidence to suggest that the right hemisphere is important for learning the passive avoidance task has come from a study that sought to examine the lateralization of glycoprotein fucosylation following training. As outlined in Chapter 2, memory for the task is associated with an increase in the incorporation of fucose into glycoproteins (Rose & Harding, 1984). Chicks can be rendered amnesic if this incorporation is inhibited by bilateral intracranial injection of 2-Deoxy-galactose (Rose & Jork, 1987). In a follow-up study, unilateral injections of 2-D-gal were given 45min pretraining into the area of either the right or the left IMHV (Barber, 1990). This treatment revealed a lateralization of effect for 2-D-gal: chicks were amnesic when tested at 1, 2 or 4hr posttraining after 2-D-gal injection into the right hemisphere,

similar injections into the left hemisphere were only amnestic when chicks were tested 4hr posttraining. This suggested that the increased fucosylation of glycoprotein required for memory of the task occurs predominantly in the right hemisphere. It may be of some significance that the first peak in bursting appears 3-4hr posttest, the approximate time at which amnesia appears after pretraining injection of 2-D-gal into either hemisphere.

The highest level of bursting occurs 6-7hr posttest. In a follow-up study to Barber (1990), chicks were given bilateral intracranial injections of 2-D-gal around this 6hr posttraining timepoint: these chicks exhibited significant amnesia at a 24hr test (Zamani & Rose, 1990). This suggests that there is a 'double wave' of glycoprotein synthesis in day-old chicks following training upon a passive avoidance task. The second wave of this synthesis is coincident with the maximum level of bursting in the IMHV. The requirement of both of these phenomena for memory formation suggests that they may be causally related. However, there is, as yet, no data concerning any lateralization of effect for these 6hr posttraining 2-D-gal injections. It would of course be of some interest if a lateralization to the right hemisphere were found, coincident with the lateralization of bursting to the right IMHV at this timepoint.

Similar time-dependent processes have been seen in other preparations. A second wave of glycoprotein synthesis is also exhibited following the induction of LTP (Mathhies, 1989). This 'late LTP' occurs 6-8hrs posttetanization and requires both the induction of protein synthesis, via the activation of PKC, plus the posttranslational fucosylation of newly synthesized proteins. Induction of these macromolecular processes involves the activation of immediate early genes, suggesting that these processes are involved with the longer-term maintenance of LTP. Activation of immediate early genes is also seen 30min after passive avoidance training in day-old M chicks (Anokhin et al., 1991). One of the 'triggers' for the activation of immediate early genes in mammalian dentate granule cells is an increase in burst-firing (Douglas

et al., 1988).

Neural cell adhesion molecules (NCAMs) have also been shown to be of importance during these extended posttraining periods. NCAMs have been implicated in the synaptic remodelling underlying memory formation (Doyle et al., 1990). Rats given intraventricular administration of an antiserum to NCAM 6-8hrs following acquisition of a passive avoidance response were amnesic for the task. Injections at any other time tested were without effect.

It appears from these various lines of evidence that the 6-8hr posttraining period is crucial for both the maintenance of LTP and the consolidation of memory for a variety of behavioural training tasks, including passive avoidance training. One of the correlated neuronal activities during this period, at least for passive avoidance training in the chick, is an increase in the frequency of burst-firing.

The results from the bursting timecourse data suggest an important role for the IMHV, especially the right IMHV, in the consolidation of memory for passive avoidance training. Although no lateralization of bursting was seen to the IMHV of the left hemisphere, increased levels of bursting may be evident in this structure during periods that were not examined in this experiment. Lesion studies have indicated that the left IMHV is a necessary structure for the acquisition and/or early processing of memory for passive avoidance training (Patterson et al., 1990a). It is possible that these processes occurring around the time of training involve increases in the levels of bursting, perhaps in the left IMHV. That bursting might have a role in acquisition of passive avoidance training is impossible to say from this data. Changes in the firing of cells following an experiential event have traditionally been time-locked to periods either during or very soon after the event itself. Such early electrophysiological changes have been associated with the initial stages of memory formation, the proposal being that this early activity in some way 'triggers' subsequent

morphological and biochemical changes. Although this initial electrical activity is almost certainly essential for the initiation of the above changes, it has not been possible to investigate in this instance because of the very nature of the preparation. The earliest that recordings can be initiated following testing is governed by the time taken for anaesthesia, surgery and location of the area to be recorded from (these issues will be dealt with more fully in the General Discussion, Chapter 7). However, as outlined in Chapter 1, burst-firing has been shown to be the most efficient of initiating stimuli for the induction of LTP (Diamond et al., 1988). Therefore, if an LTP mechanism proves to be the process underlying acquisition of passive avoidance training, then burst-firing neurons may well be involved. Evidence in support of this proposal comes from experiments in which day-old chicks were rendered amnesic by bilateral, intracranial injections of AP5 before training on a passive avoidance task, suggesting that AP5 was interfering with the acquisition of the task (Patterson et al., in preparation). As stated in Chapter 1, AP5 is a potent inhibitor of burst generation, LTP and some forms of behavioural learning. This is accomplished through its antagonism of NMDA receptors, although it does not prevent normal synaptic transmission. This raises the possibility, therefore, that the amnestic action of AP5 around the time of training may be through an antagonism of burst-firing in the (left?) IMHV, possibly in association with an antagonism of LTP-like processes.

In section 3.3 bursting in the IMHV was shown to have a distinct pattern. Bursts tend to occur in small groups, especially when the overall frequency of bursting is maximal between 6-7hr posttest. These groups of bursts appear to be separated by quite long intervals of around 2.5sec. This long latency is, to say the least, somewhat surprising. This rhythmic burst generation may be controlled by either extrinsic or intrinsic oscillators. It is difficult to envisage an extrinsic oscillator with a period of around 2.5sec. However, there is a putative intrinsic oscillator: *in vitro* intracellular recordings from the IMHV have shown that neurons in this structure exhibit an

extremely long afterhyperpolarizing potential, lasting some hundreds of milliseconds (A. Webb, pers. comm.). If these cells are of the same class as those seen to burst in this Experiment, then this intrinsic membrane property may help to explain the long interval between groups of bursts. Another possibility is that this rhythmic generation of bursting is simply correlated with the rhythmic breathing movements of the chick whilst under anaesthesia. However, the breathing cycle (inhalation and exhalation) of the Urethane anaesthetized chick is much faster than 2.5sec (personal observation). Also, although this rhythmicity of burst-firing appears to some extent in every histogram in Figure 3.10, it is hard to imagine why the pattern should be so pronounced between 6-7hr posttest if it a correlate of breathing movements. A salient point to remember here is that these results have come from multi-unit recordings. The number of cells that are contributing to this recording is impossible to gauge, a rough estimate would be a total of about 10. In other words, the neuronal activity seen in the multi-unit trace is a form of network, or population activity. These groups of bursts may, therefore, reflect the near synchronous firing of groups of cells. The possible relevance of this synchronized activity to 'group selection' and other models of memory formation will be discussed more fully in the General Discussion chapter.

The results of this Experiment suggest that bursting in M-trained chicks reaches a maximal value and becomes highly synchronized during the period 6-7hr posttest. From Table 3.11, the average interburst interval of M chicks during this posttest period is approximately 0.12sec, a burst frequency of 8.33Hz, which falls within the theta frequency range. Bursting stimulation at a theta frequency has been shown to be the most efficient means of inducing LTP (Diamond et al., 1988), which raises the possibility that a process such as LTP may be occurring between bursting cells in the right IMHV of M-trained chicks 6-7hr posttest. Such an increase in the frequency and synchrony of bursting also appears to underlie both the generation and maintenance of LTP in the mammalian hippocampus (Buzsaki, 1989).

In conclusion, the results reported in this Chapter indicate that there is a large increase in burst-firing within the IMHV of M chicks following passive avoidance training. This increase is time-dependent and exhibits a lateralization to the right hemisphere. The IMHV is one of two structures shown to exhibit increased neuronal activity following passive avoidance training (Rose and Csillag, 1985). The second structure highlighted in this investigation was the LPO. The Experiment in the following Chapter describes an investigation into burst-firing activity of the LPO following passive avoidance training.

CHAPTER 4. Recording Multi-Unit Activity from the LPO of the Day-Old Chick Following Passive Avoidance Training.

Introduction.

The establishment of a training effect upon spontaneous bursting in the IMHV of the day-old chick (Chapter 3) suggested that a similar enhancement of neural activity might also occur in the LPO after training, as these were the two regions to show enhanced neuronal activity in the 2-DG study of Rose and Csillag (1985). The Experiments in this Chapter describe an investigation to test this hypothesis. In short, spontaneous multi-unit activity from the LPO of day-old chicks was recorded after training using a similar method to that for IMHV recordings. The results for these Experiments are presented in three sections, a similar format to that used in Chapter 3: the first section describes an investigation of burst-firing episodes in the LPO; the second section provides an examination of the timecourse and lateralization of bursting in the LPO; and the third section examines the patterning of bursting episodes in the LPO.

Methods.

The behavioural, anaesthetic, surgical and recording procedures were carried out as described in Chapter 2 for LPO recordings. This method can be summarized as follows: pairs of day-old chicks were housed in pens and then trained to produce two treatment groups. One group was trained using a methylanthranilate-coated bead (M chicks) and the other using a water-coated bead (W chicks). Only birds that pecked the training bead were included in later parts of the experiment. Chicks that trained successfully were tested one hour posttraining with a dry bead. M chicks that pecked the dry bead were rejected, as were W chicks that avoided the dry bead. Each group was then assigned a code. Birds were then taken one at a time for recording. The order

Figure 4.1; Schematic representation of recording sites in LPO.

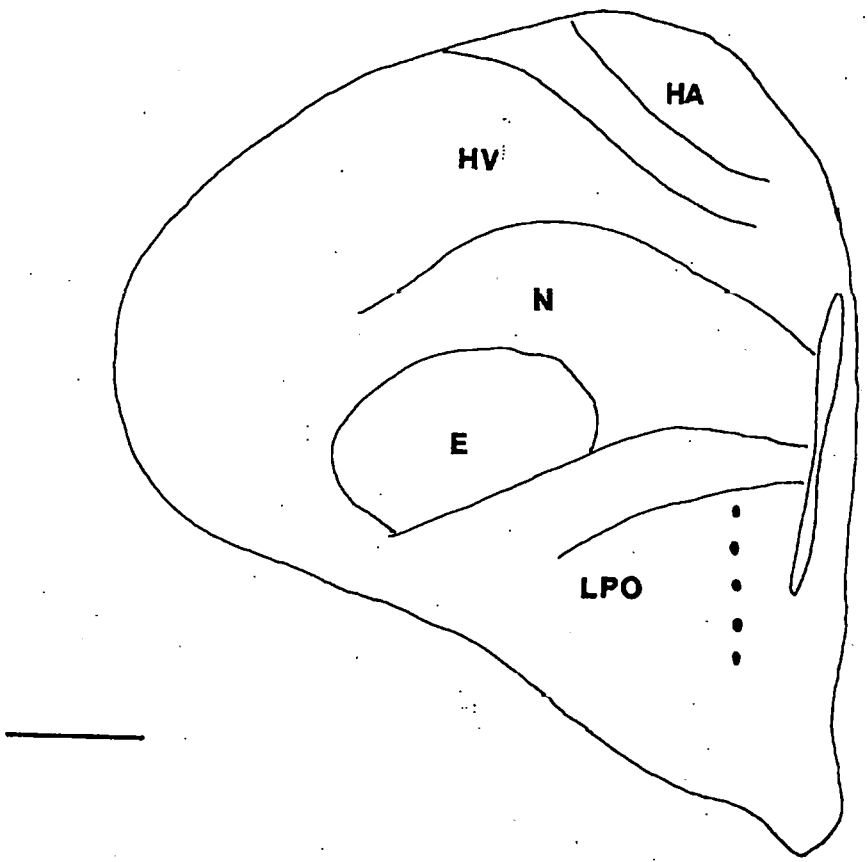


Figure 4.1; Representation of recording sites in LPO. Only the right hemisphere is shown. Filled circles represent recording sites. The section corresponds to the anterior 5.2 reference in the Youngren and Phillips (1988) atlas of the 3 day-old chick. The section indicates that recordings were made from a posterior part of the LPO. Abbreviations: HA, hyperstriatum accessorium; HV, hyperstriatum ventrale; N, neostriatum; E, ectostriatum; LPO, lobus parolfactorius, Scale bar = 1mm.

Figure 4.2(A); Multi-unit Activity Recorded from the LPO.

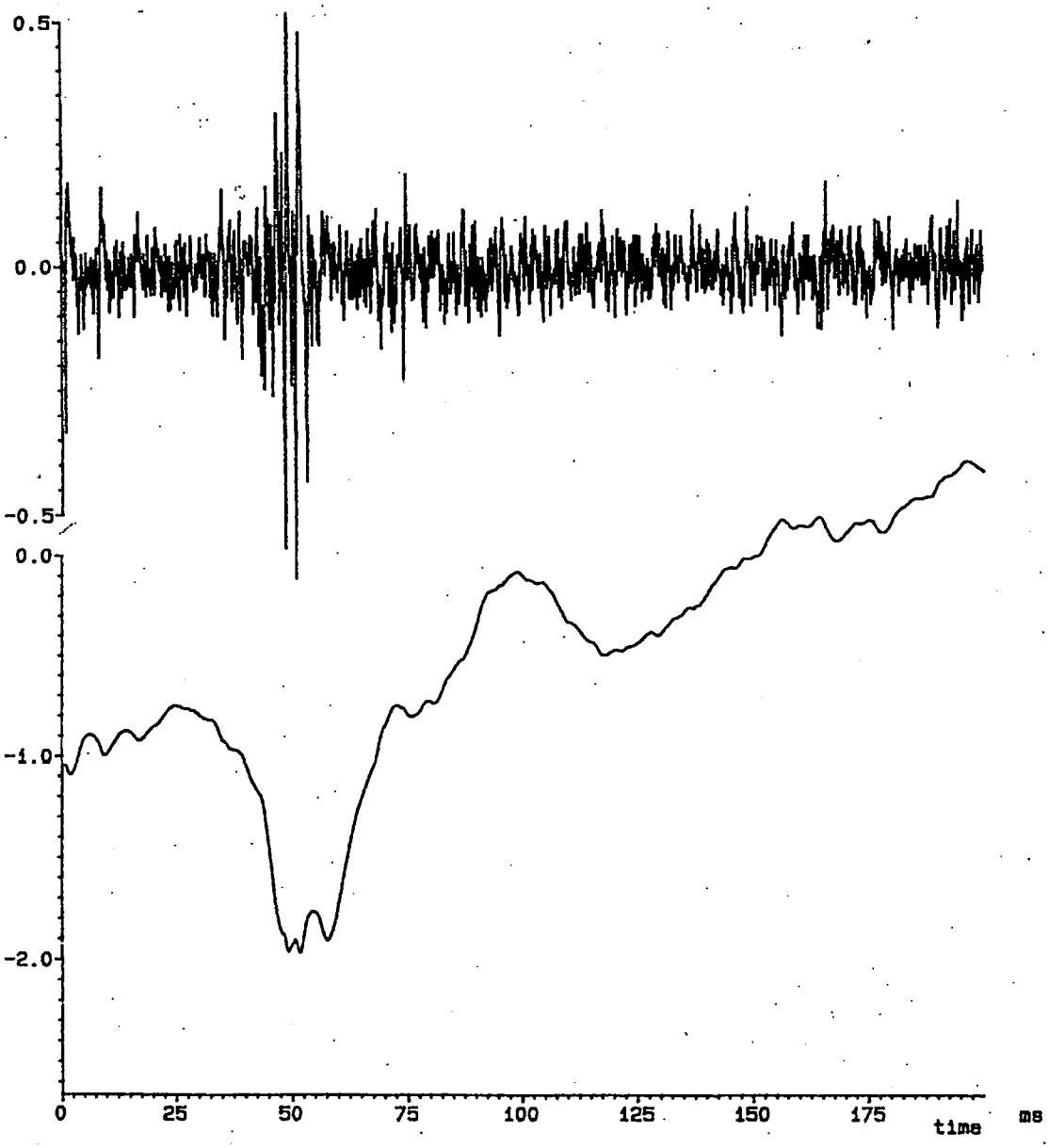


Figure 4.2(A); LPO multi-unit activity. Top trace includes a bursting epoch. Lower trace represents simultaneously recorded local field potential. Note the coincidence of bursting with a negative shift in field potential. Calibrations are 0.5mv (top trace) and 1.0mv (lower trace).

Figure 4.2(B); LPO Multi-unit Activity Using a Slower Oscilloscope Sweep Time to That Used in 4.2(A).

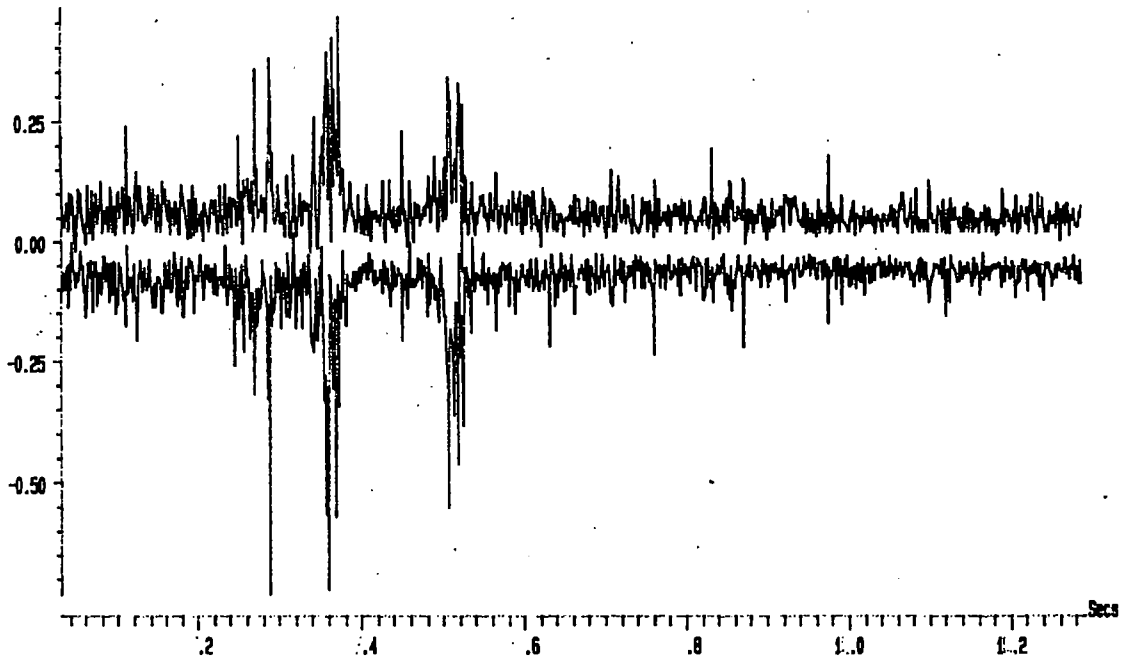


Figure 4.2(B); LPO multi-unit activity using a slow sweep time. Due to the compression of data, individual spikes cannot be discerned. The negative and positive peaks of each spike have been 'fused' together to form the two 'edges' of the spike train. A large amplitude single spike can be discerned, followed by two bursts. Calibrations are mV (vertical) and seconds (horizontal).

Figure 4.3; Mean LPO Burst Frequencies for Methyl and Water Trained Day-old Chicks over 1-10hr Posttest.

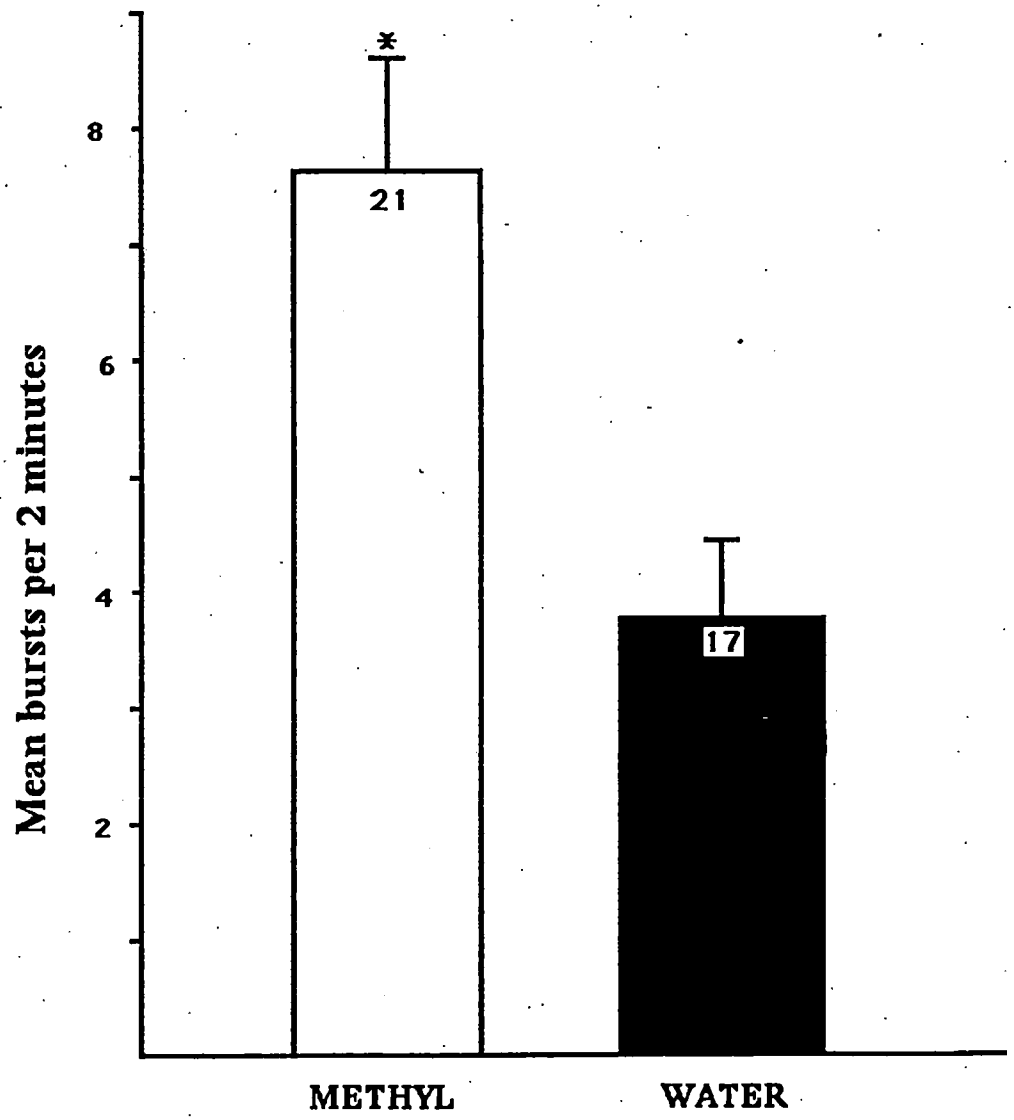


Figure 4.3; Mean bursting per 2min for the LPO of methyl and water-trained, day-old chicks over 1-10hr posttest. The level of bursting represents the mean value for left and right hemispheres. The numbers in bars represent sample size. Chicks trained on a methylanthranilate coated bead exhibit a significantly higher level of bursting when compared to chicks trained on a water coated bead ($p<0.02$). Error bars are standard error of the mean.

Figure 4.4; Hemispheric Differences in Mean LPO Burst Firing for Methyl- and Water-trained Day-old Chicks over the period 1-10hr Posttest.

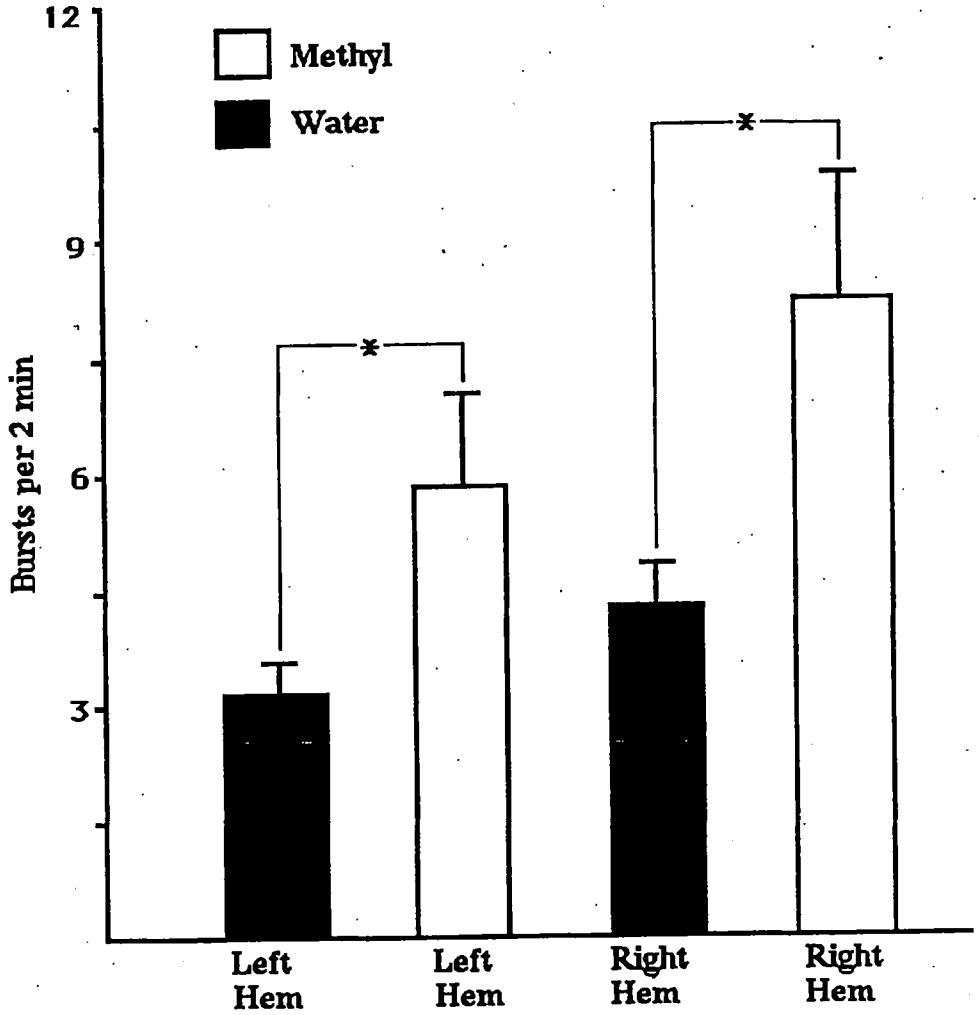


Figure 4.4; Hemispheric differences in bursting per 2min between methyl- and water-trained day-old chicks over 1-10hr posttest. The sample sizes for each group are as per Figure 4.3, that is, 21 methyl-trained chicks and 17 water-trained chicks. Although there is a trend for the left and right hemishpheres of methylanthranilate-trained chicks to exhibit higher mean LPO burst rates when compared to water-trained chicks, these differnces do not reach significance (*p=0.07). There is no significant difference in burst-firing rates between hemispheres within either group.

in which birds were taken was random, as was the order in which hemispheres were recorded from. Figure 4.1 shows a typical histological reconstruction of recording sites.

4.1 Effects of passive avoidance training on bursting recorded in the LPO over 1-10hr posttest.

Results.

A typical burst recorded from the LPO is shown in Figure 4.2. The lower trace represents the associated focal EEG. Mean burst frequencies (i.e., the mean of left and right hemispheres) for both methyl (M) and water (W) trained animals are presented in Figure 4.3. There is a significant difference between groups ($p<0.02$; Anova F-ratio=8.20). The mean bursting frequency for each hemisphere of both groups is presented in Figure 4.4. There is: (1) no significant within-group hemispheric asymmetry in LPO bursting; and (2) a non-significant trend for a between-group hemispheric difference in LPO bursting ($p=0.07$ in both cases)

Discussion

Bursting in the LPO appears quite similar to that from the IMHV (Figure 4.2). The overall burst frequencies recorded from the LPO of M birds are significantly higher when compared to those for W birds in data pooled over the 1-10hr posttest period. The overall levels of LPO bursting for both groups of chicks appears to be quite similar to those seen in the IMHV (see Chapter 3, Figure 3.3 for IMHV bursting levels). There are no hemispheric differences in LPO bursting, although there is a trend for bursting in the left and right LPOs of M birds to be higher than the respective LPOs of W birds.

In Chapter 3, a training-induced, lateralized and time-dependent increase in IMHV bursting was reported. It appears, therefore, that training day-old chicks on a one-trial

passive avoidance task induces an increase in one particular type of neuronal firing, high-frequency bursting, in (at least) two areas of the chick forebrain. To examine any timecourse of bursting in the LPO, the data presented in Figure 4.3 were plotted against time as well as treatment group. This analysis is presented in the next section.

4.2. The Timecourse and Lateralization of Bursting in the LPO following Training.

Introduction

The timecourse of bursting in the LPO was investigated by pooling the data into three equally-sized posttest periods: 1-4hr posttest; 4-7hr posttest; and 7-10hr posttest. The lateralization of LPO bursting was investigated by splitting the data set in a similar fashion.

Results.

The LPO burst frequency for each group is plotted against time in Figure 4.5. This shows the timecourse of bursting over 1-10hr posttest for both W and M-trained day-old chicks. Subjecting these data to an analysis of variance revealed that there was a significant effect of time posttest on levels of LPO bursting (Anova F-ratio=4.01, p=0.007). Subsequent *post hoc* Student's t-tests demonstrated that M-trained animals show a significant increase in bursting over W animals between 4-7hr posttest ($p<0.02$). There is no significant difference between groups during the periods 1-4hr and 7-10hr posttest.

Figure 4.6 shows the same data described in Figure 4.5, in this instance as bursting by hemisphere for M birds with time. Figure 4.7 is the counterpart for W trained animals. No asymmetry is evident during any time period for either M or W birds.

The data presented in Figure 4.8 represents the mean percentage of discriminated spikes that fall within bursts for both M and W birds, displayed once again as a

Figure 4.5; The Timecourse of LPO Bursting Measured Over 1-10hr Posttest for Methyl- and Water-trained Chicks.

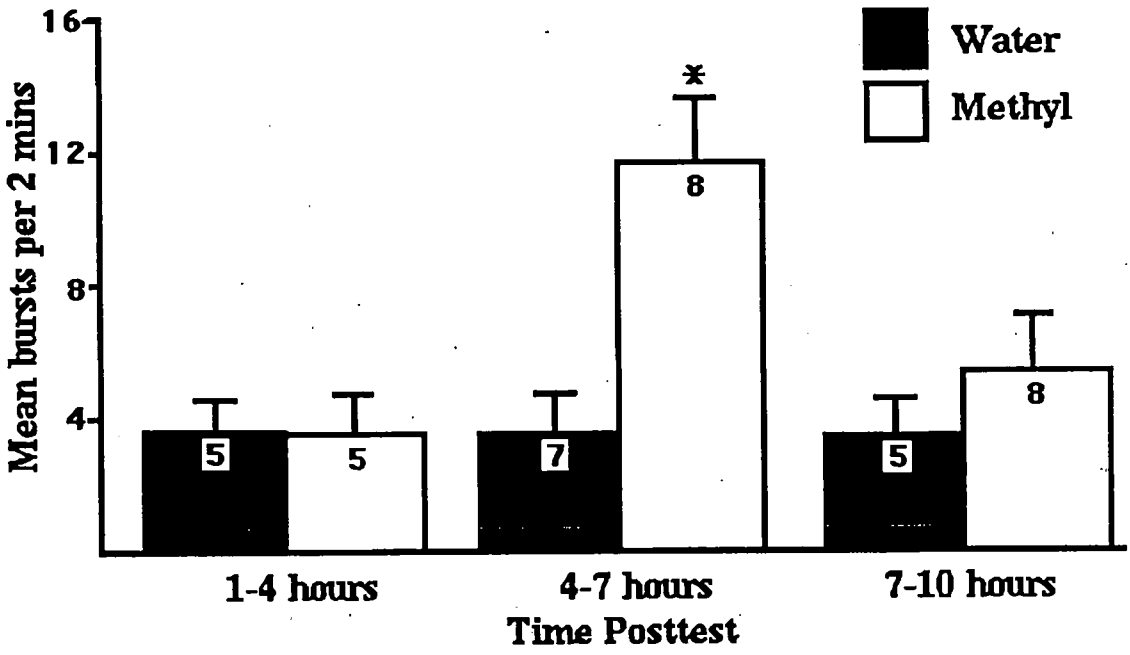


Figure 4.5; The timecourse of bursting for methyl- and water-trained chicks over the 1-10hr posttest period. The sample sizes for each group are indicated in bars. Methy-trained chicks exhibit significantly higher burst-frequencies between 4-7hr posttest when compared to water-trained chicks (*$p<0.02$). Error bars are standard error of the mean.

Figure 4.6; Mean Bursting in Left and Right LPO Measured Over 1-10hr Posttest for Methyl-trained Chicks.

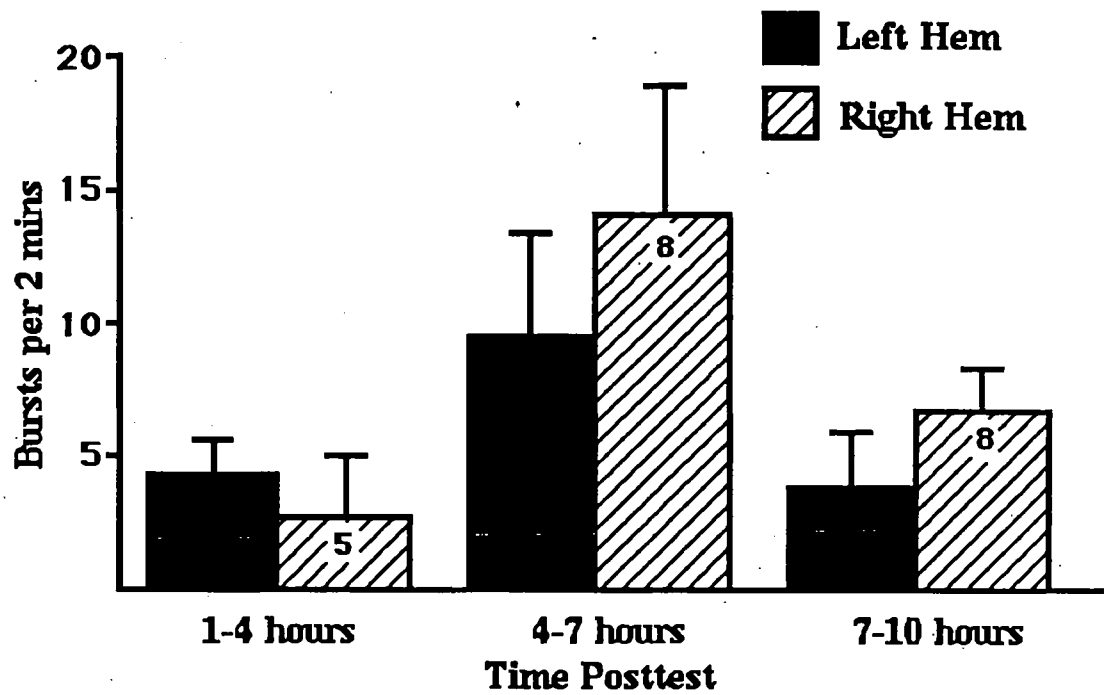


Figure 4.6; Mean bursting in the left and right LPO for methyl-trained chicks over the 1-10hr posttest period. The sample sizes for each Methyl-trained group are as per Figure 4.5. There are no significant differences between hemispheres at any time. Error bars are standard error of the mean.

Figure 4.7; Mean Bursting in Left and Right LPO Measured Over 1-10hr Posttest for Water-trained Chicks.

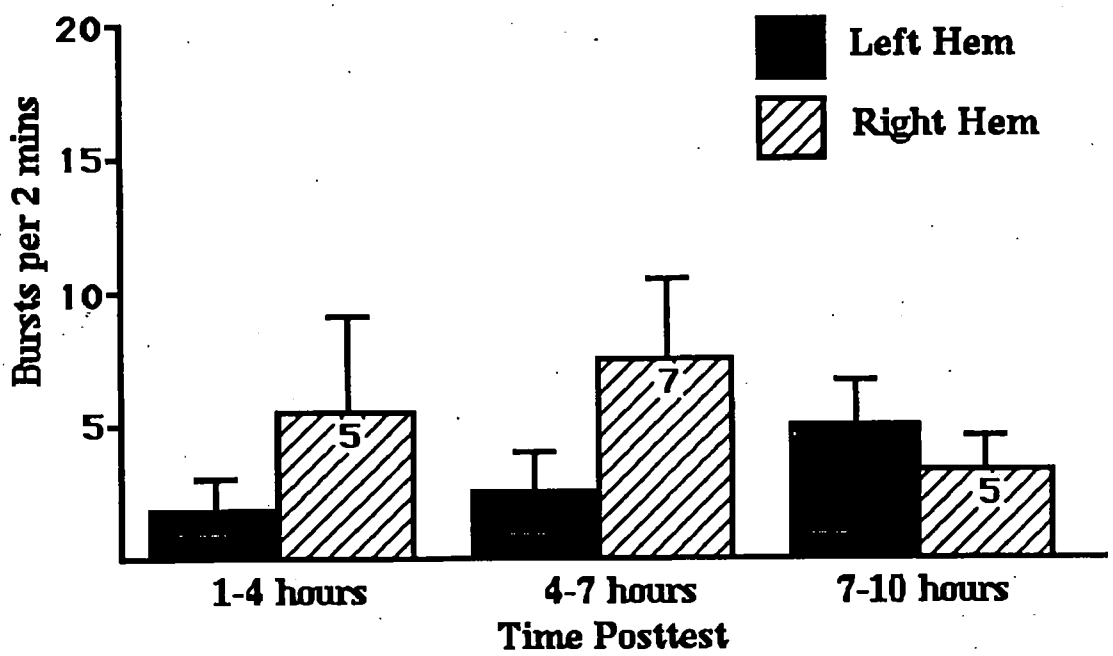


Figure 4.7; Mean bursting in the left and right LPO for water-trained chicks over the 1-10hr posttest period. The sample sizes for each water-trained group are as per Figure 4.5. There are no significant differences between hemispheres at any time. Error bars are standard error of the mean.

Figure 4.8; The Percentage of Discriminated Spikes to Fall Within Bursts for both Methyl- and Water-trained Chicks as a Function of Time Posttest.

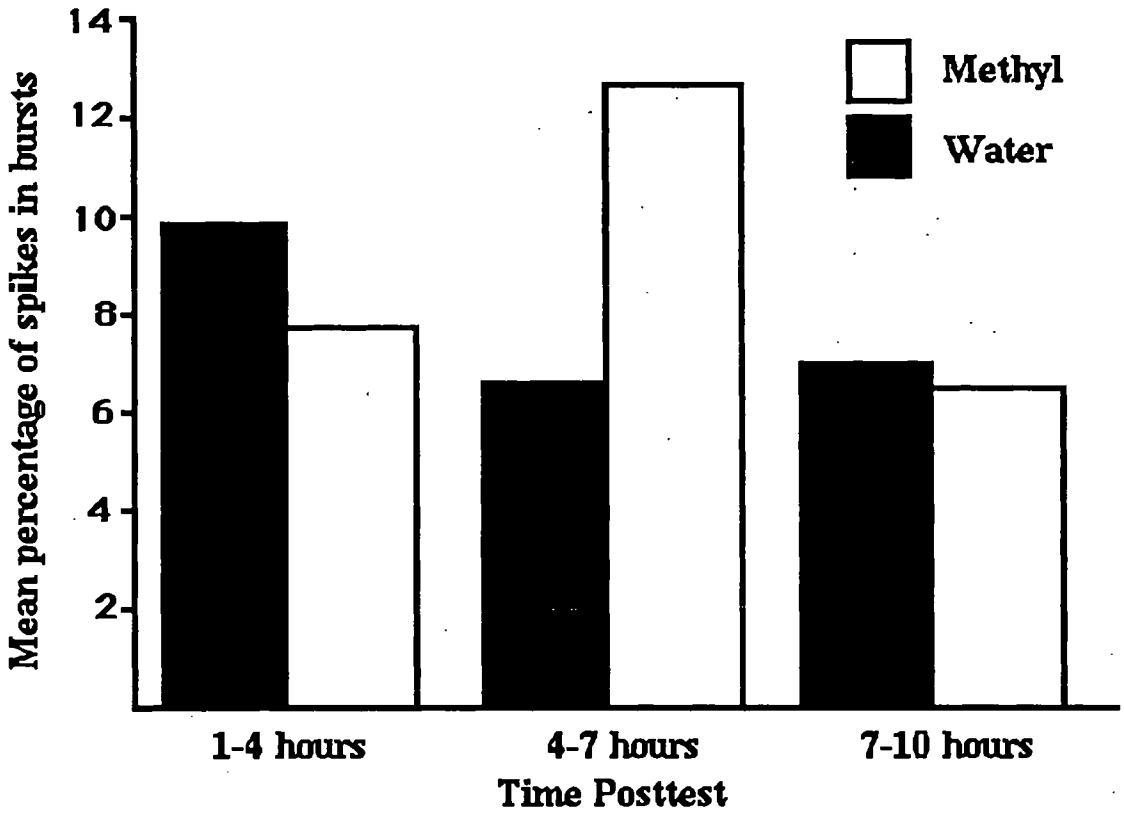


Figure 4.8; The percentage of discriminated spikes to be included within bursts for both methyl- and water-trained chicks over the 1-10hr posttest period. The sample sizes for each group are as per Figure 4.5.

Figure 4.9; The Mean Number of Spikes per Burst for both Methyl- and Water-trained Chicks as a Function of Time Posttest.

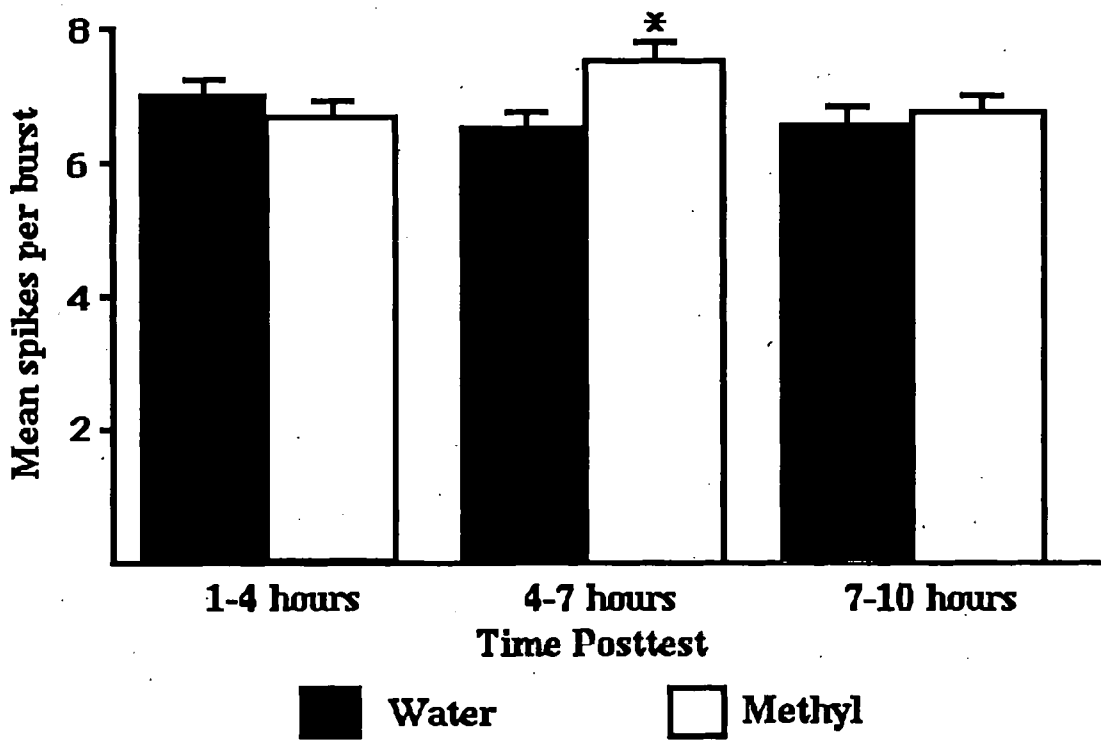


Figure 4.9; The mean number of spikes per burst for both methyl- and water-trained chicks over the 1-10hr posttest period. The sample sizes for each group are as per Figure 4.5. Bursts recorded from methyl-trained chicks 4-7hr posttest display significantly more spikes per burst than bursts recorded from water-trained chicks ($*p<0.05$). Error bars are standard error of the mean.

timecourse. Methyl-trained chicks show a much greater percentage of spikes in bursts during the period 4-7hr posttest.

The data in Figure 4.9 shows the mean number of spikes per burst for M and W birds, again as a timecourse. The number of spikes per burst is significantly higher in M birds during the period 4-7hr posttest ($p<0.05$). No such difference exists between groups either 1-4hr or 7-10hr posttest.

Discussion.

As can be seen, the increased LPO bursting seen in M chicks when compared to W chicks does not exhibit a generalized increase with time, but rather shows a distinct timecourse. The mean level of bursting in M birds is significantly higher between 4-7hr posttest, as is the mean number of spikes per burst. Although there was no significant

difference in the number of spikes per burst in data from the IMHV in Chapter 3, a significant increase in the number of spikes per burst was reported in the IMHV of M birds by Mason and Rose (1987). As Mason and Rose (1987) suggested, an increase in the number of spikes per burst could indicate that bursts were becoming longer in duration, or it might be that bursts were tending to occur with greater synchrony, that is, two bursts occurring almost simultaneously in a multi-unit recording may appear as one long burst. The percentage of spikes to occur in bursts is maximal during the 4-7hr posttest period, which suggests that the firing-pattern of large amplitude neuronal activity becomes predominantly burst-firing during this posttest period, that is, the increase in bursting during this period is not accompanied by an equal increase in the the amount of non-bursting, high amplitude activity.

In contrast to the IMHV data presented in Chapter 3, which indicated that there was an asymmetry of bursting that favoured the right hemisphere 6-7hr posttest, no

hemispheric asymmetry of bursting in the LPO appears in either M or W birds during the posttest period, although there is a trend for bursting in the LPO of the right hemisphere to be elevated in both groups.

From these results it appears that the increase in the rate and size of bursts in the LPO is confined to the period 4-7hr posttest. This time-dependency of LPO bursting is similar to that for bursting in IMHV, suggesting that bursting in/between these two structures may be subject to some form of mutual control. It has been demonstrated on the basis of lesion studies that the LPO is a necessary structure for longer-term storage of memory for passive avoidance training (Gilbert et al., in press), which raises the possibility that this time-dependent increase in LPO and IMHV bursting may form an integral part of the process that underlies this long-term storage. This will be discussed in the General Discussion chapter.

In Chapter 3, bursts recorded from the IMHV were shown to occur in definite patterns, especially when the level of bursting was maximal. The next section describes a similar investigation of the patterning of bursting in the LPO.

4.3. The Characteristics of Bursting Patterns.

Introduction

The method for the generation of burst start times was identical to that used in Chapter 3. During burst analysis, burst start times were saved in an event channel. Analysis of this channel provided inter-burst interval histograms burst autocorrelelograms. Burst analysis was then performed on burst start times to provide data which would indicate the patterning of bursts.

Results.

The pattern of bursting can be seen in the interval histograms presented in Figure

Figure 4.10; Bursting from the LPO displayed in the form of inter-burst interval histograms and burst autocorrelelograms.

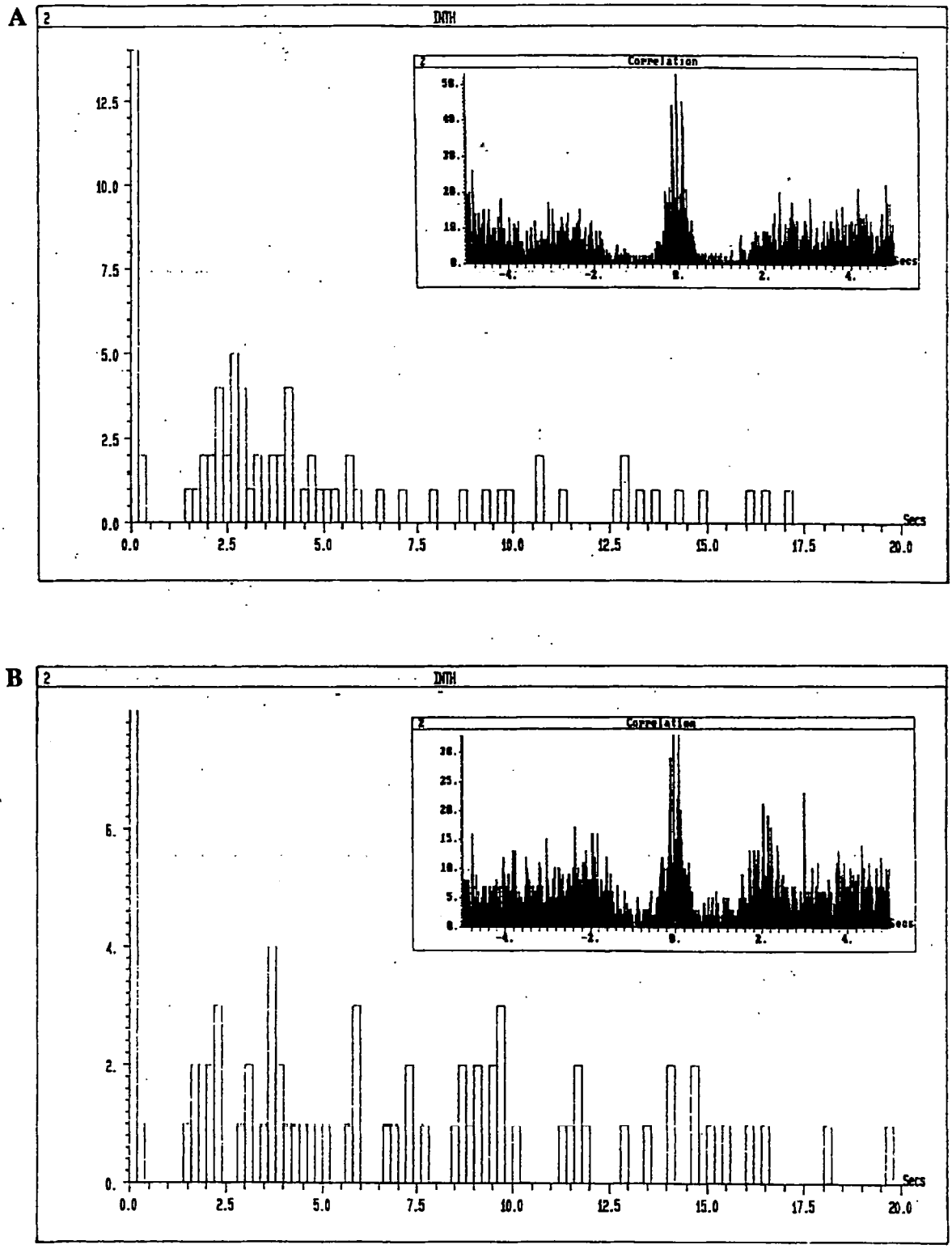
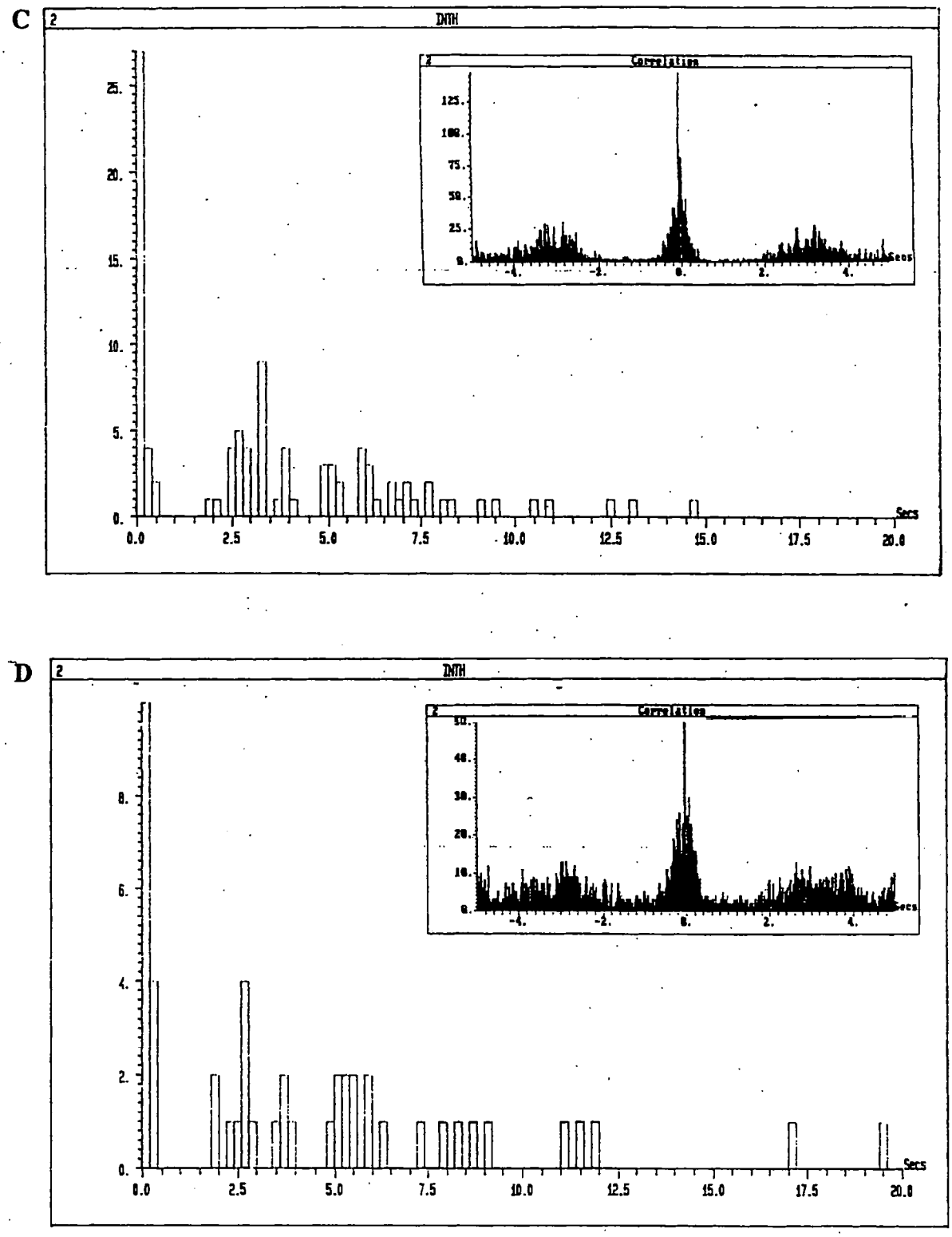


Figure 4.10; Each histogram and autocorrelelogram represents 5min of data.

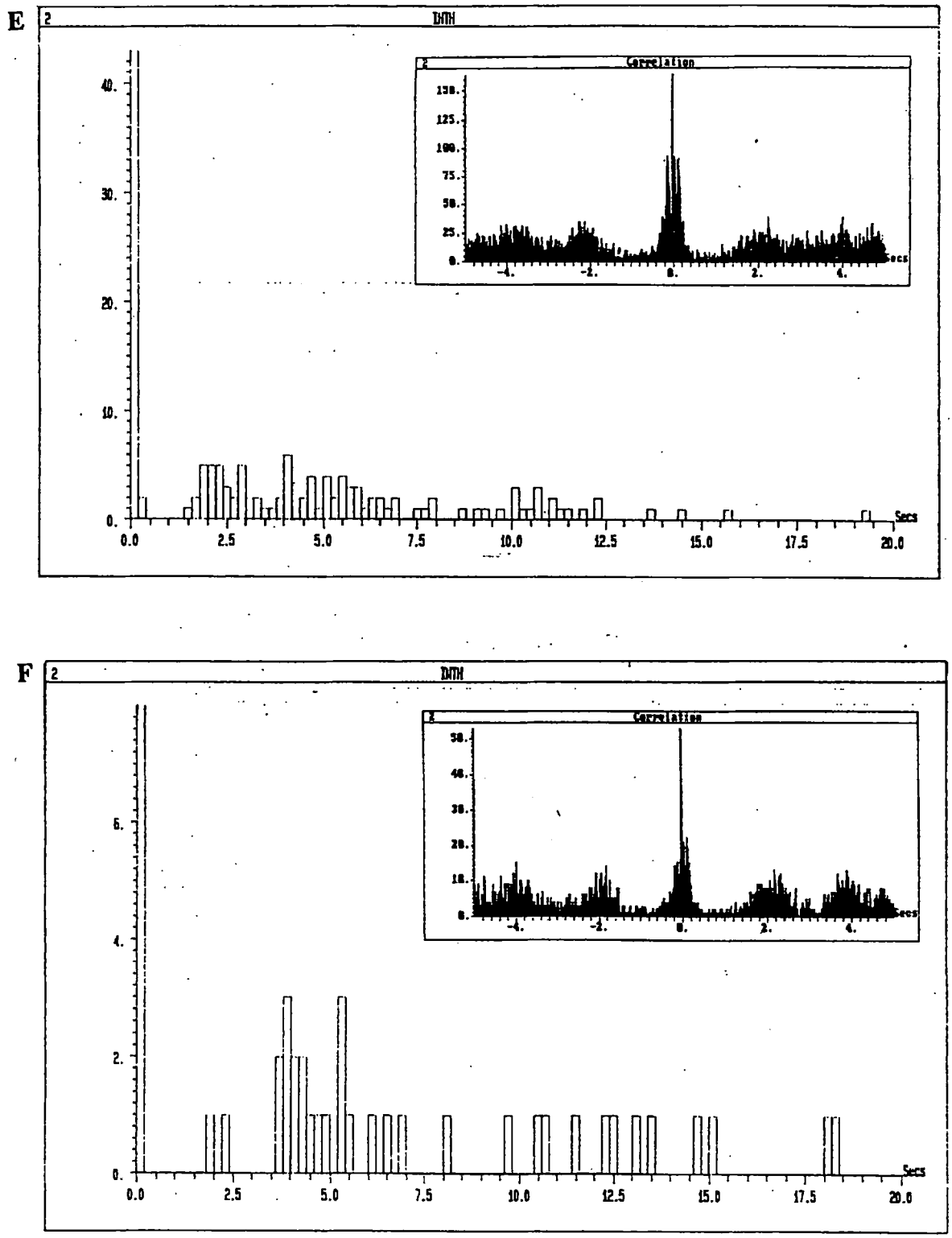
A, B. Bursting recorded form the left (A) and right (B) LPO of a methyl-trained chick between 4-5hr posttest.

Figure 4.10; C and D



C, D. Bursting recorded from the left (C) and right (D) LPO of methyl-trained

Figure 4.10; E and F.



E, F. Bursting recorded from right (E) and left (F) LPO of methyl-trained chick 6-7hr posttest.

4.10. These histograms represent the distribution of burst start times. The inset graphs are autocorellelograms of the burst start times used in the accompanying histogram. Each graph represents the results from an individual chick, recorded at the posttest time indicated. Table 4.11 shows the results of passing the start times of LPO bursts through the simple cluster analysis described in Chapter 3 for Table 3.11.

Table 4.11. Analysis of bursting recorded from the LPO of four methyl-trained chicks between 4-7hr posttest.

	Average bursts/group	Average interburst time (sec)
1.	2.14	0.129
2.	2.16	0.140
3.	2.26	0.126
4.	2.25	0.096
Average	**2.21**	**0.123**

Discussion

As can be seen from both the histograms and autocorrelations of Figure 4.10, the pattern of burst-firing in the LPO is distinctly non-random. As can be seen in Figure 4.10, bursts appear to be occurring in small groups.The histograms of Figure 4.10 exhibit short latency peaks (the 'within group' interburst intervals) and secondary, longer latency intervals (the intervals between groups of bursts). This biphasic pattern is most evident when the burst frequency is maximal, that is, between 4-7hr posttest Table 4.11 indicates that these groups are between 2-3 bursts long. This patterning of LPO bursts between 4-7hr posttest appears similar to the pattern of IMHV bursting during the same posttest period (compare Figure 4.10 A, B and C to Figure 3.10 A and B, especially the autocorellelograms), although the pattern in the IMHV seems more pronounced. It appears, therefore, that day-old chicks trained on a one-trial

passive avoidance task show a significant elevation of burst-firing during the 4-7hr posttest period in two regions of forebrain (the IMHV and LPO) and that this increased bursting follows a similar pattern (groups of bursts) in both of these structures.

General Conclusions

In Section 4.1, a significant overall increase in LPO bursting was shown in M chicks over W chicks. This increase in LPO bursting exhibited no lateralization and could only be seen as an overall increase, although there was a trend for individual hemispheres of M chicks to exhibit higher mean bursting rates when compared to the respective hemispheres of W chicks. This absence of lateralization in either group was also seen when the data were described as a timecourse in Section 4.2, although there was a trend for the right hemisphere to show a higher mean burst rate in both groups. However, when mean overall burst rates were plotted as a timecourse, there was a definite peak in bursting in M chicks between 4-7hr posttest. Bursting in the LPO of M chicks during this posttest period also exhibited: (1) a significantly higher number of spikes per burst; and (2) a greater percentage of discriminated spikes to be included in bursts. Section 4.3 further analyzed this bursting and indicated that bursts in the LPO occurred in a similar fashion to those recorded from the IMHV, that is, in small groups of 2-3 bursts with large intervals between such groups.

When taken together, these results are similar to those reported in Chapter 3 for bursting in the IMHV. It appears that bursting is elevated in both the LPO and the IMHV of M chicks following passive avoidance training, and that these increases follow a similar timecourse. Bursting in both structures is organized into small groups of bursts and this organization is most evident 4-7hr posttest. As discussed in Chapter 3, such an increase in both the occurrence and synchronization of bursting underlies the initiation and perhaps the maintenance of LTP, which suggests that an LTP-like mechanism may underlie the formation of long-term memory for passive avoidance

training (Buzsaki, 1989). The LPO is necessary for long-term memory of passive avoidance training (Gilbert et al., in press). The lack of lateralization in bursting found in the present experiments is consistent with the lack of lateralization of storage for memory in the LPO (either LPO is sufficient for recall).

The increased activity in the LPO appears temporally correlated with a similar increase in the IMHV. This suggests two possibilities: either the activity of one structure is influencing the activity of the other, or there may be a third structure which, through its projections to both IMHV and LPO, is controlling their activity. If we consider the first possibility, although it is true to say that all structures of the brain are connected together, via routes some of which are more tortuous than others, no direct pathway between the IMHV and the LPO has been demonstrated in the chicken. Indeed, this is an area of current research. This leaves us with the second possibility. Certain pathways between the IMHV and LPO that involve intermediary brain structures have been identified (see Figure 7.1). By looking at the retrograde transport of horseradish peroxidase, Boxer and Csillag (1986, chick) were able to show that the LPO receives afferent projections from four brain regions: the archistriatum; the nucleus superficialis parvocellularis; the area ventralis tegmentalis of Tsai; and the nucleus tegmenti pedunculo-pontinus. Projections from the archistriatum to the LPO have recently been confirmed in the chick with the use of lectin tracing (Davies, pers. comm.). Bradley et al. (1985) have shown that the archistriatum is reciprocally connected to the IMHV. The archistriatum of the chick, therefore, sends afferent projections to both the IMHV and the LPO. Dubbeldam and Visser (1987) have shown that, in the mallard, the HV projects to distinct regions of the neostriatum: the dorsal, ventral, and lateral portions of the neostriatum frontale. The dorsal portion of the neostriatum frontale projects to both the archistriatum and the LPO. In the study by Bradley et al. (1985) they demonstrated a projection from the neostriatum to the IMHV. From this it appears that two regions of the avian brain (the neostriatum and the archistriatum) send afferent

projections to both the IMHV and the LPO. These issues will be discussed more fully in the General Discussion.

The Experiment detailed in Chapter 5 was designed to test whether the increased levels of bursting seen in the LPO after training are direct and specific correspondent of memory formation for the passive avoidance task.

CHAPTER 5. The Effects of Electroshock on Bursting Activity Recorded from the LPO of the Day-Old Chick Following Passive Avoidance Training.

Introduction.

Although the amnesic actions of electroconvulsive shock (ECS) were first noted in humans (Zubin & Barrera, 1941), most of the experimental work on the effects of ECS on memory has been carried out with rodents as experimental subjects. The preference for rodents followed the introduction of a simple one-trial learning procedure by McGaugh (1966). The work on ECS and memory in animals has produced controversial interpretations regarding the effects of ECS (for review see Dawson & McGaugh, 1969; Gibbs and Mark, 1973). However, it is now well established that the learning of a variety of behavioural tasks is followed by a period of decreasing susceptibility to the disruptive effects of ECS, as measured by subsequent performance. Such a retention deficit, often referred to as retrograde amnesia, can also be induced by spreading cortical depression, hypoxia, hypothermia, localized brain stimulation and many other treatments (see Gibbs & Mark, 1973; Jarvik, 1972).

ECS has been shown to produce amnesia in day-old chicks. Chicks given ECS during a 45sec period after passive avoidance training are amnesic at test (Lee-Teng and Sherman, 1966). Rose and Harding (1984) studied the effects of subconvulsive ECS on the training-induced fucosylation of glycoproteins from the chick forebrain. In this experiment chicks were given ECS either immediately posttraining or 10min posttraining. Chicks that received delayed electroshock showed recall at test and a training-induced increase in fucosylation of forebrain glycoproteins; chicks that had received immediate posttraining ECS were amnesic and showed no such increase in fucosylation. This experiment demonstrated that increased fucosylation of glycoprotein was directly associated with memory, rather than with some general aspect of the task such as the taste of methylanthranilate.

A similar technique was used by Patel et al. (1988) to show that training-induced increases in dendritic spine density in the IMHV were related to memory formation. In this experiment, methyl-trained chicks were given subconvulsive ECS 5min after training. This treatment produced two groups of chicks: one group that showed recall (avoided the test bead) and another that was amnesic (pecked the test bead). Changes in spine density measured 24hr posttraining were restricted to the group that displayed recall, demonstrating that spine density changes were specifically related to memory formation.

Mason and Rose (1988) also exploited the use of subconvulsive ECS to demonstrate that increased bursting in the IMHV is also directly associated with memory formation. Only chicks that showed recall exhibited an increased IMHV burst frequency (see Chapter 2 for details of this experiment).

In Chapter 4 a training-induced increase in bursting in the LPO of the day-old chick was reported. The following Experiment describes an investigation using subconvulsive ECS to test whether training-induced increases in LPO bursting are directly associated with memory formation for the task. The rationale behind the use of ECS in this Experiment is as follows: to test whether bursting is related to memory formation, it is necessary to separate the components of the task that are specific to memory formation from those that are non-specific, e.g., the taste of methylanthranilate and the motor activity during pecking etc. One way to do this is to produce two groups of chicks that have been subjected to identical training procedures but one group remembers the task (avoids the bead) and one group that is amnesic (pecks the bead). These two groups can be recorded from and any differences in levels of bursting between the groups should reflect retention rather than, for instance, the sequelae of the taste of methylanthranilate.

Normal training and testing produces two such groups, as not all chicks remember the task. However, chicks tend to perform with a high degree of success (one of the attractions of the task): the majority of chicks will remember the task, only the minority will forget. Such unequal group sizes are disadvantageous in a 'blind' experiment that compares chicks that remember against chicks that forget: the two groups should be of roughly equal size to ensure that no experimenter bias is introduced during data capture and analysis. Therefore, to provide equally matched groups, methylanthranilate-trained chicks were subjected to ECS 5min posttraining. It is important to note that all these chicks experienced the same task, including the taste of methylanthranilate, etc. Thus, the delayed ECS procedure produced two groups of identically trained and experienced chicks, approximately equal in size, that differed in only one respect: the presence or absence of memory for the task, as measured by subsequent test.

The results of this Experiment will be presented two sections, a format similar to the preceding Chapters of this thesis.

Methods.

The behavioural, anaesthetic, surgical and recording procedures were carried out as described in Chapter 2 for LPO recordings, with the exception that chicks were subjected to subconvulsive ECS 5min posttraining. This method can be summarized as follows: pairs of day-old chicks were housed in pens and then trained using a methylanthranilate-coated bead. Only birds that pecked the training bead were included in later parts of the experiment. Chicks that trained successfully were given a brief, transcranial, subconvulsive electroshock 5min posttraining. The details of the ECS magnitude were: 12mA, 110V, 220ms duration at 50Hz. This shock was applied through hand-held, transdermal electrodes. Chicks were then tested one hour

posttraining with a dry bead. This procedure generated two groups of chicks at test: a group that pecked the bead (were amnesic) and a group that avoided the bead (displayed recall). Each bird was then assigned a code. Birds were then taken one at a time for recording over the period 1-10hr posttest. The order in which birds were taken was random, as was the order in which hemispheres were recorded from. Codes were broken at the end of all experimental procedures.

5.1. The Effect of Electroshock on Bursting Recorded from the LPO over the Period 1-10hr Posttest.

Results.

Mean LPO burst frequencies (i.e., the mean of left and right hemispheres) for both methyl-trained chicks that remembered (Avoid) and forgot (Peck) the task are presented in Figure 5.1. There is a significant difference between these groups ($p=0.05$); chicks that remember the task show significantly more bursting than amnesic chicks. The mean bursting frequency for each hemisphere of both groups is presented in Figure 5.2. There is neither no significant within-group hemispheric asymmetry in LPO bursting. Also, there is no significant between-group hemispheric asymmetry in LPO bursting (one-tailed t-test: left hem. avoid vs left hem. peck $p=0.21$; right hem. avoid vs right hem. peck $p=0.18$).

Discussion

The overall burst frequencies recorded from the LPO of chicks that remember the task are significantly higher than those for chicks that forgot the task in data pooled over the 1-10hr posttest period. There are no hemispheric differences in LPO bursting between Avoid and Peck groups. These results suggest that the training-induced increases in bursting in the LPO are direct correspondents of memory formation/consolidation for the passive avoidance task. This increase in bursting was shown in Chapter 4 to have a distinct time-dependent component: bursting in the LPO

Figure 5.1; Mean LPO Burst Frequencies for Avoid and Peck Groups of Day-old Chicks over 1-10hr Posttest.

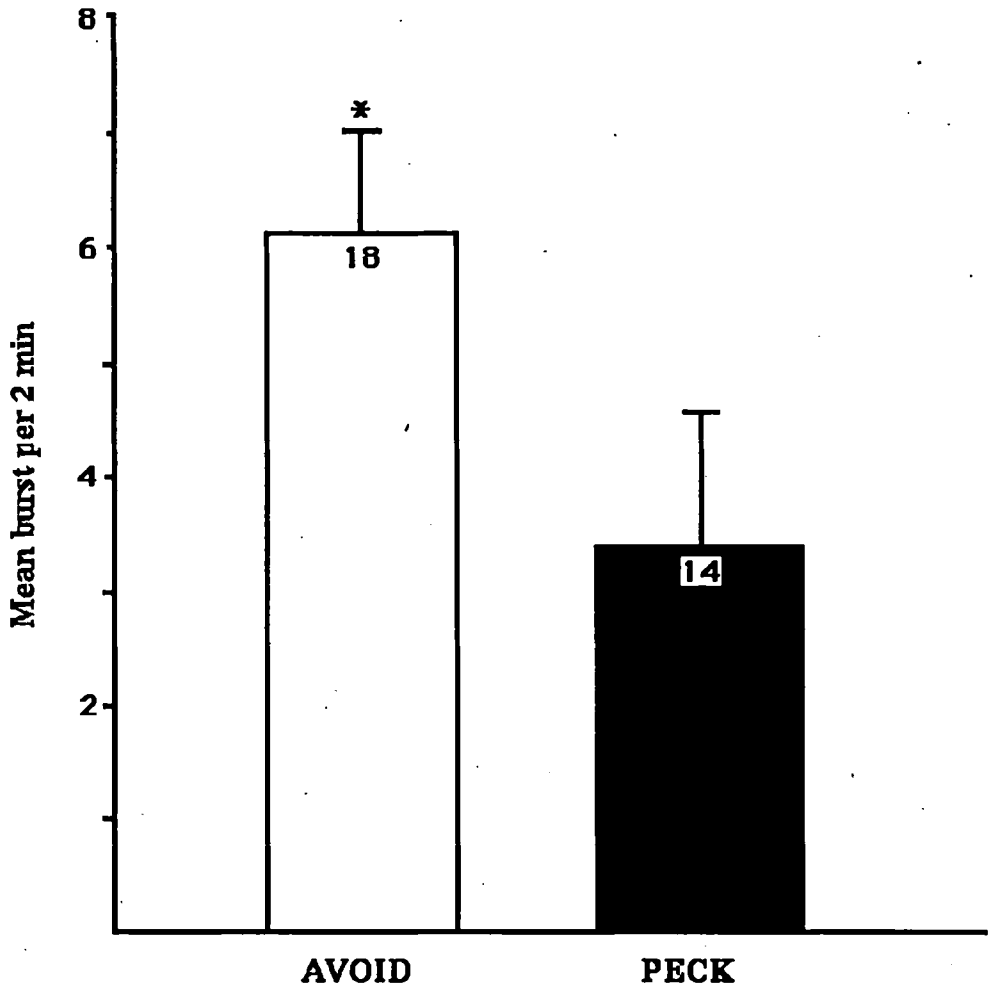


Figure 5.1; Mean LPO bursting recorded over the period 1-10hr posttest for groups of day-old chicks that at test either avoid (remember) or peck (amnesic). The level of bursting represents the mean value for left and right hemispheres (bursts per 2min). The numbers in bars represent sample size. Chicks that avoid the bead at test exhibit a significantly higher level of bursting when compared to chicks that peck at test (*p=0.05). Error bars are standard error of the mean.

Figure 5.2; Mean Bursting for Left and Right LPO of Avoid and Peck Groups of Day-old Chicks over the period 1-10hr Posttest.

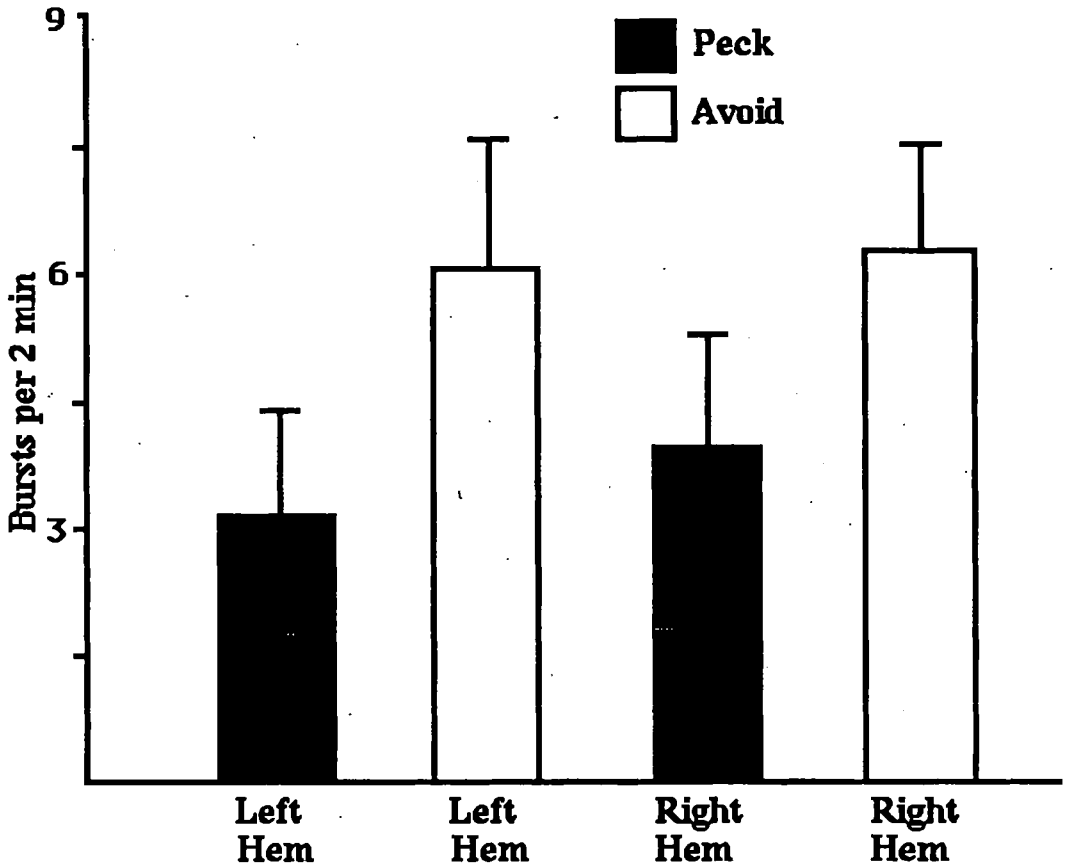


Figure 5.2; Hemispheric differences in bursting per 2min between Avoid and Peck day-old chicks over 1-10hr posttest. The sample sizes for each group are as per Figure 5.1, that is, 18 chicks that avoided and 14 chicks that pecked at test. There are no significant differences between left and right hemispheres of chicks that avoid compared to chicks that peck. Also, there are no significant differences between hemispheres in burst-firing rates within either avoid or peck groups.

of M chicks was significantly higher during the period 4-7hr posttest. To examine the timecourse of bursting following electroshock, the data presented in Figure 5.1 were plotted against time. This analysis is presented in the next section.

5.2. The Timecourse and Lateralization of Bursting in the LPO following Electroshock Recorded 1-10hr Posttest.

Introduction

The timecourse of bursting in the LPO following subconvulsive ECS was investigated by pooling the data from Avoid and Peck groups into three posttest periods: 1-4hr posttest; 4-7hr posttest; and 7-10hr posttest. These periods were chosen on the basis of the increased bursting seen in the LPO of non-electroshocked chicks 4-7hr posttest, as reported in the Chapter 4. The lateralization of LPO bursting in Avoid and Peck groups was investigated by splitting the data set in a similar fashion to provide three posttest periods.

Results.

The LPO burst frequency after ECS for Avoid and Peck groups is plotted against time in Figure 5.3. As can be seen, chicks that Avoid show a significant increase in bursting over chicks that Peck between 4-7hr posttest ($p<0.01$). There is no significant difference between groups during the periods 1-4hr or 7-10hr posttest.

Figure 5.4 shows the same data described in Figure 5.3, in this instance as bursting by hemisphere for Avoid birds with time. Figure 5.5 is the counterpart for Peck chicks. No asymmetry is evident during any time period for either Avoid or Peck chicks.

The data presented in Figure 5.6 represents the mean percentage of discriminated spikes that fall within bursts for both Avoid and Peck chicks, displayed once again as

a timecourse. Avoid chicks show a much greater percentage of spikes in bursts during the period 4-7hr posttest.

The data in Figure 5.7 shows the mean number of spikes per burst for Avoid and Peck birds, again as a timecourse. No significant difference exists between groups during any posttest period.

Discussion.

As can be seen, the increased LPO bursting evident in chicks that avoid the test bead follows a distinct timecourse. The mean level of bursting in these chicks that display recall is significantly higher between 4-7hr posttest. The percentage of spikes to occur in bursts is maximal during the 4-7hr posttest period in chicks that remember the task, suggesting that the firing-pattern of large amplitude neuronal activity becomes predominantly burst-firing during this posttest period, that is, the increase in bursting during this period is not accompanied by an equal increase in the the amount of non-bursting, high amplitude activity.The lack of asymmetry in LPO bursting in both groups is in agreement with the lack of asymmetry seen in Chapter 4.

General Conclusions

Subconvulsive ECS was used to investigate whether the increased bursting seen in the LPO after passive avoidance training was a direct correspondent of memory for the task, or was alternatively a consequence of some non-specific aspect of the training, such as the taste of methylanthranilate. The results in Section 5.1 demonstrate that the increase in the rate of bursting in the LPO after training is directly associated with memory formation for the task. Section 5.2 demonstrates that this increase in bursting is predominantly confined to the period 4-7hr posttest. This time-dependency of LPO bursting in chicks that remember the task is similar to that for training-induced bursting from both the IMHV (Chapter 3) and the LPO of non-electroshocked chicks

Figure 5.3; The Timecourse of LPO Bursting Measured Over 1-10hr Posttest for Avoid and Peck Chicks.

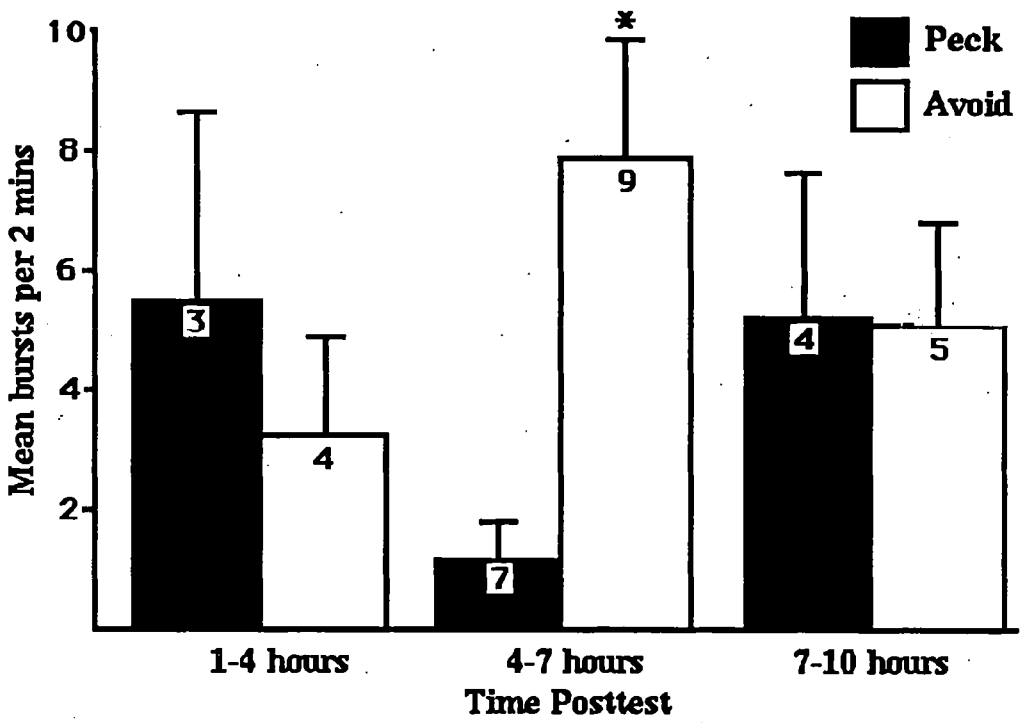


Figure 5.3; The timecourse of bursting for Avoid and Peck chicks over the 1-10hr posttest period. The sample sizes for each group are indicated in bars. Avoid chicks exhibit significantly higher burst-frequencies between 4-7hr posttest when compared to Peck chicks (*$p<0.01$). Error bars are standard error of the mean.

Figure 5.4; The Lateralization of LPO Bursting Measured Over 1-10hr Posttest for Avoid Chicks.

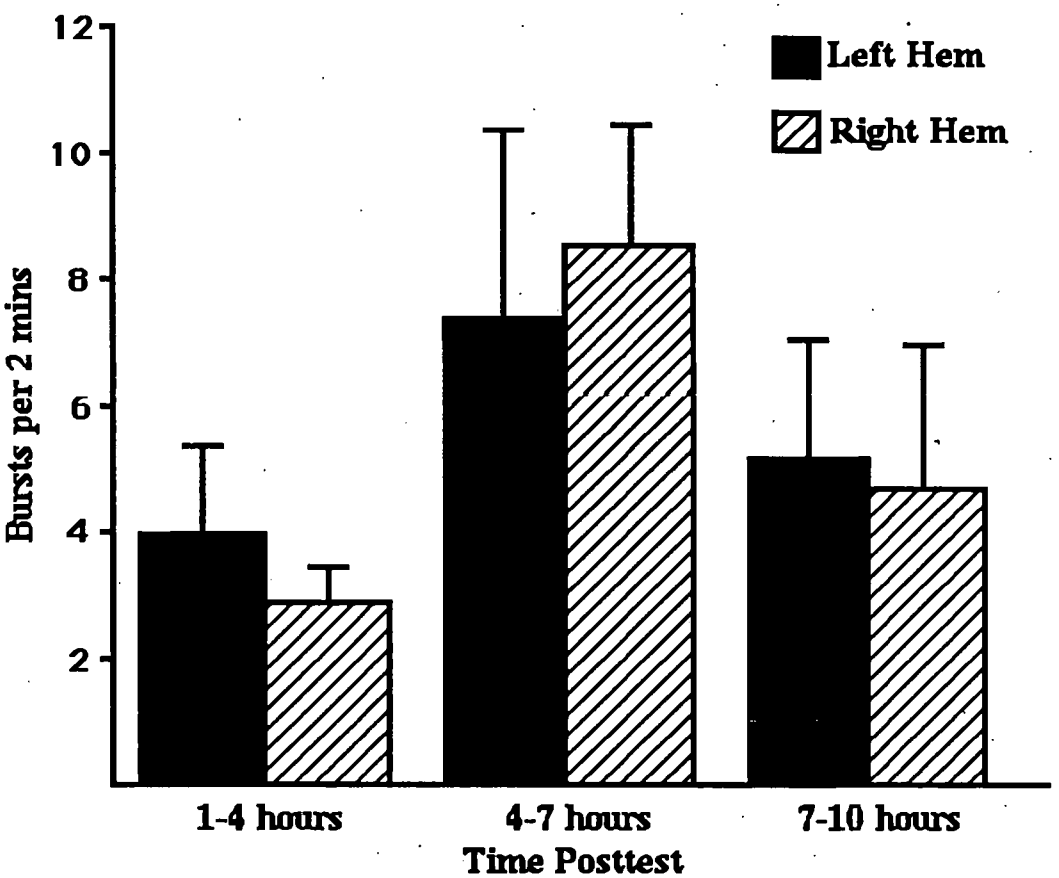


Figure 5.4; The lateralization of bursting in the LPO for Avoid chicks over the 1-10hr posttest period. The sample sizes for each Avoid group are as per Figure 5.3. There are no significant differences between hemispheres at any time. Error bars are standard error of the mean.

Figure 5.5; The Lateralization of LPO Bursting Measured Over 1-10hr Posttest for Peck Chicks.

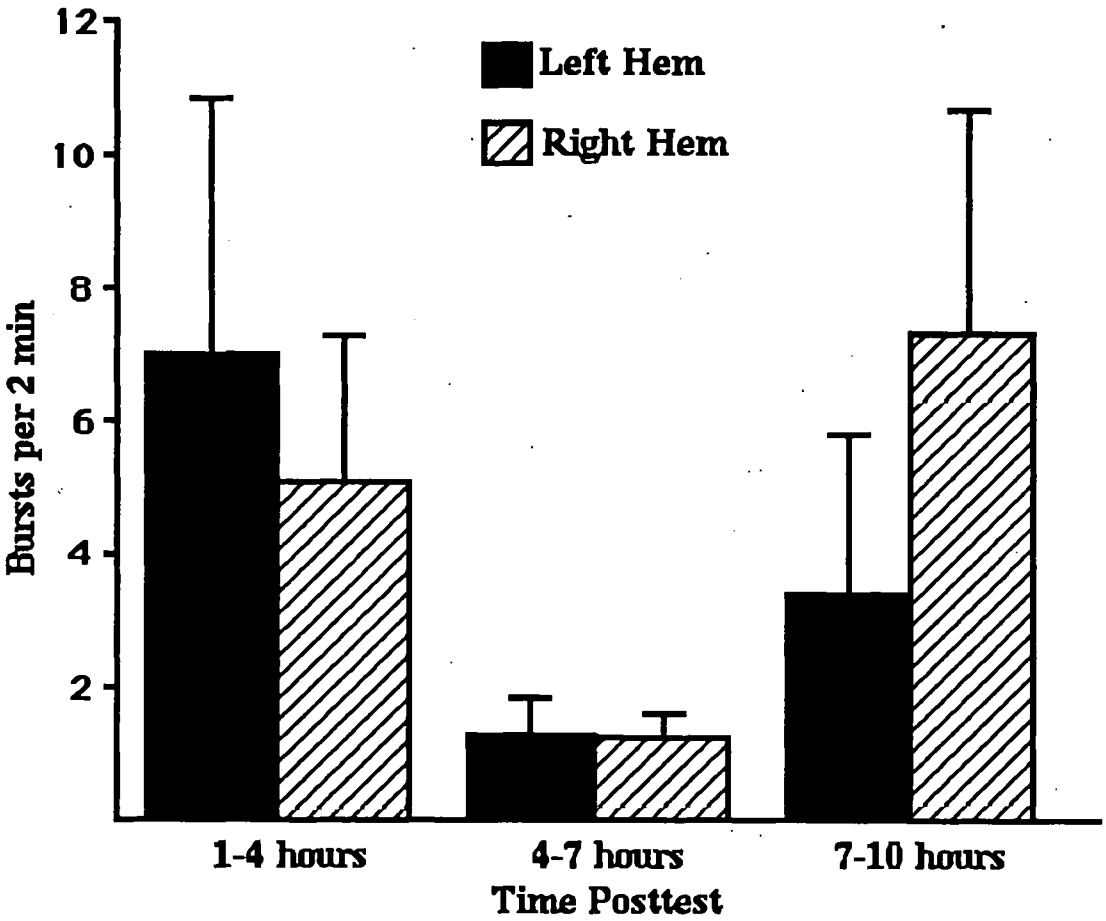


Figure 5.5; The lateralization of bursting in the LPO for Peck chicks over the 1-10hr posttest period. The sample sizes for each water-trained group are as per Figure 5.3. There are no significant differences between hemispheres at any time. Error bars are standard error of the mean.

Figure 5.6; The Percentage of Discriminated Spikes to Fall Within Bursts for both Avoid and Peck Chicks as a Function of Time Posttest.

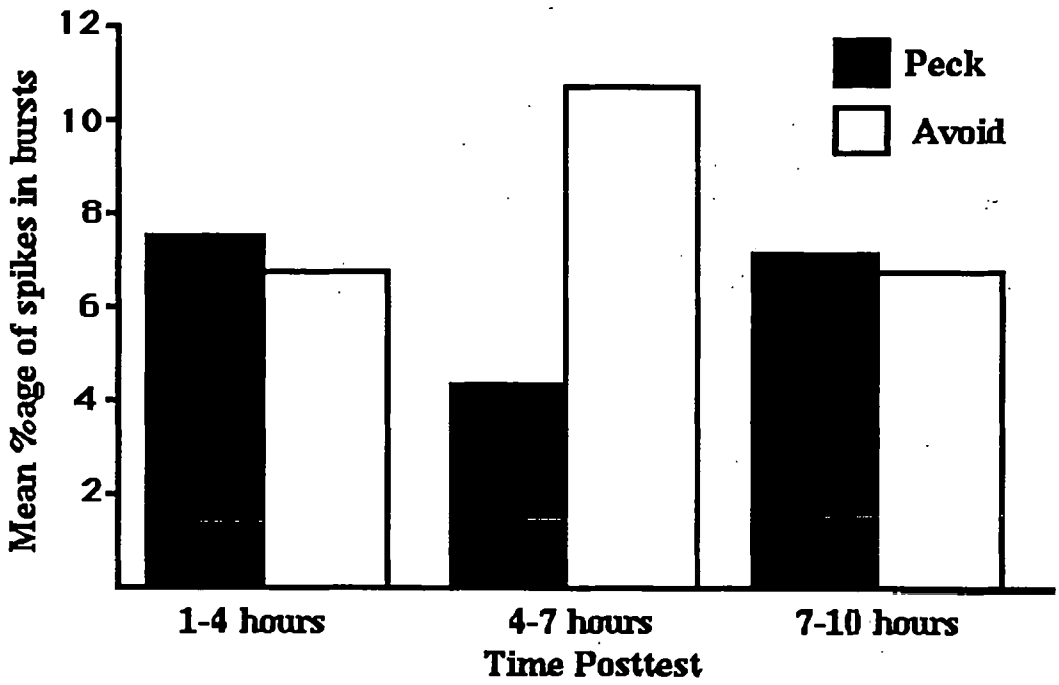


Figure 5.6; The percentage of discriminated spikes to be included within bursts for both Avoid and Peck chicks over the 1-10hr posttest period. The sample sizes for each group are as per Figure 5.3.

Figure 5.7; The Mean Number of Spikes per Burst for both Avoid and Peck Chicks as a Function of Time Posttest.

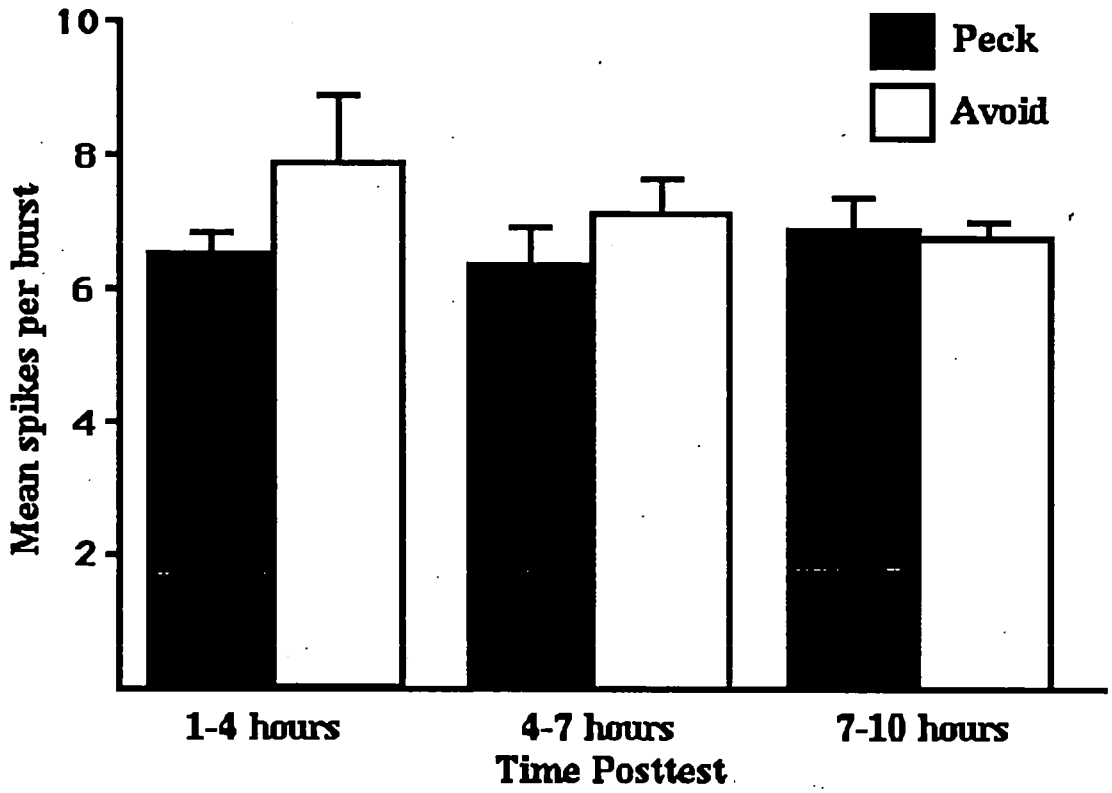


Figure 5.7; The mean number of spikes per burst for both Avoid and Peck chicks over the 1-10hr posttest period. The sample sizes for each group are as per Figure 5.3. There are no significant differences between groups. Error bars are standard error of the mean.

(Chapter 4). Thus, the ECS data presented here provides more evidence to suggest that bursting during the period 4-7hr posttest in both the IMHV and the LPO is crucial to the learning of the task and that the neuronal activity in/between these two structures may be subject to some form of mutual control.

There are some slight differences between the LPO bursting data from normally trained chicks (Chapter 4) and the data from trained-ECS chicks presented here. The overall level of bursting in chicks that avoid after ECS is slightly lower than the overall bursting level for methyl-trained chicks. However, the mean bursting levels of water-trained chicks and chicks that peck after ECS are quite similar. This may explain why, in contrast to normally trained chicks, there is no significant difference in the number of spikes per burst between chicks that avoid and chicks that peck after ECS; a lower mean burst rate reduces the chances of bursts in the avoid group occurring synchronously, one of the factors proposed in Chapter 4 as underlying an increase in spikes per burst. Perhaps the most marked difference between normally trained chicks and chicks given posttraining ECS is seen in burst frequencies recorded during the period 4-7hr posttest. Chicks that peck at test (are amnesic) exhibit a distinctly lower level of bursting during the 4-7hr posttest period when compared to the 1-4 and 4-7hr periods. This 4-7hr value is also lower than the level of bursting seen in non-electroshocked, water-trained animals during the same posttest period (see Chapter 4). This implies that there may be an increasing graduation of LPO bursting, running from amnesic chicks (with the lowest bursting levels) to water-trained chicks and then to methyl-trained chicks with the highest level of bursting. The proposal that water chicks should exhibit a higher LPO burst frequency than amnesic chicks suggests that W chicks learn about the task. This is supported by data showing that chicks that peck a dry (non-reinforcing) bead form a memory of that experience: a memory that is sensitive to protein synthesis inhibition (Barber et al., 1990). Memory formation for the passive avoidance task has been shown here to be accompanied by an increase in

burst firing in the LPO, which suggests that the level of bursting in water trained chicks should be higher than that of amnesic/naive chicks. The inclusion of a water-trained, electroshocked group in the Experiment detailed above might have helped to clarify this situation. However, it must be emphasised that it is impossible to assess amnesia in water-trained animals: they peck both upon training and testing. Whether they remember or forget their initial training cannot be ascertained, as they would peck at test in both instances.

A similar pattern of bursting to that seen in the LPO after ECS is also seen for bursting in the IMHV after ECS (Mason, pers. comm.). Bursting in the IMHV of chicks that have been rendered amnesic by ECS is reduced to an extremely low level during the period 4-7hr posttest, after which bursting rises to a level similar to that of chicks that remember; precisely the same sequence of events reported here for bursting in the LPO after ECS. This provides more evidence to suggest that the activities of the IMHV and LPO are 'linked' in some way during memory formation for the task.

In conclusion, the enhancement of bursting in the LPO seen after training is a direct correspondent of memory formation for the task and is not due to non-specific aspects of the task such as the taste of methylanthranilate.

Lesion studies have indicated that day-old chicks show recall for passive avoidance training despite being subjected to bilateral, pretraining lesions of the LPO (Gilbert et al., in press). However, if similar LPO-lesioned chicks are methyl-trained and then subjected to right IMHV lesions, placed 1hr posttraining, they are subsequently amnesic at a 24hr test. Chicks given pretraining LPO lesions followed by posttraining lesions of the left IMHV still show recall at test. Similarly treated water-trained chicks will peck at test. It appears, therefore, that in the absence of the LPO the right IMHV becomes a necessary structure for long-term memory of the task. The next Chapter

describes an experiment in which chicks were given bilateral LPO lesions and then trained as normal. The spontaneous activity of the IMHV of both hemispheres was subsequently recorded to examine any electrophysiological consequences of the capacity of the right IMHV to become a necessary structure for memory in the absence of the LPO.

CHAPTER 6. The Effects of Pretraining, Bilateral LPO Lesions on Bursting Activity Recorded from the IMHV of the Day-Old Chick After Passive Avoidance Training.

Introduction.

Day-old chicks show recall for passive avoidance training despite being subjected to bilateral, pretraining lesions of the LPO (Gilbert et al., in press). However, if similar LPO-lesioned chicks are methyl-trained and then subjected to right IMHV lesions, placed 1hr posttraining, they are subsequently amnesic at a 24hr test. It appears, therefore, that under these conditions the right IMHV becomes a necessary structure for long-term memory of the task.

The following Experiments describe the effect of bilateral LPO lesions on the spontaneous activity of the IMHV. Multi-unit recordings were made after passive avoidance training from the IMHV of chicks that had previously been given pretraining, bilateral LPO lesions. As reported above, similar LPO lesions have been shown to be non-amnestic. Therefore, the activity recorded from the IMHV of chicks with pretraining, bilateral LPO lesions will still be from chicks that remember the task. Evidence to suggest that this IMHV activity may be different from that seen in the IMHV of chicks without LPO lesions comes from the experiment described above; the right IMHV becomes a necessary structure for memory of the task in chicks that lack an intact LPO (Gilbert et al., in press). This altered capacity of the IMHV may be seen in changes in multi-unit activity. Also, the experiments described in earlier Chapters of this Thesis have provided some physiological evidence to suggest that the activities of neurons in the IMHV and the LPO are closely correlated following training on a passive avoidance task, that is, there may be some functional connection between the two structures. From this, it might be expected that any influence that the activity of the IMHV may have from/over the activity of the LPO will be severely disrupted in

chicks with LPO lesions.

Methods.

The behavioural, anaesthetic, surgical and recording procedures were carried out as described in Chapter 2 for IMHV recordings. All lesioning and training procedures were carried out by Dr. T.A. Patterson. Chicks were given either bilateral LPO lesions or bilateral sham lesions on the day before training. These chicks were allowed to recover overnight. On the day of training pairs of day-old, LPO-lesioned chicks and sham-lesioned chicks were housed in pens. They were then pretrained using a white bead and trained using either a methylanthranilate-coated or a water-coated bead. Only birds that pecked the training bead were included in later parts of the experiment. Chicks that trained successfully were tested one hour posttraining with a dry bead. This procedure generated four groups of chicks: LPO-lesioned M chicks, LPO-lesioned W chicks, sham-lesioned M chicks and sham-lesioned W chicks. Each of these groups was assigned a code. Birds were then taken one at a time for recording over the period 1-10hr posttest. These recordings were carried out by J. Gigg. The order in which birds were taken was random, as was the order in which hemispheres were recorded from. Codes were broken at the end of all experimental procedures.

6.1. The Effect of Pretraining, Bilateral LPO Lesions on Bursting Recorded from the IMHV over the Period 1-10hr Posttest.

Results.

A representative histological reconstruction of an LPO lesion is presented in Figure 6.1. Histological procedures were carried out by J. Gigg. Mean IMHV burst frequencies (i.e., the mean of left and right hemispheres) for LPO-lesioned and sham-lesioned M and W chicks are presented in Figure 6.2. Sham-lesioned M chicks show

Figure 6.1; A Schematic Representation of Typical Bilateral LPO Lesions.

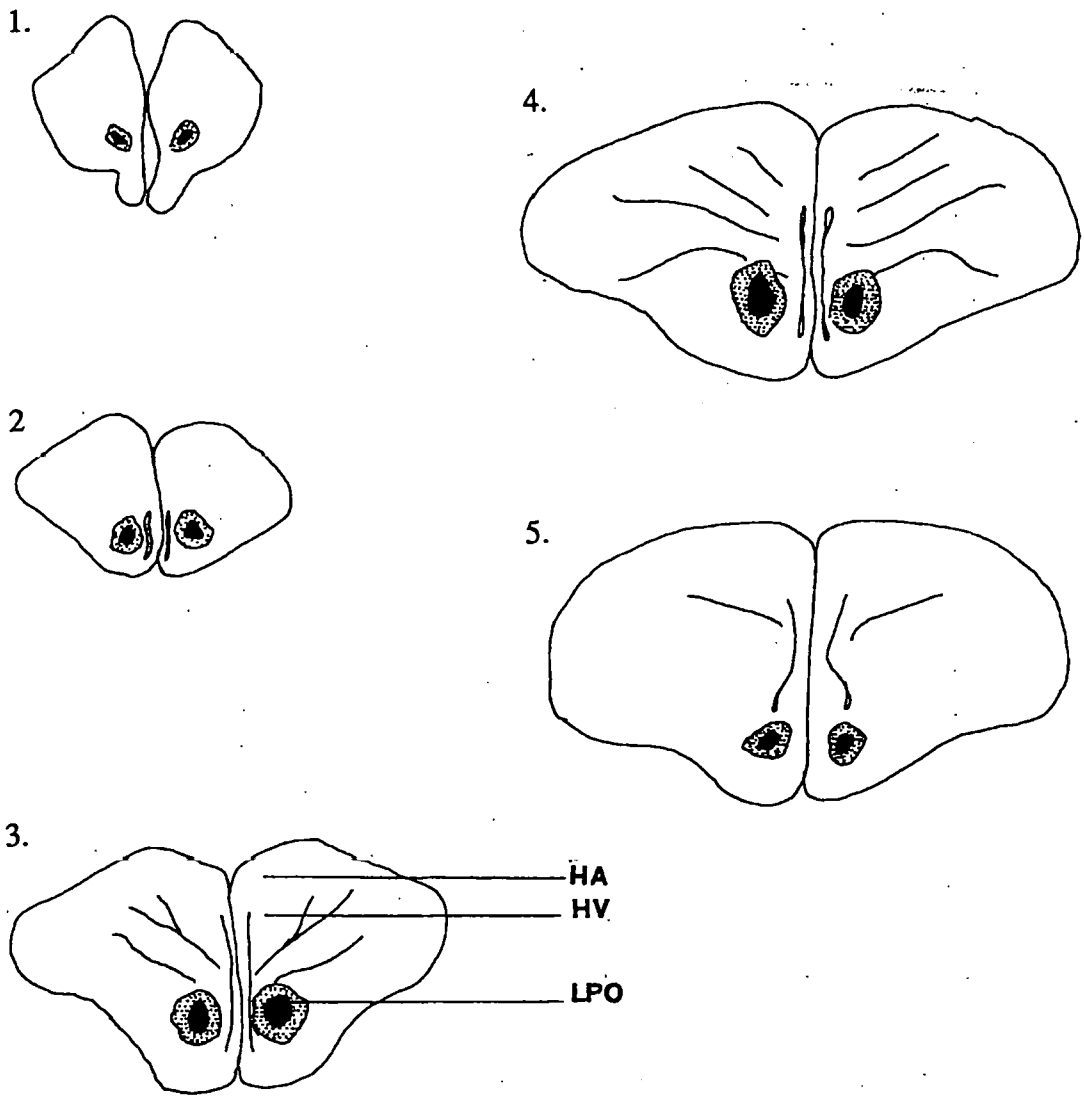


Figure 6.1; Schematic representation of typical LPO lesions. Sections 1, 2, 3, 4 and 5 move anterior-posterior and correspond to the anterior 6.5, 6.0, 5.0, 4.5 and 4.0 references in Youngren and Phillips' (1978) stereotaxic atlas of the 3 day-old chick. Filled areas represent extent of lesions. Black-filled areas indicate tissue destroyed in 90% of brains, stippled areas indicate tissue destroyed in more than 25% of brains. No such damage was seen in sham-lesioned chicks. Abreviations: HA, hyperstriatum accessorium; HV, hyperstriatum ventrale; LPO, lobus parolfactorius.

Figure 6.2; Mean IMHV Burst Frequencies for LPO-lesioned and Sham LPO-lesioned Groups of Day-old Chicks Over the Period 1-10hr Posttest.

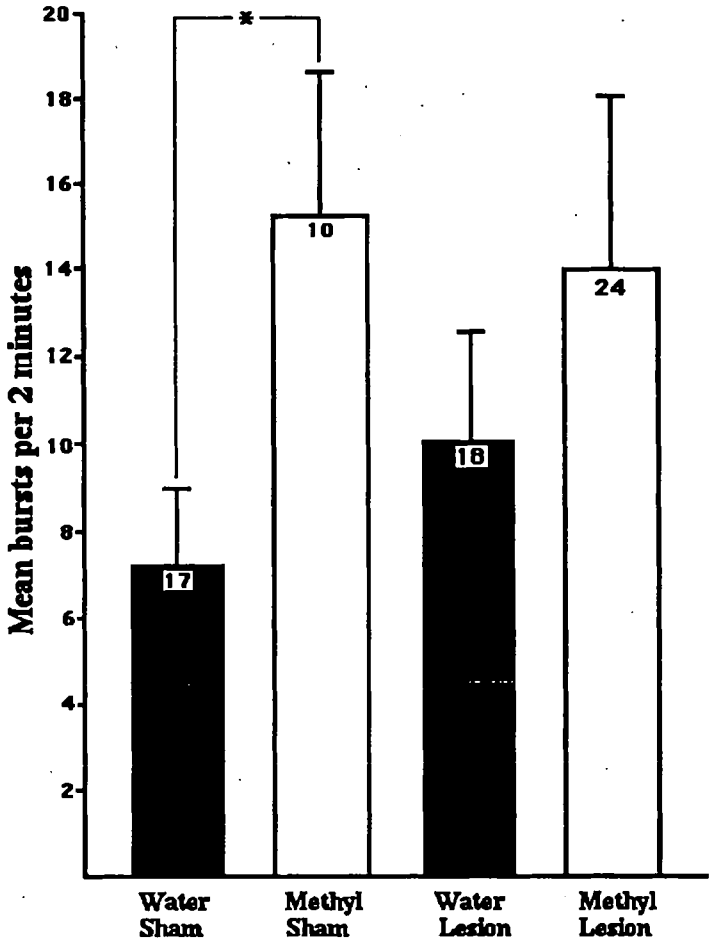


Figure 6.2; Mean IMHV bursting recorded over the period 1-10hr posttest for four groups of day-old chicks: pretraining LPO-lesioned M and W chicks; and pretraining sham LPO-lesioned M and W chicks. The level of bursting represents the mean value for left and right hemispheres (bursts per 2min). The numbers in bars represent sample size. Methyl-trained chicks that received sham lesions exhibit a significantly higher level of bursting when compared to water-trained sham-lesioned chicks (*p=0.05). Bursting levels between LPO-lesioned M and W groups are not significantly different, although the trend is in the same direction as that for shams. Error bars are standard error of the mean.

Figure 6.3; Hemispheric Differences in Mean IMHV Burst Firing for LPO-lesioned and Sham LPO-lesioned Day-old Chicks over the period 1-10hr Posttest.

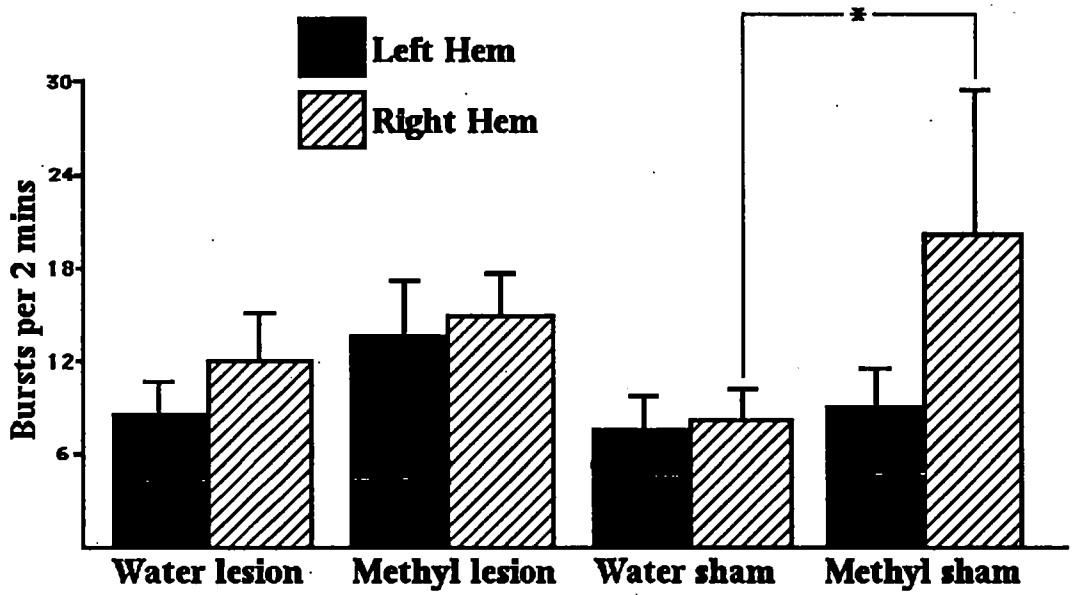


Figure 6.3; Hemispheric differences in bursting per 2min between LPO-lesioned M and W chicks and sham LPO-lesioned M and W chicks over 1-10hr posttest. The sample sizes for each group are as per Figure 6.2, that is, 17 sham-lesioned W chicks, 10 sham-lesioned M chicks, 18 W lesioned chicks and 24 M lesioned chicks. There is a significant difference in mean bursting levels between the right IMHVs of M and W sham-lesioned chicks ($*p<0.05$) There is no significant difference between hemispheres in burst-firing rates within or between any other group(s).

significantly more bursting than sham-lesioned W chicks ($p<0.05$); there is no significant difference in mean burst levels between LPO-lesioned M chicks and LPO-lesioned W chicks.

The mean bursting frequency for each hemisphere of all four groups is presented in Figure 6.3. The right IMHV of sham M chicks displays a higher mean burst rate when compared to the right hemisphere of sham W chicks ($*p<0.05$). There are no other significant within-group hemispheric differences in IMHV bursting.

Discussion

The overall burst frequency recorded from the IMHV of M chicks with sham LPO lesions is significantly higher than that from similarly treated W chicks. This replicates the effect seen in Chapter 3 and reported by Mason and Rose (1987) and suggests that these sham groups provide adequate controls for the effects of surgery and placement of lesion electrodes on the activity of the IMHV. The increased bursting in sham-lesioned M chicks is not bilateral, as seen in non-lesioned M chicks; only the right hemisphere of sham-lesioned M chicks displays a significantly elevated mean burst rate. This lack of effect between the left hemispheres of sham-lesioned M and W chicks may be a product of the sham-lesioning procedure, or might be due to sampling bias, as the number of sham M chicks recorded from is very small (n=10).

There is no significant difference in overall burst frequencies measured in lesioned M chicks compared to lesioned W chicks. Bursting between the right and left IMHVs of lesioned M chicks also appears to be quite similar, in contrast to sham-lesioned M chicks and non-operated M chicks (Chapter 3). These results suggest that the posttest bursting activity of the IMHV of M-trained chicks is severely disrupted in the absence of the LPO. However, the overall levels of bursting between lesioned and sham M chicks are quite similar. One possible reason for the lack of any significant difference between lesioned M and W groups may be that the overall level of bursting in lesioned

W chicks is higher than that of sham W chicks. Although there is no significant difference between bursting in sham and lesioned W chicks, there is a trend for bursting in lesioned W chicks to be higher.

As indicated in the Introduction, the right IMHV becomes a necessary structure for the storage of memory for passive avoidance training in the absence of the LPO at training (Gilbert et al., in press). This presumed change of function of the right IMHV is not evident in posttest levels of bursting activity in lesioned M chicks; the levels of bursting between the right and left IMHV are very similar in lesioned M chicks, especially when compared to the hemispheric differences in bursting in sham-lesioned M chicks where the right IMHV displays a higher level of bursting. If changes in neuronal firing actually underlie this altered function of the right IMHV, then it may be that increased levels of bursting occur in the right IMHV during times closer to training, that is, at times not included in this analysis. The proposed importance of such early activity after training will be discussed further in the General Conclusions section.

6.2. The Timecourse and Lateralization of Bursting in the IMHV of Chicks with Pretraining, Bilateral LPO Lesions or Bilateral Sham LPO Lesions, Recorded over the Period 1-10hr Posttest.

Introduction

The timecourse of bursting in the IMHV of the four groups of chicks described in section 6.1 was investigated by pooling the data into the three posttest periods used in Chapters 4 and 5: 1-4hr posttest; 4-7hr posttest; and 7-10hr posttest. The lateralization of IMHV bursting was investigated by splitting the data set in a similar fashion to provide three posttest periods.

Figure 6.4; The Timecourse of IMHV Bursting Measured Over 1-10hr Posttest for Sham-lesioned M and W Chicks.

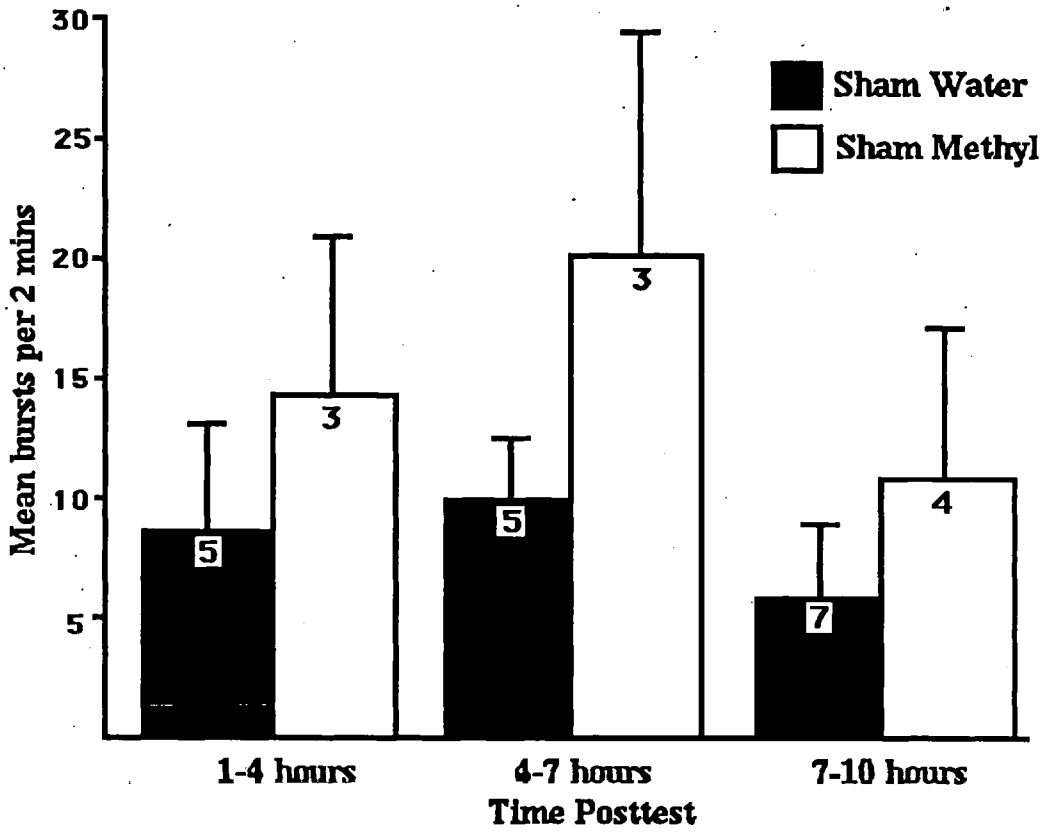


Figure 6.4; The timecourse of bursting for sham-lesioned M and W chicks over the 1-10hr posttest period. The sample sizes for each group are indicated in bars. Although the means appear quite different, there is no significant difference between groups during any posttest period. This is probably due to the small sample sizes. Error bars are standard error of the mean.

Figure 6.5; The Timecourse of IMHV Bursting Measured Over 1-10hr Posttest for LPO-lesioned M and W Chicks.

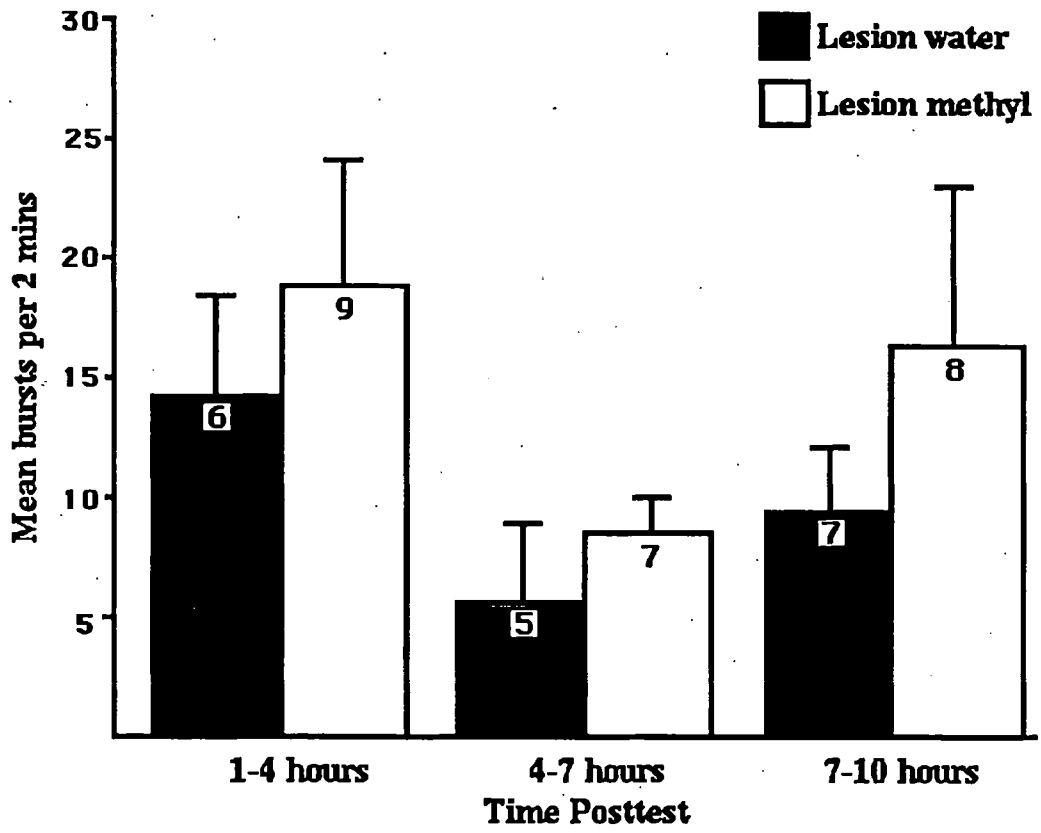


Figure 6.5; The timecourse of bursting for LPO-lesioned M and W chicks over the 1-10hr posttest period. The sample sizes for each group are indicated in bars. There is no significant difference between groups during any posttest period. Error bars are standard error of the mean.

Figure 6.6; The Lateralization of IMHV Bursting Measured Over 1-10hr Posttest for Sham-lesioned M Chicks.

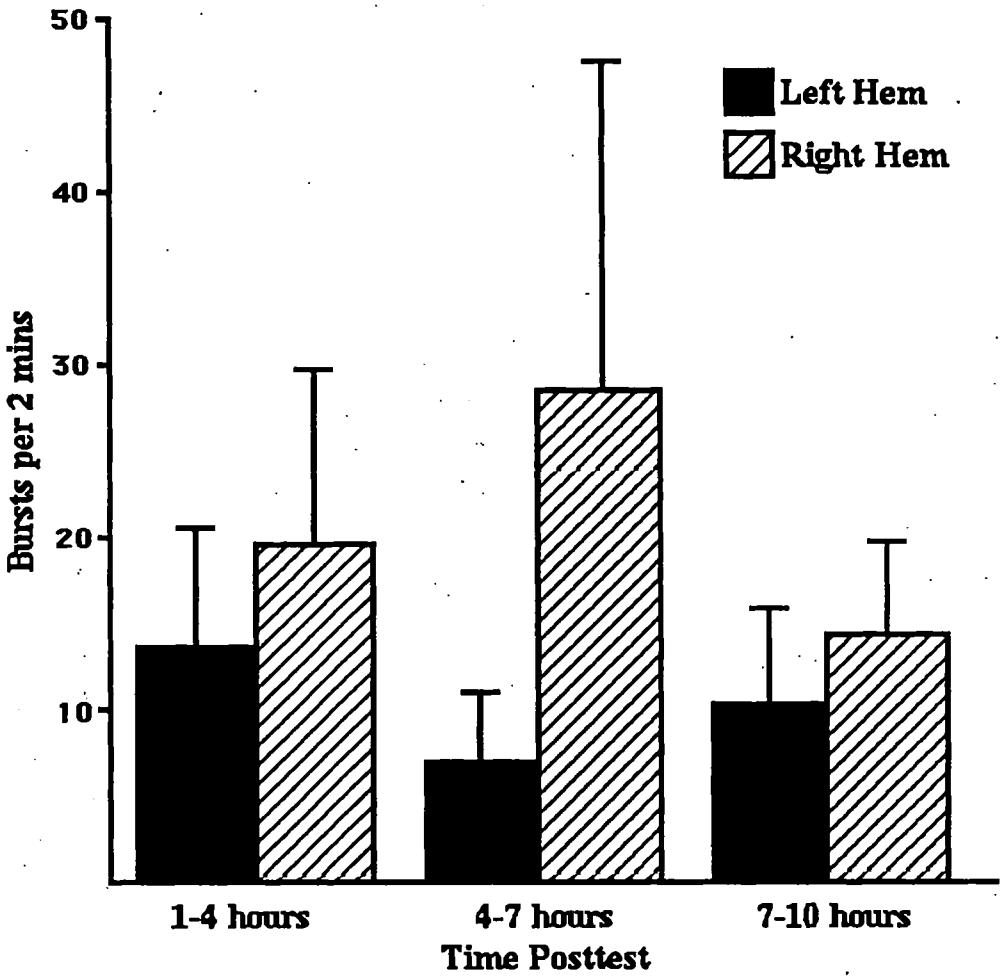


Figure 6.6; The lateralization of bursting in the IMHV for sham-lesioned M chicks over the 1-10hr posttest period. The sample sizes for each methyl-trained group are as per Figure 6.4. Although the means appear quite different, especially during the 4-7hr posttest period, there are no significant differences between hemispheres at any time. This is probably due to the small sample sizes. Error bars are standard error of the mean.

Figure 6.7; The Lateralization of IMHV Bursting Measured Over 1-10hr Posttest for Sham-lesioned W Chicks.

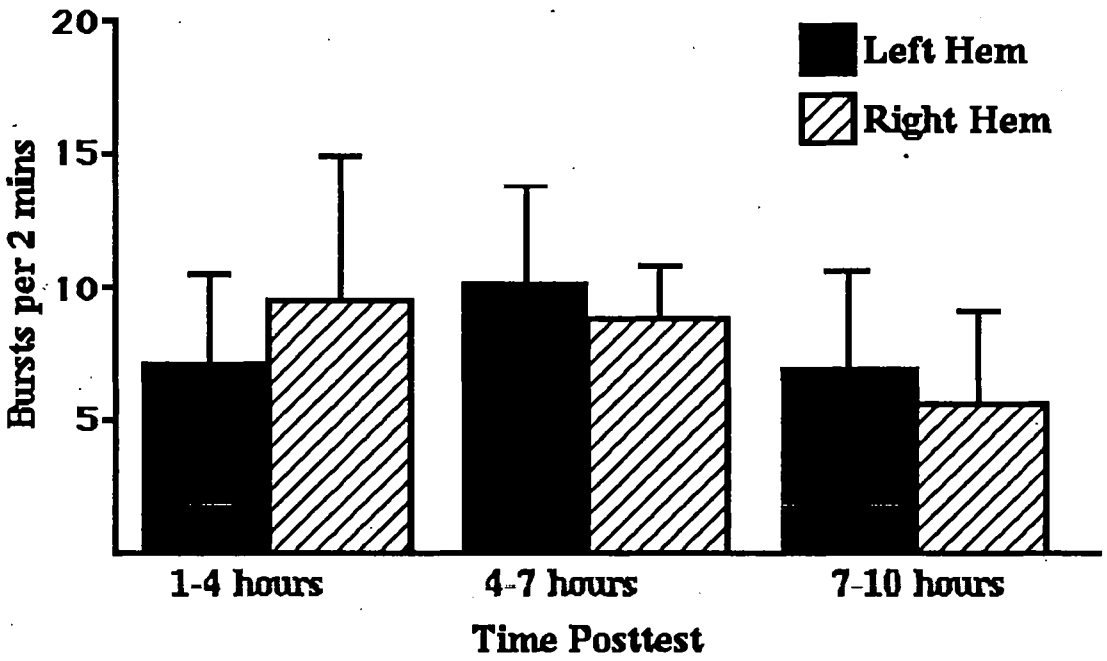


Figure 6.7; The lateralization of bursting in the IMHV for sham-lesioned W chicks over the 1-10hr posttest period. The sample sizes for each water-trained group are as per Figure 6.4. There are no significant differences between hemispheres at any time. Error bars are standard error of the mean.

Figure 6.8; The Lateralization of IMHV Bursting Measured Over 1-10hr Posttest for LPO-lesioned M Chicks.

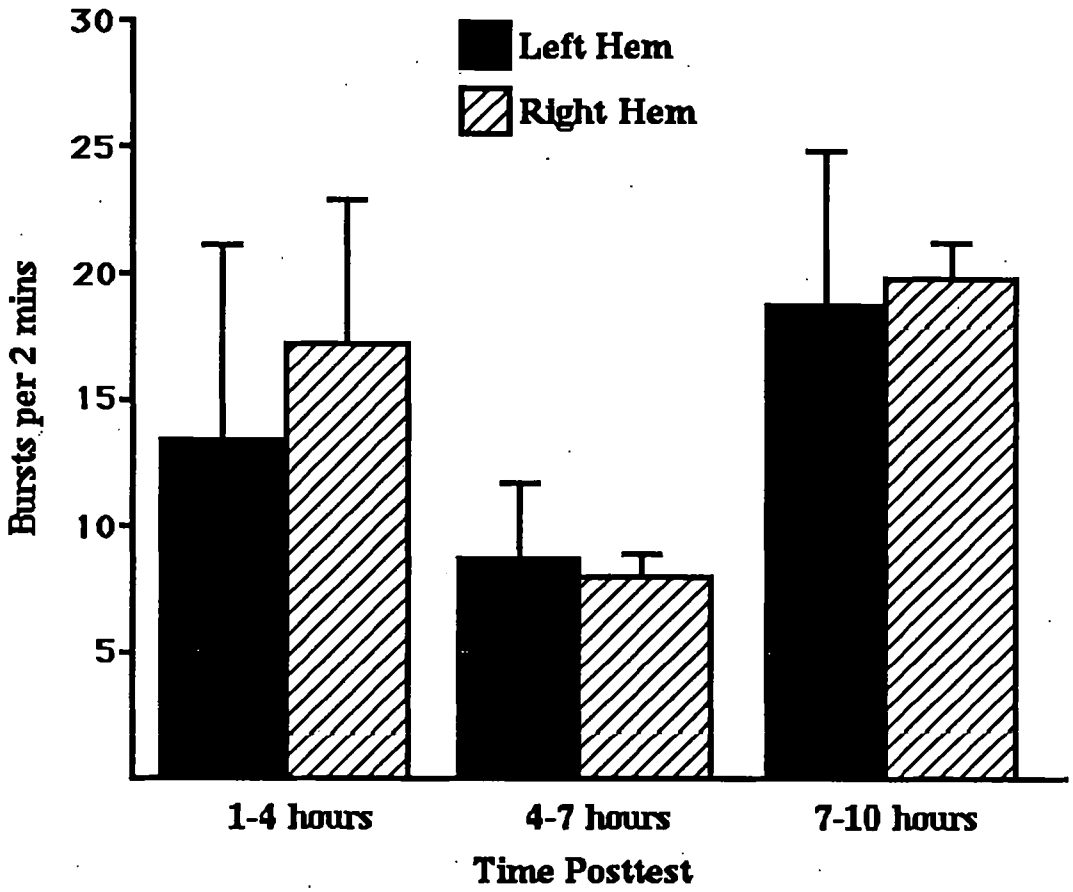


Figure 6.8; The lateralization of bursting in the IMHV for LPO-lesioned M chicks over the 1-10hr posttest period. The sample sizes for each methyl-trained group are as per Figure 6.5. There are no significant differences between hemispheres at any time. Error bars are standard error of the mean.

Figure 6.9; The Lateralization of IMHV Bursting Measured Over 1-10hr Posttest for LPO-lesioned W Chicks.

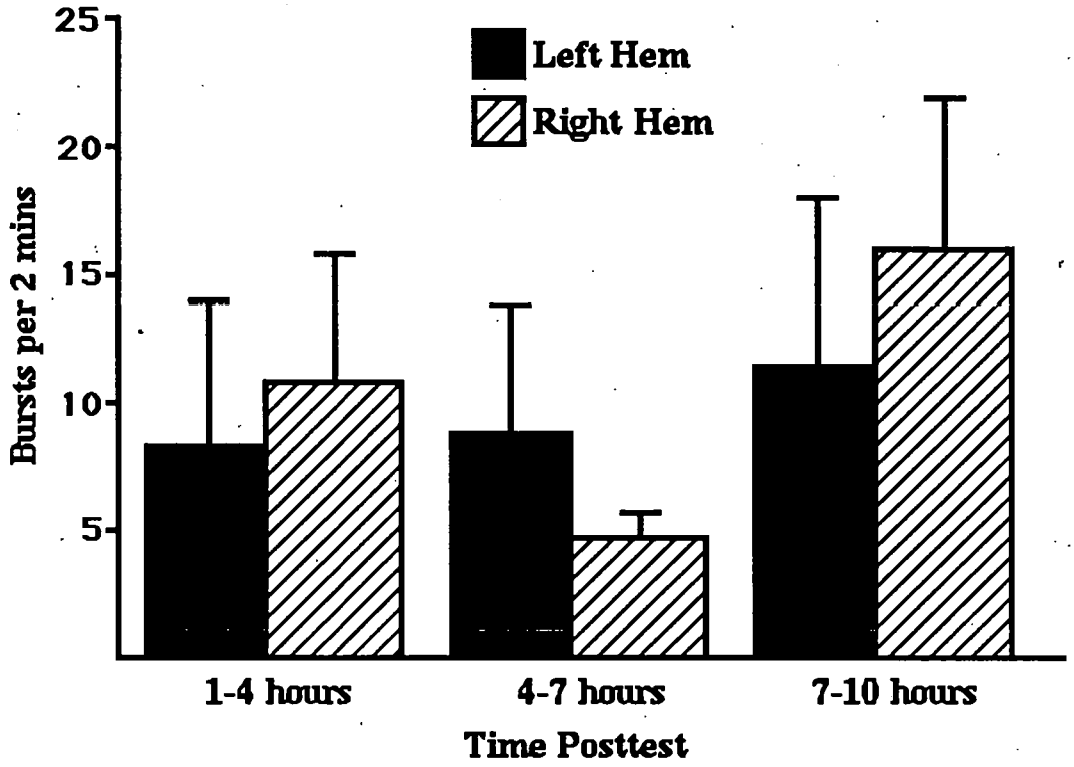


Figure 6.9; The lateralization of bursting in the IMHV for LPO-lesioned W chicks over the 1-10hr posttest period. The sample sizes for each water-trained group are as per Figure 6.5. There are no significant differences between hemispheres at any time. Error bars are standard error of the mean.

Results.

The mean IMHV burst frequencies for sham-lesioned M and W groups is plotted against time in Figure 6.4. There is no significant difference between groups during any of the three posttest periods, probably because of the small sample sizes involved. Figure 6.5 is the counterpart for lesioned M and W chicks. There are no significant differences between groups.

Figure 6.6 shows the same data described in Figure 6.4, in this instance as bursting by hemisphere for sham-lesioned M birds with time. Figure 6.7 is the counterpart for sham-lesioned W chicks. No asymmetry is evident during any time period for either group of sham-lesioned chicks. This also applies for lesioned M chicks (Figure 6.8) and lesioned W chicks (Figure 6.9).

Discussion.

As can be seen, bursting in sham-lesioned M and W chicks follows a similar timecourse to that seen in non-operated chicks (Chapter 3), although there is no significant difference between sham groups during any posttest period. This lack of significance is probably due to the small sample sizes involved. The timecourse of bursting in lesioned M chicks appears different to that of sham-lesioned M chicks, especially during the 4-7hr period when LPO-lesioned M chicks exhibit a drop in bursting when compared to sham M chicks.

General Conclusions

Experiments were performed to examine the effects of pretraining, bilateral LPO lesions on subsequently recorded multi-unit activity of the IMHV. Section 6.1 demonstrated that M chicks with sham LPO lesions exhibit a significantly higher overall mean burst frequency when compared to sham W chicks over the period 1-10hr posttest. This indicated that, so far as was possible to tell, the surgical

procedures used to produce sham treated chicks did not interfere with the normal pattern of bursting seen in the IMHV posttest. In contrast to the burst data for sham chicks, there is no difference in mean burst rates between LPO-lesioned M chicks and LPO-lesioned W chicks, as measured over the same posttest period.

The initial interpretation of the data presented in this Experiment is that an increased level of bursting in the IMHV is not required for memory formation; a conclusion inconsistent with the data presented in Chapter 3. However, it must be remembered that the data presented in this Chapter represents the activity of the IMHV over the period 1-10hr posttest. A more accurate interpretation of these results is, therefore, that chicks that lack an intact LPO do not exhibit increased IMHV bursting during the period 1-10hr posttest, but still show retention for the task. This is, perhaps, a simplistic view. The results shown in Figure 6.2 indicate that the overall bursting levels between lesioned M chicks and sham M chicks are quite similar, suggesting that there may be little difference between these groups in terms of posttest bursting levels. However, there is a trend for bursting in lesioned W chicks to be higher than that for sham-lesioned W chicks. This suggests that the reason why there is no significant difference in mean bursting levels between lesioned M chicks and lesioned W chicks is not that that the mean level of bursting in lesioned M chicks is reduced (compared to sham-lesioned M chicks), but rather that bursting in lesioned W chicks is increased (above that of sham W chicks).

Why might lesioned W chicks display a higher level of bursting? One possibility is that lesioning the LPO causes some disinhibition of burst-firing in the IMHV, thereby increasing the spontaneous burst rate. If this were the case, then it might be expected that levels of bursting between lesioned M and W chicks would be: (1) very similar; and (2) higher than those levels seen in sham-lesioned and non-lesioned chicks. This is plainly not the case, as lesioned M chicks display a higher mean burst rate compared to lesioned W chicks and these bursting levels are similar to non-lesioned M and W

chicks (Chapter 3). An alternative explanation for the enhanced level of bursting in lesioned W chicks is that, in the absence of an intact LPO, an increased 'demand' is placed upon structures such as the IMHV for memory formation; it must be remembered at this juncture that chicks do remember the act of pecking a dry bead (Barber et al., 1990). It is conceivable that such an increased demand is evidenced as an increase in IMHV bursting levels in lesioned W chicks when compared to sham-lesioned W chicks. There is, of course, the possibility that increased posttest bursting in lesioned W chicks is a combination of the reasons discussed above.

Figure 6.2 demonstrates the burst frequencies of all four groups as timecourses of activity after testing. This analysis provides some further insight into why lesioned M chicks do not display significantly higher mean burst rates compared to lesioned W chicks. This is presented below (it must be stressed at this point that the following discussion is based upon very small sample sizes). The level of bursting in lesioned M chicks appears reduced, when compared to sham M birds, during the period 4-7hr posttest. As reported earlier, bursting during this posttest period is maximal in both the IMHV and the LPO of unoperated M-trained chicks (Chapters 3 and 4). It is also during this 4-7hr posttest period that bursting in both the LPO (Chapter 5) and the IMHV (Mason, pers. comm.) is reduced in chicks rendered amnesic by subconvulsive ECS. These experiments provide evidence to suggest that the IMHV and LPO may be (at least) physiologically connected and that this connection is manifest during the period 4-7hr posttest as a near simultaneous increase in both the rate and synchrony of bursting in/between the two structures. Lesioning the LPO pretraining will severely disrupt these (putative) connections with the IMHV, thereby compromising any information flow between the two structures. This compromised flow is evidenced as a drop (or lack of increase) in IMHV bursting during the period 4-7hr posttest.

As discussed in earlier parts of this thesis, lesion studies have indicated that the left

IMHV is a necessary structure for the acquisition and/or early processing of information for the passive avoidance task (Patterson et al., 1990a). The LPO is a necessary structure for the long-term retention of memory for the task (Gilbert et al., in press). In the General Conclusion of Chapter 3, a rather general scheme was put forward to describe the neuronal events that may occur at or around the time of passive avoidance training, based upon theories of mammalian learning. This scheme involved an initial, weak potentiation of connections between cells that would ultimately form the neural 'representation' of memory for the task (a 'priming' effect), followed by a later consolidation of this memory trace. The initial priming was proposed to occur during and shortly after a period of 'theta' rhythm, coincident with the actual performance of training, that is, during pecking. This initial priming may be controlled by the IMHV, perhaps in particular the left IMHV, and may involve neural circuits between the IMHV, the LPO and other as yet unidentified cortical areas. It is implicit in this scheme that these priming events take place very soon after training, that is, during periods in which recordings have not been made from anaesthetized chicks.

As discussed in Section 6.1 there is no increase/difference in bursting between the right and left hemispheres of lesioned M chicks after training. Lesion studies have indicated that the right IMHV becomes a structure necessary for storage of memory for the task in the absence of the LPO at training (Gilbert et al., in press). The results from this Chapter suggest that this capacity of the right IMHV to become a necessary structure/store for memory is not reflected in increased levels of bursting. However, such changes in activity may occur during the above priming period, that is, recording IMHV activity 1-10hr posttest may be too late to see any changes in burst-firing patterns of the right IMHV.

Evidence to indicate that the LPO may be involved in early stages of information processing after passive avoidance training comes from two sources; studies which

have examined the effects of inhibitors of memory formation when injected into the area of the LPO and studies to examine the accumulation of 2-DG within the brain after training. Serrano et al. (1988) showed that injections of ouabain into the region of the LPO 5min before training produced amnesia at a 24hr test. Patterson et al. (1986) and Gibbs & Ng (1977) have shown that similar injections of ouabain disrupt the formation of ITM when placed in the region of the IMHV. Also, Serrano et al. (1990) demonstrated that injections of the muscarinic antagonist scopolamine into the LPO produced maximal amnesia by 30min after training; the IMHV displays a similar timecourse of susceptibility to the injection of scopolamine. It must be emphasised that Serrano et al. (1988; 1990) did not examine the extent to which their injected substances diffused from the site of injection. It is possible that their injections may not only have affected the LPO but also areas such as the IMHV. The increased 2-DG metabolism seen in the left LPO also suggests that increased activity occurs in this region at times close to training; 2-DG uptake is increased in the left LPO if the injections of 2-DG are given either 5min pretraining or 10min posttraining, as measured 30min posttraining (Rose & Csillag, 1985). This early activity of the IMHV and LPO may be part of the priming effect postulated above. The increased bursting seen in IMHV and LPO during the period 4-7hr posttest may represent the neural activity responsible for the consolidation of this memory trace into long-term memory ('hard-wiring' the memory trace). In the absence of the LPO at training, a different (compensatory?) circuit might be established between the IMHV and other forebrain areas during perhaps the first two hours posttraining. This would be a circuit in which the IMHV becomes a necessary store for memory. This scenario explains, to a certain degree, why the IMHV does not exhibit an increase in bursting during the period 4-7hr posttraining; there is no LPO at training so the circuit that would normally be formed is compromised. With no LPO, the long-term consolidation proposed to occur between IMHV and LPO during the period 4-7hr posttraining would be absent, evidenced as a lack of increased activity in the IMHV.

Another possible mechanism for the formation of memory for passive avoidance training in the chick is that different areas of the chick brain are responsible for encoding different aspects of the training task, e.g., colour of the bead, taste of the bead etc. Whilst this scheme and the one outlined above are not necessarily mutually exclusive, the proposal that different brain regions are responsible for different parts of the representation does allow for these 'parts' of the memory trace to be formed sequentially. For instance, the IMHV might initially code for visual components of the task, whilst the LPO could code some other, perhaps emotional, components of the task. This activity of the LPO could occur, perhaps under the influence of the IMHV, at some later period, e.g., 4-7hr posttest. If this were indeed the case, then the absence of the LPO at training would imply that the representation of memory for the task within the chick brain would be 'different' in lesioned chicks to that for non-lesioned chicks, that is, whatever it is that chicks remember about the task differs between lesioned and non-lesioned chicks. This scheme also explains why: (1) there is no overall difference in bursting between lesioned M and W chicks; and (2) why there is an apparent drop in bursting in lesioned M chicks during the period 4-7hr posttest. The part of the representation encoded by the LPO/IMHV (4-7hr posttest?) cannot be formed in the absence of an intact LPO and hence no increased activity is seen in the IMHV.

It must be reiterated that the above proposals are based upon the results from only a small number of animals, further work would need to be undertaken to establish their validity. Lesion studies are notoriously difficult to interpret and the Experiment reported here is no exception. However, it has suggested further experiments and has also provided some further evidence to suggest that the IMHV and LPO are functionally connected.

The following General Discussion reiterates some of the points discussed so far.

CHAPTER 7. General Discussion and Future Directions

This Thesis was primarily aimed at an investigation into the effects of learning on the spontaneous electrical activity of neurons in two regions of the chick forebrain; the IMHV and the LPO. Such an experimental approach to the study of learning and memory in the chick can be rightly criticized for trying to "run before it has learned to walk", as very little is known regarding the intrinsic firing repertoires of chick forebrain neurons. This means that when a multi-unit recording from the chick is studied, the various components of the trace cannot be confidently associated with known neuronal generation sites, for example, IMHV bursting cannot be said to be of dendritic origin. Nevertheless, the results from the various Experiments detailed in this Thesis are important in that they describe an effect of learning/memory on (at least) one type of spontaneous neuronal firing; bursting. The remainder of this Chapter will deal with both a review of the work presented in this Thesis and also suggestions for further work to be carried out on the basis of these findings.

Chapter 3 described an Experiment in which the spontaneous neural activity of the IMHV of the day-old chick was recorded after passive avoidance training. Recordings were made with low-impedance glass microelectrodes to allow the simultaneous recording of a number of neurons, a technique called multi-unit recording. Using this technique, Mason and Rose (1987; 1988) have shown a significant, memory-specific elevation in burst-firing in the IMHV following training. The Experiment detailed in Chapter 3 sought to both replicate this training-induced effect on IMHV bursting and also to extend the previous findings to include a timecourse of bursting after training. To this end, groups of chicks were trained to either peck a water-coated bead (W chicks), or to avoid a methylanthranilate-coated bead (M chicks). Multi-unit recordings were made from the right and left IMHV of both groups of chicks over the period 1-9hr posttest. Analysis of the results from this Experiment demonstrated that M chicks displayed a higher mean burst rate, when compared to W chicks, in data pooled over

the posttest period. The overall burst rates for the IMHV of both the left and right hemispheres of M chicks were also higher than the respective values for W chicks. These findings essentially replicate the original effect described by Mason and Rose (1987). This replication proves just how strong an effect training has on bursting activity in the IMHV, especially when one considers that perhaps only 10 cells are recorded from at any one time using the multi-unit technique. This means that if, for example, 6 sequential recordings are made from the left or right IMHV, then only about 60 cells will contribute to the multi-unit recordings taken. The cells that contribute to the multi-unit trace are recorded from on a random basis, that is, there is not (and probably cannot be) any systematic sampling of readily identifiable cells as, for instance, is possible when recording from *Aplysia* ganglia. The number of neurons in the IMHV is probably in the order of 120,000 (T. Doubell, pers. comm.). This means that recording the activity of 60 cells represents a sampling of only 1/2000 of the total neuronal population of the IMHV. When one also considers the rather strict definition of what, for the purposes of analysis, constitutes a burst, then the total number of units that contribute to the final analysis is made even smaller.

To summarize the above, the ability to replicate an effect based upon the recording of spontaneous activity from random populations of cells within the IMHV indicates two things: (1) that the effect is both very reliable (i.e., easily reproducible); and (2) that, many cells in the IMHV must, theoretically, contribute to the effect. It is difficult to confidently state whether the effect of training on burst-firing is to: (a) produce bursting discharges from more cells, that is, to recruit more bursting cells; (b) increase the burst-firing rate of a stable population of cells; or (c) produce an increased number of bursting cells that exhibit an enhanced level of bursting.

The second aim of the Experiment in Chapter 3 was to describe the timecourse of bursting in the IMHV after training. From the discussion above it would seem that a

large percentage of the population of cells in the IMHV of M chicks show enhanced levels of bursting after training. Is this a general increase in the discharge rate of cells, or are increases in bursting isolated to certain posttest periods? From the results of the timecourses presented in Chapter 3, the latter seems to be the case for IMHV bursting. Bursting is significantly higher in M chicks during the periods 3-4, 5-6 and 6-7hr posttest. Also, the right IMHV of M chicks displays a significantly higher mean burst rate when compared to the left during 6-7hr posttest. This suggests that large numbers of neurons in the IMHV show a high level of burst-firing during specific posttest periods. These increases in burst firing have been shown to be memory-specific, as chicks rendered amnesic with the use subconvulsive ECS show no increase in bursting above levels seen in W chicks (Mason & Rose, 1988).

What does this increase in bursting represent? As discussed in Chapter 3, bursting in the IMHV may be under the influence of afferents arising from the medial septal nuclei. Another possibility is that bursting is intrinsic to the IMHV. Bursts in IMHV are associated with large negative deflections in field potential (Fig. 3.2), a similar association to that seen between bursts and sharp-waves in the CA fields of the hippocampus (Buzsaki, 1989). Sharp-waves appear to be intrinsic to the hippocampus, that is, they are not induced by extra-hippocampal input (Buzsaki et al., 1988; 1989). Subcortical afferents may actually exert a tonic suppressive action on burst-synchronization mechanisms of the hippocampus; when these subcortical inputs are temporarily antagonized the reduction of inhibition allows the occurrence of sharp-wave associated bursting in the hippocampus (Buzsaki et al., 1983). It is possible, therefore, that increased (synchronized?) bursting in the IMHV seen during the posttest periods outlined above arises from some reduction in afferent inhibition. Indeed, studies using a chick brain slice preparation have indicated that neurons in the IMHV are subject to high levels of tonic inhibition (A. Webb pers. comm.). GABAergic activity, as measured by (^{3}H) muscimol binding (Stewart et al., 1988), is very high in the IMHV of chicks. Work by Muller and Scheich (1988) has shown that

GABAergic inhibition in the HV and neostriatum (caudal auditory telencephalon) increases the neuronal selectivity to natural sounds. What relationship has this to bursting activity? In the hippocampus, GABAergic inhibition has a controlling effect upon bursting. Pyramidal cells are sequentially excited when GABAergic inhibition is reduced. Normally, GABAergic 'tone' will limit the communication between neurons. However, blockade of this inhibitory tone allows cells to fire bursts of action potentials in response to a single synaptic input (Miles & Wong, 1987). If inhibition is decreased to some threshold level, then the neuronal population may exhibit epileptic, seizure-like cascading excitation.

The mechanism by which inhibitory tone is decreased is unclear. Repeated electrical stimulation will rapidly decrease GABAergic inhibition (Ben-Ari et al., 1979). A number of mechanisms possibly underlying 'GABA fading' have been proposed. These include : an increase in GABA uptake from the extracellular medium; GABA receptor desensitization; and an increase in extracellular potassium (see Taylor, 1988 for review). The activation of NMDA receptors in CA1 pyramidal neurons has been found to block GABAergic inhibition (Stelzer et al., 1987). A decrease in inhibition alone, therefore, seems to be insufficient for the spread of bursting type activity. What does appear to be necessary, according to recent work by Yaari and Jensen (1987), is an increase in the extracellular potassium concentration. This potassium rise could be produced by gradual depolarizations of neurons and glia, elevations which have been seen prior to seizure-like activity in the hippocampus (Taylor, 1988). What these observations propose is that, for burst firing to occur, at least two processes must be in evidence: (1) an increase in the overall excitability of the population via an increase in the concentration of extracellular potassium, regulating the threshold for burst firing; and (2) a modulation of excitatory and inhibitory synapses, possibly regulating focal bursting discharges.

What is the possible role of bursting in memory formation? As mentioned earlier, bursting in the chick is 2-APV sensitive, i.e., microinjection of 2-APV into the IMHV will prevent bursting in this structure (Mason, pers. comm.). Intracerebral injection of 2-APV into the area of the IMHV has also been shown to produce amnesia for passive avoidance training in chicks (Patterson et al., in prep.). This amnestic action of 2-APV is only evident if injections are given around the time of training. If the amnestic action of 2-APV in this instance is to antagonize burst firing in the IMHV, then it can be inferred that bursting in the IMHV is elevated around the time of training. This is of some interest in the light that during bursting in the hippocampus the intracellular calcium concentration increases via depolarization and/or activation of NMDA receptors. If we consider bursting to be involved in some way with memory formation, possibly via routes outlined in the General Introduction, then this sharp rise in intracellular calcium concentration could have some functional aspect in the establishment of a memory trace. A well established route for this could be via phosphorylation of specific structural proteins. It has been suggested that calcium influx activates both calcium/calmodulin dependent protein kinases 1 and 2 as well as protein kinase C. This activation of multiple calcium regulated protein phosphatases and dephosphatases may underly pleiotrophic physiological actions of calcium at the nerve terminal (Wang et al., 1988). These changes in biochemical regulatory kinases etc. could underly the morphological changes seen after passive avoidance training. M chicks show significant increases in spine density of multi-polar projection neurons in both the IMHV and LPO 24-26hr posttraining (Patel & Stewart, 1988; Patel et al., 1988; Lowndes & Stewart, 1990). It is of some interest that similar changes can be induced in the mammalian hippocampus 15min following the initiation of bursting activity by the application of kainate (Petit et al., 1989). Whilst the increase in IMHV bursting around the time of training is entirely speculative, the Experiment in Chapter 3 indicated that bursting is elevated in the IMHV during the period 3-4hr and 5-7hr posttest. As will be discussed later, similar increases in bursting are seen in the LPO

4-7hr posttest (Chapter 4). If similar biochemical processes to those described above occur during these periods in IMHV and LPO, then this may be the posttest period in which the increase in spine density is initiated. However, as mentioned previously, it is unknown whether (a) bursting is produced by the dendrites/axons of single cells and (b) whether these presumed bursting neurons are of the same class as those shown to undergo spine density changes, that is, large multi-polar projection neurons. Further experiments in which neurons in IMHV and LPO are recorded from intracellularly and then filled with horseradish peroxidase should provide the answers to these questions.

Which area(s) of neuronal membrane are responsible for bursting in the IMHV and LPO? Bursting in both of these structures seems to occur in a time-locked fashion with negative shifts in the focal EEG activity. From morphological investigations it appears that the IMHV and the LPO are homogeneous structures with no clear layering of cell processes as, for instance, is seen in the hippocampus. It can be inferred, therefore, that when multi-unit recordings are made from IMHV and LPO, the chances are that the electrode tip will be predominantly surrounded with, and electrically affected by, dendrites. If this is the case, then these negative shifts in potential are occurring predominantly in dendritic fields. This, together with the fact that bursts may occur with a spike frequency of over 1000Hz (pers. observation), approaching the absolute refractory period for action potential generation, suggests that bursting may be of dendritic origin. However, it must be emphasised that there is no direct proof for the presence of areas of active dendritic membrane in the IMHV or the LPO of the chick. Until recordings can be made from identified dendritic regions of neurons in these areas of the chick brain the source of bursting discharges remains unresolved.

Bursting has also been associated with other models of memory formation. Buzsaki (1989) has proposed a two-stage model for memory formation in which theta activity and sharp-wave associated bursting play prominent roles. This model was briefly

outlined in Chapter 3 and will be more fully discussed here. During the first stage of the model, information is acquired and stored in a relatively labile form during exploratory behaviour (theta behaviours). In terms of hippocampal activity, it is during this period that CA3 pyramidal cells receive converging excitatory input from fast-firing granule cells. This excitatory input transiently potentiates synaptic connections between granule cells and CA3 pyramidal cells. During the second stage of the model this labile trace is then converted into a long-lasting form. This is suggested to occur at the termination of exploration, when sharp-wave associated bursting occurs in CA3 at the termination of theta. This synchronous bursting induces a long-term enhancement of synaptic efficacy in CA3 neurons and in some of their CA1 target neurons. Specificity of information is proposed to be ensured by the weakly potentiated CA3 cells (stage one) becoming initiator neurons of the subsequent sharp-wave associated bursts (stage two). Buzsaki (1989) hypothesizes that these CA3 initiator cells receive maximum convergence of excitation during sharp-wave associated bursting. This is proposed to occur by a reverberatory action; initiator CA3 cells fire, triggering the sharp-wave burst, which causes less excitable cells to fire. As the population burst spreads, the maximum effect of the reverberatory activity converges on the initiator cells. In essence, the 'function' of the second stage of the model is to select a group of neurons (burst-initiators) and to strengthen the synaptic connections between them. Recall will occur if the same subset of cells are reactivated.

A similar scenario in which groups of neurons (functional units) are selected forms the basis for a theory of cortical function developed by Edelman (1987). Edelman's 'group selection' theory proposes that: (1) specific intrinsic and extrinsic cortical connections, developed during ontogeny, provide the basis for the selection of neurons into isofunctional groups, or units; (2) these units provide the basis for neuronal representation (coding of information) and that different units interact with each other; and (3) repeated activation of a cortical unit results in alterations of the

efficacy of synaptic interactions between members of that unit, such that the probability of the selection of the unit would be increased relative to that of other units.

An alternative hypothesis has been proposed by Abeles (1988). According to this hypothesis, information is coded not by the firing rates of neurons but rather by the combination of neurons that fire in synchrony. The recruitment of neurons into a circuit does not involve the sequential activation of single cells, but results from the synchronized firing of groups of neurons. The chain of neurons that fires in synchrony (what Abeles calls a 'synfire chain') transfers information by activating a second synfire chain, which in turn excites another chain and so on. The process of recruiting neurons into a synfire chain is dynamic, that is, at different times a single cell may participate in different synfire chains. Statistical analyses (Abeles, 1982) have shown that neurons are activated 10 times more efficiently by synchronous presynaptic firing, compared to postsynaptic integration of asynchronous inputs. The former of these might be represented as synchronous, perhaps bursting dendritic spikes at a number of different dendritic loci. Such bursting may act to consolidate the connections between cells in the synfire chain by potentiating groups of dendritic synapses. As such, bursting can be envisaged as an extremely efficient means of communication between cells. Other evidence to support the role of bursting in the formation of new functional connections between cells has come from a study by Miles and Wong (1987). In this study pairs of burst firing CA3 pyramidal cells were simultaneously recorded from in a hippocampal slice preparation. After tetanic stimulation of the mossy fibre input to CA3, polysynaptic excitatory pathways between previously unconnected cells became apparent. The efficacy of recurrent inhibitory circuits was also reduced after tetanization. This treatment led to the formation of groups of synaptically associated cells, seen as an enhanced synchronous firing of groups of CA3 cells, and the ability of some cells to initiate synchronous firing in a larger group of cells through recurrent pathways. This is consistent with the

hypotheses presented above in relation to group selection of neurons.

Hoffman and Haberly (1989) have also shown that long-term electrophysiological changes can be produced by bouts of bursting activity. This work was conducted in slices of olfactory cortex. Bursting was induced by either removing extracellular magnesium, or decreasing the extracellular chloride concentration. Upon return to normal artificial cerebrospinal fluid, afferent stimulation induced a new long-lasting, high-amplitude depolarizing potential that followed the normal graded postsynaptic potential. The authors suggest that this late potential is the result of enhanced synchronous activity amongst a large number of cells.

The selection of groups of neurons has also been associated with neural network models of memory formation. Whilst a review of neural network research is beyond the scope of this Thesis, some recent work is quite relevant to the results presented in earlier Chapters. The operation of neural networks can be thought of as a parallel action of neurons, or classes of neurons, each with potentially different output/input relationships and intrinsic capabilities, interconnected by synapses with complex properties. For any given set of inputs to a neural network, cells are thought to fire in a cyclical manner. If a given configuration of firing neurons has repeated itself once, it will repeat itself indefinitely, as the very same postsynaptic potentials are generated. In other words, the dynamics of the network can lead from some initial configuration (the initial network state), to an attractor state at which the activity of the network remains (essentially a stationary, or fixed state). Amit (1989) has postulated that elementary cognitive phenomena (such as memory retrieval and recognition) are represented by patterns of activity in large networks of neurons, containing 10^3 to 10^4 cells, and that these patterns are fixed point attractor states. It may be of some significance that Amit (1989) proposes that when a network reaches an attractor state, some neurons will be firing maximally (in bursts), whilst other neurons are silent. A pattern (memory) is recalled if, under the influence of external stimuli, the network drifts rapidly into an

attractor such as a fixed point. However, although the process by which such attractors can provide associative memory recall appears to be well founded, the means by which memories are learnt and stored as patterns of altered synaptic efficacies between cells in the networks is unclear. Amit (1989) proposes that learning may occur in a network through a process that appears to be similar to that proposed by Buzsaki (1989) for hippocampal pyramidal cells, as described earlier. According to Amit (1989), 'training' starts with a network that has random connections. There is then a persistent imposition of an external stimulus into a subset of the neurons in the network, constraining that subset into either active or inactive states (similar to the 'stage one' dentate input to CA3 proposed by Buzsaki). The rest of the neurons are left to their natural threshold dynamics. In other words, the network is set in a random configuration and allowed to vary according to usual neural dynamics, with the fields of the training stimulus added to the afferent network postsynaptic potentials. This is continued until the neural system enters an attractor; a fixed point. Recognition is a rapid convergence into an attractor when the same stimulus is presented at a future occasion. This transition to a fixed point may be similar to the 'stage two' synchronized, reverberatory activity proposed by Buzsaki (1989) to occur amongst groups of CA3 cells and their target cells in CA1 at the termination of theta-associated activity.

Gray and Singer (1989) have proposed that local neuronal populations in the (visual) cortex engage in stimulus-specific synchronous oscillations resulting from an intracortical mechanism. They measured multi-unit activity and local field potentials from areas 17 and 18 of the cat visual cortex and discovered a transient, rhythmic firing of neurons when they were presented with their preferred stimulus. It is of some interest that these neurons fired in bursts of spikes, with each burst closely correlated with a negative shift in field potential. This negative field potential was thought to represent the activity of a larger group of neurons than that sampled by the multi-unit

electrodes. Because of of the close correlation between multi-unit activity and field potential, Gray and Singer (1989) postulate that the field potential must reflect the synchronous activity of a population of cells. This close correlation between multi-unit activity and field potential is also seen in the IMHV of the chick (Fig. 3:2). Gray et al. (1989) have suggested that the synchronous activity of neuronal populations in the visual cortex may serve as a mechanism for the extraction and representation of global and coherent features of a pattern. They further suggest that these synchronous responses may have a more general function in cortical processing by acting to establish cell assemblies that are characterized by the phase and frequency of their coherent oscillations.

To summarize the above, it appears that memories may be represented in the brain as certain patterns of connections between groups of neurons. Patterns of connections may be formed/modified by an increased level of synchronized neuronal bursting within and between such networks of cells. This highly synchronized activity may best be seen in local field potentials (Gray & Singer, 1989). This raises the possibility that the increased level of bursting seen in the IMHV of M chicks after training may represent the emergence/modification of neuronal groups (attractors?). Increased bursting may represent some consolidation of long-term memory for passive avoidance training, via the strengthening of connections between member cells of such networks. As was discussed in the General Introduction, bursting appears to be the most favourable of firing modes for the induction of LTP, especially when bursts occur at a theta periodicity (Douglas, 1977; Staubli & Lynch, 1987; Diamond et al., 1988; 1990). When burst rates are highest in IMHV, during the period 6-7hr posttest, groups of bursts tend to occur with a between-burst interval that falls within the theta range (Table 3.11), suggesting that a similar mechanism of potentiation may be occurring in the IMHV. During this posttest period, bursting appears to be highly synchronized within the IMHV, in particular the IMHV of the right hemisphere (Fig.

3.10). The groups of IMHV bursts mentioned above appear to occur with quite a regular oscillation of about 2secs, as shown by the interburst interval histograms of Figure 3.10. It is important to emphasize that these histograms represent the accumulated bursting activity from different depths within the IMHV, that is, it appears that the bursting activity of the right IMHV during the period 6-7hr posttest is highly synchronous throughout the entire vertical electrode penetration. This proposed synchrony of bursting in IMHV is supported by the burst-associated large negative shifts in field, which may represent the synchronous activation of large numbers of bursting cells in the IMHV. In addition to some within-IMHV consolidating role, the increased level of bursting in IMHV may also serve to 'output' locally processed information regarding memory for the task to other areas of the brain. This may lead to both the formation of new associations and also the consolidation of previous associations with respect to different components of the task.

From the 2-DG study of Rose and Csillag (1985) two areas of the chick brain were seen to exhibit a significant increase in neuronal activity following training on a passive avoidance task; the IMHV and the LPO. The effect of training on the spontaneous activity of the IMHV suggested that a similar training-induced increase in spontaneous activity might also occur in the LPO. Chapter 4 described such an investigation into the spontaneous multi-unit activity of the LPO after training. Recordings were taken from M and W chicks over the period 1-10hr posttest. The results indicated that the LPO of M chicks displayed a significantly higher burst rate when compared to the LPO of W chicks. Although this increase in LPO bursting in M chicks exhibited no lateralization, that is, it could only be seen as an overall increase, there was a trend for the LPO of individual hemispheres of M chicks to exhibit higher mean bursting rates when compared to the respective hemispheres of W chicks. When bursting levels were described as a timecourse, a significant elevation in LPO bursting was seen to occur during the period 4-7hr posttest; precisely the same time as the posttest increase in IMHV bursting. Bursts recorded from the LPO during the 4-7hr

posttest period were also seen to occur in similar patterns to those seen for bursts in the right IMHV, that is, groups of bursts with a between-burst interval within the theta range. The between-group interval for bursts in LPO during the 4-7hr posttest period was also similar to that for bursts in right IMHV during the 6-7hr period. This between-group patterning was most evident in the burst autocorrelelograms. These results suggest that when levels of bursting are maximal in the LPO and the right IMHV the patterns of bursts are very similar. From the above discussion for IMHV bursting, it follows that similar mechanisms may be controlling the generation of bursting in the LPO. In other words, large populations of cells in the LPO and right IMHV appear to be synchronously active during the same posttest period. This suggests that there may be a functional, if not anatomical, connection between the IMHV and the LPO. It also suggests the emergence/consolidation of neuronal groups both within and between the two structures.

Chapter 5 described an Experiment which examined whether the increase in LPO bursting seen in M chicks was a direct correspondent of memory formation for the task. In this Experiment chicks were trained using a methylanthranilate coated bead. Chicks that pecked the bead and showed a disgust response were then given subconvulsive ECS 5min after training. When these chicks were tested one hour later they could be split into two groups, based upon their behaviour at test; a group that remembered the task (avoided the bead) and a group that forgot the task (pecked the bead). As both groups had been subjected to similar experiences of housing and training etc., the only difference between the groups was the presence, or absence of memory for the task. Multi-unit recordings were then made from the LPOs of these chicks. Analysis of the mean levels of bursting between these two groups of chicks demonstrated that the group that avoided at test exhibited a significantly higher mean level of bursting. This suggested that increased levels of LPO bursting were directly associated with memory for the task, that is, they were not due to aspects of the task

non-specific to memory formation such as the taste or the smell of the bead etc.

When levels of LPO bursting between groups of chicks that either remembered or forgot the task were compared as a timecourse the increased bursting in the group that remembered the task was predominantly confined to the 4-7hr posttest period. This time-dependent increase in bursting is similar to that for training-induced bursting from both the LPO (Chapter 4) and the IMHV (Chapter 3) of non-electroshocked chicks. This provides more evidence to suggest that bursting during the 4-7hr posttest period in both the IMHV and the LPO is crucial to learning of the task and that the neuronal activity in/between these two areas may be subject to some form of mutual control.

As suggested in the General Conclusion of Chapter 6, memory for passive avoidance training may exist as a 'structural engram', produced by the potentiation of connections both between and within certain areas of the chick forebrain. These areas may include the IMHV and the LPO. During training, the chick visually orients to the bead. Such behaviour has been shown to induce EEG within the theta frequency range in the hyperstriatum. The left IMHV has been shown to be important for the learning and/or early processing of information for the task (Davies et al., 1988; Patterson et al., 1990a). It is possible, therefore, that during orientation to the bead certain cell(s) within the left IMHV (and LPO?) are excited, and their connections transiently potentiated. This may occur in a similar manner to which CA3 cells and their CA1 targets are excited in the first stage of Buzsaki's (1989) two-stage model. At the termination of theta-behaviour (after pecking?) the potentiated cells in the left IMHV become the initiator cells for population, sharp-wave associated bursting. This may involve a mechanism similar to that suggested to occur during stage two of Buzsaki's (1989) model, that is, excitatory reverberatory activity may then ensue in the IMHV such that the initiator cells receive maximal excitation. Possible reverberatory activity

has been seen in stimulus-evoked field potentials in the left IMHV (Mason & Gigg, in prep.). Such activity may then cause the emergence of groups of interconnected, potentiated cells in the left IMHV. This increased activity within the left IMHV may also aid the enhancement of synaptic bonds in target structures of the left IMHV. These targets may include the right IMHV and the LPO, amongst others. The enhancement of connections in the LPO may be crucial, as the LPO appears to be a necessary store of memory for the task (Gilbert et al., in press).

Assuming that sharp-wave associated bursting underlies the formation of neuronal groups responsible for the storage of memory traces, then incidences of sharp-wave associated bursting subsequent to the task should enhance the retention of memory (Buzsaki, 1989). This is possibly what is occurring during the period 4-7hr posttest in the IMHV and the LPO of methyl-trained chicks. Synchronized, field potential-associated bursting may enhance the connections both within and between burst initiator cells of the IMHV and LPO. How might such a 'faithful' communication between areas such as IMHV and LPO come about? Evidence to demonstrate that synchronized activity may occur between widely separated groups of cells has come from one of the studies described earlier, that of Gray et al. (1989). In this study neurons in primary visual cortex that were separated by as much as 7mm were seen to fire synchronously, suggesting that large distances between cells does not hinder their synchronous activity. Miller (1989) has proposed that the emergence of connections both between and within networks of neurons may be under the control of temporal aspects of connectivity, that is, axonal conduction delays. He proposes that loops of connections which carry neural activity resonating at theta rhythm can become preferentially selected. This might occur by means of Hebbian processes of synaptic modification that act within loops of connections that exhibit a variety of axonal conduction delay times. This would provide a means by which connections between different cortical structures could become strengthened. If, as described above, the firing of cells in the left IMHV becomes synchronous after training, then this may

provide the means for the emergence of one or more local cell assemblies within the left IMHV. This synchronous activation, which may exhibit a similar theta periodicity to that seen in the right IMHV 6-7hr posttest and LPO 4-7hr posttest, could provide for the enhancement of reciprocal synaptic connections between the left IMHV and its target structures. These connections, which take the form of a variety of long delay lines, may become established as follows. Assume that a regular cycle of rhythmic neural activity is imposed at one end of such a series of loops, in this case synchronous bursting in the left IMHV. For those pathways with a total loop time that approximates the period of the imposed rhythm, signals generated by one 'beat' of the oscillator (left IMHV) will return to the oscillator in time for the next beat. This is exactly the circumstance in which the Hebbian mechanism is recruited, that is, cells will fire action potentials at the same time as they receive return excitation (coincident pre and postsynaptic activation). If Hebbian mechanisms do operate at both ends of the loop, then the synaptic modifications that occur will tend to strengthen only those connections which form loops whose delay times approximate the oscillator period.This process of 'phase-locked loop' conduction allows for the emergence of 'global cell assemblies'. Such a 'global' structure may be resistant to the posttraining removal of certain constituent parts, e.g., the IMHV. Long-term consolidation of memory in such an assembly could be induced by posttraining periods during which the pattern of connections is 'reactivated'. This proposal is similar to that of Buzsaki (1989) in his description of a two-phase model of memory formation. Bouts of synchronized, sharp-wave associated bursting that occur after an experiential event would tend to aid the 'hard-wiring' of connections between groups of burst-initiator cells, thereby consolidating the connections between cells in the assembly. Such a consolidation of connections may be occurring between the IMHV and LPO during the 4-7hr posttest period via an increase in the frequency and synchrony of burst-firing.

As suggested in Chapter 6, an alternative hypothesis to the 'global' representation of

memory is that different parts of the representation may be sequentially registered. For example, the IMHV may initially be important for the storage of visual aspects of the task, e.g., colour of the bead, whilst the LPO may be important for the later registration of more emotive task aspects. If this is the case, then the 'function' of increased bursting during the 4-7hr posttest period may be to 'code' for a certain aspect of the task.

Another possible means by which memory for passive avoidance training may be stored in the chick forebrain has been proposed on the basis of lesion studies (Patterson et al., 1990a; Gilbert et al., in press). According to this proposal memory for the task transfers between the IMHV and the LPO. The left IMHV has been shown to be necessary for the acquisition and/or early processing of memory for the task (Patterson et al., 1990a). However, neither the left nor the right IMHV is necessary for longer-term storage of memory for the task. Is memory stored elsewhere in the forebrain? Bilateral posttraining LPO lesions have demonstrated that the LPO may be such a long-term store as this procedure is amnestic (Gilbert et al., in press). However, bilateral pretraining lesions of the LPO are non-amnestic. Does the IMHV 'become' a necessary store for memory in the absence of the LPO at training? It appears that this is the case. Pretraining bilateral lesions of the LPO together with a posttraining lesion of the right (but not the left) IMHV are amnestic, suggesting that the right IMHV becomes a necessary store for memory in the absence of the LPO at training (Gilbert et al., in press). This sequence of experiments led the authors to suggest the following: memory is initially stored in the left IMHV. Memory then transfers to the right IMHV. From the right IMHV memory then transfers to the left and right LPO for long-term storage. All posttraining lesions were made 1hr posttraining. Therefore, the transfer of memory is assumed to occur during the first hour posttraining. Do the bursting data agree with such a memory transfer proposal? Although the activity of the IMHV and LPO has not been sampled during the first hour

posttraining, the period assigned to the proposed transfer, the bursting data during the 4-7hr posttest period does 'fit in' to a certain degree with the idea of memory transference. According to the model, the establishment of long-term memory requires the combined activities of the right IMHV together with the left and right LPO. From the preceding discussion it appears that the most efficient way in which this memory flow could occur is by an increase in the frequency and synchronicity of burst firing in/between these structures. This is precisely what can be seen during the period 4-7hr posttest; bursting is increased in the right IMHV and both LPOs. This suggests that these bursting increases may reflect the consolidation/'read out' of memory for the task.

The capability of the right IMHV in LPO-lesioned chicks to become a necessary structure for memory suggested that this changed capacity might be reflected in its spontaneous neural activity. Chapter 6 described an Experiment designed to investigate the effects of pretraining bilateral LPO lesions on posttest IMHV bursting levels. On the day before training two groups of chicks were prepared; one group received bilateral radiofrequency LPO lesions, the other group received bilateral sham lesions. Sham operated chicks underwent identical surgical procedures to the lesioned chicks with the exception that the lesioning current was not turned on when the lesion electrode was in place. On the day of training each of these groups was split into two. One half was methyl-trained, the other water-trained. This training procedure generated a final total of four groups of chicks; sham M and W chicks and lesioned M and W chicks. The spontaneous activity of the IMHV over the period 1-10hr posttest was recorded in chicks from all four of the above groups.

The results from this experiment were quite surprising. Methyl-trained sham-lesioned chicks showed a significant increase in mean bursting levels over sham-lesioned water-trained chicks, which suggested that sham operated chicks were good controls for the surgical and anaesthetic procedures associated with the lesion protocol.

However, there was no significant difference in bursting levels between lesioned M chicks and lesioned W chicks. By examining the overall bursting levels for all four groups, it appeared that bursting levels for lesioned M and sham M chicks were similar. One possible reason for the lack of significant difference between lesioned M and W groups appeared to be that that the overall bursting level for lesioned W chicks was raised in comparison to that for sham W chicks. Previous work has showed that chicks learn and remember pecking a dry (non-reinforcing) bead (Barber et al., 1990). In the absence of the LPO at training, an increased demand for memory storage may be placed on remaining structures of the brain such as the IMHV. If, as seems likely, IMHV and LPO bursting is associated with memory for pecking a methylanthranilate-coated bead, then in the absence of the LPO at training, such an increased demand may be seen as an increase in bursting in the IMHV.

Another interpretation of this data is suggested from the above memory transference proposal. According to the model, if the LPO is absent at training, memory for the task is first registered in the left IMHV and then passes to the right IMHV. Because the LPO has been lesioned memory cannot pass out of the right IMHV, which now becomes a necessary store for memory. According to the model this process occurs during the first hour posttraining. It seems likely that the increased activities of the LPO and right IMHV 4-7hr posttest are associated with long-term memory consolidation. It is not surprising, therefore, that in chicks with pretraining bilateral LPO lesions there is no increased bursting activity in the IMHV during the period 1-10hr posttest: there is no LPO to receive any information output from the right IMHV. Long-term memory formation has occurred in the right IMHV well before this posttest period. According to this, the lack of increased bursting posttest in the IMHV of the pretraining LPO-lesioned chick supports the memory transference model.

As will be discussed below, the LPO may be involved in circuits controlling motor

behaviour. These circuits may be especially involved with the inhibition of ballistic head movements such as pecking. When chicks with LPO lesions are housed in pens they appear to be far less vocal when compared to non-operated chicks, suggesting that these lesions may in some way reduce the amount of subjective stress (fear?) to environmental changes (personal observation). The general pecking behaviour of chicks also appears to be affected by LPO lesions; lesioned chicks will peck far more readily compared to non-operated chicks, which may explain why these lesioned chicks are 'easier to train', that is, peck more readily at training (Patterson, pers. comm.). It appears, therefore, that LPO lesions affect the general behaviour of chicks, perhaps through some disinhibition of pecking. As stated above, chicks with pretraining LPO lesions can still learn a passive avoidance task, however, the LPO is a necessary structure for memory posttraining (Gilbert et al., in press). If the LPO is involved in memory for the task via some role in the inhibition of pecking, then the rise in bursting in the IMHV and LPO of non-operated chicks 4-7hr posttest may represent some consolidation of a neuronal circuit responsible for the association of the chrome training bead with inhibition of pecking. In the absence of the LPO at training, bursting levels in M chicks are very low during the period 4-7hr posttest; the posttest period during which maximal bursting in M unoperated M chicks is evident. If the LPO and IMHV are indeed involved in the representation of memory for the task, then the absence of an increase in IMHV bursting during the period 4-7hr posttest may indicate that the representation of the memory trace is different in lesioned chicks.

What might be the functions of the LPO and the IMHV? The IMHV is thought to have a role as a polysensory processing centre, possibly analogous to mammalian association cortex, as it receives information from a number of sources including the visual, auditory and somatosensory systems (Horn, 1985; Bradley et al., 1985). The LPO may form part of the avian olfactory system, as primary olfactory afferents project to the LPO from the olfactory bulb (Reike & Wenzel, 1978). As indicated

above, the LPO has also been associated with motor control, as it appears to form part of the paleostriatal complex, the avian equivalent of the mammalian basal ganglia (Karten & Dubbeldam, 1973).

Certain pathways between the IMHV and LPO that involve intermediary brain structures have been identified (Figure 7.1). Boxer and Csillag (1986, chick) have shown that the LPO receives afferent projections from four brain regions: the archistriatum; the nucleus superficialis parvocellularis; the area ventralis tegmentalis of Tsai (AVT); and the nucleus tegmenti pedunculo-pontinus (TP). Projections from the archistriatum to the LPO have recently been confirmed in the chick with the use of lectin tracing (Stewart & Csillag, pers. comm.). Kitt and Brauth (1981) have described afferent connections from the LPO and PA to the AVT and TP, which together with the results of Boxer and Csillag (1986) suggests that the PA and LPO are reciprocally connected to these structures: Kitt and Brauth (1986) consider the AVT and TP to be similar to the A10 and A9 components of the mammalian nigral complex. As the archistriatum of the chick is reciprocally connected to the IMHV (Bradley et al., 1985), it is in a position to influence the activities of neurons in both the IMHV and the LPO via its efferent projections.

The HV of the mallard duck projects to distinct regions of the neostriatum: the dorsal, ventral, and lateral portions of the neostriatum frontale (Dubbeldam & Visser, 1987). The dorsal portion of the neostriatum frontale projects to both the archistriatum and the LPO. Bradley et al. (1985) have demonstrated a projection from the neostriatum to the IMHV. From this, it appears that (at least) two regions of the avian brain (the neostriatum and the archistriatum) send afferent projections to both the IMHV and the LPO. This provides two candidate brain regions for the role of synchronizing the activities of the IMHV and the LPO. Another candidate is the PA, which is reciprocally connected to the IMHV (Bradley et al., 1985). However, there is no clear morphological boundary between the PA and the LPO, which makes it is difficult to

Fig 7.1; Schematic Diagram to Show Some of the Connections Between the HV and the LPO.

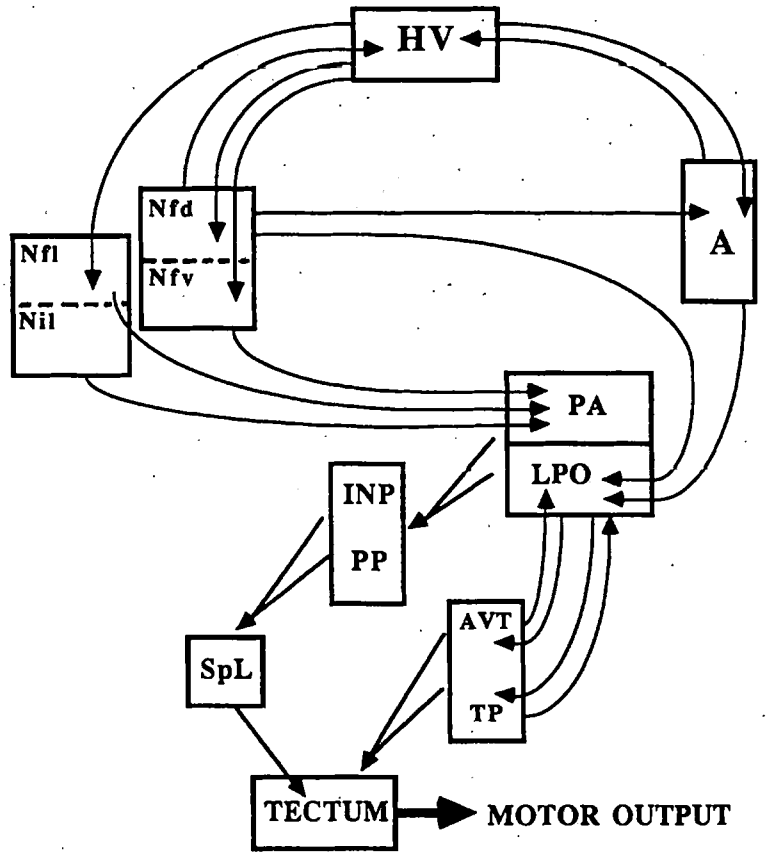


Figure 7.1. Diagram to show some of the principal connections between the HV and the LPO in relation to tectal motor output. By the nature of their connections with the LPO and HV, two areas of the chick forebrain emerge as candidate regions with the ability to control and possibly synchronize the activities of the IMHV and the LPO. These candidate areas are the neostriatum and the archistriatum. For simplicity, the reciprocal connection between the HV and the PA is not shown. Abbreviations: A, archistriatum; Nfl, Nfd and Nfv are the lateral, dorsal and ventral aspects of the neostriatum frontale; Nil, lateral aspect of the neostriatum pars intermedia. Other abbreviations are included in the text. The diagram is based upon data from the following publications: Boxer and Csillag (1986); Bradley et al. (1985); Dubbeldam and Visser (1987); Kitt and Brauth (1981; 1986) and the review by Reiner et al. (1984a).

perform tracing studies between the two areas (Stewart, pers. comm.). Nevertheless, it seems likely that the PA and LPO are anatomically connected.

As mentioned above, the LPO may have a role in the control of motor activity. The IMHV has reciprocal projections with the PA (Bradley et al., 1985). The PA and LPO together comprise the avian striatum, which sends projections to the avian pallidum (Reiner et al., 1984b). Karten and Dubbeldam (1973) were able to show a projection from the avian pallidum (paleostriatum primitivum and nucleus intrapeduncularis), via the lateral forebrain bundle to the nucleus spiriformis lateralis (SpL). Reiner et al., (1984a) suggest that the SpL may represent the major projection target by which the basal ganglia influence motor functions. The SpL has only one afferent projection, which terminates in the those tectal layers that produce the afferent projections of the tectum. This suggests that the tectal input from SpL may modulate the activity of tectal neurons. These neurons in turn affect the activity of brainstem and spinal motor neurons thought to be responsible for visuo-motor integrative functions. Reiner et al. (1982) have provided evidence to support this theory. They showed that pigeons with bilateral SpL lesions were severely impaired in the initiation and execution of tasks involving motor responses to objects moving in their visual space. On the basis of this, Reiner et al. (1984a) suggest that the avian tectum is likely to be involved in the initiation and control of ballistic head movements such as pecking and general orientation, approach and avoidance movements. This suggests that there is an information pathway from the sensory/integrative areas of the avian forebrain, including IMHV, via the avian basal ganglia (PA, PP, INP and LPO) to motor regions that have been shown to be involved in the initiation and execution of pecking responses to visually conspicuous objects such as those used in passive avoidance training. This suggests that the increased neural activity seen in the IMHV and LPO after training may represent the establishment and/or consolidation of an inhibitory tectal input to prevent pecking of the aversive bead.

Perhaps the biggest criticism of the experimental work discussed in this Thesis is that the data has come from an anaesthetized preparation. It is unknown whether the training-induced effects described in this Thesis occur in the awake, freely behaving chick. Although pilot experiments carried out at the Open University by Dr T.A. Patterson and myself have shown that bursting can be recorded from the IMHV of the awake chick, technical problems have meant that no long-term recordings of behaving chicks have been possible. One of the hypotheses presented in the above General Discussion is that increased neuronal activity, perhaps of the bursting type, should occur in the IMHV and LPO around the time of training. As described in the General Introduction, changes in the firing of cells have been closely associated with presentations of training/conditioning stimuli. It is difficult to imagine how the biochemical and morphological consequences of memory for passive avoidance training could be produced without some initial increase in the firing activity of neurons during the acquisition of the learned response. One inhibitor of behavioural acquisition has proved to be 2APV. This NMDA receptor antagonist has been shown to prevent: (1) place-learning in rats (Morris et al., 1986); (2) fear-potentiation of the acoustic startle reflex in rats; and (3) passive avoidance training in chicks (Patterson et al., in prep.). In none of these cases did 2APV prevent the expression of learning, which suggests that the effect of 2APV was to block the acquisition of learning. As 2APV is a potent inhibitor of burst-firing in CA1 pyramidal cells, the amnestic effect of 2APV in the chick may be mediated by a suppression of bursting in areas such as IMHV around the time of training. The only means by which such an effect could be observed is in the awake, freely behaving chick. Whilst it is possible to record from anaesthetized chicks at times closer to training than used in the experiments reported here, it would be difficult to interpret the results. This is because there is a 30min posttraining period during which anaesthesia is itself amnestic (Patterson, pers. comm.).

Another criticism of the experimental work in this Thesis was levelled at the beginning

of the General Discussion. Extremely little is known regarding the functional neuronal connectivity both between and within chick brain nuclei. This has meant that the results presented throughout this thesis have been constantly compared to mammalian studies in which far more is known regarding neuronal physiology and morphology/functional anatomy. Further neurophysiological studies in the chick should, therefore, attempt to define the mechanisms of burst generation in both the IMHV and the LPO. Specifically, the neurotransmitter substances and receptor/ion channel complexes responsible for spontaneous burst generation/modulation in these two regions should be elucidated. Another important investigation would be to identify the neuroanatomical areas of the chick brain that control burst generation in the IMHV and LPO via the transmitter/receptor systems implied above. One of the implications of the results presented in this Thesis is that the IMHV and LPO are functionally connected. Simultaneous multi-unit recordings from various sites within both the IMHV and LPO, in tandem with evoked potential recording, will clarify the existence of this proposed functional connection. From the results of Chapters 3 and 4 bursting appeared to be occurring in a synchronous fashion throughout both the LPO and the IMHV. Experiments using multi-electrode arrays, arranged both vertically and horizontally, would allow spontaneous recordings to be taken simultaneously from a number of different sites within either structure to further examine this synchronous activity.

Another important consideration is the very nature of the training effect on bursting. Do other learning paradigms produce similar patterns of burst firing in the IMHV and LPO, or is increased bursting a phenomenon restricted to passive avoidance learning? One way to test whether bursting in the chick forebrain has a more general role in memory formation would be to record from the IMHV and LPO of chicks trained on the sickness-aversion task developed by Alistair Barber (Barber et al., 1990). Is the association between induced sickness and the prior pecking of the dry bead evident in

increased bursting in these two structures? Once again, the ideal means of recording in this instance would be from the awake chick.

In a final summary, the experiments described in this Thesis have shown that training chicks on a one-trial passive avoidance task produces a time-dependent, focal elevation of neuronal bursting in two regions of the chick forebrain; the IMHV and the LPO. The increased IMHV bursting is lateralized to the right hemisphere during the period 6-7hr posttest. The increased bursting in IMHV has been shown to be directly associated with memory for the task (Mason & Rose, 1988), as has the increased LPO bursting in Chapter 5 of this Thesis. The peak of bursting in these two structures occurs during the same posttest period; 4-7hr posttest. During this posttest period bursting in both structures becomes increasingly synchronous. It can be suggested from this that (a) the IMHV and LPO are functionally connected and that (b) the synchronous activity in the IMHV and LPO may reflect the emergence/consolidation of neuronal cell assemblies in/between these two structures that are fundamental to the formation of long-term memory for the task. Further experiments, including the recording of multi-unit activity from the awake, freely behaving chick, should provide clear evidence as to both the validity of these proposals and also the dynamics of bursting in the behaving animal.

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Appendix.

```
'Demonstration of bursts and derived data from bursts
'Author Greg Smith 24/Apr/88
'This will run on the file DEMO.SMR or any sensible event file

'Variables used
'a      Accumulated time in bursts
'b      data channel used to hold burst times
'c      Number of bursts found
'f      Printer output device
'm      Total events in the bursts in time range
'n      Total events in the time range
'p      Number of bursts with a spike following in analysis period
'q      Total of times of spikes after bursts
'r      Total inter-burst time
't      Start of time period to be analysed for bursts
'u      End of time period to be analysed
' Others are used as temporary variables

'Other variables used as work space
CLEAR
VIEW 1; NORMAL                  'In case user has been using other script
WINDOW 0 0 100 50-CHARH         'set display window for time data
DRAW 0 Maxtime
Spikedat
v:=0;                           'This is used as our exit flag
PRINTTO "BSTATS.PRN"            'set name of output print file
f:=0; PRINTTO f                 'send printer output to the screen
FKEY 0                          'Initialise function keys
FKEY 1 2 DoBurst "Burst"        'Set function key definitions
FKEY 1 3 BSTATS  "Stats"
FKEY 1 4 DoPrint "CmdLine"
FKEY 1 5 DoFile "NewFile"
FKEY 1 6 Spikedat "Sparams"
FKEY 1 7 BstIntH "IntBst"
FKEY 1 8 AUTO  "AutoCr"
FKEY 1 10 Stop "Quit"
REPEAT
  FKEY 5                        'do what the user says
UNTIL v                         'until they choose F10:Quit
END                             'Replace with QUIT to exit SPIKE2

PROC DoPRINT    'Choose which output device to use
DOCASE
  CASE f=0
    FKEY 1 4 DoPrint "Screen";f:=1
  CASE f=1
    FKEY 1 4 DoPrint "LPT1:"; f:=2
  CASE f=2
    FKEY 1 4 DoPrint "File"; f:=3
  CASE f=3
    FKEY 1 4 DoPrint "CmdLine"; f:=0
ENDCASE
PRINTTO f
RETURN

PROC Spikedat
INPUT h "How many spikes make up a burst ?" 2 15
INPUT k "ISI to constitute beginning of new burst (sec)" .001 5
INPUT g "Max. interval between spikes to make a burst (sec)".0009 k
DoBurst                         'do initial burst analysis
RETURN
```

```
PROC Stop       'set the stop flag
v:=1                          'simply set the flag to request stop
RETURN                        'and return to the caller

PROC DoBURST    'Do a simple-minded burst analysis of data
CLEAR 1; NORMAL
WINDOW 0 0 100 50-CHARH    'set display window for time data
DRAW 0 MAXTIME            'show all the data

MOVETO 10 95; PRINT 1 "Intraburst ISI %8.5d (Sec)" g
MOVETO 60 95; PRINT 1 "Interburst time %8.5d (Sec)" k

  REPEAT
  INPUT e "Channel to analyse for bursts" 1 15
  IF ChanKind[e]<2              'must be an event channel
    Message "Channel %d is not event data" e
  ENDIF
  UNTIL ChanKind[e]>1

  REPEAT
  INPUT b "Channel to store bursts in" 1 15
  z:=1
  IF ChanKind[b]>0              'Warn user if channel is in use
    QUERY "That channel is already in use!" "Really overwrite?" "Y" z
  ENDIF
  UNTIL Z

VIEW 1
OFF 1 2 3 4 5 6 7 8 9
OFF 10 11 12 13 14 15
ON e b
DRAW

CURSORS 2;                    'show cursors to select area
INTERACT "Please select area to be analysed and press Enter"
DRAW c1 c2-c1
t:=c1; u:=c2                  'take cursors as analysis positions
MAKEBRST                      'now make up a burst
RETURN

PROC MAKEBRST   'Divide data into bursts based on time intervals
VIEW 1                        'back to the initial view
NEWEVENT 1 9000              'make space for bursts
y:=g; z:=k
BURSTS 0 e t u z y            'Make bursts based on times for demo file
IF EVENT                      'Any events made?
  NEWEVENT 5 b 1              'save as channel b
  ON b;DRAW t u-t             'display this as our data area
ELSE
  Message "That produced no bursts! Nothing saved"
ENDIF
RETURN

PROC NXTBST     'set x,y to start and end of next bursts after y or x=-1
REPEAT
  NEXTTIME b y y x            'find the start of next burst
UNTIL (y<0)|(x=0)             'until no times, or a burst start
IF y<0                        'if no more times
  x:=-1; RETURN               'flag that we failed
ENDIF
x:=y                          'set start time
NEXTTIME b x y                'get end time
IF y<0
  x:=-1                       'return no time if no end time
ENDIF
RETURN
```

```
PROC BSTNMORE
REPEAT
  NXTBST
  COUNT e x y d
UNTIL (d)=h)|(x<0)
IF d)=h
 NEWEVENT 2 x
ENDIF
RETURN

PROC BstIntH
CLEAR; VIEW 1; WINDOW 0 0 100 45; DRAW 0 MAXTIME
INPUT i "Input channel to produce Interburst Histogram" 1 15
ON i; VIEW 2; WINDOW 0 45 100 90; DRAW 0 MAXTIME
VIEW 1; SETINTH 2 i 100 0.1
VIEW 2; OFF TRAM; ON TITLE; MINMAX 1 0 MAXTIME-1 X Y
YRANGE 1 0 Y
VIEW 2; DRAW
RETURN

PROC AUTO
INPUT j "Channel to perform Auto Correlation on " 14
CLEAR; VIEW 1; WINDOW 0 0 100 45; DRAW 0 MAXTIME
VIEW 2; WINDOW 0 45 100 90; DRAW 0 MAXTIME
VIEW 1; SETCROSS 2 j 1000 0.01 5 1
VIEW 2; [498]:=0;[499]:=0; [500]:=0; [501]:=0; OFF TRAM; ON TITLE;
MINMAX 1 0 MAXTIME-1 x y
YRANGE 1 0 y
VIEW 2; DRAW
RETURN


PROC BSTATS     't=start time, u=end time, b=burst chan, e=event chan
COUNT e t u n                   'n=total number of events in range
p:=0; q:=0                      'init for post burst spike latency
r:=0                            'sum of times between bursts
m:=0                            'm=total events in bursts
a:=0                            'a=total burst time
f:=0
c:=0                            'c=number of bursts
y:=t                            'set start position
CLEAR
MOVETO 10 10
 NEWEVENT 0
 NEWEVENT 1 400
BSTNMORE                        'get the first burst of z num into x y
WHILE x>0
  c:=c+1                        'increment burst count
  COUNT e x y d                 'get number of events in the burst
  m:=m+d
  l:=(y-x);                     'length of the burst in seconds
  f:=f+(1/(l/d))                'increment total intraburst freq.
  a:=a+l                        'total time of bursts
  NEXTTIME e y o                'get next spike after burst
  IF o>0                        'check we have one
    o:=o-y                      'latency of next spike
    p:=p+1                      'increment count
    q:=q+o                      'increase sum of latencies
  ENDIF
```

```
  ' **** Delete next line if you don't want details of each burst
  o:=y                          'save old end of burst time
  BSTNMORE                      'get next burst times
  IF x>0                        'if not the end
    r:=r+(x-o)                  'add inter burst time

  ENDIF
WEND
  IF f=1                        'Is output to screen?
    CLEAR                       'if so, clear it for final information
    MOVETO 10 10                'move to near the top
  ENDIF
  INPUT q "Channel to store burst start times in"
  NEWEVENT 4 q 1
  ON 15
  PRINT ""
  PRINT "Start of analysis period                          :%d" t
  PRINT "End of analysis period                            :%d" u
  PRINT "Max. interval between spikes to constitute a burst :%d" g
  PRINT "ISI to constitute beginning of new burst          :%d" k
  PRINT "Number of spikes which make up a burst            :%d" h
  PRINT "Number of bursts found                            :%d" c
  IF c>0
    PRINT "% of spikes in bursts                 :%5.1d%" a/n*100
    PRINT "Mean spikes per burst                 :%d" a/c
    PRINT "Mean burst interspike                 :%d" a/(a-c)
    PRINT "Mean inter burst time                 :%d" r/c
    PRINT "Average interspike interval           :%d" f/c
    PRINT "Burst frequency (bursts per 2 min)    :%d" c/(u-t)*120
    PRINT ""
    PRINT ""
  ENDIF
f:=0
RETURN

PROC DoFile
FILE
DoBurst
RETURN
```