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LIMITATION OF EXERCISE PERFORMANCE BY ACTIVITY OF INTRACEREBRAL DOPAMINERGIC SYNSPSES

BY

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A Thesis

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STRIATAL DOPAMINE LIMITS EXERCISE CAPACITY

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ABSTRACT

Evidence from the literature suggests that a limiting factor to the performance of exhaustive exercise is the ability of the brain to maintain the stimulation of the alpha-motor neuron. This thesis examined the hypothesis that this 'central fatigue' results from a deficit in the transfer of information across striatal dopaminergic synapses. Decreases in striatal dopaminergic activity should impair exercise capacity whereas increases in dopaminergic activity should therefore improve exercise capacity. The rat was chosen as the experimental animal because of the availability of techniques to alter dopaminergic activity and to quantify exercise capacity. Striatal dopaminergic activity was reduced by depletion of striatal dopamine by an intracerebroventricular injection of 200 µg of 6-hydroxydopamine. Dopaminergic activity was increased by an intraperitoneal injection of the specific dopamine receptor agonist apomorphine. Exercise capacity was quantified with treadmill-grid system by measuring the time taken for a running rat to become exhausted. Striatal dopamine depletion decreased exercise capacity whereas apomorphine increased exercise capacity. In contrast, striatal and hypothalamic

norepinephrine depletion had no effect on exercise capacity.

The limitation of exercise capacity by the activity of striatal dopaminergic synapses was not due to a failure of these synapses to maintain the release of dopamine, but may be due to these synapses releasing amounts of dopamine which are insufficient to transmit all the information arriving at the presynaptic terminal to the postsynaptic neuron. I propose that the release of dopamine is limited in exhausted rats by the activitation of presynaptic dopamine receptors located on the terminal and that the purpose of these receptors is to maintain the concentration of dopamine in the functional pool of the terminal.

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CHAPTER 1.

HISTORICAL AND FUNCTIONAL PERSPECTIVE OF INFORMATION TRANSFER WITHIN THE CENTRAL NERVOUS SYSTEM.

The modern view of the brain is that of a processor of 'information' (Pinsker and Willis 1980). This model proposes that the brain is an organ which "...receives information, elaborates and perceives it, and makes decisions" (Kuffler and Nicholls 1976). Information can be described as that which increases the awareness or state of knowledge of the organism (Saltzberg 1963). This includes information from sense organs such as the eye, carotid chemoreceptors and muscle spindles which inform the brain of events in the external environment, the status and composition of the internal environment and body posture and movement. The brain also sends out information in the form of instructions; for example, to initiate muscle contractions and maintain a particular body posture.

MECHANISM OF INFORMATION TRANSFER IN THE NERVOUS SYSTEM.

Neurons.

Following the development of the microscope at the beginning of the Renaissance period, Leeuwenhoek, Nissl and Golgi discovered that the brain was composed of individual nerve cells (see Williams and Warwick 1975). Nerves have long been recognized as the message carriers in the brain but it was the pioneering work by Galvani, Volta, Muller, DuBois Reymond and Helmholtz (see Kandell and Schwartz 1981) which led to the concept of information transferred along nerve axons in the form of coded stereotyped electrical signals (action potentials).

Information is coded by three mechanisms. The intensity of the stimulus is coded for by the frequency of action potentials (pattern coding) and the number of neurons recruited by the stimulus. The modality of the signal is coded for by the destination of the signals (place coding).

Synapses

With the development of techniques to stain individual nerve cells (see Williams and Warick 1975), it became clear that neurons were not continuous but existed as separate units. Cajal and Golgi were the first to describe the structure of the neuron in detail and identify the site where neurons communicated with each other. Sherrington

referred to this site as the synapse. The synapse is the site where information is transferred from the nerve terminals of one neuron to the dendrites and cell body of the next (Kandell and Schwartz 1981). The question as to how information was transferred across the synapse sparked the now famous debate between Sir Henry Dale, a proponent of the chemical transmission hypothesis, and Sir John Eccles a proponent of the electrical transmission hypothesis. The model of chemical neurotransmission was derived from the study of peripheral synapses and was first suggested by Du Bois-Reymond in the mid-1880's. The argument was resolved in favour of the chemical transmission hypothesis by the work of Loewi, Brown and co-workers and Dale himself. But, there are examples of electrical synapses, for example in the retina.

In the chemical transmission model, action potentials arriving at the presynaptic terminal stimulate release of a chemical messenger molecule (neurotransmitter) from vesicles into the synaptic cleft. Over 30 such molecules have been identified in the brain (Iversen 1979). The amount of neurotransmitter released is proportional to the number of action potentials arriving at the terminal. In the cleft the neurotransmitter diffuses to the postsynaptic neuron binds to a specific receptor on the postsynaptic membrane and affects the ion conductance of small area of membrane

surrounding the receptor. Changes in membrane potential are conducted along the cell body and dendrites by the passive cable properties of the membrane and determine the membrane potential of the axon hillock. The axon hillock has the lowest threshold for the generation of an action potential. Therefore, temporal and spatial summation of the excitatory and inhibitory input at this site determines the firing frequency along the axon (Carpenter and Reese 1981). In this way neurotransmitters function to transfer information from one nerve cell to the next.

The axon hillock is also a site where information from one or more neurons is integrated. The presynaptic neuron may make many synaptic connections with the postsynaptic neuron and there may be thousands of different neurons making contact with the dendrites and cell body of any given neuron. The terminals of one neuron appear to release the same neurotransmitter. However, the different neurons making synapses with a given neuron may use different neurotransmitters: either excitatory or inhibitory (see Hokfelt, Johansson, Ljungdahl, Lundberg and Schultzberg 1980).

The mechanism of integration of information at the synaptic level is complicated (Redman 1976) by the fact that synaptic contacts are found over much of the area of the dendrites and cell body. The summation and intregration of

the different synaptic inputs and the changes in membrane potential at the axon hillock is determined by the site of action of the neurotransmitter relative to the distance from the axon hillock (spatial summation: which is dependant on the space constant and time constant of the membrane). In addition, because the effect of neurotransmitters on membrane potentials are transient in nature, interaction of neurotransmitters action is determined by how close to the changes in membrane potential due to one neurotransmitter are followed by changes in membrane potential due to the next neurotransmitter (temporal summation: determined by the time constant of the membrane).

This model of information transfer across chemical synapses has progressed still further by the finding that a number of molecules which are released from nerve terminals do not have direct effects on postsynaptic neurons but are able to alter the response of postsynaptic neurons to other neurotransmitter (for example TRF; see Carpenter and Reese 1981). The term 'neuromodulators' has been used to describe the action of these molecules.

'FATIGUE' OF INFORMATION TRANSFER WITHIN THE BRAIN?

One of the most obvious functions of the brain is to control movement and locomotion which enables the organism to search for food, a mate and escape enemies. Movement

requires the accurate, graded and coodinated contraction and relaxation of motor units in skeletal muscles. The activity of the alpha-motor neuron (Sherrington's 'final common pathway' in the control of muscle contration) is determined by the balance between the excitatory and inhibitory inputs from the brain itself and peripheral receptors such as the muscle spindles. A review of the neuroanatomical connections to the alpha-motor neuron and the control of its activity is beyond the scope of this review. Suffice to say that the control of movement demands acurate and rapid transfer of information from the brain to the muscles and feedback information concerning the actual movements performed, so that course corrections can be made.

It is common experience that muscular exercise cannot be performed indefinately, but eventually 'fatigue' and ultimately 'exhaustion' limit exercise performance in both man and animals. In man, fatigue and exhaustion are associated with athletic performance, disease states and may be side-effects of drug therapy (Lancet 1980). By the terms 'fatigue' and 'exhaustion', I am not referring to the subjective feeling, but that defined in terms of work output, that is: Fatigue: a reduced capacity and ability to perform work; Exhaustion: An inability to perform work (see Appendix I for further discussion).

Fatigue and exhaustion have been investigated by exercise physiologists who have looked for the so-called 'limiting factor of exercise performance'. Much of this research has focused on changes in muscle and blood biochemistry, such as depletion of energy stores and accumulation of inhibitors of muscle contractility such as lactic acid. However, very little attention has been paid the the role of the central nervous system in fatigue and exhaustion. This is surprising because very few of the physiological responses during exercise are not under the control of the brain. Nevertheless, there is evidence that events within the brain may be in part responsible for the development of fatigue and exhaustion: a phenomena referred to as 'Central Fatigue'.

'Central fatigue'.

One of the first workers to describe central fatigue was the Russian physiologist I. M. Setchenov at the turn of the 20th century. He showed that following exhaustive exercise with the right arm, subsequent work output by that arm could be improved if the subject performed work with the left arm rather than if both arms were passively rested (Setchenov 1935; see Asmussen and Mazin 1978a). This 'Setchnov phenomenon' has been replicated by the substitution of other 'diverting activities' such as

dorsiflexion of the foot (Alpert 1969), pain and solving mathematical problems (Asmussen and Mazin 1978a), opening the eyes during the resting period (Asmussen and Mazin 1978b) and encouragement by an audience (Rube and Secher 1981).

Although many workers have suggested that the mechanisms responsible for the 'Setchenov phonomenon' reside in the central nervous system, Alpert (1969) proposed that the phonomenon occurs because exercise by other muscle groups increases blood pressure and subsequent blood flow in the fatigued muscles. Experiments to test this hypothesis have been equivocal (see Asmussen and Mazin 1978a).

Bigland-Ritchie and co-workers (Bigland-Ritchie, Jones, Hosking and Edwards 1978) have found evidence for central fatigue during fatigue of the human quadriceps muscle during a sustained maximum voluntary contraction. The group noted that the force generated during a maximum voluntary contraction declined with time. In half of their subjects, force was increased by direct stimulation of the quadriceps by surface electrodes or localized stimulation of the femoral nerve at a time when electromyograph activity had declined in parallel with the force of contraction. The group concluded that up to 30% of the loss of contractile force during maximum voluntary contraction of the quadriceps could be attributed to 'central fatigue'.

The mechanisms responsible for central fatigue have not been determined. However, there is evidence, discussed below, which suggests that exercise performance can be improved by drugs which mimic the effect of dopamine, a major neurotransmitter of the basal ganglia.

I propose that a limiting factor to the performance of exercise and a mechanism that may be responsible for central fatigue, is an inability of striatal dopaminergic synapses to maintain the transfer of information at high rates of activity, (where 'activity' refers to the amount of information arriving at the presynaptic terminal and the rate of dopamine released by the terminal).

The purpose of my Introduction is to summarize the evidence for this hypothesis and to present the experimental approach I have used to test it. The Introduction includes an in-depth review of mechanisms that dopaminergic synapses use to maintain an adequate supply of available neurotransmitter and identify potential mechanisms for defficiencies in the transfer of information across the synapse. This information will form the basis of the discussion of my results.

CHAPTER 2.

INTRACEREBRAL DOPAMINERGIC MECHANISM FOR FATIGUE DURING MUSCULAR EXERCISE: AN HYPOTHESIS.

Dopamine is a major neurotransmitter in the central neural circiuts involved in the control of movement (Marsden 1982; Penney and Young 1983) and locomotor behaviour (Robbins and Everitt 1982). If the performance of physical exercise is limited by the ability of dopaminergic synapses to maintain the transfer of information across the synapse, it should be possible to improve performance by the administration of agents which increase stimulation of postsynaptic dopamine receptors.

Marshall and Berios (1979) have addressed this question. They observed the effects of dopamine receptor activation on the swimming ability of either young or aged rats (Figure 1). They observed that when young and aged rats were placed in a tank of water, the 'vigour' with which the animals swam and the 'success' with which the animals kept their heads above water both decreased as the swim continued (see legend to Figure 1); the decline was quickest in the aged animals.

Administration of the dopamine receptor agonist apomorphine slowed down the loss of both vigour and success

in the aged rats. Marshall and Berios (1979) reasoned that the loss of motor function in aged animals was the result of the decline in dopaminergic function because the aging process is associated with decreases in the concentration of cerebral dopamine, acivity of tyrosine hydroxylase and the sensitivity and number of dopamine receptors in the brain (Pradham 1980). Furthermore, intracerebral dopamine depletion by an intrcerebral injection of 6-hydroxydopamine is also associated with impairments of motor function similar to those seen in aged animals (Marshall, Levitan and Stricker 1976).

The group also observed that high doses of apomorphine improved the vigor and success of swimming in young animals, (but did not comment on this finding). It is possible that swimming had induced a decrease in the stimulation of post synaptic dopamine receptors, hence the ability of apomorphine to delay the decline in swim performance. I interpret this study as evidence for the existence of fatigue in dopaminergic synapses during movement.

FIGURE 1. Effects of Apomorphine and L-Dopa on the swim performance of aged and young rats. Photograph of Figure 2 in Marshall and Berios (1979).

Footnote.

"The following rating scales were used. For vigor: 3, continuous movement of all four limbs; 2.5, occcasional floating; 2, floating more than swimming; 1.5, occasional swimming using all four limbs;1, occasional swimming using hind limbs only; and 0, no use of of limbs. For success: 3, entire head always above water; 2.5, ears but not eyes usually below water; 2, eyes but not nose usually below water; 1, entire head below water for periods of 6 seconds or longer; and 0 animal on bottom of tank for 10 seconds or longer."

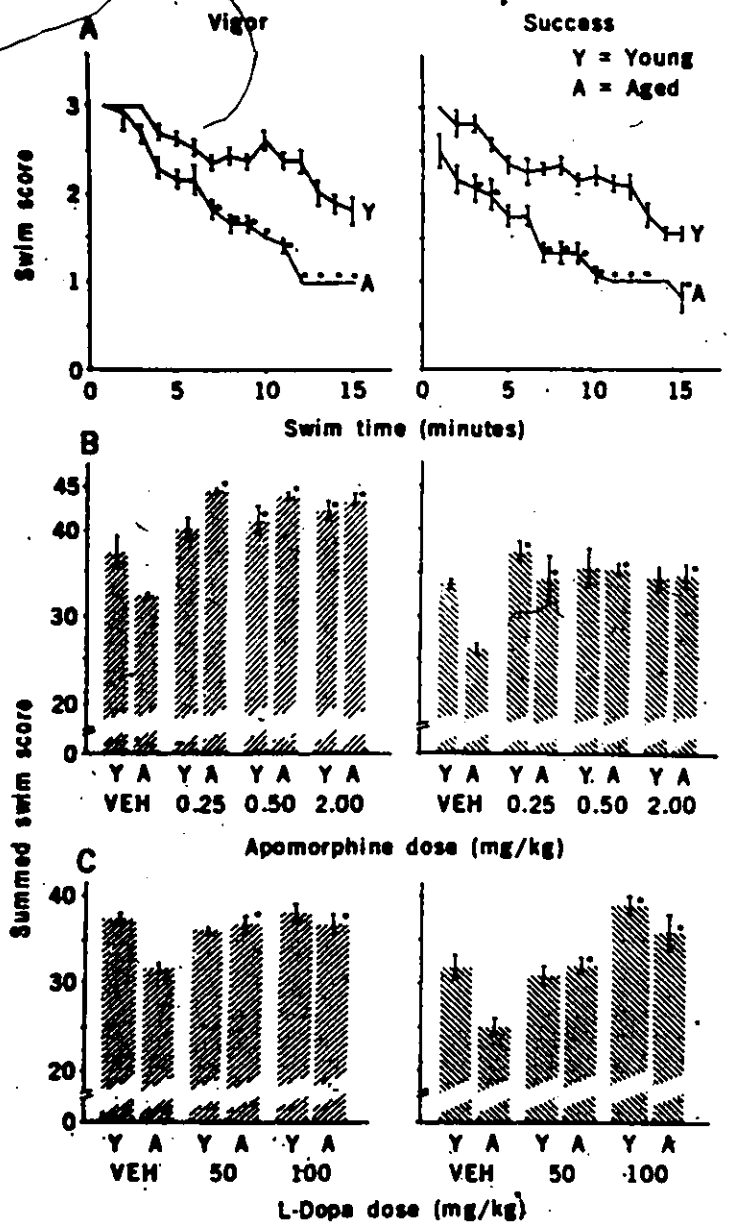


Fig. 2. Mean (± 1 standard error) swim performance of aged (*A*) and young adult (*Y*) rats that received no drugs (A) or were treated with apomorphine (B) or L-dopa (C). (*A*) Dots indicate significant differences ($P < .05$, Scheffé tests) between 6 aged rats and 13 young adult rats. (B) The effects of age and of drug dose are significant for both measures of swim performance, as are the group-by-dose interactions (analyses of variance, $P < .002$). Dots indicate significant differences (Dunnett's *t*-tests, $P < .05$) from respective vehicle (*VEH*) control condition. Each group contained from 6 to 25 rats. (C) The effects of age and of drug dose are significant for both measures of swim performance, as are the group-by-dose interactions (analyses of variance, $P < .005$). Dots indicate significant differences (Dunnett's *t*-tests, $P < .05$) from respective vehicle control conditions. Each group contained 6 to 10 rats.

Amphetamine has been used by athletes and military personal in an effort to increase athletic and military performance with significant success. Amphetamine is known to release dopamine itself from nerve terminals (Raiteri, Bertollini, Angelini and Levi 1975), block the re-uptake of dopamine (Azzaro, Ziance and Rutledge 1974) and retard the metabolism of dopamine by monoamine oxidase (Green and El Hait 19780.) In man, amphetamine has been shown to improve the performance of various forms of exercise including treadmill exercise (Heyhodt and Weissenstein 1940), bicycle exercise (Cuthbertson and Knox 1947), marching (Cuthbertson and Knox 1947; Borg, Edstrom, Linderholm and Marklund 1972), swimming (Smith and Beecher 1959, 1960) and also short-term ballistic sports such as weight throwing (Smith and Beecher 1960) and grip strength (Hurst, Radlaw and Bagley 1968). Improvements in swimming and treadmill running have also been reported in animals treated with amphetamine (Bhagat and Wheeler 1973; Gerald 1978). The improvements in work performance are usually small (1 to 5%), hence the title of a recent review on the subject: 'The amphetamine margin in sports' (Laites and Weiss 1981).

On the other hand, it is clear that disruption of the integrity of dopaminergic synapses has detrimental effects on the ability of animals and man to perform motor tasks. For example, the performance of the conditioned

avoidance by rats can be disrupted by the inhibition of tyrosine hydroxylase (Hanson 1965) or depletion of catecholamines by 6-hydroxydopamine (Laverty and Taylor 1970). In man loss of dopaminergic neurons in the nigro-striatal tract in Parkinson's disease results in impairments of motor function that can be restored by drugs that enhance dopaminergic activity (Tera Vainen and Calne 1979; Marsden 1982). There is also evidence that certain of the deleterious effects of hypoxia on cerebral performance, such as the conditioned avoidance response are the result of a defficiency in dopamine receptor stimulation (Brown, Kehr and Carlsson 1975; Boismare, Le Poncin and Rapin 1980).

These reports are consistent with there being dopaminergic synapses, which by some mechanism have not been able to maintain the transfer of information, and thereby maintain the performance of the of motor tasks. It is not clear which dopaminergic system(s) may be responsible for this apparent 'fatigue'. However, bearing in mind the role for the dopaminergic nigrostriatal system in the recruitment of motor programs (Marsden 1982) I suggest that the synapses of this pathway may be involved.

It is possible that norepinephrine, which is sensitive to the effects of 6-hydroxydopamine and amphetamine (Kostrzewa and Jacobowitz 1974; Cooper, Bloom and Roth 1978), is also involved in the effects of amphetamine on

exercise capacity. Noradrenergic neurons project from to the locus coeruleus to brain areas involved in movement including the striatum (Moore and Bloom 1979). This possibility is addressed in Experiments 1 parts C and D.

Mechanisms for fatigue of dopaminergic synapses.

As discussed in Chapter 3 of the Introduction, dopaminergic synapses have evolved a number of feedback systems which attempt to maintain an adequate supply of available dopamine. These feedback systems include the increase in the enzymatic activity of tyrosine hydroylase and mobilization of dopamine stores within the terminal. It is not known whether these systems are always able to provide an adequate supply of available dopamine.

Evidence cited above suggest that dopaminergic terminals may not be able to maintain an adequate supply of available neurotransmitter during the performance of motor tasks. If loss of dopamine exceeded the maximum capacity for dopamine re-synthesis the concentration of dopamine in the terminal would be expected to fall. Eventually the amount of dopamine released per action potantial would decrease. Alternatively, the synapse may limit the release of dopamine such that the rate of dopamine loss does not exceed the maximum capacity for dopamine resynthesis and dopamine

depletion would not occur. Both responses result in a discrepancy between the amount of dopamine required to be released and the amount of dopamine actually released. Functionally, the synapse would fail as an information transducer.

CHAPTER 3.

THE DOPAMINERGIC SYNAPSE.

The purpose of this chapter is to describe in detail the systems within the dopaminergic terminal which attempt to maintain the supply and release of dopamine. Much of this work has used the rat striatum as a model dopaminergic system.

In the late 1950's the catecholamine dopamine was first identified biochemically as dopamine in the brain of the rat (Montagu 1957; Carlsson, Lindqvist, Magnusson and Waldek 1958; Bertler and Rosengren 1959) and in the early 1960's was demonstrated to be localized within the neurons by the formaldehyde fluorescent technique of Falck and Hillarp (Carlsson, Falck and Hillarp 1962). This technique showed that the pattern of dopamine distribution throughout the brain was quite different from that of norepinephrine. These observations raised the possibility that dopamine was a neurotransmitter in its own right separate from its role as a precursor molecule of brain norepinephrine. Over the subsequent 20 years extensive research established that dopamine has fulfilled all the criteria for a molecule to be defined as a neurotransmitter in the central nervous system (Orrego 1979). This research can best be summarized as a

general description of how dopamine is thought to behave as a neurotransmitter.

RELEASE AND METABOLISM OF DOPAMINE

When an action potential arrives at the presynaptic nerve terminal, calcium ions enter the terminal and promote the fusion of vesicles containing dopamine to the presynaptic membrane and release their contents into the synaptic cleft (Raiteri, Cerrito, Cerroni, del Carmine, Ribera and Levi 1978). The amount of dopamine released (quanta) is proportional to the intensity of stimulation (Besson, Cheramy, Feltz and Glowinski 1973). In the cleft dopamine diffuses to the postsynaptic membrane where it binds reversibly to a specific receptor molecule (Seeman 1980; Creese, Morrow, Leff, Sibley and Hawblin 1982). Activation of this receptor affects the electrical activity of the postsynaptic neuron. In this way dopamine functions to transfer information from one neuron to the next (Figure 2A).

Unbound dopamine in the cleft follows one of two fates: It is either taken back into the cytoplasm of the presynaptic neuron by an active transport mechanism (Paton 1980) or is metabolized outside the neuron.

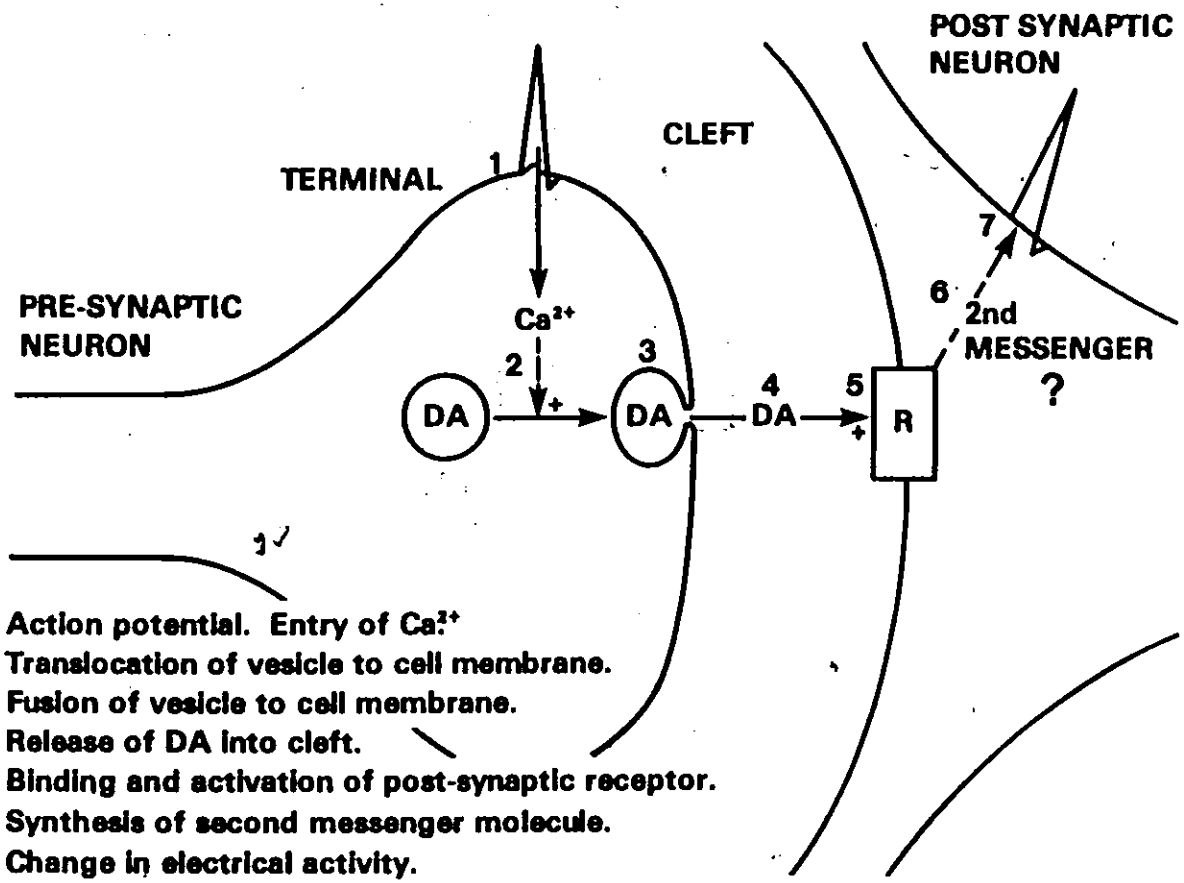
Dopamine taken back into the terminal is either stored in vesicles for subsequent re-use or is oxidized by

the enzyme monoamine oxidase (MAO), located on the outer membrane of the mitochondria (Weiss and McCauley 1979), to form dihydroxyphenylacetic acid (DOPAC). This deaminated molecule may then diffuse out of the neuron unchanged. Alternatively, DOPAC may be further metabolized to homovanillic acid (HVA) by the enzyme catechol-O-methyl transferase (COMT) "...located at sites distinct from the dopaminergic terminal" (Kaplan, Hartman and Creveling 1979; Westerink 1979). Dopamine remaining in the cleft may also be metabolized by COMT to form 3-methoxytyramine (3-MT) and then to HVA by MAO (Figure 2B). The amounts of DOPAC and HVA synthesized during activation appear to be proportional to the intensity of stimulation (Roth, Murrin and Walters 1976; Glowinski 1981). Consequently, the concentrations of DOPAC and HVA are often used as an index of brain dopaminergic activity. In unstimulated striatal dopaminergic neurons, Westerink and Spaan (1982) have estimated that 80% of HVA in the striatum is formed via DOPAC and 20% is synthesized via 3-MT. However, it is not known whether this proportionality remains the same during periods of increased activation.

FIGURE 2A. Mechanism of information transfer across intracerebral dopaminergic synapses.

When an action potential arrives at the presynaptic terminal, calcium ions enter the cell (1) and induce the exocytocis of vesicles containing the stores of dopamine into the cleft (2, 3 and 4). Dopamine in the cleft then diffuses to the postsynaptic neuron and binds to a specific dopamine receptor located on the post synapstic neuron (5). Activation of this receptor results in a change in the electrical activity of the postsynapstic neuron (7), an event which may depend on the synthesis of a second messenger molecule (6).In this way, dopamine functions to transfer information from one nerve cell to the next.

MECHANISM OF INFORMATION TRANSFER ACROSS DOPAMINERGIC SYNAPSES



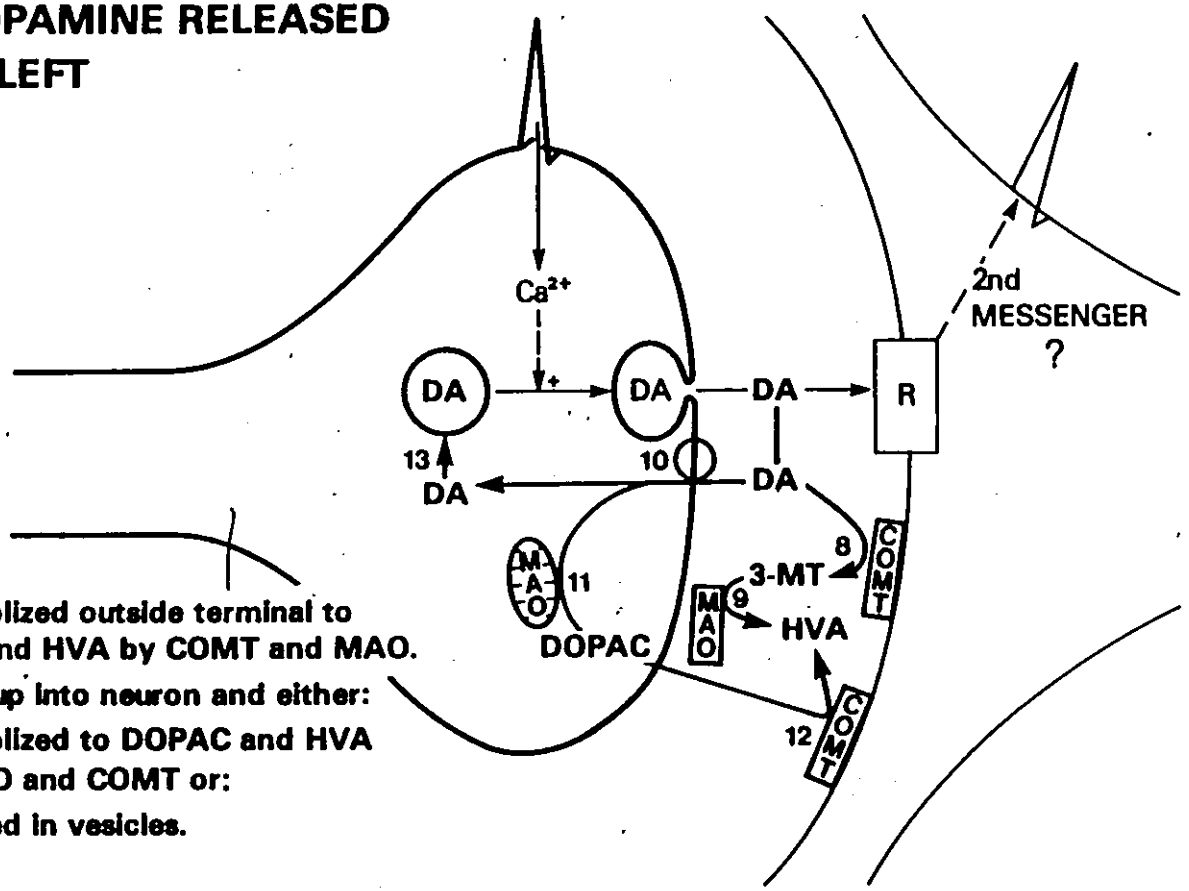
1. Action potential. Entry of Ca^{2+}
2. Translocation of vesicle to cell membrane.
3. Fusion of vesicle to cell membrane.
4. Release of DA into cleft.
5. Binding and activation of post-synaptic receptor.
6. Synthesis of second messenger molecule.
7. Change in electrical activity.

FIGURE 2B. Fate of dopamine released into the cleft.

The two major metabolic by-products of dopamine are dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Dopamine may be metabolized outside the terminal by catechol-O-methyl transferase (COMT) to 3-methoxytyrosine (8) and then to HVA (9) by monoamine oxidase (MAO). Alternatively, dopamine may be taken back up into the terminal (10) by an active transport mechanism (Patton 1980). Dopamine taken back in the terminal may either be placed back into vesicles for subsequent release (13) or metabolized to DOPAC by MAO (11). DOPAC may then either leave the brain unchanged or be metabolized further by COMT to form HVA (12).

The rate of formation of DOPAC and HVA is proportional to the rate of dopamine release and can be used as an index of the rate of dopamine release into the cleft.

FATE OF DOPAMINE RELEASED INTO THE CLEFT



8. & 9. Metabolized outside terminal to 3-MT and HVA by COMT and MAO.

10. Taken up into neuron and either:

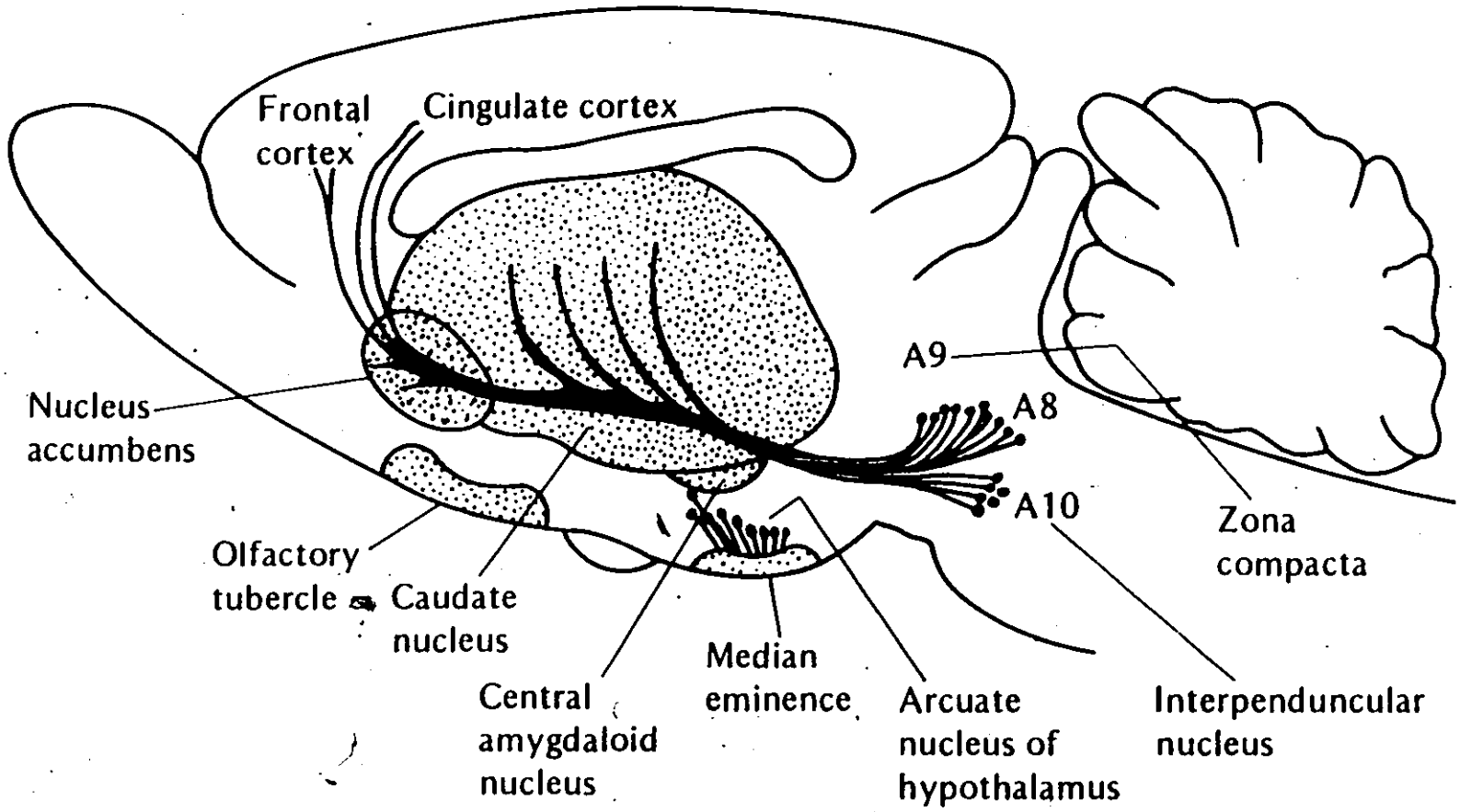
11. & 12. Metabolized to DOPAC and HVA by MAO and COMT or:

13. Restored in vesicles.

Neuroanatomy of dopaminergic neurons.

Dopamine is found in a number of neuronal pathways in the brain as shown in Figure 3 (Lindvall and Bjorklund 1978; Dray 1979). The nigrostriatal system has cell bodies located in the zona compacta of the substantia nigra and the ventral tegmental area which projects via the median forebrain bundle to the caudate nucleus, putamen and the nucleus accumbens (neostriatum). In fact, the striatum contains the highest concentrations of dopamine in the brain. The mesocortical system has cell bodies again in the substantia nigra and projections in the median forebrain bundle to a variety of higher structures including amygdala, and entorhinal cortex. There are also at least three other dopaminergic systems located almost entirely within the hypothalamus. The nigrostriatal pathway, the mesocortical system and the intrahypothalamic pathways are involved in a wide variety of brain functions including the control of movement, behaviour, emotion and the regulation of visceral homeostasis including hormonal secretion patterns and body temperature regulation.

FIGURE 3. Neuroanantomy of intracerebral dopaminergic neurons in the rat. The major pathway is the nigrostriatal pathway from the zona compacta of the substantia nigra to the striatum (caudate nucleus and putamen). The substantia nigra also sends fibres to the cerebral cortex. There are also intrahypothalamic dopaminergic tracts. The diagram is reproduced from Cooper, Bloom and Roth (1978).


Frontal cortex
Cingulate cortex
A9
A8
A10
Nucleus accumbens
Olfactory tubercle
Caudate nucleus
Central amygdaloid nucleus
Median eminence
Arcuate nucleus of hypothalamus
Interpenduncular nucleus
Zona compacta

The ability of these intracerebral dopaminergic pathways to carry out their functions is dependent on the accurate transfer of information across the synapse. As already noted, some dopamine is metabolized to DOPAC and HVA. If the terminal did not replace the degraded dopamine the supply of dopamine in the terminal would become depleted. In this case the terminal would fail as a transducer of information. In order to replenish the supplies of dopamine the rate of dopamine synthesis increases.

REGULATION OF DOPAMINE SYNTHESIS

Dopamine is synthesized in nervous tissue from tyrosine by the action of tyrosine hydroxylase and aromatic amino acid decarboxylase. Tyrosine hydroxylase is an intraneuronal enzyme and is found in the brain with a distribution similar to that of dopamine (Bacopoulis and Bhatnagar 1977). It catalyses the conversion of l-tyrosine to l-dihydroxyphenylalanine (l-Dopa). This reaction requires molecular oxygen, ferrous ions and a reduced tetrahydropteridine cofactor. The co-factor is oxidized during the reaction and is reduced back to tetrahydropteridine by the enzyme dihydropteridine reductase. Aromatic amino acid decarboxylase is found not only in the neurons but also in blood vessel walls. This enzyme catalyzes the conversion of l-Dopa to dopamine.

A number of endogenous factors have been demonstrated to affect the rate of dopamine synthesis over short perids of time and appear to act by changing the catalytic activity of tyrosine hydroxylase (Figure 4). These include: end-product inhibition by dopamine itself, the adenylate cyclase system and dopamine autoreceptors on the presynaptic terminal. Some of these factors may operate to link the activity of tyrosine hydroxylase to the activation of the terminal and the release of dopamine. Increases in the amounts of tyrosine hydroxylase increase dopamine synthesis over the long term (Nagatsu 1979).

End-product inhibition by dopamine.

Tyrosine hydroxylase does not normally operate at its maximum rate, (ie Vmax), in the resting neuron. Early studies of tyrosine hydroxylase demonstrated that the enzyme was subject to end-product inhibition by dopamine (Udenfriend 1966). This suggested that tyrosine hydroxylase activity was increased simply by removal of end-product inhibition. This picture has been complicated by the finding that compounds not found in the biosynthetic pathway of dopamine synthesis have profound effects on the activity of tyrosine hydroxylase. These external regulators include the adenyl cyclase system, dopamine autoreceptors and calcium ions.

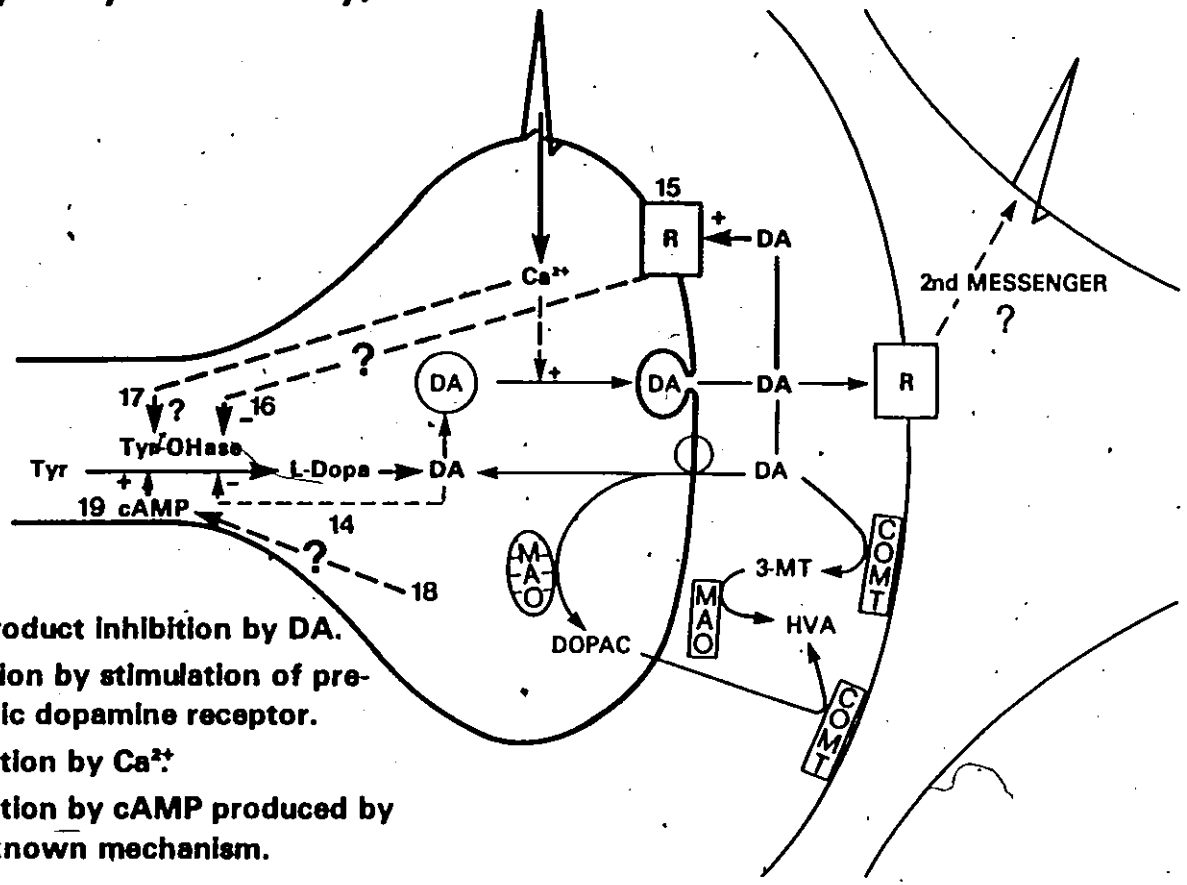
FIGURE 4. Regulation of dopamine synthesis.

In order to maintain the supply of dopamine in the terminal, dopamine synthesis increases in response to an increase in the rate of dopamine metabolism in an activated synapse. Dopamine is synthesized de novo in the terminal by the conversion of L-tyrosine to dihydroxyphenylalanine (L-DOPA) by the action of tyrosine hydroxylase, and the conversion of L-DOPA to dopamine by the action of amino acid decarboxylase. The activity of tyrosine hydroxylase is much less than the concentration of amino acid decarboxylase. Consequently the rate of dopamine formation is determined by the catalytic activity of tyrosine hydroxylase. It is not known whether the amount of tyrosine in the terminal is ever a limiting factor to the rate of dopamine synthesis.

Tyrosine hydroxylase is sensitive to a number of compounds including cyclic AMP (19), calcium ions (17) and dopamine (14) itself. However, the role of these and other compounds in the linking of tyrosine hydroxylase to the rate of dopamine catabolism is unclear. There is also a dopamine receptor located on the presynapstic membrane which when activated decreases the activity of tyrosine hydroxylase (15). The precise mechanism which links the activation of this receptor by dopamine in the cleft to tyrosine hydroxylase activity is not known.

REGULATION OF DOPAMINE SYNTHESIS

(Tyrosine Hydroxylase Activity)



14. End-product inhibition by DA.
15. & 16. Inhibition by stimulation of pre-synaptic dopamine receptor.
17. Regulation by Ca^{2+}.
18. & 19. Regulation by cAMP produced by an unknown mechanism.

Adenyl cyclase system

The effects of the adenyl cyclase system on tyrosine hydroxylase activity have been studied in a variety of preparations including tissue slices, synaptosomes(de Belleroche and Bradford 1978; De Langen and Maulder 1979; De Langen, Maulder and Stoof 1979) and purified protein extracts. By the mid-1970's it was clear that addition of analogues of cAMP to synaptosomes prepared from striatum, cerebral cortex, mesolimbic structures, forebrain, septum, hippocampus and brain stem produced a dose-dependent increase in the activity of tyrosine hydroxylase of 50% to 800%. (Harris, Morgenroth, Roth and Baldessarini 1974; Harris, Baldessarini, Morgenroth, and Roth 1975; Lovenberg, Bruckwick and Hanbauer 1975; Goldstein, Bronaugh, Ebstein and Roberge 1976; Boarder and Fillenz 1978; Bustos, Simon and Roth (1980); Pradham, Alphs and Lovenberg 1981; Vrana, Allhiser and Roskoski 1981). Cyclic AMP itself was first reported to have inhibitory effects on tyrosine hydroxylase (Harris et al 1974; Goldstein et al 1976) until it was shown that magnesium ions and ATP were required in the incubation media before cAMP increased tyrosine hydroxylase activity (Lovenberg et al 1975; Morgenroth, Hegstrand, Roth and Greengard 1975; Goldstein et al 1976). Cyclic AMP mediates its effects on tyrosine hydroxylase through the synthesis of a protein kinase molecule (Lloyd and Kauffman 1975;

Lovenberg et al 1975; Morgenroth et al 1975; Goldstein et al 1976). There is currently debate as to whether the enzyme is phosphorylated by cAMP, as it appears to be in the adrenal medulla (Letendre, Macdonnell and Guroff 1977; Yamauchi and Fujisawa 1979a) and the heart (Yamauchi and Fujisawa 1979b). Tyrosine hydroxylase has been reported to be phosphorylated in some experiments (Joh, Park and Reis 1978) but not others (Lloyd and Kauffman 1975; Lovenberg et al 1975).

Cyclic AMP does not appear to have any effects on the activity of aromatic amino acid decarboxylase (Harris et al 1975, 1976). Cyclic GMP is ineffective in increasing tyrosine hydroxylase activity (Harris et al 1975; Lovenberg et al 1976).

Cyclic AMP affects the kinetic properties of tyrosine hydroxylase. Studies have consistently demonstrated decreases in the Km for tyrosine but no change in the Vmax for tyrosine (Harris et al 1974, 1975; Lovenberg et al 1975) and a reduction in the Km for the reduced pteridine co-factor but no change in its Vmax (Harris et al 1975; Lloyd and Kauffman 1975; Lovenberg et al 1975; Goldstein et al 1976; Ames, Lerner and Lovenberg 1978; Prahham et al 1981; Vrana et al 1981). The affinity of the enzyme for dopamine is reduced as indicated by an increase in the Ki value (Harris et al 1974, 1975; Lovenberg et al 1975; Goldstein et al 1976; Ames et al 1978). Thus, at any given

concentration of tyrosine, the rate of dopamine synthesis is increased by cAMP, in part because of an increased affinity of the enzyme for tyrosine and the pteridine co-factor and in part because of a decrease in the potency of the inhibitory affect of dopamine on the enzyme.

Dopamine receptors

Certain forms of the dopamine receptor are able to modulate the activity of brain adenylate cyclase and the production of cAMP (Kebabian and Calne 1979; Seeman 1980). For example, agonists of the D-1 receptor (classified according to the system of Kebabian and Calne 1979) increase cAMP production in the striatum (Stoof and Kebabian 1982). However, agonists of dopamine receptors which increase the production of cyclic AMP do not have stimulatory effects on tyrosine hydroxylase as predicted by these observations (Waggoner, McDermed and Leighton 1980; Pradham et al 1981; Bitran and Bustos 1982) in fact, tyrosine hydroxylase activity is decreased. Dopamine antagonists on the other hand results in an increase in the activity of tyrosine hydroxylase (Waggoner et al 1980).

In vivo studies where the activity of tyrosine hydroxylase is increased by either blockade of the presynaptic dopamine receptors (see below) or alterations in neuronal activity are associated with a decrease in the Km

for the reduced co-factor (Lerner et al 1977) and an increase in the Ki for dopamine (Zivlovic and Guidotti 1974; Zivkovic, Guidotti and Costa 1974, 1975). Because these changes are qualitatively similar to the effects of cAMP, it has been suggested the cAMP may have a physiological role in vivo in the regulation of tyrosine hydroxylase (Murrin, Morgenroth and Roth 1976) perhaps through an adenyl cyclase system linked to dopamine receptors.

The issue of whether dopamine receptors linked to adenyl cyclase have any role in the regulation of dopamine metabolism has been further clouded. Destruction of post synaptic neurons with kainic acid results in a complete loss of dopamine sensitive adenyl cyclase (Di Chiara, Porceddu, Spano and Gessa 1977). This indicates that dopamine receptors linked to adenyl cyclase are located on the postsynaptic membrane. However, inhibition of tyrosine hydroxylase by dopamine receptor agonists and activation of the enzyme by dopamine receptor antagonists could still be evoked, suggesting a cAMP-independant receptor mechanism regulating tyrosine hydroxylase (Di Chiara et al 1977).

Calcium ions

A further factor which has potent effects on tyrosine hydroxylase is the calcium ion. In norepinephrine-containing neurons, calcium ions increase the activity of

tyrosine hydroxylase (See Kaptos and Zigmond 1982). It has been suggested that because entry of calcium ions occurs with the arrival of action potentials at the terminal, calcium ions may have a role in the regulation of tyrosine hydroxylase. Studies of tyrosine hydroxylase obtained from dopamine-rich-areas of the brain have revealed inconclusive results with calcium ions reported to be inhibitory (Morgenroth, Boadle-Biber and Roth 1976), or without effect (Lerner, Ames and Lovenberg 1977; Kaptos and Zigmond 1982). Calcium ions do, however, appear to be necessary for certain processess involved in the release of dopamine from the terminals and the synthesis of cAMP. Therefore calcium may be essential for the activation of tyrosine hydroxylase by other mechanisms (Kaptos and Zigmond 1982). Recently, Bitran and Bustos (1982) have suggested that calcium ions are involved in the inhibitory effects of dopamine autoreceptor activation.

Summary

The influence that cAMP, dopamine and calcium have on tyrosine hydroxylase has mostly been investigated in in vitro preparations. It is not clear what importance these factors have in the regulation of dopamine synthesis in the intact neuron and what mechanism(s) link rate of dopamine release to the rate of dopamine synthesis.

ROLE OF TYROSINE AVAILABILITY IN THE DETERMINATION OF DOPAMINE SYNTHESIS.

There is a consensus that the rate of dopamine synthesis is determined by the catalytic activity of tyrosine hydroxylase. As discussed above, a number of factors have been identified which affect the activity of tyrosine hydroxylase and may be responsible for increasing the rate of dopamine synthesis in activated terminals. Recent studies indicate that the availability of the precursor molecule tyrosine may determine how much the rate of dopamine synthesis can be increased (Wurtman, Hefti and Melamed 1981).

Textbooks and review articles, including Cooper, Bloom and Roth (1978) and Coyle and Snyder (1981), state that tyrosine hydroxylase has a Km for tyrosine well below the endogenous concentration of tyrosine and the enzyme is therefore virtually saturated with substrate. However, Carlsson and Lindqvist (1978) have reported that the concentration of tyrosine in the brain is actually close to the Km value. It is possible therefore that the rate of dopamine synthesis is determined not only by the activity of tyrosine hydroxylase but also by the availability of tyrosine in the terminal. If this is the case the rate of dopamine synthesis can be increased by increasing the availability of tyrosine in the brain. The following studies

suggest this is the case.

Scally, Ulus and Wurtman (1977) have shown that the concentration of DOPAC and HVA in the striatum is increased by haloperidol (a specific blocker of the presynaptic dopamine receptors, which results in an increase in the release of dopamine from the terminal). If rats were pretreated with tyrosine, the amount of DOPAC and HVA produced in response to haloperidol was accentuated. Similar results have been observed if reserpine is substituted for haloperidol (Sved, Fernstrom and Wurtman 1979).

In rats with unilateral lesions of the nigrostriatal pathway, the remaining ipsilateral neurons become hyperactive. When tyrosine was administered the release of dopamine was enhanced (Melamed, Hefti and Wurtman 1980). However, in normal rats, the administration of tyrosine alone has no effect on the concentrations of HVA. These results indicate that in resting neurons, the availability of tyrosine is not a limiting factor of dopamine synthesis. However where the rate of dopamine release is increased, a limiting factor in the synthesis of dopamine is the availability of tyrosine.

Administration of tyrosine to patients with Parkinson's disease results in an increase in the concentrations of tyrosine and HVA in the cerebrospinal fluid, indicating that tyrosine has crossed the blood-brain

barrier and been converted to dopamine and the rate of dopamine release increased (Growdon and Melamed 1980). In Parkinsonian patients the activity of the surviving nigrostriatal dopaminergic neurons is increased, as indicated by an increase in the HVA:dopamine ratio in the striata of post mortem brains (Bernheimer, Birkmayer, Hornykiewicz, Jellinger and Spitelberger 1973). It is possible that in such patients the rate of dopamine synthesis is in part limited by the availability of tyrosine.

Wurtman et al (1981) reasoned that the lack of facilitation of dopamine synthesis by the administration of tyrosine to normal animals may be due to the presence of end-product inhibition by dopamine itself on tyrosine hydroxylase and also the activation of dopamine autoreceptors on the presynaptic membrane and dendrites which also suppress the activity of tyrosine hydroxylase.

Although these studies indicate the importance of the availability of tyrosine in determining the rate of dopamine synthesis in activated neurons no study has addressed whether in the normal intact animal tyrosine is ever a limiting factor to the synthesis of dopamine which in turn has detrimental effects on the ability of the synapse to transmit information.

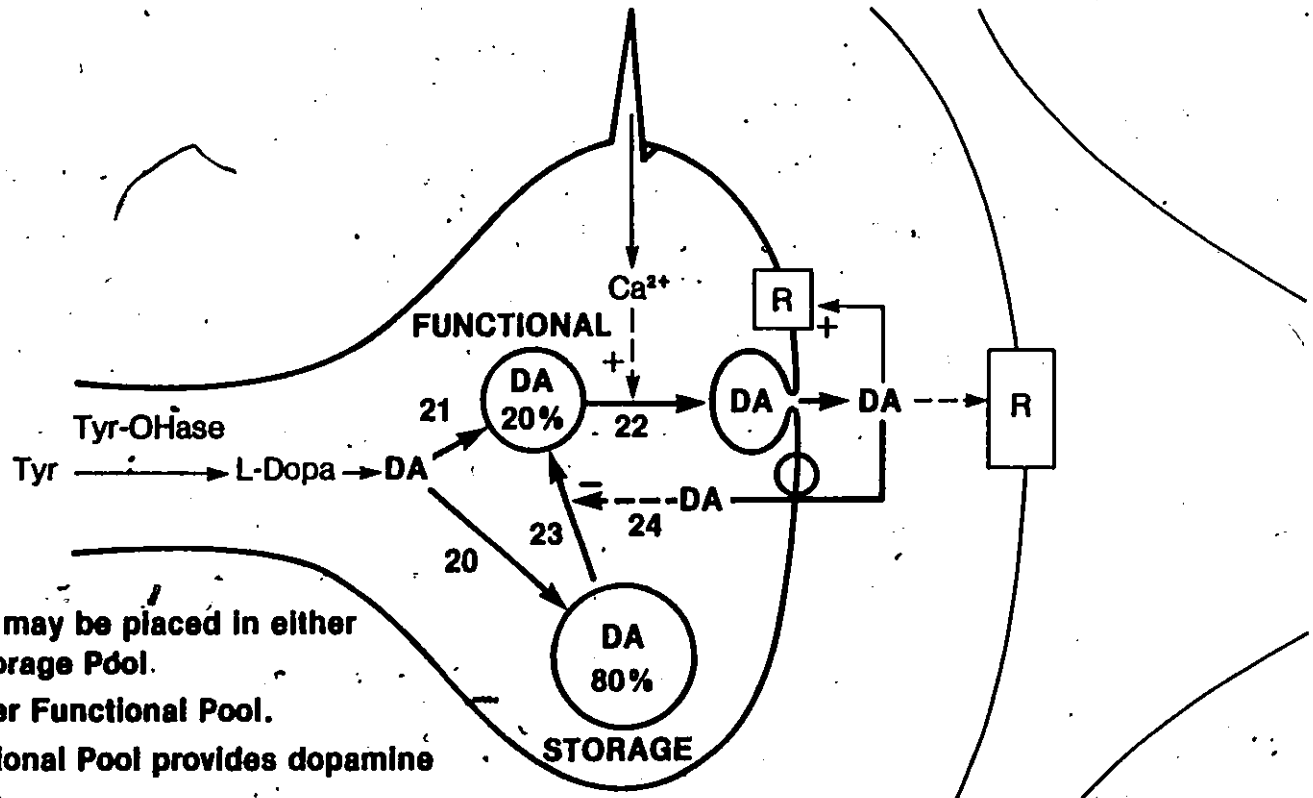
INTRANEURONAL STORES OF DOPAMINE.

Once dopamine has been synthesized it is stored in vesicles ready for subsequent release. The relation between stored dopamine and release is complicated by the fact that dopamine appears to exist in two compartments inside the nerve terminal: a small 'functional' pool which is preferentially released and a larger 'storage' pool (Figure 5).

FIGURE 5. Storage of dopamine in the presynaptic terminal: The Functional Pool and the Storage Pool.

Dopamine appears to exist in two pools in the terminal: a large storage pool (20) and a smaller functional pool (21) which provides dopamine for release into the cleft (22). Dopamine may be transferred from the storage pool to the functional pool (23). The rate of this transfer is inversely proportional to the concentration of free dopamine in the cytoplasm of the presynaptic terminal (24), which in turn is determined by the rate of dopamine synthesis, the rate of dopamine reuptake and the rate at which dopamine is placed in the two pools (Shore et al 1978; McMillen et al (1980).

STORAGE OF DOPAMINE IN THE PRESYNAPTIC TERMINAL



20 Dopamine may be placed in either a large Storage Pool.

21 or a smaller Functional Pool.

22 The Functional Pool provides dopamine for release.

23 Dopamine may be transferred from the Storage Pool to the Functional Pool.

24 The rate of this transfer is inversely proportional to the concentration of free dopamine in the cytoplasm.

The existence of two pools was suggested by the observation that after inhibition of tyrosine hydroxylase by alpha-methyltyrosine, the concentration of dopamine within the brain declined rapidly at first then subsequently more slowly (Neff and Costa 1968; Glowinski 1970). When dopaminergic neurons were supplied with labelled dopamine precursors or labelled dopamine itself, the specific activity of the dopamine which was released initially was greater than the specific activity of the total dopamine pool. Later the specific activity of the dopamine released was identical to that of the total dopamine pool (Sedvall, Weise and Kopin 1968; Glowinski 1970; Thierry, Blanc and Glowinski 1970; Schildkraut, Droskoczy and Lo 1971; Besson, et al 1973). Similarly, the specific activity of dopamine metabolites in the stimulated striatum was also highest immediately after stimulation compared to later (Gropetti, Algeri, Cattabeni, DiGiulio, Galli, Ponzio and Spano 1977). It has been calculated based on wash-out studies of dopamine loss following alpha-methyl tyrosine treatment that 20% of striatal dopamine is found in the functional pool and 80% is found in the storage pool.

The anatomical site for the two pools of dopamine within the terminal have so far not been identified. However, Hartman and Halaris (1980) have found in rat striatal synaptosomes that dopamine was found in two places,

the cytoplasm and in synaptic vesicles. The ratio of this dopamine was 4:1 in favour of cytoplasm. It is unclear whether these two locations of dopamine correspond to the storage and functional pools respectively.

The presence of two dopamine pools has not been unchallenged however. Doteuchi, Wang and Costa (1974) suggested that the apparent biphasic decline in the concentrations of dopamine in the striata of rats treated with alpha-methyltyrosine may be due to the rapid synthesis of p-hydroxyamphetamine and p-hydroxynorephedrine (from alpha-methyltyrosine) two compounds which can increase the efflux of dopamine from the striatum (Paden 1979).

Relationship between the functional and storage pools.

Although the two pool hypothesis for dopamine storage and release in the terminal has not been clearly proven, it has proved a useful experimental model. Shore and co-workers have extended the model to include mechanisms which regulate the transfer of dopamine between the two pools and the functional significance (Shore, McMillen, Miller, Sanghera, Kiser and German 1978; McMillen, German and Shore 1980). The model will be used in the present thesis and is described in detail below.

Dopamine in the functional pool is preferentially released into the cleft. However, dopamine may be transferred from the storage pool to the functional pool for release as indicated in Figure 5. The evidence for this is derived from indirect studies. Blockade of dopamine receptors by haloperidol results in immobility which is quantified as a 'cataleptic score' (Shore and Dorris 1975). If tyrosine hydroxylase is inhibited with alpha-methyl-tyrosine no catalepsy is observed although dopamine levels in the brain are depleted by over 50%, that is by an amount greater than the amount of dopamine in the functional pool as estimated from wash-out studies. This indicates that dopamine stores can be mobilized for release to prevent catalepsy.

The transfer of dopamine from the storage pool to the functional pool appears to be regulated by the concentration of free dopamine within the terminal cytoplasm (Shore et al 1978; McMillen et al 1980). The concentration of free dopamine in the cytoplasm is determined by the balance between the rates of dopamine synthesis and re-uptake into the terminal, which tend to increase the concentration, and the rates of dopamine catabolism and sequestration into vesicular storeswhich tend to decrease the concentration. Again, the evidence for this is derived from indirect studies. Amfonelic acid (AFA) and amphetamine

have similar effects on behaviour, including increases in locomotor activity and intracranial self-stimulation. Although AFA is a potent inhibitor of dopamine reuptake into the terminal Shore et al (1978) have argued that AFA is a behavioural stimulant by facilitating neurogenic dopamine release rather than simply increasing the concentration of dopamine in the cleft by preventing its removal. The mechanism by which AFA does this is by first preventing dopamine reuptake, leading to a decrease in the concentration of dopamine in the terminal cytoplasm. Shore et al (1978) propose that the rate of transfer of dopamine from the storage pool to the functional pool is inversely proportional to the concentration of dopamine in the cytoplasm; as this concentration decreases more dopamine is transferred to the functional pool and released per impulse arrriving at the terminal. This model is proposed as an explanation of why the effects of AFA can be prevented by the depletion of total dopamine stores by reserpine but cannot be prevented by the inhibition of alpha-methyltyrosine. In this situation newly synthesized dopamine is not essential for the effects of AFA because the transfer of dopamine from the storage pool to the functional pool is at a sufficient rate to maintain an adequate supply of available dopamine for release into the cleft. In contrast, amphetamine which appears to act through the release of dopamine into the

cleft and terminal itself can be prevented from having an effect by inhibition of tyrosine hydroxylase but not depletion of dopamine stores. In this situation the transfer of dopamine from the storage pool to the functional pool is prevented by the increase in the concentration of dopamine in the cytoplasm (by dopamine taken back up into the terminal and freshly synthesized dopamine), hence the dependance on newly synthesized dopamine in the functional pool for the effects of amphetamine.

CHAPTER 4.

SCOPE OF THE PRESENT THESIS.

The question which has been raised is: Are the mechanisms which synthesize dopamine and the mechanisms which make dopamine available for release into the cleft, able to do so when the terminals are activated intensely or for prolonged periods, such that the transfer of information across the synapse is not a limiting factor to the performance of physical exercise? In other words: Do intracerebral dopaminergic synapses ever 'fatigue'. The experimental results reviewed in my Introduction suggest that this may be the case. The purpose of my thesis was to propose a hypothesis to further investigate whether dopaminergic synapses fatigue.

HYPOTHESIS.

A. Exercise increases the turnover rate of intracerebral dopamine in the neural pathways involved in the control of muscular exercise.

B. The demand for dopamine release exceeds the supply and capacity for synthesis, resulting in a reduced availability or amount of dopamine released into the cleft and an inadequate degree of receptor stimulation.

C. This defficiency of dopaminergic function limits exercise performance.

There are four consequences and predictions of this hypothesis.

CONSEQUENCES.

1. If brain dopamine levels were reduced before exercise subsequent exercise performance should be decreased. Exercise performance should be restored in dopamine-depleted animals by dopamine receptor agonists.

2. If exercise performance was being limited in normal animals by the amount of dopaminergic activity, exercise performance of normal animals should be improved by dopamine receptor agonists.

3. In an animal performing exercise the turnover rate of dopamine should be increased as indicated by increases in the regional concentrations of DOPAC and HVA.

4. If exercise performance was being limited by an inadequacy of dopamine release from the terminal due to a depletion of dopamine stores, an exhausted animal would have significantly lower concentrations of dopamine in the brain than animals which had not exercised.

My thesis has examined whether these predictions derived from my hypothesis can be verified in an experimental model I have developed.

The first step was to design a system that enabled quantitative measurements of exercise capacity to be made. The most frequently used measure of the capacity of an animal to perform exercise is the time taken for the animal to become 'exhausted'. This measure was used in my experiments. For the reasons outlined in Appendix 1, treadmill running was used as the form of exercise. A full description of the properties of this system is given in the Material and Methods section and Appendix 1.

EXPERIMENTAL PROTOCOLS.

Experiment 1: Do decreases in the availability of striatal dopamine decrease exercise capacity?

Consequence 1 predicts that if the availability of dopamine was reduced prior to the exercise, exercise capacity should be reduced. Intracerebral dopamine was depleted by an injection of the neurotoxin 6-hydroxydopamine (6-OHDA) into the lateral ventricles of the brain (See Appendix 2). This drug results in a significant depletion of dopamine within the brain (Urtetsky, 1970; Kostrzewa and Jacobowitz 1974). However, postsynaptic neurons and their dopamine receptors are left intact. Experiments outlined in Chapter 2 of the Introduction concerning improvements in exercise capacity following administration of amphetamine could also be accounted for by a norepinephrine as well as a

dopamine related mechanism. Also, 6-OHDA results in a significant depletion of norepinephrine as well as dopamine. Therefore, in 6-OHDA treated rats, dopamine or norepinephrine were selectively replaced by specific receptor agonists to see if one or both reversed the effects of 6-OHDA. Apomorphine was used as dopamine receptor agonist (Anden, Rubenson, Fuxe and Hokfelt 1967; Ernst 1967) and clonidine as norepinephrine receptor agonist (Timmermans, Schoop, Kwa 1981; McLennan 1981). Both drugs cross the blood-brain barrier.

An x-ray technique was developed to identify rats which had received a correctly located intracerebroventricular injection (Heyes, Garnett and Coates 1983). The exerimental protocol of teaching the rats to run on the treadmill and running them to exhaustion was a lengthy process. Therefore, only animals with correctly located injections were accepted for further study.

Experiment 2: Do increases in dopaminergic activity increase exercise capacity?

Consequence 2 predicts that if exercise capacity is limited by the degree of postsynaptic dopamine receptor activation, dopamine receptor agonists should improve exercise capacity in normal rats. To examine this prediction, normal rats were treated with the dopamine receptor agonist apomorphine and run to exhaustion.

Experiment 3: When rats are exhausted is there evidence of a failure of striatal dopaminergic transmission?

Consequence 3 predicts that animals run to exhaustion on the treadmill would demonstrate increases in the turnover of dopamine as indicated by increases in the brain concentrations of DOPAC and HVA. The striatum and hypothalamus contain the terminals of the major dopaminergic systems in the brain which appear to be involved in the regulation of movement and peripheral homeostasis respectively. I therefore measured the changes in the concentration of DOPAC and HVA. Furthermore, because the brainstem contains the cell bodies and dendrites of the major ascending dopaminergic systems which can also release dopamine from themselves, this area was also examined. An opportunity was also taken to evaluate the intramuscular concentrations of carbohydrates and high energy phosphate compounds to understand better the energy substrate profile of these animals at a time when exercise performance was being increased by apomorphine.

MATERIALS AND METHODS.

CHAPTER 1.

ANIMALS.

Studies were performed on adult male Sprague Dawley rats (300-450g) obtained from the Montreal Branch of Canadian Breeding Farms. Animals were housed in perspex cages, (3 per cage) in an air conditioned room (21-22°C) and fed Purina rat chow and water ad libitum. The lighting schedule was 12 hours light (fluorescent):12 hours dark. Experiments were performed during the animals dark cycle (Nocturnal) unless otherwise stated. Animals were allowed at least seven days of acclimatization before beginning the studies.

DRUGS AND REAGENTS.

The following compounds were obtained from Sigma Chemical Co. St. Louis Missouri USA: 6-hydroxydopamine, apomorphine, dopamine, homovanillic acid (HVA), dihydroxy-phenylacetic acid (DOPAC), norepinephrine. Clonidine and enzymes for energy substrate analysis were obtained from Boehringer Mannheim of Canada Ltd., Dorval, Quebec.

EXERCISE TREADMILL SYSTEM.

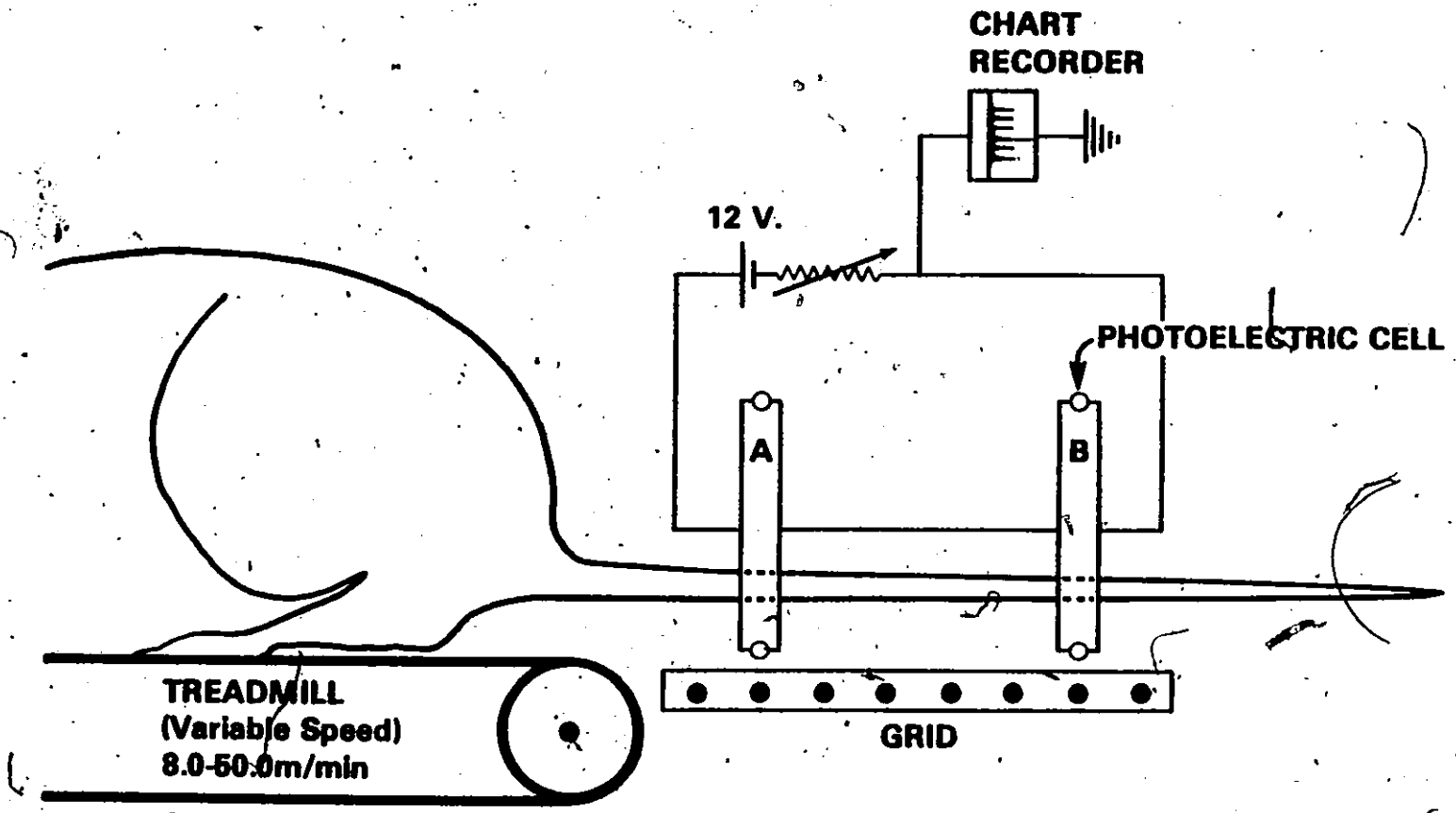
The capacity to perform physical exercise was quantified by measuring the time required for a running rat to become exhausted. This next section provides a detailed description of the apparatus and experimental protocol of how this was done. Appendix I gives a detailed description of how the definition of 'exhaustion' was developed and the characteristics of the system described here.

Apparatus.

The apparatus (Fig 6) is a modified variable speed treadmill (Quinton Instruments, Seattle, Washington; Model 14-44A). The running belt is separated into three lanes 25 cm long, 11 cm wide and 12 cm high by plywood suspended over the belt by a wooden frame bolted to the treadmill. Each lane is covered by a wire mesh. The proximal 10 cm of the track is kept in shadow while the rest of the treadmill is exposed to room lighting. Immediately behind each lane and 2 cm below the running plane is a grid consisting of 12 steel rods 0.3 cm in diameter and 0.8 cm apart. The grid is connected to a current generator (Grason Gstadler W Concorde, Mass.; Model E1064 GS).

FIGURE 6. Treadmill exercise system.

The apparatus consists of a variable speed treadmill, with an electric grid system at the distal end of the treadmill belt. Photoelectric cells placed over the grid system are used to produce a permanent chart record of the position of the animal on the apparatus. The time of exhaustion is defined as the time required for a running animals to stop running and rest on the grid for 10 seconds.



RAT TREADMILL EXERCISE MODEL

Two pairs of photoelectric cells (A and B) are placed over the grid in one of the tracks, pair A 2.5 cm behind the end of the belt and pair B a further 4.5 cm behind pair A. The vertical distance between the photoelectric cells is 2 cm, and the bottom photoelectric cell is in line with the plane of the treadmill. The photoelectric cells within each pair are wired in parallel and the pairs themselves wired in series. Four light emitting diodes, wired in series, are placed directly opposite the photoelectric cells. Voltage changes across the photoelectric cells are recorded on a pen chart recorded. A maximum voltage change only occurs when one or both pairs of photoelectric cells are occluded; the voltage change produced by occlusion of only one photoelectric cell is less the 60% maximum. This spacial and electrical arrangement of the photoelectric cells allows the differentiation of occlusions caused by the animal's tail from those caused by the animals body. The tail is thick enough to occlude only one photoelectric cell of each pair, which produces a sub-maximal voltage change, whereas if the animal touches the grid both photoelectric cells within either pair A or pair B are occluded and a full scale deflection is produced. An 'occlusion' is defined as a full scale deflection produced by the animal's body becoming interposed between one or both pairs of photoelectric cells and their corresponding light emitting diodes.

Familiarization Runs.

The following protocol was used to familiarize the animals to running on the treadmill (Table 1).

Animals were brought from their housing quarters and placed in a dark room until used. Animals were run in groups of three, one rat to each lane, beginning 2 hours after lights off (Nocturnal). The animals were allowed between one to three minutes to explore the apparatus and then the treadmill was switched on. The speed and duration of the run was gradually increased from 9.0 m/min for 0.5 minutes on Day 1 to 33.0 m/min for 1.5 minutes on Day 8. Four learning runs were done on each of Days 1, 2 and 3 and three learning runs were done on each of Days 4, 5, 6, 7 and 8, with 0.5 minute rest allowed between each run.

During the last run on each day the rats were given a subjective score from zero to four points on the basis of the criteria outlind in Table 2. These criteria were designed to identify animals which were willing and able to run on the treadmill.

For the reasons given in Appendix 1, only animals which scored consistently 3 or 4 points on each of Days 2, 3, 4 and 5 of the learning period were used.

TABLE 1. Summary of the protocol of the Learning Schedule.

DAY	EXPLORATION TIME (Min)	TREADMILL SPEED m/Min	NUMBER OF RUNS	DURATION OF RUNS (Min)
1	3	9.0	4	0.50
2	2	12.0	4	0.50
3	1	16.0	4	0.50
4	1	20.0	3	0.75
5	1	24.0	3	0.75
6	1	28.0	3	1.00
7	1	31.0	3	1.25
8	1	34.0	3	1.50
9	1	EXPERIMENTAL	1+Ex	0.50

Experience shows that animals which have been familiarized to running on the treadmill have less variability in their times of exhaustion compared to animals which have not previously been run on the apparatus (See Appendix 1).

Ex: Exhaustion run.

TABLE 2. Learning Score Criteria used to identify animals which are willing and able to run on the treadmill.

LEARNING SCORE	CHARACTERISTICS OF RUNNING PERFORMANCE DURING THE LAST RUN OF THE LEARNING RUN.
4	Runs at the proximal end of the treadmill. Head-down running posture. PEC Occlusion Frequency <2 occl/15s.
3	Runs largely at the proximal end of the treadmill. Head-down running posture; Occasionally looks around. PEC Occlusion Frequency <3 Occl/15s.
2	Runs at variable places on the treadmill. Occasionally 'hops'; Frequently looks around. PEC Occlusion Frequency >2 Occl/15s.
1	Occasionally stops running. PEC Occlusion Frequency >5 Occl/15s.
0	Frequently stops running. PEC Occlusion Frequency > 5/15s.

Experience has shown that animals that are poor runers on the treadmill during the learning period will not run to exhaustion. By rejecting from further experimentation those animals which scored 3 points or less during the first 5 days of the Familiarization period it proved possible to appreciably reduce the variability in the times of exhaustion.

Exhaustion Run.

On Day 9 the animals were weighed and placed on the treadmill belt, beginning two hours after lights off. After one minute the treadmill was switched on for a 'warm-up' run of 0.5 minute at the designated experimental speed (35.0 m/min to 37.0 m/min). This speed corresponds to a work output equal to approximately 80% of the maximun oxygen consumption of untrained rats (Patch and Brooks 1980). After a 5 minute wait the treadmill and photoelectric cell systems were switched on and the animal was run to a point of exhaustion defined as the time required before the animals ceases to run and elects to rest on the grid for longer than 10 seconds. Only one exhaustion run was performed on each rat.

INJECTION OF 6-HYDROXYDOPAMINE (6-OHDA) INTO THE LATERAL CEREBRAL VENTRICLES.

To deplete intracerebral catecholamines the neurotoxin 6-hydroxydopamine (6-OHDA) was infused into the ventricular system of the rat brain (Figure 7). The radio-opaque contrast medium thorium dioxide was used as carrier. The animal's heads were x-rayed in lateral view to identify rats with a thorium shadow characteristic of a correctly located infusion cannula. A full description of the characteristics of this technique is given in Appendix 2 and has been published in the Journal of Neuroscience Methods (Heyes, Garnett and Coates 1983).

Animals were anaesthetized with sodium pentobarbital (50 mg/Kg i.p.) and immobilized in a stereotaxic frame (David Kopf Instruments, Tujunga, California; Model 900) After drilling a 2 mm diameter burr hole through the skull, a 25-gauge cannula was inserted into the left lateral cerebral ventricle accoring to co-ordinates in the neuro-anatomical text of Pelligrino and Cushman (1967) using Bregma as zero point: L=1.6 mm, A-P=3.2 to 3.6 mm, D-V=0.0 mm. Rats were then infused at a rate of 1.8 µl/min from a 50 ul Hamilton syringe by a pump (Sage Instruments, White Plains, New York; Model 255 W-1) with either 200 µg of 6-OHDA dissolved in 20 µl of a 25% suspension of thorium dioxide (6-OHDA; with 0.4 mg/ml of ascorbic acid as

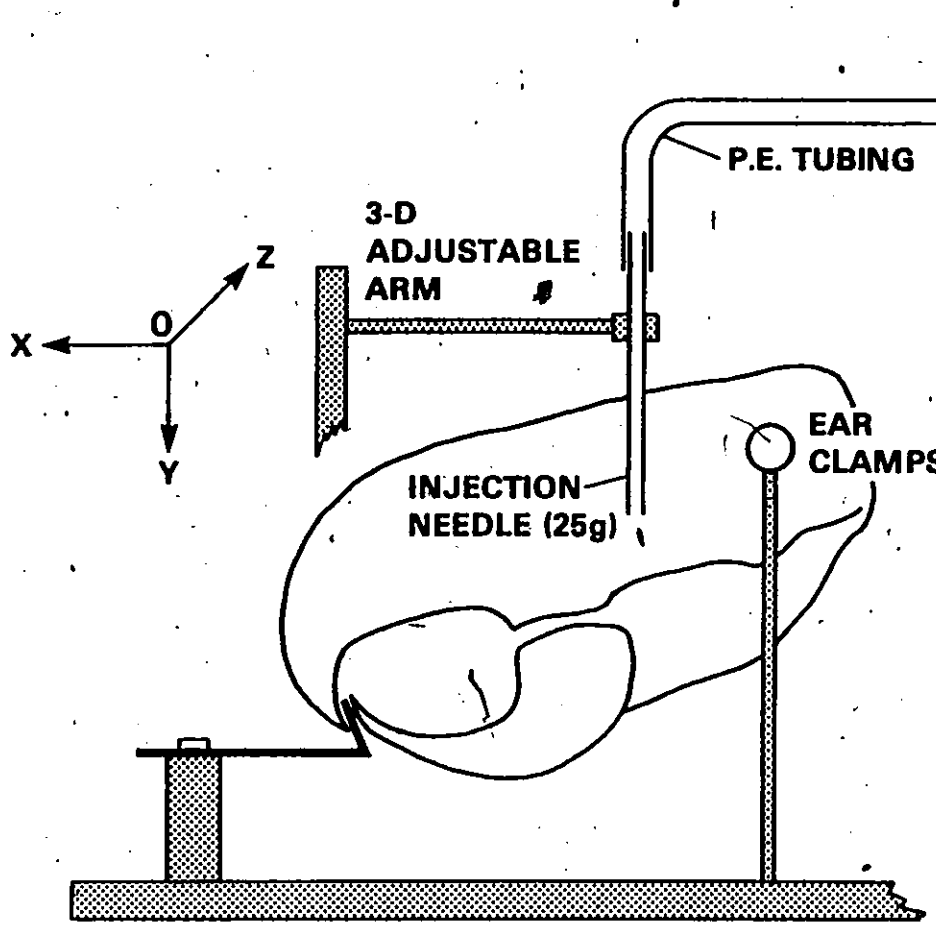
antioxidant) or 20 µl of carrier alone (Sham; thorium dioxide suspension and ascorbate). Each solution of 6-OHDA was prepared no longer than 10 minutes before actual injection and was kept on ice. Three minutes after completion of the infusion the cannula was removed, the burr hole was filled with bone wax and the skin sutured with silk thread.

Anaesthetized animals were then x-rayed in lateral view with a Siemens Gigantos x-ray generator with a small focus on 0.2 mm at a magnification of X3 to identify animals which had received a correctly located ventricular injection. The x-ray film was Kodak X-Omat using an ultradeltoid screen.

The degree of spreading of thorium throughout the ventricular system was evaluated using a subjective score system relying the criteria indicated in Table 3. Only animals with an x-ray score of one or above were accepted into the study.

FIGURE 7. Stereotaxic method for the injection of 6-hydroxydopamine into the lateral ventricle of the brain.

Animals are first anaesthetized and secured into the stereotaxic frame. A 25 gauge infusion cannula is then introduced into the left lateral cerebral ventricle. A dose of 200 µg of 6-hydroxydopamine is then infused into the ventricle dissolved in 20 µl of a 25% suspension of thorium dioxide at a rate of 1.8 µl/min. The animals are then x-rayed in lateral view to identify animals which show a ventricular thorium shadow characteristic of correctly injected rats. Animals which failed to exhibit this shadow were rejected.



6-HYDROXYDOPAMINE:

10µg/µl IN 25% SUSPENSION OF THORIUM DIOXIDE (ThO_2) WITH 0.4mg/ml ASCORBIC ACID

CO-ORDINATES:

X=0.0mm
Y=3.2 TO 3.6mm
Z=1.6mm

X-RAY:

LATERAL VIEW (Mag X3)

STEREOTAXIC METHOD FOR INTRAVENTRICULAR INJECTIONS OF 6-HYDROXYDOPAMINE

TABLE 3. Criteria of x-ray scores.

SCORE	CRITERIA
3	Complete spreading of thorium throughout the ventricular system (Figure 2-1).
2	Some spreading of thorium
1	Small amount of spreading
0	No shadow visible at all

Histological examination of brain tissue.

In order to demonstrate that the x-ray technique could identify animals which had a correctly located intracerebroventricular injection, ten animals were operated upon and their brains removed for histological examination to verify the actual site of injection. The results of this experiment are presented in Appendix 2. Following removal, the brains were frozen to -20°C in a freezer. The cerebellum was removed, the brain mounted on a metal stage using embedding medium (Tissue-Tek II) and placed in a cryostat at -20°C. Serial frontal sections 30 µm in thickness were cut and every second to fifth sample was placed on a microscope slide. The slides were then dried on a hot plate and then stained with Paragón fast blue (toluidine blue 3.6 mg/ml and basic fuchsin 1.4 mg/ml dissolved in 30% ethanol). Permanent mounts were made using Permount (Fischer Sci. Co. New Jersey).

CHAPTER 2.

EXPERIMENTAL PROTOCOLS.

EXPERIMENT 1: Effects of intracerebral catecholamine depletion on exercise performance.

This experiment consisted of four parts. The first part measured the regional depletion of catecholamines following an injection of 6-OHDA into the left lateral cerebral ventricles (Experiment 1A). The second part measured the effects of this depletion on exercise capacity (Experiment 1B). The third and fourth parts determined whether the effects of catecholamine depletion were the result of a dopaminergic mechanim (Experiment 1C) or a noradrenergic mechanism (Experiment 1D). A summary of the procedures performed in this experiment are presented in Figures 8, 9, 10, 11, 12 and 13).

Basic protocol.

Animals were exposed to the 8 day learning schedule. On day 5 animals scoring consistently 3 or 4 points were randomly allocated to one of three groups and treated as follows:

6-OHDA Animals were given an intracerebroventricular injection of 6-OHDA dissolved in 20 µl of thorium dioxide suspension and x-rayed.

Sham Animals received an intracerebroventricular injection of thorium dioxide suspension alone and x-rayed.

Intact No operation or x-ray studies performed

Animals which failed to exhibit any characteristic ventricular shadow (see Appendix 2) were eliminated from further study.

Injections of saline, apomorphine (APO) at 1.0 mg/Kg body weight and clonidine (CLON) at 0.5 mg/Kg body weight were given one hour prior to the animal's exhaustion run on Day 9.

EXPERIMENT 1A: Effects of icv 6-OHDA on regional brain monoamine content.

This experiment determined the effects of 6-OHDA on the concentrations of catecholamines in striatum, brain stem and hypothalamus. The protocol is summarized in Figure 8.

Nine animals were exposed to the 8 day learing schedule. On Day 5, five animals were given 6-OHDA and four animals were given carrier alone. (One rat was rejected because of low learning scores). On day 9, 6-OHDA animals which displayed thorium shadow scores of 0, 1, 2, 3 and 4 were weighed and decapitated. The striatum, hypothalamus and brain stem were dissected out and homogenized in 0.05 M perchloric acid. The four Sham animals had thorium shadow scores of 1, 1, 3 and 4. The extract of monoamines was isolated by centrifugation.

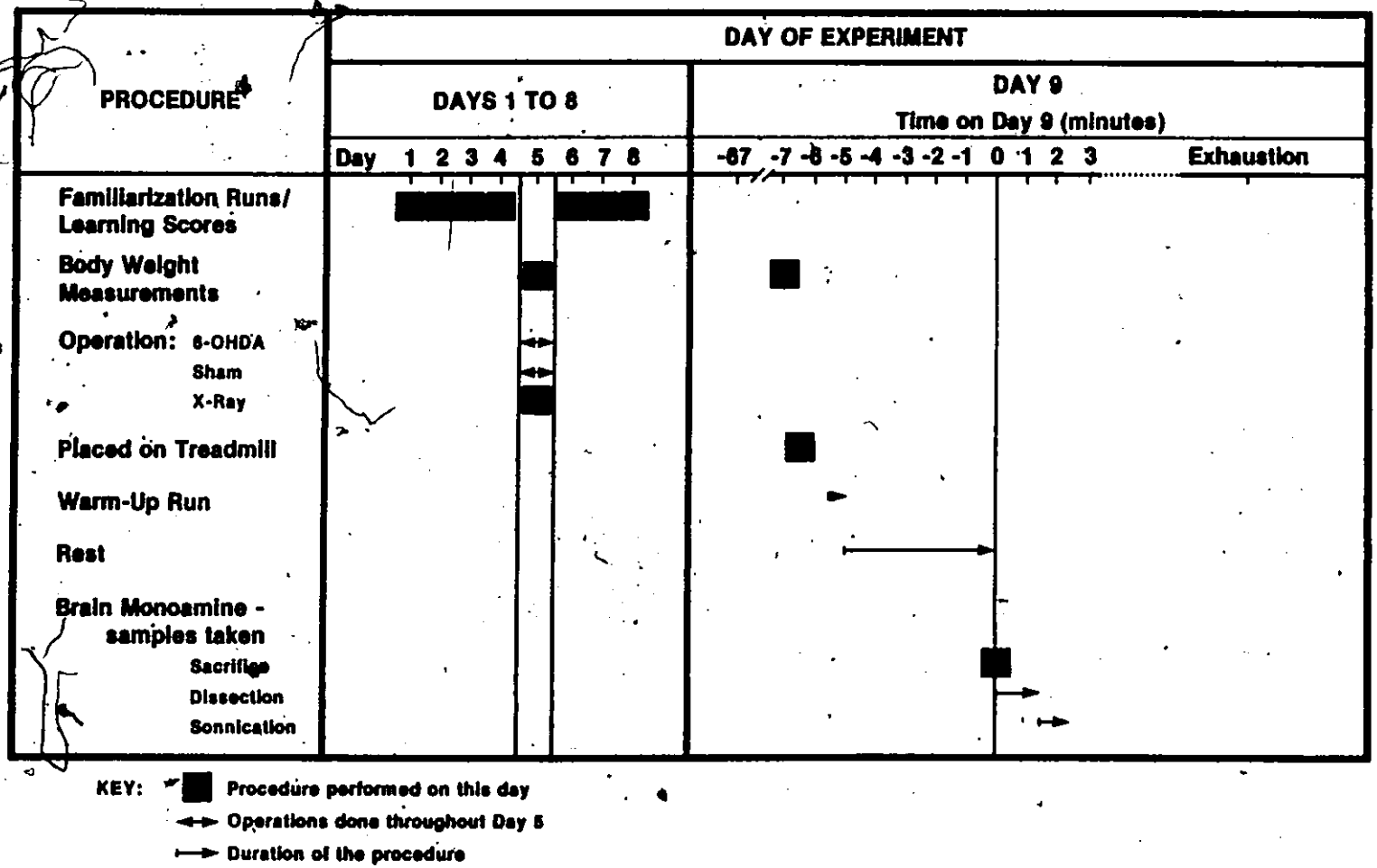
FIGURE 8. Protocol for Experiment 1A. Effects of intracerebroventricular 6-OHDA on regional brain monoamine content.

The object of theis experiment was to measure the degree of depletion of dopamine and norepinephrine in the striatum, brain stem and hypothalamus following an injection of 200 µg of 6-OHDA into the left lateral cerebral ventricle.

All rats were exposed to the 8-day learning schedule. On day 5 the animals received an injection of either 6-hydroxydopamine (6-OHDA) or carrier alone (Sham). x-ray views were taken to identify rats with a correctly located injection.

On day 9 the rats were sacrificed, and the brain regions dissected. Samples were placed in cold perchloric acid and sonicated to extract the monoamines. Levels of monoamines were measured by high pressure liquid chromatography.

Protocol for Experiment 1A:
Effects of Intracerebroventricular 6-OHDA on Regional Brain Monoamine Content



EXPERIMENT 1B: Effects of intracerebral catecholamine depletion on exercise performance.

This experiment determined whether exercise performance was impaired by depletion of intracerebral dopamine and norepinephrine. A summary of the experimental protocol is presented in Figure 9.

Eighteen animals were exposed to to the 8-day learning schedule. On day 5, four animals recived an icv injection of 6-OHDA, four animals were given the sham operation and six animals were left intact. The remaining four animals were eliminated because of low learning scores or x-ray scores of 0. On Day 9 the animals were run to exhaustion at a speed of 35 m/min.

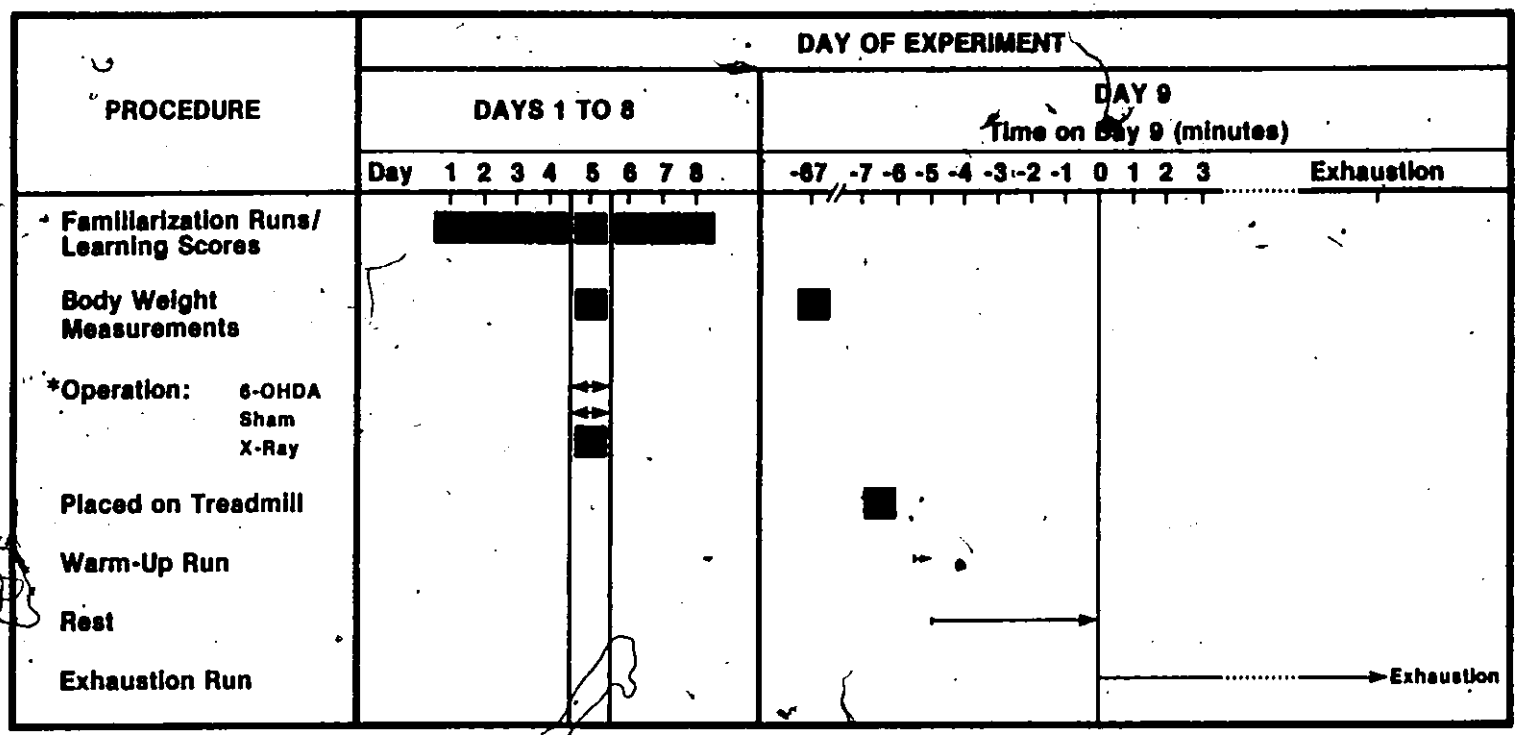
FIGURE 9. Protocol for Experiment 1B. Effects of intracerebral catecholamine depletion on exercise performance.

The object of this experiment was to determine whether the availability of intracerebral catecholamine can limit exercise capacity.

The protocol was the same as that of Experiemnt 1A (Figure 8) except that a group of rats which was not operated on (Intact) was included.

On day 9 the rats were run to exhaustion.

Protocol for Experiment 1B:
Effects of Intracerebral Catecholamine Depletion on Exercise Performance



KEY:
- ■ Procedure performed on this day
- ←→ Operations done throughout Day 5
- ——→ Duration of the procedure

* One group of animals was exposed to the above protocol except that no operation was performed (intact)

EXPERIMENT 1C: Effects of apomorphine on the exercise performance of 6-OHDA-treated rats.

To see whether the effects of 6-OHDA can be reversed by apomorphine. A summary of the experimental protocol is presented in Figure 10.

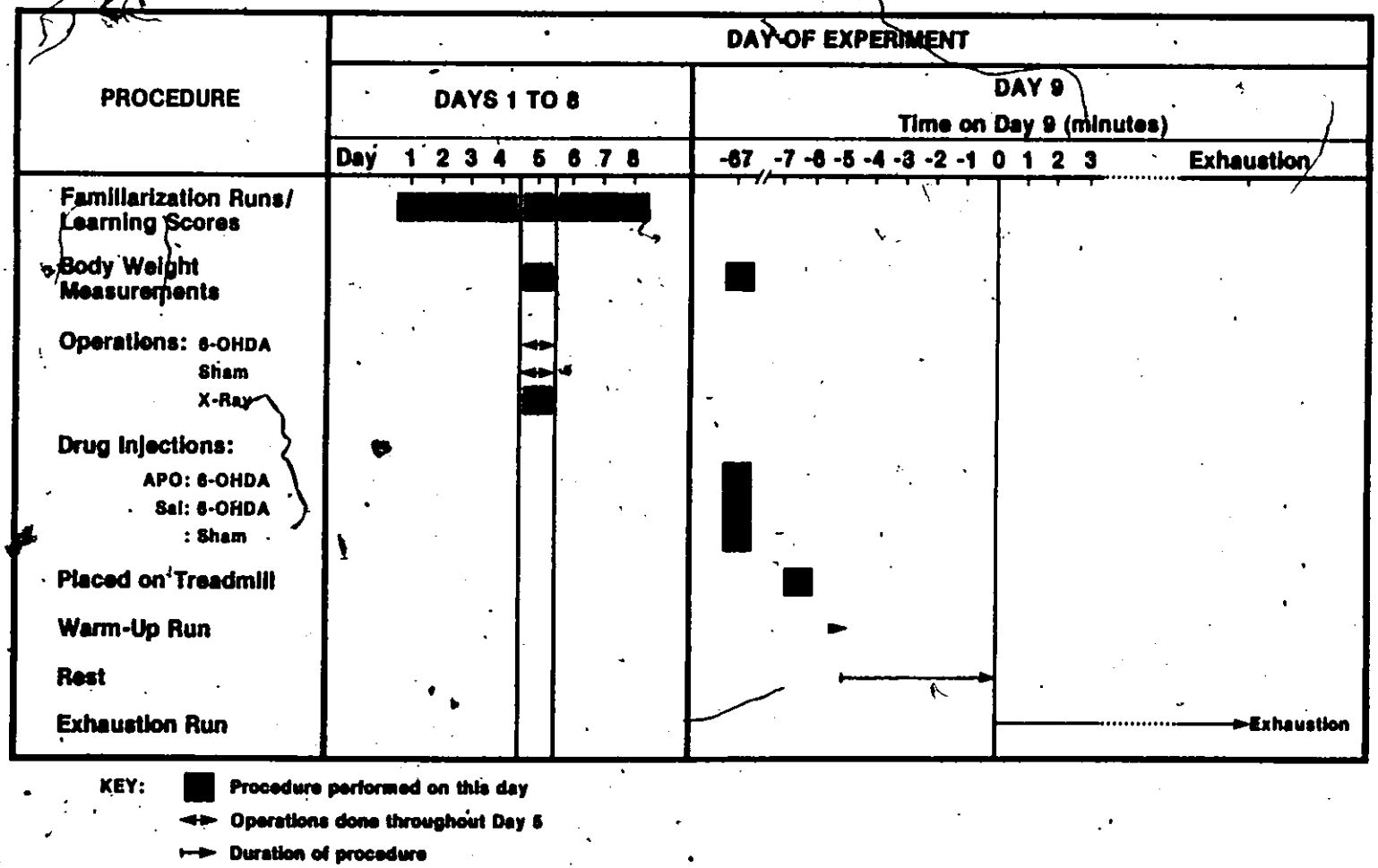
Thirty rats were exposed to the 8-Day learning schedule. On Day 5, 8 animals were given the Sham operation and 16 animals received an icv injection of 6-OHDA. Six animals were rejected on the basis of low learning scores and four animals were rejected because of x-ray scores of 0. On day 9, the rats treated with 6-OHDA were matched in pairs on the basis of their learning score patterns after the 6-OHDA injection. One of each pair was given an intraperitoneal (i.p.) injection of apomorphine (1 mg/Kg) and the other animal was given an injection of saline carrier alone (i.p.) one hour prior to being run to exhaustion.

FIGURE 10. Protocol for Experiment 1C. Effects of apomorphine on exercise performance in 6-OHDA animals.

The object of this experiment was to determine whether the detrimental effects of catecholamine depletion could be reversed by selective replacement of dopamine using the postsynaptic dopamine receptor agonist apomorphine (APO).

The protocol was the same as Experiment 1B (Figure 9) except that a group of 6-OHDA treated rats were given an intraperitoneal injection of apomorphine (1mg/Kg body weight); Sham and a group of 6-OHDA treated rats were given saline alone i.p.

Protocol for Experiment 1C:
Effects of Apomorphine on Exercise Performance in 6-OHDA Animals



EXPERIMENT 1D: Effects of clonidine on exercise performance of 6-OHDA-treated rats.

To see whether the effects of 6-OHDA could be reversed by clonidine. A summary of the experimental protocol is presented in Figure 11.

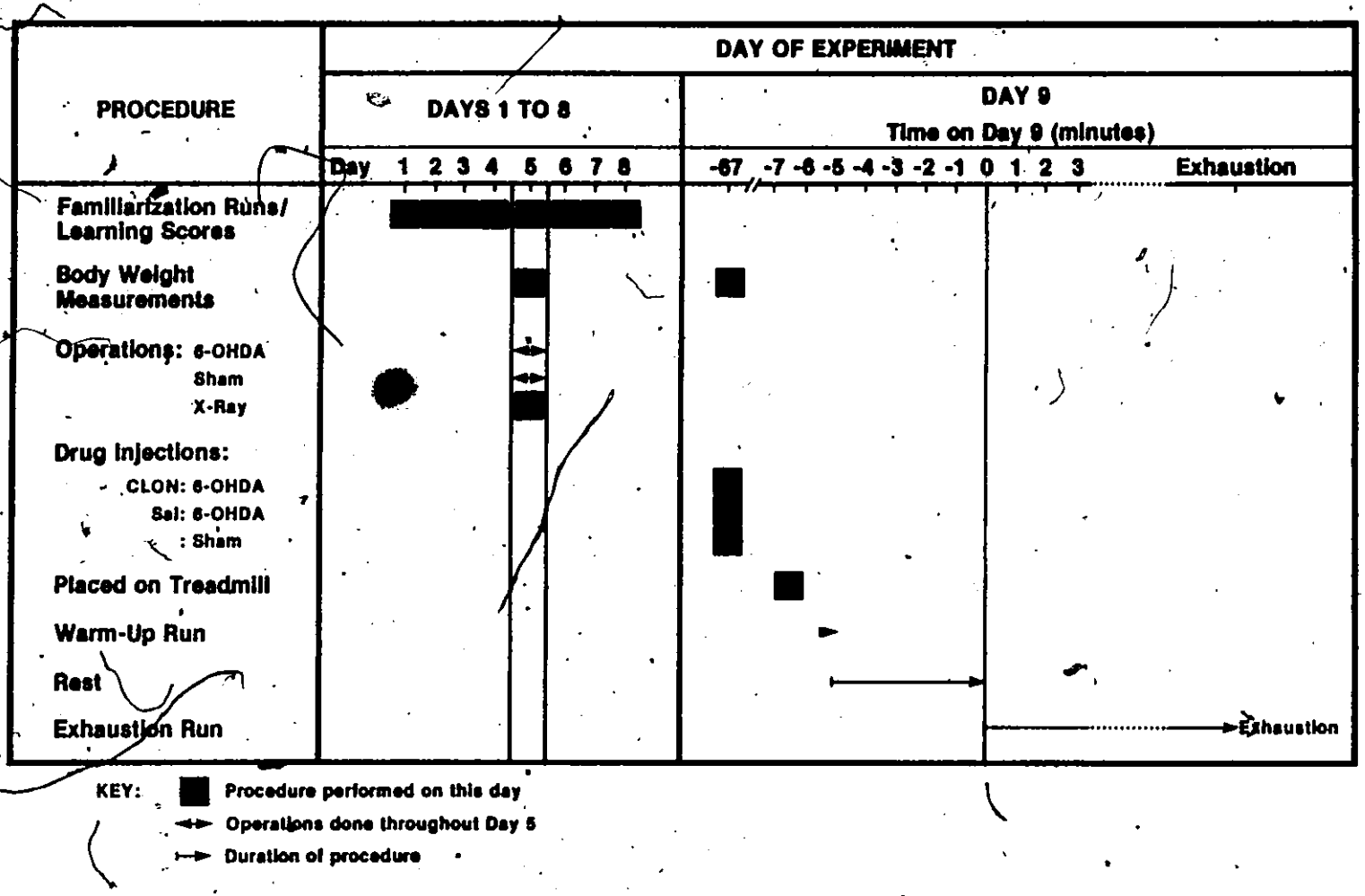
Animals were treated as in Experiemnt 1C except that clonidine (0.5 mg/Kg i.p.) was used instead of apomorphine.

FIGURE 11. Protocol for Experiment 1D. Effects of Clonidine on exercise performance in 6-OHDA animals.

The object of theis experiment was to determine whether the detrimental effects of catecholamine depletion on exercise performance could be reversed by the specific norepinephrine agonist clonidine (CLON).

The protocol was the same as that for Experiment 1C (Figure 10) except that clonidine (500 mg/Kg) was used instead of apomorphine.

Protocol for Experiment 1D:
Effects of Clonidine on Exercise Performance in 6-OHDA Animals



EXPERIMENT 2: Effects of apomorphine on exercise performance of intact animals.

This experiment determined whether exercise capacity was increased by the dopamine receptor agonist apomorphine. A summary of the experimental protocol is presented in Figure 12.

Thirty aniamls were exposed to the 8 day learning schedule. On day 9 the animals were randomly allocated to one of the following groups:

Apomorphine 1 (APO 1). Received 1.0 mg APO/Kg i.p.
Apomorphine 2 (APO 2). Received 2.0 mg APO/Kg i.p.
Apomorphine 5 (APO 5). Received 5.0 mg APO/Kg i.p.
Control (CON). Received saline carrier alone i.p.

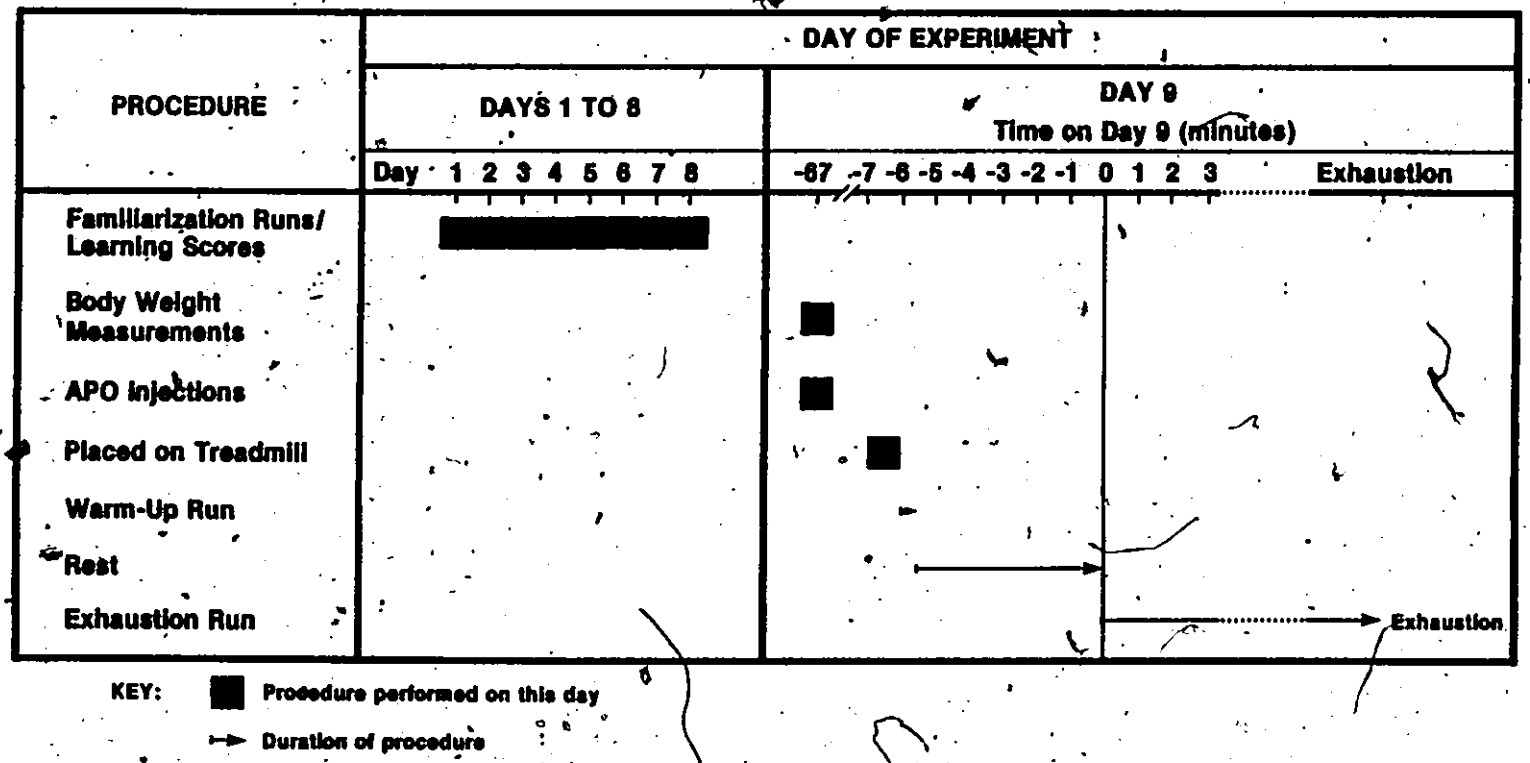
The injections were given one hour prior to being run to exhaustion.

FIGURE 12. Protocol for Experiment 2. Effects of apomorphine on exercise capacity in normal rats.

The object of this experiment was to determine whether apomorphine could increase exercise capacity in normal rats.

The animals were exposed to the 8-day learning schedule. On day 9 the animals were given and injection of apomorphine i.p. (1 mg/kg, 2 mg/Kg or 5 mg/Kg) one hour before being run to exhaustion.

Protocol for Experiment 2



EXPERIMENT 3: Effects of exhaustive exercise on regional brain dopamine turnover and dopamine concentrations.

This experiment determined whether the point of exhaustion was associated with failure of dopaminergic synaptic transmission, such as a depletion of dopamine or a decrease in the turnover of dopamine, (as indicated by the concentrations of DOPAC and HVA). I also measured indices of muscle metabolism in soleus, vastis lateralis white portion and vastus lateralis red portion to determine whether exercise capacity was limited by the depletion of muscle energy stores or accumulation of lactic acid. The metabolic characteristics of these muscles are:

1. Soleus: high oxidative, 80% slow twitch red and 20 % fast twitch red.
2. Vastus lateralis white: high glycolytic,100% fast twitch white.
3. Vastus lateralis red: high oxidative\high glycolytic 70% fast twitch red and 30% slow twitch red.

These muscles were chosen because they are activated during treadmill running and they represent the muscle fibre types found in this species (Barnard, Edgerton, Furukawa and Peter 1971; Baldwin, Klinkerfuss, Terjung, Mole and Holloszy

1972; Ariano, Armstrong and Edgerton 1973; Clarke and Conlee 1979).

Thirty three animals were exposed to the 8-day learning schedule. On day 9 the animals were weighed and placed on the treadmill. After one minute they were given a 0.5 minute warm-up run. Five minutes later the animals were randomly allocated to one of five groups and treated as follows (Figure 13):

Exhaustion.	Run to exhaustion at 36.0 m/min then removed.
Late.	Run until the photoelectric cell cell occlusion frequency was approximately half the maximum value of exhausted animals then removed.
At.	Run until the occlusion frequency had just begun to increase above 2 occl/15s then removed.
Early.	Run for a time that was half that of the At group then removed.
Control.	Immediately removed from the treadmill.

When removed from the treadmill the animals were immediately decapitated and trunk blood collected into vaccutainers containing sodium citrate. Brains were removed

from the crania and striatum, hypothalamus and brain stem were dissected out for quantitation of monoamines. Samples of soleus, vastus lateralis white and vastus lateralis red were obtained for energy metabolite analysis.

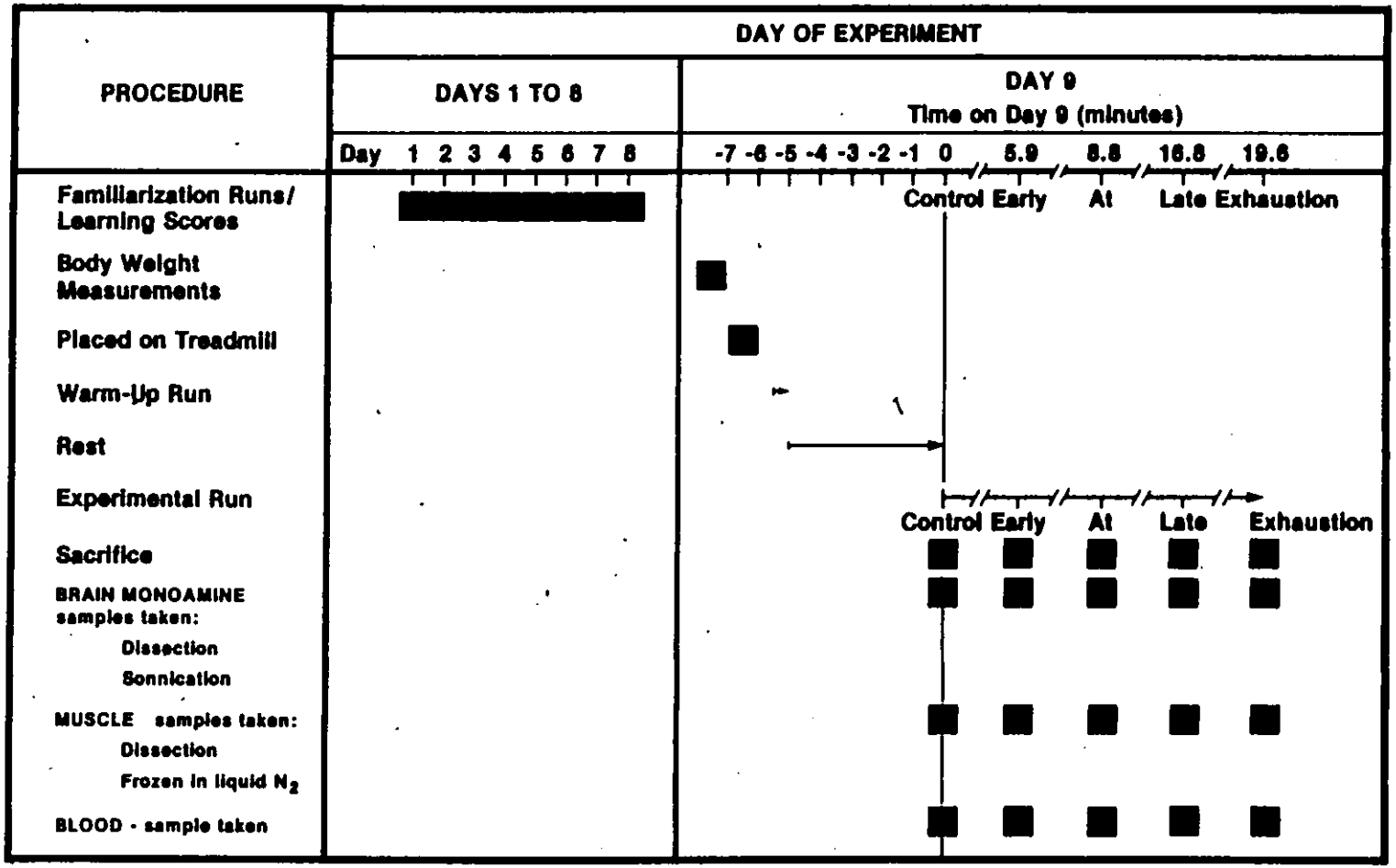
FIGURE 13. Protocol for Experiment 3. Effects of exhaustive exercise on the turnover of intracerebral dopamine.

The object of this experiment was to determine whether at the time of exhaustion there was any evidence of a failure of intracerebral dopaminergic transmission.

Rats were exposed to the 8-day learning schedule. On day 9 the rats were placed on the treadmill belt. After one minute rats were given a 30 second warm-up run and then allowed to rest for 5 minutes. The rats were then either immediately removed from the treadmill or run to various stages of exhaustion. Rats were removed either 'Early' during the run, 'At' the time when the photoelectric cell occlusion frequencies had just increased above 3 occlusions/15 s, 'Late' in the exercise when the occlusion frequencies were approximately 50% of the maximum value or run until 'exhaustion'.

After removal from the treadmill, rats were killed by decapitation and trunk blood collected for glucose and lactate measurements. The brain was dissected into striatum, brain stem and hypothalamus and a perchloric acid extract obtained for monoamine measurements. Samples of soleus, vastus laterlis white and vastus lateralis red were taken for energy substrate analysis.

Protocol for Experiment 3:
Effects of Exhaustive Exercise on the Turnover of Intracerebral Dopamine



CHAPTER 3.

BIOCHEMICAL ASSAYS.

REGIONAL BRAIN CATECHOLAMINE MEASUREMENTS.

Brain Dissection and Preparation of Samples.

The striatum, hypothalamus and brain stem were dissected according to the proceedure of Glowinski and Iversen (1966). The brain was removed from the cranium as quickly as possible after decapitation (within one minute) and immediately place on a chilled Petri dish over ice. The cerebellum and brainstem (medulla oblongata and pons) were separated from the dienchphalon and the cerebellum separated from the brain stem. The white matter of the pyramidal tract was then removed from the brain stem. A complete transverse section was then made at the level of the optic chiasm. The hypothalamus was dissected using a small dental scoop after first making an incision using the anterior commisure as horizontal reference and the line between the posterior hypothalamus and mamillary bodies as the caudal limit. The striatum was then dissected from both of the remaining portions of the brain using a dental scoop after first circumscribing the outer limits of the striatum with lateral ventricles as internal limits and the corpus callosum as external limits. The total time for the complete dissection

was between 1 to 2 minutes.

Brain sample treatment.

Each brain part was immediately added to an accurately weighed volume (2.0 ml) of ice cold 50 mM perchloric acid (pH=1.5) and re-weighed. The samples were homogenized using a Bronwill Biosnik sonicator, (Bronwill Scientific, Rochester New York, USA; Model 11A), for five minutes at <5°C. The perchloric acid extracts containing the monoamines were then isolated by centrification at 12000 g for 20 minutes at 4°C and stored at -70°C in Eppendorf polypropylene tubes.

Samples were sent to Bioanalytical systems on dry ice Measurement of dopamine, DOPAC, HVA, norepinephrine, 5-HT and 5-HIAA by high pressure liquid chromatography (Mayer and Shoup 1982). The measurements of catecholamines were not performed by the author on the advice of my thesis committee for two main reasons. First the equipment required for these measurements were not available to me at the time. Secondly the committee considered that to set-up such a technique was beyond the scope of the thesis.

MUSCLE AND BLOOD ENERGY SUBSTRATE MEASUREMENTS.

Muscle Dissection.

Samples of soleus, the red portion of the vastus lateralis and the white portion of the vastus lateralis were obtained from the right leg. First the skin around the ankle was cut with scissors and the skin and connective tissue peeled away from the gastrocnemius muscle group. The Achilles tendon was isolated by blunt dissection and cut close to the heel. The soleus was then removed after pulling the tendon away from the heel and the gastrocnemius from the tibia. The rectus femoris was then cut at its distal end and the vastus medialis separated from the vastus lateralis. A portion of the white vastus lateralis was then taken. This portion contains almost exclusively fast-twitch glycolytic fibres. The intermediate fibres of vastus lateralis were then removed and a sample of the red slow-twitch oxidative fibres was then taken. As the muscle samples were obtained, they were immediately wrapped an aluminium foil and plunged into liquid nitrogen. All samples were stored at -70^{o}C until assayed.

Measurements of muscle, liver and plasma metabolites.

Muscle glycogen, glucose, glucose-6-phosphate (G-6-P), lactate, adenosine triphosphate (ATP), and creatine phosphate (CrP); and plasme glucose and lactate concentrations were measured by the author by enzymatic fluormetric assays. All samples were measured in duplicate against standards run in the same assay. Fluorescence was measured in 10 mm X 75 mm glass test tubes.

Preparation of muscle, liver and plasma samples.

Muscle samples were ground in a stainless steel mortar and pestle with 10 ml of liquid nitrogen. Connective tissue and blood vessels were removed with forceps. Samples for glycogen, glucose and G-6-P measurements (100 mg of muscle) were taken, accurately weighed and placed in polyethylene tubes containing 1.1 ml of acetate buffer (0.2 M) at 0°C. Samples were homogenized (Polytron, Brinkman Instruments, Rexdale, Ontario, Canada; Model RT10). Following centrifugation at 5000 g for 10 minutes at 0°C, 100 µl of the supernatant was removed for glucose and glucose-6-phosphate measurements. To the remaining sample was added 20 µl of amyloglucosidase, the tube vortexed, covered with parafilm and then incubated in a water bath at 40°C for two hours to hydrolyse the glycogen in the sample to free glucose which could then be assayed. The reaction

was stopped by the addition of 0.4 ml of a 0.3 M Trizma base solution at 0°C. Samples were then centrifuged at 5000 g at 0°C for 10 minutes and the supernatant containing the glucose stored at -70°C until assayed.

Samples for muscle (80 mg) lactate, ATP and CrP were weighed and homogenized in 2.0 ml of 6% (PCA) at 0°C in polyethylene tubes. Following centrifugation at 5000 g and 0°C the supernatant was removed and stored at -70°C until assayed.

Plasma glucose and lactate were assayed in samples diluted 1 in 3 with 6% PCA.

ASSAY PROCEEDURES.

Muscle glycogen, glucose, G-6-P, and plasma glucose.

Glycogen was assayed as total glycosyl units liberated by amyloglucosidase (Keppler and Recker 1974). Background glucose and G-6-P was measured in aliquots of acetate buffer removed prior to the addition of amyloglucosidase. The quantitation of glucose and G-6-P depends on the enzymatic production of NADPH according to the following reactions (catalysed by hexokinase and glucose-6-phosphate dehydrogenase respectively) and its subsequent quantitation by fluorimetry.

$$\text{Glucose} + \text{ATP} \longrightarrow \text{G-6-P} + \text{ADP}$$

$$\text{G-6-P} + \text{NADP}^{+} \longrightarrow \text{6-Phosphogluconolactone} + \text{NADPH} + \text{H}^{+}$$

The reaction mixture contains 25 µl of sample plus:

Tris buffer (pH 7.5) 0.1M	1.0 ml
Sodium EDTA (pH 7.4) 0.1M	10.0 µl
Mercaptoethanol	1.0 µl
Magnesium chloride	2.0 mg
NADP	0.6 mg
ATP	0.8 mg
Water	To 2.0 ml

The tubes were read at 30/50 before any enzyme was added. Two µl of glucose-6-phosphate dehydrogenase were added to each tube, the tubes then shaken and after 15 minutes read to measure the amount of NADPH produced from the G-6-P already in the sample. Two µl of hexokinase were then added to each tube, the tubes shaken and after a further 15 minutes the increase in fluorescence produced by NADPH from glucose shuttled through both of the above reactions was measured.

Muscle and plasma lactate.

This assay depends on the production and quantitation of NADH according to the following reaction catalysed by lactate dehydrogenase.

$$\text{Lactate} + NAD^+ \longrightarrow \text{Pyruvate} + NADH + H^+$$

The reaction equilibrium lies well to the right because of the removal of pyruvate by hydrazine added to the

reaction mixture and the prevention of proton accumulation by incubation in a buffer system at pH 8.5.

The reaction mixture is 200 µl of muscle extract plus buffer containing:

Tris Hydrazine (ph 8.5)	1.0 ml
Sodium EDTA (pH 7.4)	10.0 µl
Mercatoethanol	1.0 µl
NAD	0.7 mg
Water	To 2.0 ml.

Samples were incubated at 25°C for 3 hours following the addition of 5 µl of lactate dehydrogenase and then read.

Muscle ATP and CrP.

The measurement of ATP and CrP was done as a coupled assay. ATP is measured first by the production and quantitation of NADPH according to the following reactions catalysed by hexokinase and glucose-6-phoasphate dehydrogenase respectively:

$$ATP + Glucose \longrightarrow G\text{-}6\text{-}P + ADP$$

$$G\text{-}6\text{-}P + NADP^{+} \longrightarrow 6\text{-phosphogluconolactone} + NADPH + H^{+}$$

CrP is reacted with ADP to form ATP catalysed by creatine phósphokinase:

$$Cr\text{-}P + ADP \longrightarrow Cr + ATP$$

ATP is then quantitated as described above.

The reaction mixture contains 50 µl of sample plus 2.0 ml of buffer containing the following:

Tris buffer (pH 7.5)		1.0 ml
Magnesium chloride (0.1M)		0.1 ml
Glucose (0.1M)		10.0 µl
Mercaptoethanol		1.0 µl
Sodium EDTA (0.1M)		10.0 µl
NADP (0.1M)		10.0 µl
Water	To	1.0 ml

Two µl of glucose-6-phosphate dehydrogenase were added to the tubes and the baseline fluorescence measured after 15 minutes at 30/50. Then 2 µl of hexokinase were added and the change in fluorescence due to the production of NADPH was measured. Ten µl of a 0.1M solution of ADP were added and the background fluorescence measured. To start the reaction 10 µl of creatine phosphokinase were added and the change in fluorescence measured after 15 minutes.

STATISTICAL ANALYSIS.

All formulae used were obtained from Bailey (1959), Brown and Hollander (1977) and Klienbaum and Kupper (1978).

Mean values were compared using one-tailed and two-tailed un-paired and paired 't'-tests where appropriate using a Hewlet-Packard HP-25 calculator. One way- and two way-analyses of variance were done using an Apple computer. For groups whose 'F' value was significant mean values were compared by Scheffe's method. Linear regression analysis was done by the method of least squares. Pearson's correlation coefficient (r) was calculated using a Hewlett-Packard HP-25 calculator.

Group values are presented as Mean+1SEM unless otherwise stated.

A p value of <0.05 was considered to be significant. Non significance is indicated by NS.

RESULTS

CHAPTER 1.

EXPERIMENT 1. EFFECTS OF INTRACEREBRAL CATECHOLAMINE DEPLETION ON EXERCISE PERFORMANCE.

Experiment 1A. Effects of icv 6-OHDA on regional brain monoamine content.

This experiment measured the degree of catecholamine depletion following the injection of 6-OHDA. The experimental protocol is summarized in Figure 8.

Catecholamine measurements.

The concentrations of dopamine, DOPAC, HVA, norepinephrine, 5-hydroxytryptamine (5-HT) and 5-hydroxy-indoleacetic acid (5-HIAA) in the striatum, hypothalamus and brain stem of Sham and 6-OHDA animals four days after their respective injections are presented in Table 4.

Four days after the injection of 200 μg 6-OHDA into the left lateral cerebral ventricle there was a significant decrease in the concentrations of dopamine (-40%, $p<0.05$), DOPAC (-50%, $p<0.01$) and HVA (-33%, $p<0.05$) in the striatum when compared to Sham. The degree of dopamine depletion was similar to that predicted by Kostrzewa and Jacobowitz (1974). A significant correlation was noted between the

striatal content of: dopamine and DOPAC, ($r=0.93$, $p<0.01$) and between dopamine and HVA, ($r=0.99$, $p<0.001$) in 6-OHDA-treated animals. These correlations are consistent with there being a decrease in dopaminergic activity. A significant correlation was noted between the x-ray score (degree of spreading of thorium dioxide) and the striatal contents of: dopamine, ($r=0.88$, $p<0.05$); DOPAC, ($r=0.90$, $p<0.01$); and HVA, ($r=0.90$, $p<0.01$). This finding represents the first time that the effects of 6-OHDA can be predicted by a measure other than direct catecholamine measurement. No significant changes in the content of dopamine DOPAC or HVA were observed in either hypothalamus or brain stem.

A significant decrease in the norepinephrine content of both the striatum (-60%, $p<0.001$) and the hypothalamus (-86%, $p<0.02$). No change in the brain stem norepinephrine content was observed in 6-OHDA rats compared to Sham. The degree of norepinephrine depletion was greater than the depletion of dopamine; a phenomenon which has been noted previously (see Kostrzewa and Jacobowitz 1974).

No significant changes in the concentrations of 5-HT or 5-HIAA in any brain region were observed except for a significant decrease in the 5-HIAA content of the hypothalamus of 6-OHDA animals (-47%, $p<0.05$).

TABLE 4. EXPERIMENT 1A. Effects of 6-hydroxydopamine on regional brain catecholamine concentrations.

	CONCENTRATION OF CATECHOLAMINE (ng/g Wet Weight)					
	DA	DOPAC	HVA	NE	5-HT	5-HIAA
STRIATUM						
Sham	6746+584	744+ 86	612+ 65	107+6	98+ 7	82+11
6-OHDA	4052+900*	344+119**	409+110*	40+7**	92+20	75+24
BRAIN STEM						
Sham	40+4	27+4	21+3	303+43	42+6	46+ 5
6-OHDA	50+8	24+2	21+5	253+75	32+9	44+18
HYPOTHALAMUS						
Sham	295+101	111+25	74+20	1973+335	116+28	136+13
6-OHDA	285+ 33	86+ 7	64+ 4	270+ 47*	82+10	74+25*

Values are Mean+1SEM.

*$p<0.05$

**$p<0.01$

Samples were obtained four days after an injection of 200 µg of 6-OHDA into the left lateral cerebral ventricle.

The weights of the brain parts used are given in Table 5. The weights of the brain parts were the same in both groups.

Learning scores.

Sham animals maintained their learning scores at control values (Days 2 to 5) following the operation (Days 6 to 8). In contrast, 6-OHDA showed a significant decrease in their learning scores on the first and second day after the operation (Day 6) compared to Sham and the groups's own control value. However by the third day after the operation (Day 8) their learning scores were not significantly different from either pre-operation values or the scores of Sham animals (Figure 14).

Body weight changes.

Both sham and 6-OHDA-treated animals showed significant decreases in body weights following the operation ($p<0.05$) with a trend for 6-OHDA animals to have larger decreases than Sham animals (Table 6; Sham = -5.9% and 6-OHDA -8.1%).

TABLE 5. EXPERIMENT 1A. Weights of striatum, brain stem and hypothalamus.

	WEIGHT OF TISSUE (g)		
	STRIATUM	BRAIN STEM	HYPOTHALAMUS
Sham	44.9+6.5	199.5+ 5.2	88.5+7.2
6-OHDA	49.6+3.2	221.6+13.2	84.2+4.2

FIGURE 14. EXPERIMENT 1A. Effects of intracerebral catecholamine depletion on learning scores.

Animals were exposed to the 8-day learning schedule (Table 1). Their run performances were given a score of between 0 to 4 based on the criteria outlined in Table 2. Any animal which scored two points or less on one or more days during the first five days of the learning schedule are rejected from further study.

Control learning scores are the average score of days 2 to 5 inclusive. On day 5 animals were randomly allocated to either a 6-OHDA or Sham group and received an injection of either 6-hydroxydopamine (6-OHDA) or carrier alone (Sham) into the left lateral cerebral ventricle. The animals were then x-rayed in lateral view and animals which failed to show a thorium shadow were rejected from further study.

The Sham operation was without significant effect on the learning scores. Catecholamine depletion (see Table4) resulted in a significant decrease in learning scores on day 6 and 7 (the first and second day post-operative) compared to either the animals own Control values or the scores of equivalent Sham animals. On days 8 and 9, learning scores were not significantly different from either the animals own control values or the values of Sham animals.

COMPARISON OF LEARNING SCORES IN SHAM AND 6-OHDA-TREATED RATS

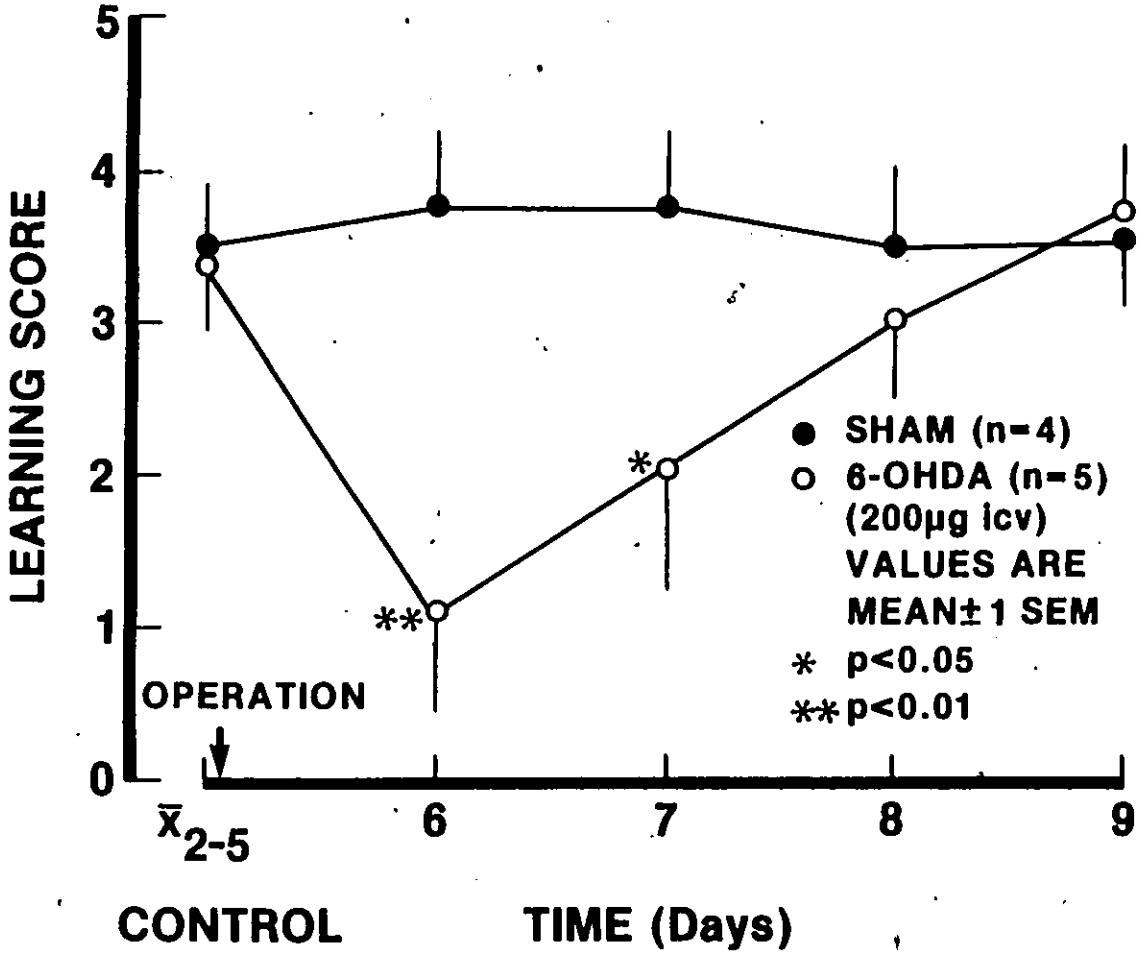


TABLE 6. EXPERIMENT 1A. Changes in body weight in Sham and 6-OHDA animals.

	BODY WEIGHT (g)		
DAY	5	9	CHANGE
GROUP			
Sham	411.0+4.2	387.2+5.7	-24.2+3.6
6-OHDA	379.7+11.9	348.9+18.7	-30.8+5.7

Experiment 1B. Effects of catecholamine depletion on exercise performance.

This experiment was designed to test the prediction that depletion of intracerebral dopamine would decrease exercise capacity. A summary of the protocol used is shown in Figure 9.

Learning scores.

The learning scores of both Intact and Sham animals were maintained above three throughout the experiment indicating both groups of rats were able to run on the treadmill without any apparent motor problems. In contrast, 6-OHDA animals decreased their learning scores on day 6 indicating that these rats had difficulty in running to avoid the treadmill. When the animals did run, the animals were quite able to perform the stepping motions of running. Therefore the decrease in learning score was not due to a problem of motor coordination per se. When the rats were run to exhaustion on day 9, however, the learning scores were indistinguishable from those of Intact and Sham animals and the rats own pre-operative values (Figure 15).

Exercise performance: Time of exhaustion and photoelectric cell occlusion frequency pattern.

Intact and Sham animals ran for a time that was not significantly different from each other. These results indicate that the operation per se was without effect on exercise capacity. In contrast, 6-OHDA animals ran for significantly less time than Sham animals ($p<0.01$; Figure 16). This experiment indicates that dopamine and/or norepinephrine depletion can limit exercise capacity. Experiments 1C and 1D were performed to determine which catecholamine was responsible.

Photoelectric cell occlusion frequency of both Intact and Sham animals remained below 2 occlusions/15 s during the first 10 minutes (33%) of the total run duration. The rats ran with no difficulty in avoiding the grid or keeping pace with the treadmill belt. After this time the photoelectric cell occlusion frequency increased throughout the remainder of the run (Figure 17) indicating that the rats were having difficulty in keeping pace with the pace of the treadmill.

Catecholamine-depleted rats also had no difficulty in keeping pace with the treadmill during the early part of the exercise. Furthermore there was no discernable difference between the running posture of catecholamine-depleted rats and either Intact or Sham animals. The pattern

of occlusion frequencies shown by 6-OHDA animals was qualitatively similar to that of intact and Sham animals. However, the time taken to execute the pattern was different. In 6-OHDA animals the occlusion frequency remained below 2 occlusions /15 s for the first 4 minutes (21%) of the run, before increasing progressively throughout the remainder of the run. The occlusion frequency increased above 3 occlusions/15s significantly sooner in 6-OHDA rats compared to Intact or Sham (8.1+1.1 min vs 14.8+1.8 min; $p<0.01$). The peak occlusion frequency was the same in all three groups (Intact: 12.8+1.7 occl/15s; Sham: 12.7+1.6 occl/15s; 6-OHDA: 12.5+2.7 occl/15s).

Body weight changes.

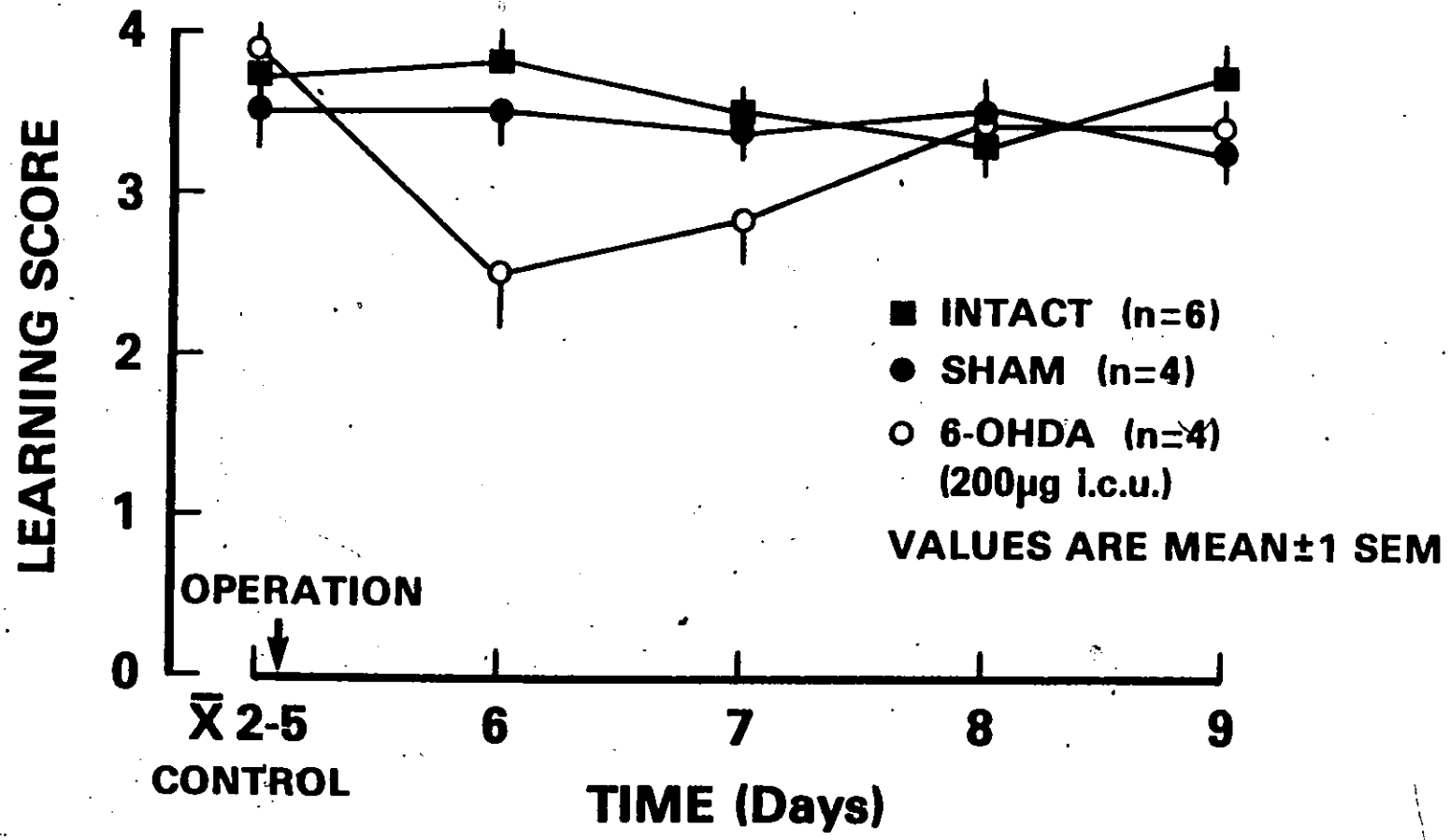
All three groups showed significant decreases ($p<0.05$) in their body weights from Day 5 to Day 9 (Table 7; Intact: -6.5%; Sham: -4.5%; 6-OHDA: -8.5%.).

FIGURE 15. EXPERIMENT 1B. Comparison of learning scores of Intact (no operation), Sham and 6-hydroxydopamine (6-OHDA) animals.

The experimental protocol was identical to that in Figure 14 Experiment 1A.

The learning scores of Intact and Sham animals remained constant throughout the 8-day learning schedule. Catecholamine depletion resulted in a significant decrease in learning scores on day 6, although they had returned to Control values by days 7, 8 and 9.

COMPARISON OF LEARNING SCORES IN INTACT, SHAM AND 6-OHDA TREATED RATS



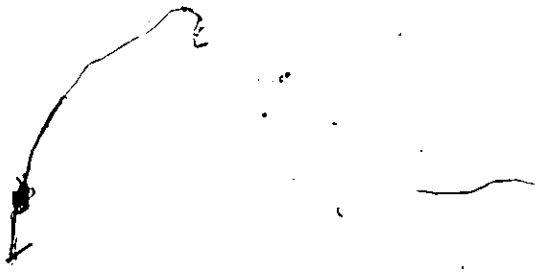


FIGURE 16. EXPERIMENT 1B. Effects of intracerebral catecholamine depletion on the time of exhaustion.

Animals were exposed to the 8-day learning schedule. Their learning scores are presented in Figure 15.

On day 5 the animals received an injection of 200 µg of 6-hydroxydopamine into the left lateral cerebral ventricle dissolved in 20 µl of a 25% suspension of thorium dioxide (6-OHDA) or carrier alone (Sham). Another group of animals were not operated upon (Intact). On day 9 the animals were run to exhaustion at a speed of 35 m/min.

Intact and Sham animals ran for the same length of time. In contrast, catecholamine depleted animals had times of exhaustion that were significantly less than the control animals.

INTRAVENTRICULAR INJECTIONS OF 6-HYDROXYDOPAMINE SIGNIFICANTLY IMPAIRS EXERCISE PERFORMANCE IN THE RAT

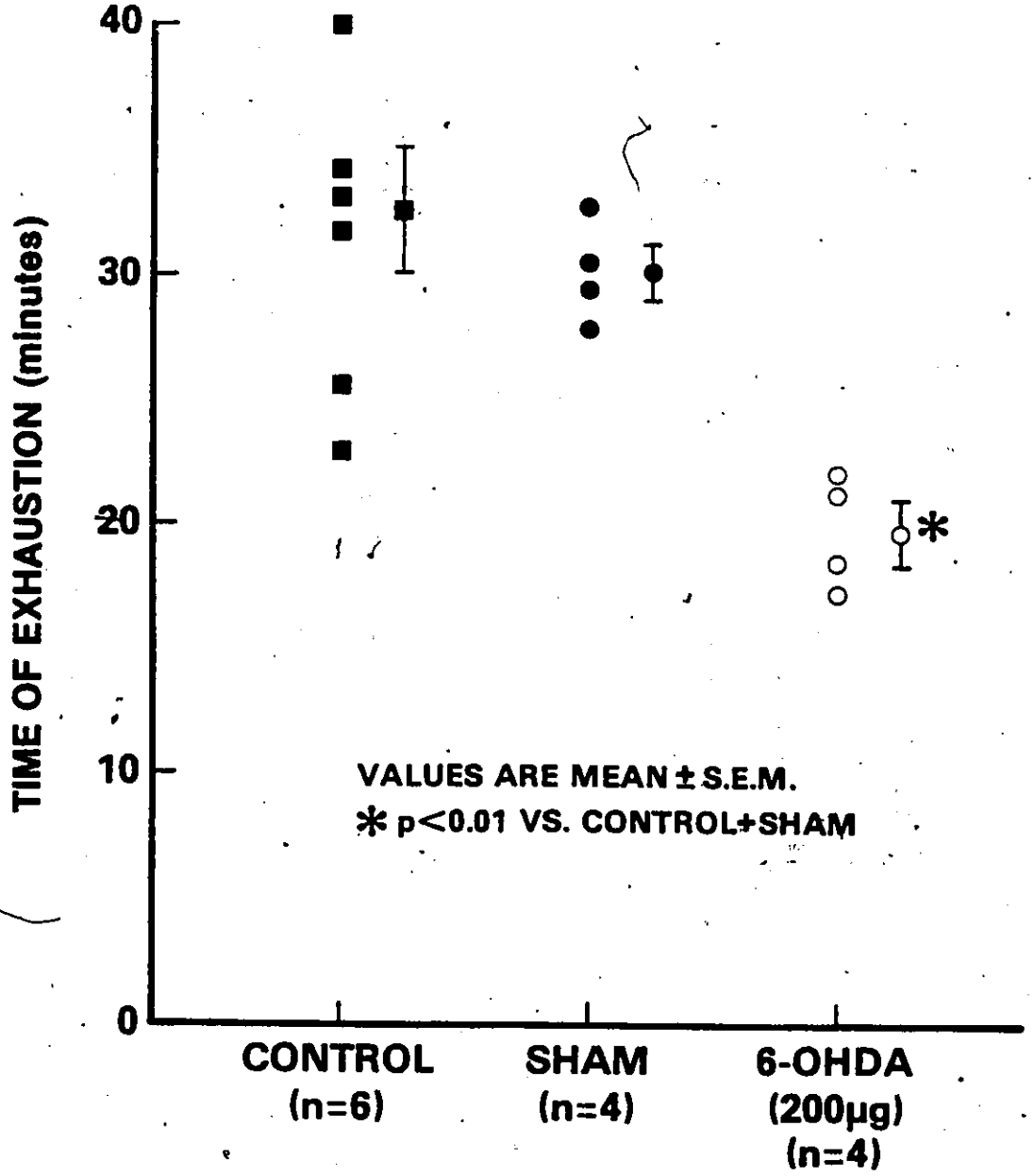


FIGURE 17. EXPERIMENT 1B. Photoelectric cell occlusion frequency during exhaustive exercise in Intact, Sham and 6-OHDA animals. Values were obtained from the animals described in Figure 16.

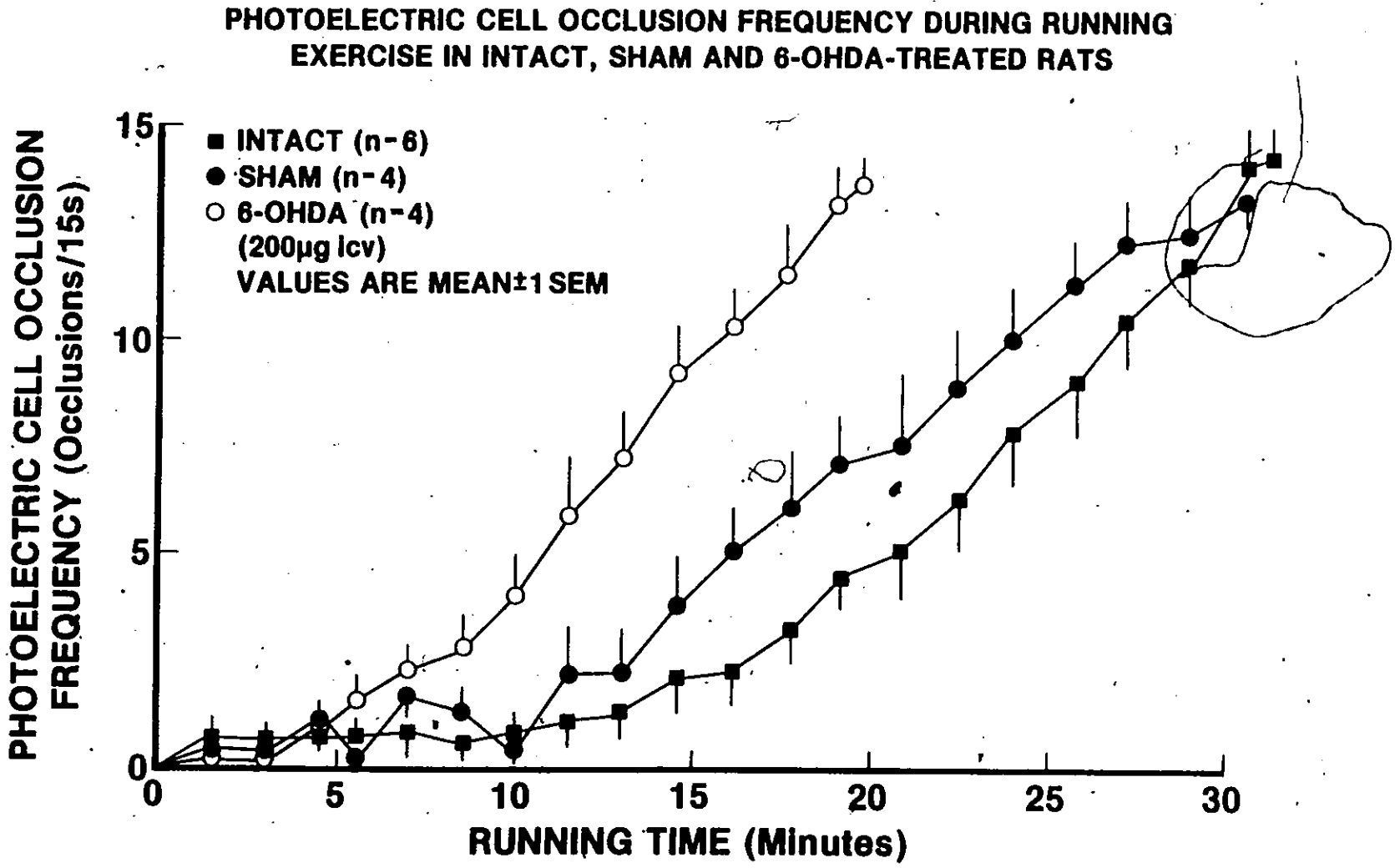

PHOTOELECTRIC CELL OCCLUSION FREQUENCY DURING RUNNING EXERCISE IN INTACT, SHAM AND 6-OHDA-TREATED RATS
■ INTACT (n-6)
● SHAM (n-4)
○ 6-OHDA (n-4)
(200µg icv)
VALUES ARE MEAN±1 SEM
PHOTOELECTRIC CELL OCCLUSION FREQUENCY (Occlusions/15s)
0
5
10
15
0
5
10
15
20
25
30
RUNNING TIME (Minutes)

TABLE 7. EXPERIMENT 1B. Body weight changes in Intact, Sham and 6-OHDA animals.

	BODY-WEIGHT (g)		
DAY	5	9	CHANGE
GROUP			
Intact	382.3+11.3	357.4+14.3	-24.9+3.9
Sham	369.2+ 3.6	352.7+ 5.5	-16.5+2.8
6-OHDA	383.3+ 4.4	359.1+12.1	-24.1+2.4

Experiment 1C. Effects of apomorphine on exercise performance in 6-OHDA animals.

Learning scores.

Sham animals maintained their learning scores above 3 throughout the experiment. In contrast both groups of 6-OHDA animals (+APO and +Sal), learning scores were significantly reduced by catecholamine depletion on day 6 and 7. By day 8 their learning scores were not significantly different either from their own control values or from Sham animals (Figure 18).

Exercise performance: Time of exhaustion and occlusion frequency patterns.

Sham animals ran for 21.6+1.4 minutes. Animals treated with saline (6-OHDA+sal) ran for a significantly shorter time than Sham, ($p<0.01$) a finding comparable with that of Experiment 1B. However 6-OHDA rats given apomorphine (6-OHDA+APO) ran for a time that was actually significantly longer ($p<0.05$) than Sham animals (Figure 19).

The pattern of occlusion frequencies during the exhaustion run are presented in Figure 20. This pattern was qualitatively similar to that of animals in Experiment 1B (Figure 17). During the early part of the run, the occlusion frequencies of the three groups were indistinguishable from

each other and remained below 3 occlusions/15 s. However, 6-OHDA animals begin to increase their occlusion frequencies above 3 occlusions/15 s sooner than either the Sham or 6-OHDA+APO animals. Linear regression analysis showed, that once the slopes of the occlusion frequencies increased above 3 occlusions/15 s the slope of the line in 6-OHDA+Sal animals was not parallel to the line of either Sham+Sal or 6-OHDA+APO ($p<0.05$). This indicates that once the animals were having difficulty avoiding the grid, the performance of the 6-OHDA+Sal animals deteriorated faster than the other two groups. The slopes of the Sham+Sal and 6-OHDA+APO animals were however parallel. Thus apomorphine was also effective in restoring the pattern of occlusion frequencies back to control values.

Body weight changes.

All three groups decreased their body weights ($p<0.05$) from day 5 to day 9; (Table 8; Sham: -3.1%; 6-OHDA+Sal: -6.1%; 6-OHDA+APO: -7.0%).

FIGURE 18. EXPERIMENT 1C. Comparison of learning scores of Sham, 6-OHDA and 6-OHDA-Apomorphine treated rats.

The experimental protocol was the same as in Figure 14. The decrease in the learning scores on days 6 and 7 were the same in both 6-OHDA and 6-OHDA-Apomorphine animals. (Note: Apomorphine was not given during the period in which learning scores were evaluated, except on day 9 when Apomorphine was given one hour prior to the run).

COMPARISON OF LEARNING SCORES IN 6-OHDA-SALINE AND 6-OHDA-APOMORPHINE TREATED RATS

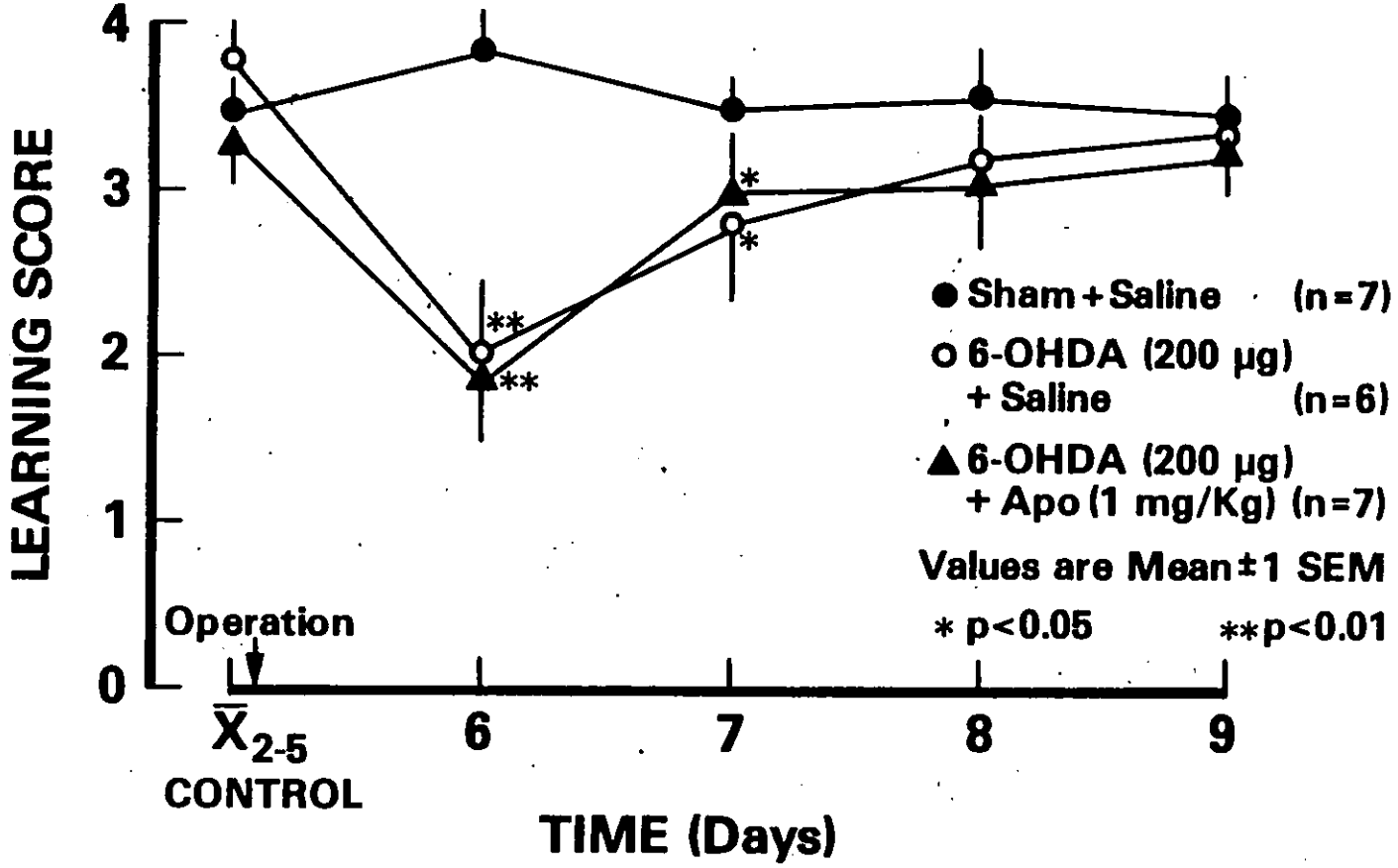


FIGURE 19. EXPERIMENT 1C. Effects of Apomorphine on the time of exhaustion in 6-hydroxydopamine-treated rats. Animals were exposed to the experimental protocol described in Figure 14, except that 6-OHDA animals were split into two groups. The rats were then given an injection of Apomorphine (1.0 mg/Kg i.p., dissolved in 0.9% saline) (Apo) or saline alone (Sal) and run to exhaustion at a speed of 37 m/min. As control a group of Sham animals were given an injection of saline alone.

THE DECRIMENTS IN EXERCISE PERFORMANCE BY 6-HYDROXYDOPAMINE ARE PREVENTED BY APOMORPHINE

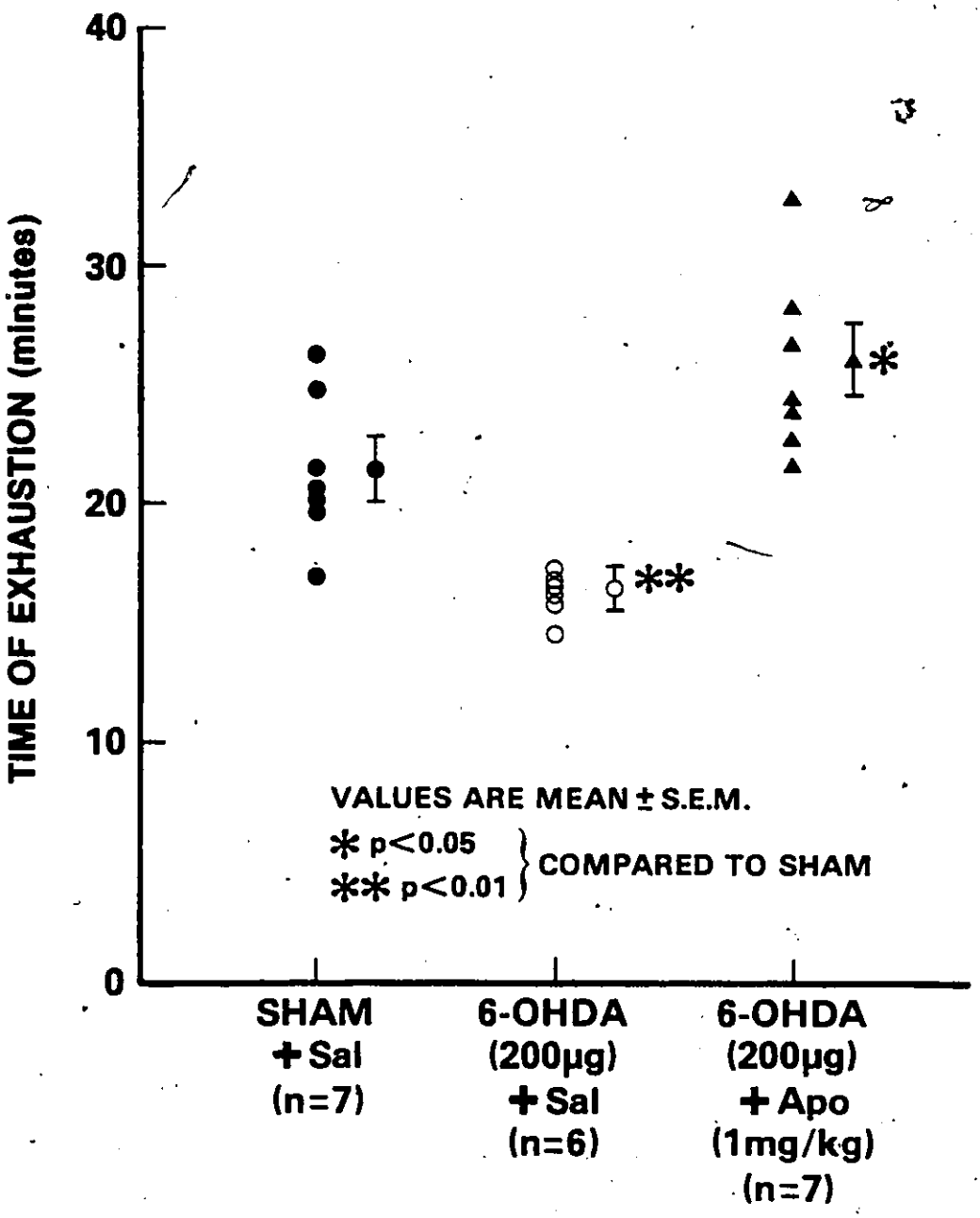


FIGURE 20. Photoelectric cell occlusion frequencies during the exhaustion run in Sham, 6-OHDA and 6-OHDA+Apomorphine animals.

The values were obtained from the animals described in Figure 19.

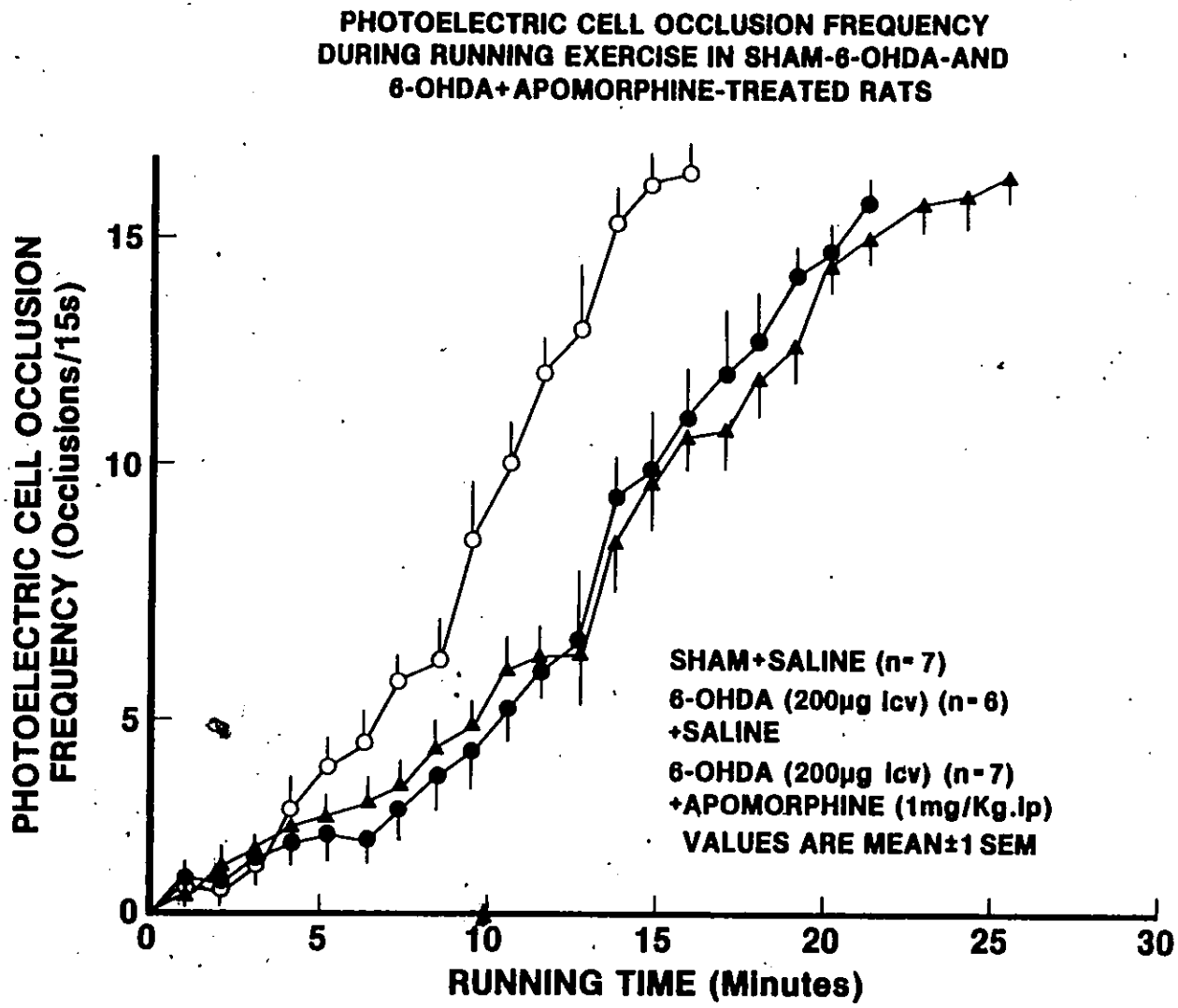

PHOTOELECTRIC CELL OCCLUSION FREQUENCY DURING RUNNING EXERCISE IN SHAM-6-OHDA-AND 6-OHDA+APOMORPHINE-TREATED RATS
PHOTOELECTRIC CELL OCCLUSION FREQUENCY (Occlusions/15s)
0
5
10
15
0
5
10
15
20
25
30
RUNNING TIME (Minutes)
SHAM+SALINE (n=7)
6-OHDA (200µg Icv) (n=6) +SALINE
6-OHDA (200µg Icv) (n=7) +APOMORPHINE (1mg/Kg.Ip)
VALUES ARE MEAN±1 SEM

TABLE 8. EXPERIMENT 1C. Body weight changes in Sham, 6-OHDA and 6-OHDA+Apomorphine animals.

	BODY WEIGHT (g)		
DAY	5	9	CHANGE
GROUP			
Sham	406.9+6.3	394.3+6.1	-12.6+3.2
6-OHDA	409.3+6.2	384.2+8.7	-25.1+4.9
6-OHDA+APO	406.0+4.6	377.7+6.7	-28.3+9.2

EXPERIMENT 1D. Effects of Clonidine on exercise performance in 6-OHDA animals.

Learning scores.

Sham rats maintained learning scores above 3 throughout the learning schedule. However, learning scoes were significantly decreased on day 6 in both groups of 6-OHDA animals to the same degree but were back to control and Sham values by day 8 (Figure 21).

Exercise performance: Time of exhaustion and occlusion frequency patterns.

Catecholamine depletion resulted in a significant decrease in exhaustion time compared to Sham ($p<0.05$). Clonidine was ineffective in restoring exercise performance in 6-OHDA animals because their time of exhaustion was not significantly different from 6-OHDA+Sal rats (Figure 22) but were significantly less than that of Sham rats ($p<0.05$).

The occlusion frequency patterns of Sham and 6-OHDA animals was very similar to the pattern showed by the Sham and 6-OHDA aniam animals in Experiment 1B and 1C (Figures 17 and 20). The occlusion frequency patterns in 6-OHDA+CLON animals was not significantly different from 6-OHDA+Sal at any time.

Body weight changes.

Body weights were decreased in all groups (Table 9; Sham: -4.5%; 6-OHDA+Sal: -5.8%; 6-OHDA+CLON: -5.4%).

FIGURE 21. EXPERIMENT 1D. Comparison of learning scores of Sham, 6-OHDA and 6-OHDA-Clonidine treated rats.

The experimental protocol was the same as in Figure 14.

The decrease in learning scores on day 6 was the same in both 6-OHDA and 6-OHDA-Clonidine animals. (Note: Clonidine was not administered during the period in which learning scores were evaluated except for day 9, when Clonidine was given one hour prior to the run).

COMPARISON OF LEARNING SCORES IN 6-OHDA-SALINE AND 6-OHDA-CLONIDINE TREATED RATS

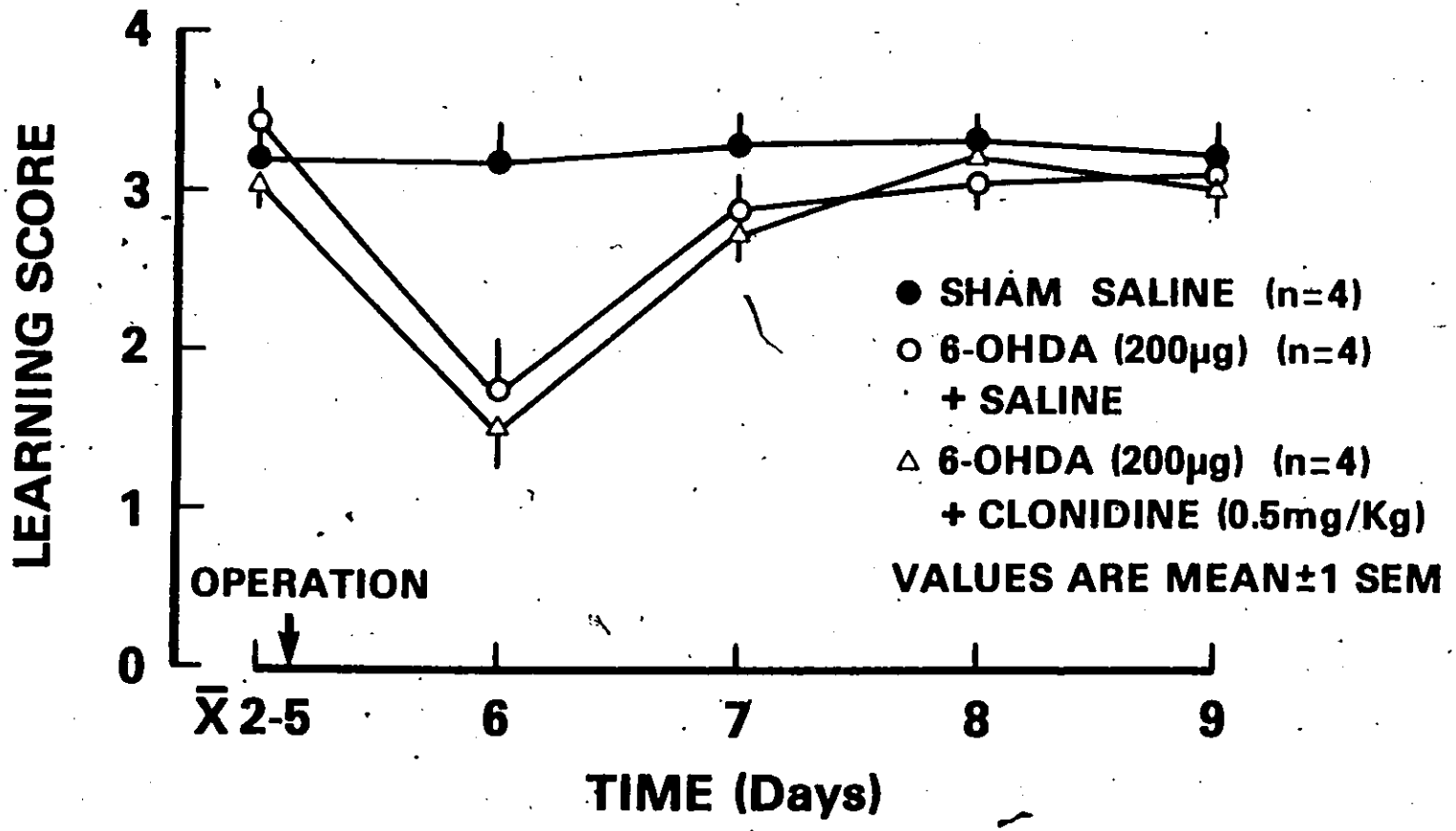


FIGURE 22. EXPERIMENT 1D. Effects of Clonidine on the time of exhaustion of 6-hydroxydopamine-treated rats.

The animals were exposed to the same experimental protocol as the animals described in Figure 14 and 19 except that Clonidine (0.5 mg/Kg i.p.) was used instead of Apomorphine and the treadmill speed was 36.0 m/min.

THE DECRIMENTS IN EXERCISE PERFORMANCE BY 6-OHDA CANNOT BE PREVENTED BY CLONIDINE

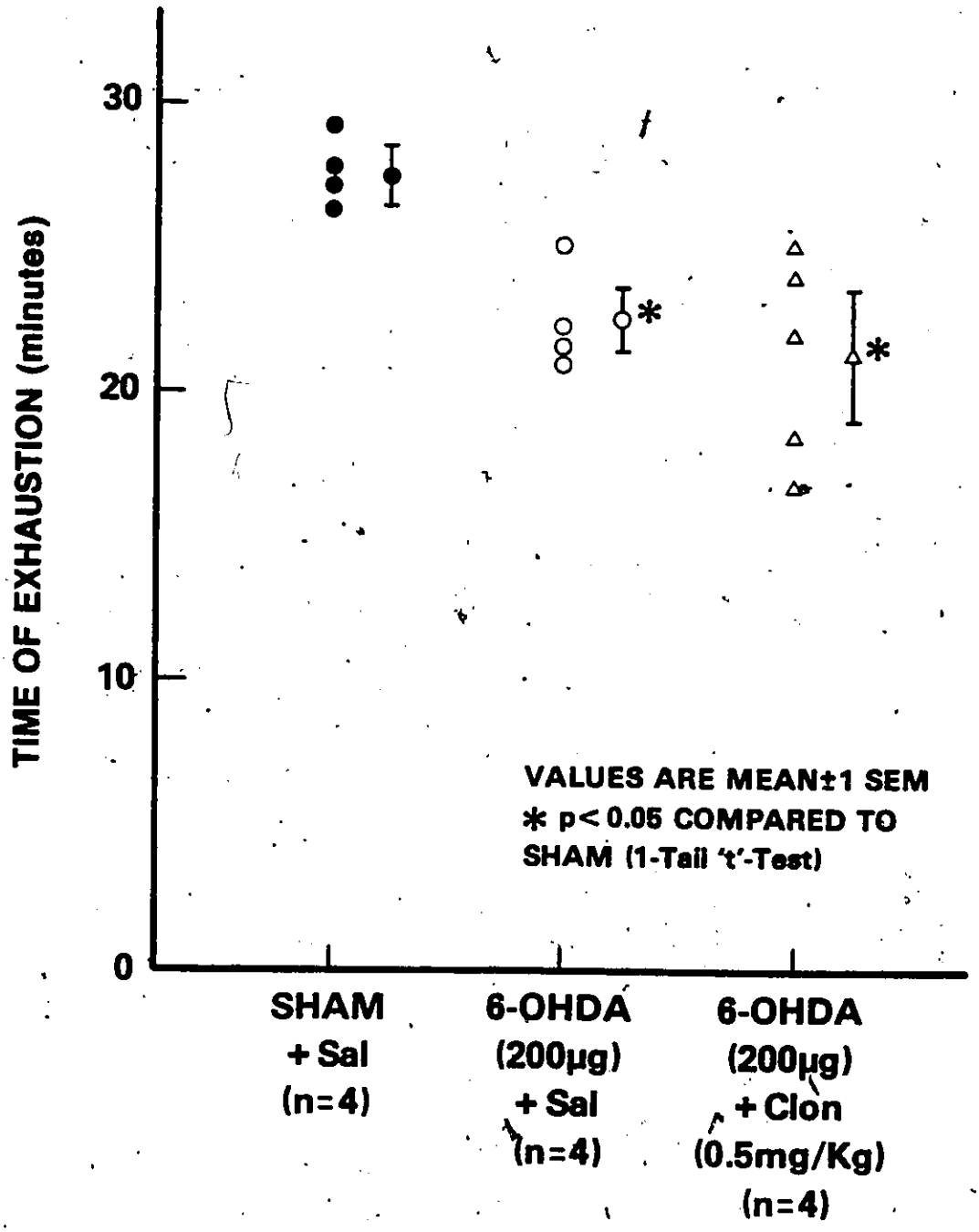


TABLE 9. EXPERIMENT 1D. Body weight changes in Sham, 6-OHDA and 6-OHDA+Clonidine animals.

	BODY WEIGHT (g)		
DAY	5	9	CHANGE
GROUP			
Sham	381.8+4.6	363.3+5.5	-18.5+5.7
6-OHDA	390.5+7.3	367.9+6.9	-22.6+5.5
6-OHDA+CLON	388.4+8.1	368.4+8.4	-20.8+7.1

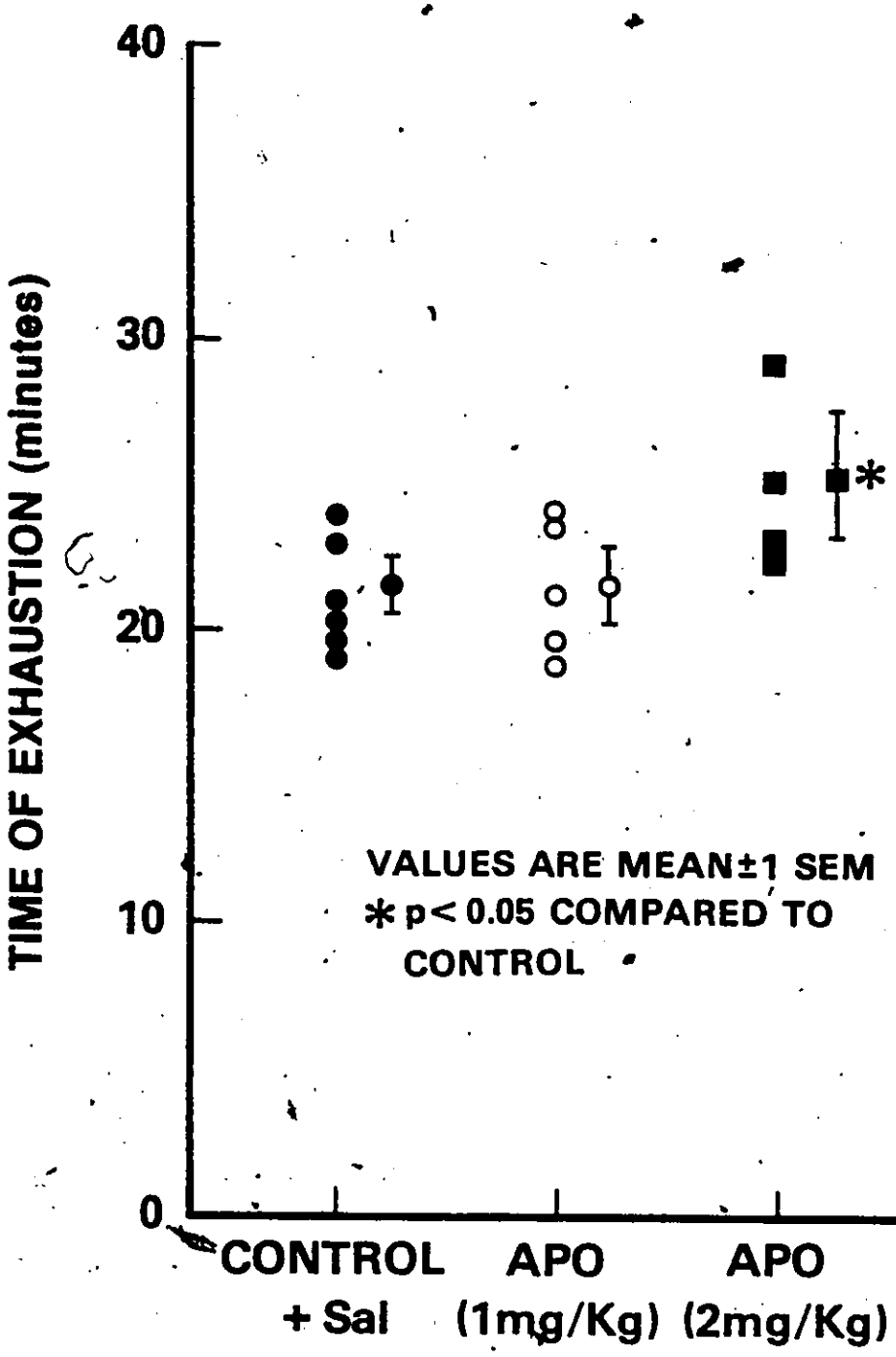
EXPERIMENT 2. Effects of Apomorphine on exercise performance in normal rats.

The times of exhaustion are presented in Figure 23. Apomorphine significantly increased the time of exhaustion at a dose of 2.0 mg/Kg compared to Control animals treated with saline ($p<0.05$). Apomorphine at a dose of 1.0 mg/Kg was ineffective in improving exercise capacity.

Of the six animals treated with 5.0 mg/Kg of apomorphine four of these animals either refused to run at all on the treadmill or else stopped running within five minutes of beginning the run. These animals were clearly not exhausted (See Appendix 1). However the two remaining animals ran for 25.1 minutes and 34.5 minutes. Efforts to induce another group of six rats treated with 5.0 mg/Kg of apomorphine to run were met with similar results.

FIGURE 23. EXPERIMENT 2. Effects of apomorphine on exercise capacity. Animals were exposed to the 8-day learning schedule. On day 9 the rats were given an injection of either Apomorphine (1 or 2 mg/Kg in 0.9% saline, i.p. APO) or saline alone (Control) one hour before being run to exhaustion at a speed of 36 m/min.

APOMORPHINE INCREASES EXERCISE CAPACITY OF NORMAL RATS



EXPERIMENT 3. Effects of exhaustive exercise on the turnover of intracerebral dopamine.

The object of this experiment was to determine whether at the time of exhaustion there was any evidence of a failure of dopaminergic transmission.

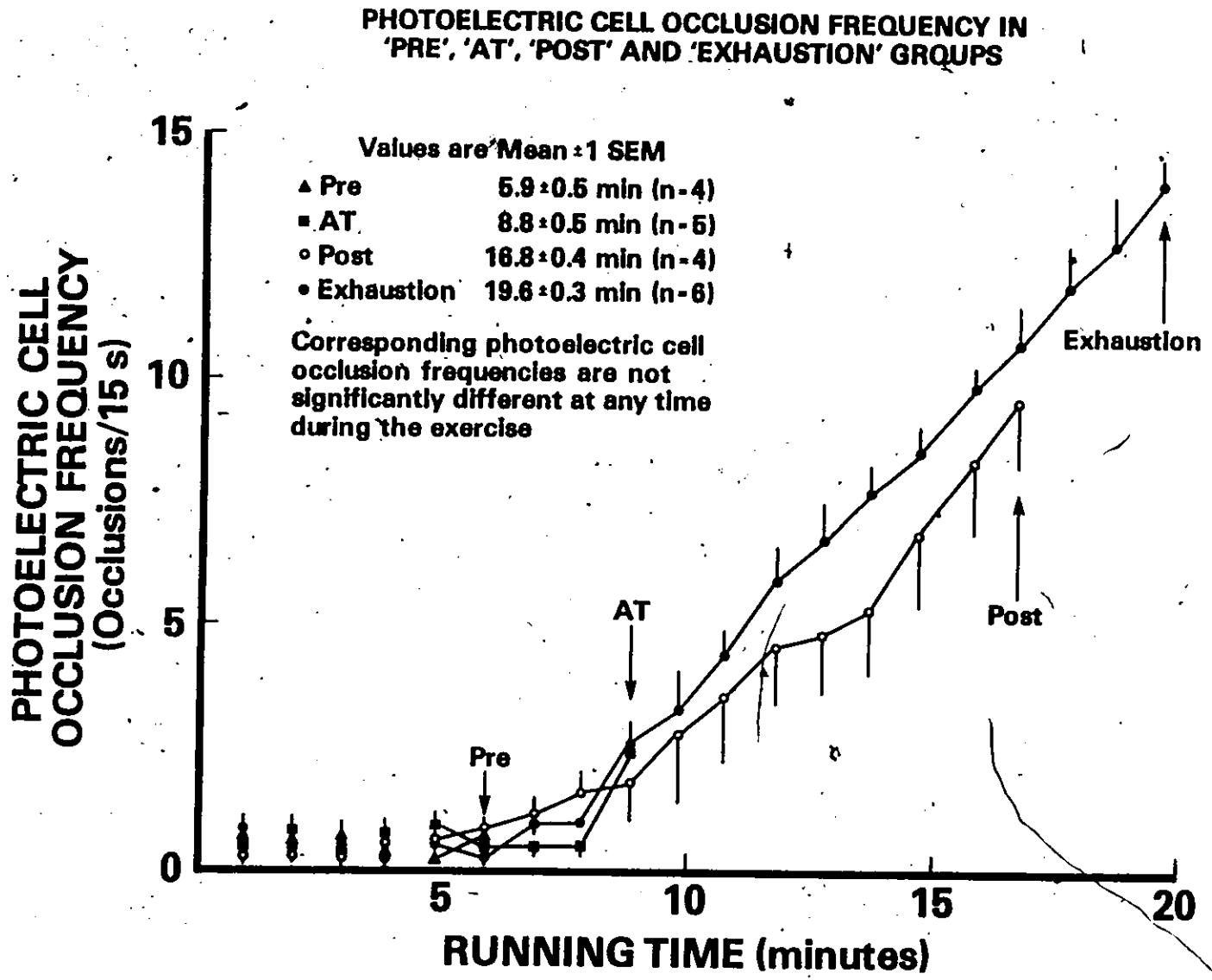
Exercise perfomance: Time of exhaustion and occlusion frequency patterns.

Rats were removed from the treadmill at varying times during the exercise performance to represent increasinig degrees of fatigue. The occlusion frequency of these rats is presented in Figure 24. The Exhaustion group ran for 19.6+0.3 minutes. Their occlusion frequency remained below 2 occlusions/15 s for the first 8 minutes, after which it increased steadily. Animals in the Early, At and Late groups were run for 5.9+0.5 minutes, 8.8+0.5 minutes and 16.8+0.4 minutes respectively. Their occlusion frequencies closely paralleled the pattern seen in the Exhaustion group and at no time were they significantly different. In addition the point when running posture was noted to change and become more sprawled was the same in the At, Post and Exhaustion groups, ie 8.0+0.7 min; 10.2+2. min; and 9.1+0.8 min respectively.

FIGURE 24. EXPERIMENT 3. Photoelectric cell occlusion frequency in 'Early', 'At', 'Late' and 'Exhaustion' groups.

Animals were exposed to the 8-day learning schedule. The animals were removed from the treadmill at varying times according to their photoelectric cell occlusion frequencies and allocated to the following groups:

Exhaustion:	Run to exhaustion.
Late:	Run until the occlusion frequency was approximately 50% of the maximum occlusion frequency exhibited by the Exhaustion group.
At:	Run until the photoelectric cell occlusion frequency had just increased to 2 occlusions/15s.
Early:	Run for approximately 50% of the time that the At group was run.
Control:	Animals were removed 5 minutes after the warm-up run.


PHOTOELECTRIC CELL OCCLUSION FREQUENCY IN 'PRE', 'AT', 'POST' AND 'EXHAUSTION' GROUPS
Values are Mean ±1 SEM
▲ Pre 5.9±0.5 min (n-4)
■ AT 8.8±0.5 min (n-5)
○ Post 16.8±0.4 min (n-4)
● Exhaustion 19.6±0.3 min (n-6)
Corresponding photoelectric cell occlusion frequencies are not significantly different at any time during the exercise
PHOTOELECTRIC CELL OCCLUSION FREQUENCY (Occlusions/15 s)
15
10
5
0
Pre
AT
Post
Exhaustion
5
10
15
20
RUNNING TIME (minutes)

Regional cerbral monoamine metabolism during exhaustive exercise.

The concentrations of dopamine, DOPAC, HVA, norepinephrine, 5-HT and 5-HIAA in striatum, hypothalamus and brain stem of the running animals are presented in Table 10.

There was an increase in the turnover rate of dopamine in the striatum of Exhausted animals. In the striatum there was a significant increase in the concentrations of both DOPAC (+37%) and HVA (+35%) (Figure 25). No significant changes in dopamine, norepinephrine, 5-HT or 5-HIAA were observed in the striatum at any time.

In the brain stem, the concentration of DOPAC was increased in Exhausted animals (+132%) and HVA was increased in Post (+96%) and Exhausted (+71%) animals (Figure 26). No significant changes were observed in the concentrations of any other compound at any time in the brain stem.

In the hypothalamus no significant change in any compound was observed at any time during the exercise.

TABLE 10. EXPERIMENT 3. Effects of exhaustive exercise on the concentrations of Dopamine, DOPAC, HVA, Norepinephrine, 5-HT and 5-HIAA in the Striatum, Brain Stem and Hypothalamus.

REGIONAL CONCENTRATION OF CATECHOLAMINE
(ng/g Wet Weight)

GROUP	CONTROL	EARLY	LATE	EXHAUSTION
TIME	0	5.9	16.8	19.6
STRIATUM				
Dopamine	8867+910	8225+750	8531+424	9776+455
DOPAC	1140+104	1112+112	1192+ 68	1557+ 65*
HVA	768+ 85	653+ 87	944+ 25	1034+ 65*
NE	193+ 25	229+ 23	183+ 26	218+ 29
5-HT	274+ 18	295+ 18	307+ 15	285+ 18
5-HIAA	293+ 20	279+ 16	477+ 47	374+ 37
BRAIN STEM				
Dopamine	68+ 7	95+17	84 +5	94+14
DOPAC	38+ 3	53+ 6	60+ 4***	90+ 8***
HVA	31+ 6	26+ 8	61+17	63+ 5**
NE	590+45	535+30	517+48	462+29
5-HT	365+29	234+61	319+43	362+27
5-HIAA	293+33	153+54	280+36	325+41
HYPOTHALAMUS				
Dopamine	548+113		351+32	587+157
DOPAC	116+ 23		128+ 8	218+ 54
HVA	91+ 17		272+40	126+ 27
NE	2382+400		1912+73	1618+200
5-HT	378+ 83		401+20	300+ 66
5-HIAA	290+108			307+ 73

* $p<0.05$

** $p<0.02$

*** $p<0.001$

Values are Mean+1SEM.

FIGURE 25. EXPERIMENT 3. Concentrations of Dopamine, DOPAC and HVA in the Striatum of animals run to exhaustion.
Samples were obtained from the animals described in Figure 24.

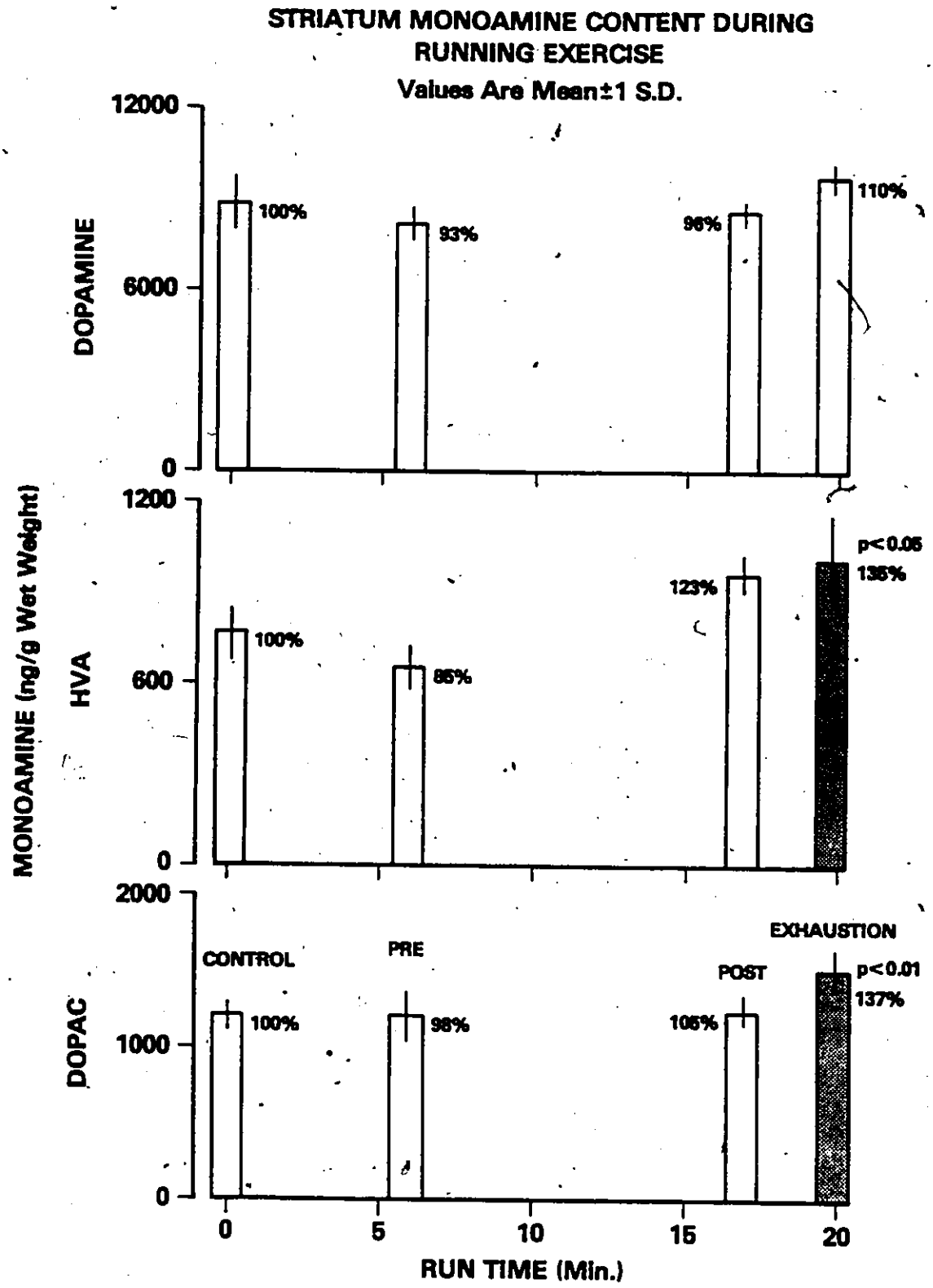
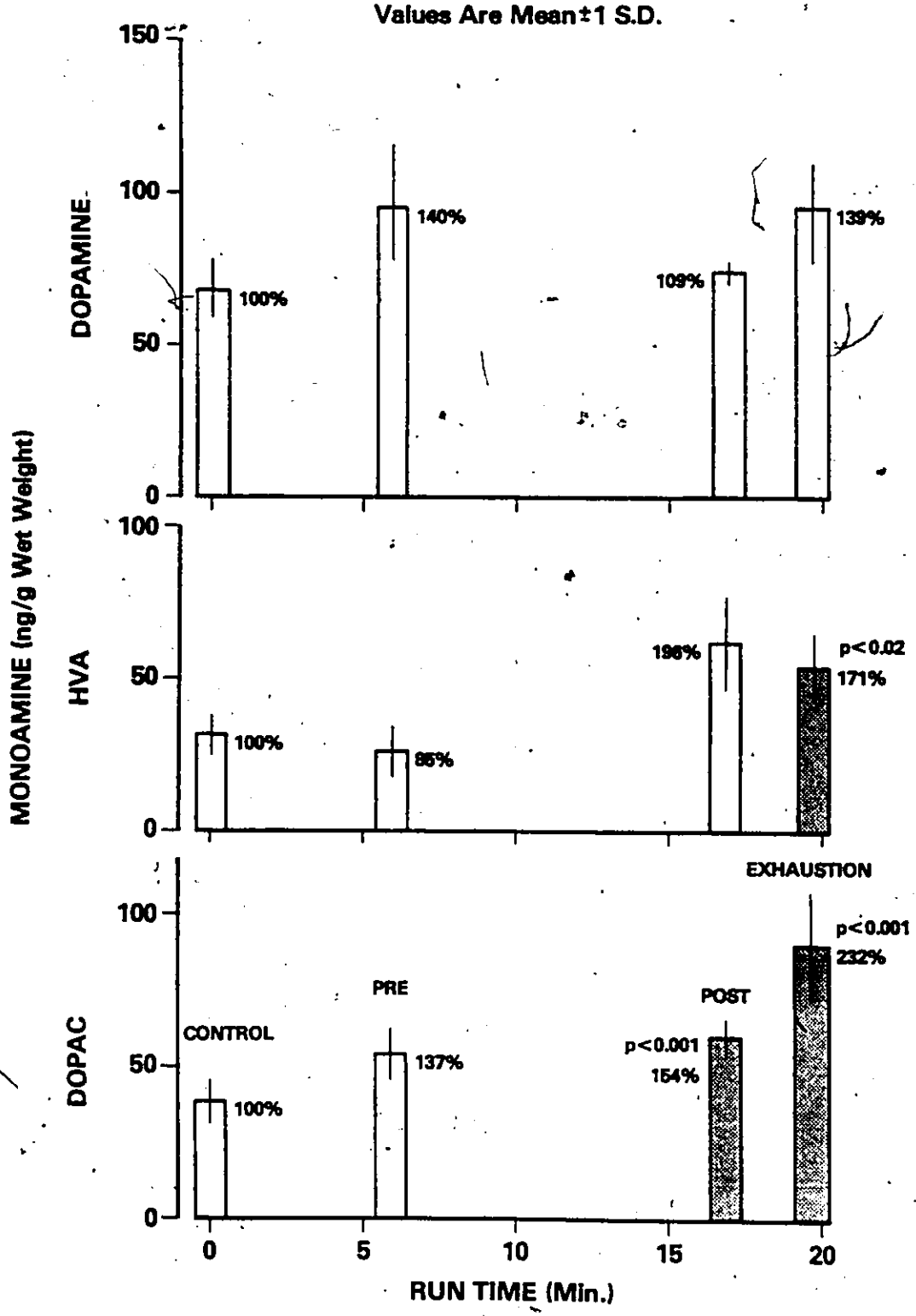

STRIATUM MONOAMINE CONTENT DURING RUNNING EXERCISE
Values Are Mean±1 S.D.
MONOAMINE (ng/g Wet Weight)
DOPAMINE
12000
6000
0
100%
93%
96%
110%
HVA
1200
600
0
100%
86%
123%
p<0.05
135%
DOPAC
2000
1000
0
CONTROL
PRE
POST
EXHAUSTION
100%
98%
106%
p<0.01
137%
0
5
10
15
20
RUN TIME (Min.)

FIGURE 26. EXPERIMENT 3. Concentrations Dopamine, DOPAC and HVA in the Brain Stem of animals run to exhaustion.
Samples were obtained from the animals described in Figure 24.

BRAIN STEM MONOAMINE CONTENT DURING RUNNING EXERCISE

Values Are Mean±1 S.D.



Muscle energy substrate profiles.

A summary of the changes in the intramuscular energy substrate stores in the three muscles examined are presented in Table 11.

Total muscle carbohydrate content decreased rapidly in soleus, vastus lateralis red and vastus lateralis white, being depleted by 75%, 60% and 72% in Early animals respectively (Figure 27). The carbohydrate stores did not deplete much further in any muscle during the remainder of the exercise. By exhaustion, depletion was 80% in soleus, 75% in vastus lateralis white and 92% in vastus lateralis red.

The lactate concentrations were unchanged in soleus and vastus lateralis white although there was a trend for lactate levels to decrease in vastus lateralis red throughout the exercise perfomance (Figure 28).

There was a significant decrease in ATP levels in Pre values in soleus, vastus lateralis white and vastus lateralis red, that remained decreased to the same degree throughout exercise performance (Figure 29).

Creatine phosphate levels remained low throughout the exercise (Table 11).

TABLE 11. EXPERIMENT 3. Effects of Exhaustive exercise on the concentrations of energy substrates in Soleus, Vastus Lateralis White and Vastus Lateralis Red.

	MUSCLE SUBSTRATE CONCENTRATION (umol/g Wet Weight)				
SAMPLE	CONTROL	EARLY	AT	LATE	EXHAUSTION
TIME	0.0	5.9	10.5	16.6	19.6
SOLEUS					
Glycogen	20.1+3.4	4.8+1.1**	4.9+1.7**	4.8+2.6**	4.6+1.0**
G-6-P	6.1+1.0	4.5+0.5	5.2+0.6	4.5+0.5	6.4+1.0
Glucose	3.3+1.0	2.0+0.7	1.0+0.4*	1.2+0.5*	0.8+0.3*
Lactate	2.5+1.3	2.6+0.9	2.1+0.4	1.5+1.0	2.6+1.1
ATP	7.0+2.1	3.5+1.1*	2.4+1.1*	3.1+1.3*	3.2+2.1*
Cr-P	2.4+1.5	4.4+2.9	4.3+2.1	3.4+1.8	3.0+1.6
VASTUS LATERALIS-WHITE					
Glycogen	8.4+0.9	4.2+1.1*	4.2+1.1*	2.4+0.5**	2.1+0.4**
G-6-P	30.5+4.8	18.5+3.3**	11.1+2.4**	9.9+1.8**	8.8+1.6**
Glucose	2.5+0.3	2.9+0.9	1.7+0.3	1.8+0.3	1.2+0.2
Lactate	6.5+0.8	5.2+1.9	1.7+0.3	1.8+0.3	1.2+0.2*
ATP	8.2+0.7	4.1+1.1*	3.4+1.4*	4.1+1.0*	3.5+1.8*
Cr-P	4.2+3.7	3.7+1.8	2.5+1.4	2.0+1.3	3.1+2.5
VASTUS LATERALIS-RED					
Glycogen	10.0+0.9	2.0+1.0**	1.2+0.3**	1.0+0.5**	1.0+0.7**
G-6-P	29.1+3.2	4.5+0.5**	4.5+1.0**	4.2+1.3**	4.0+2.1**
Glucose	1.8+0.4	1.0+0.6	1.0+0.5	0.5+0.5	0.6+0.4
Lactate	6.0+1.4	4.1+1.4	2.8+0.4*	2.1+0.5*	3.1+0.7*
ATP	7.4+1.1	2.4+0.7*	3.0+1.7*	2.6+1.1*	2.7+0.5*
Cr-P	2.3+2.2	2.0+1.6	1.6+1.1	2.8+0.6	1.6+1.3

*P<0.05

**P<0.01

FIGURE 27. EXPERIMENT 3. Concentrations of Glycogen, Glucose and Glucose-6-Phosphate in the Soleus, Vastus Laterlis White and Vastus Laterlis Red of animals run to Exhaustion.
Samples were obtained from the animals described in Figure 24.

MUSCLE CARBOHYDRATE CONTENT DURING EXHAUSTIVE EXERCISE

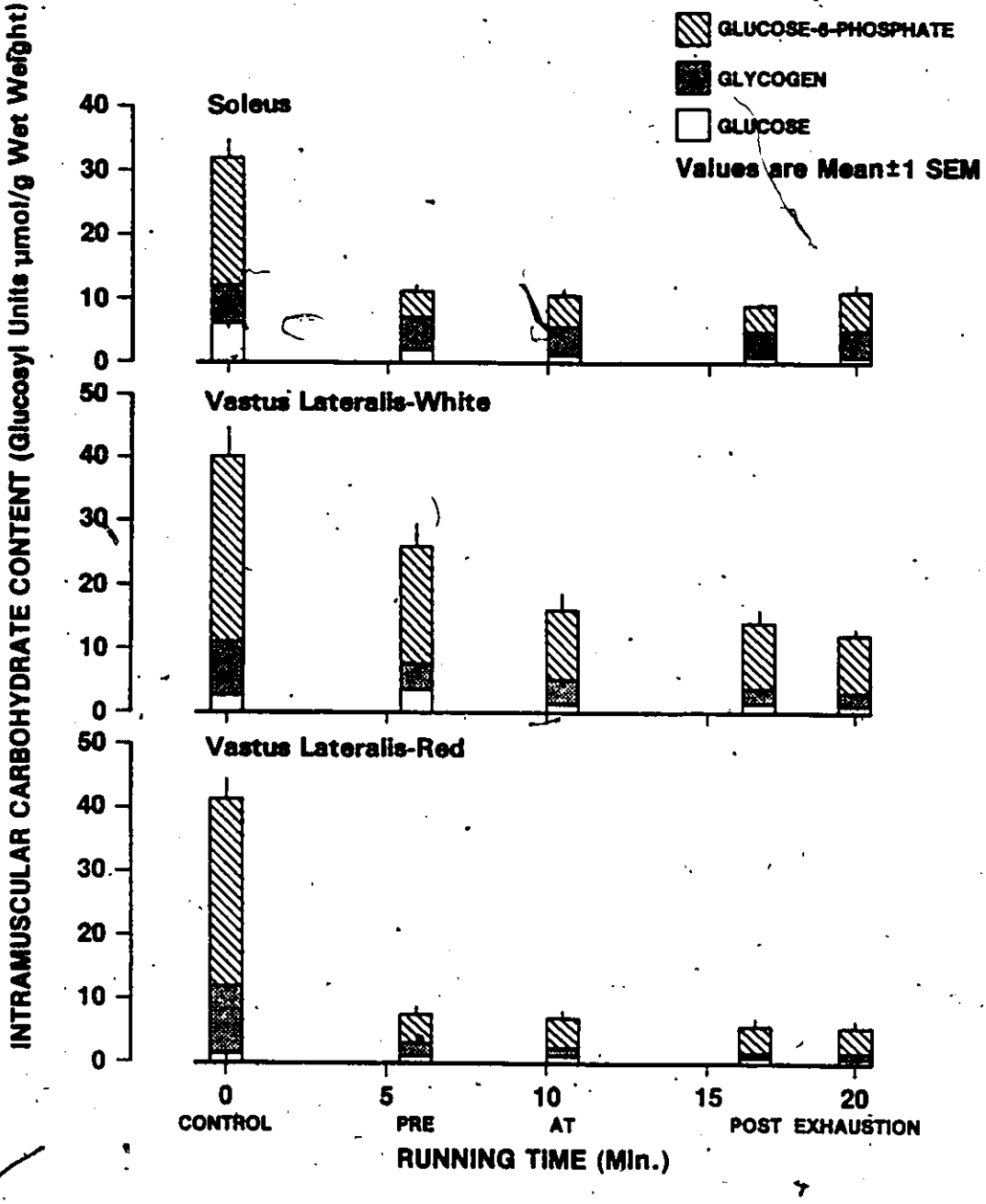


FIGURE 28. EXPERIMENT 3. Concentrations of Lactate in the Soleus, Vastus Lateralis White and Vastus Lateralis Red of animals run to exhaustion.

Samples were obtained from the animals described in Figure 24.

MUSCLE LACTATE CONCENTRATIONS DURING EXHAUSTIVE EXERCISE

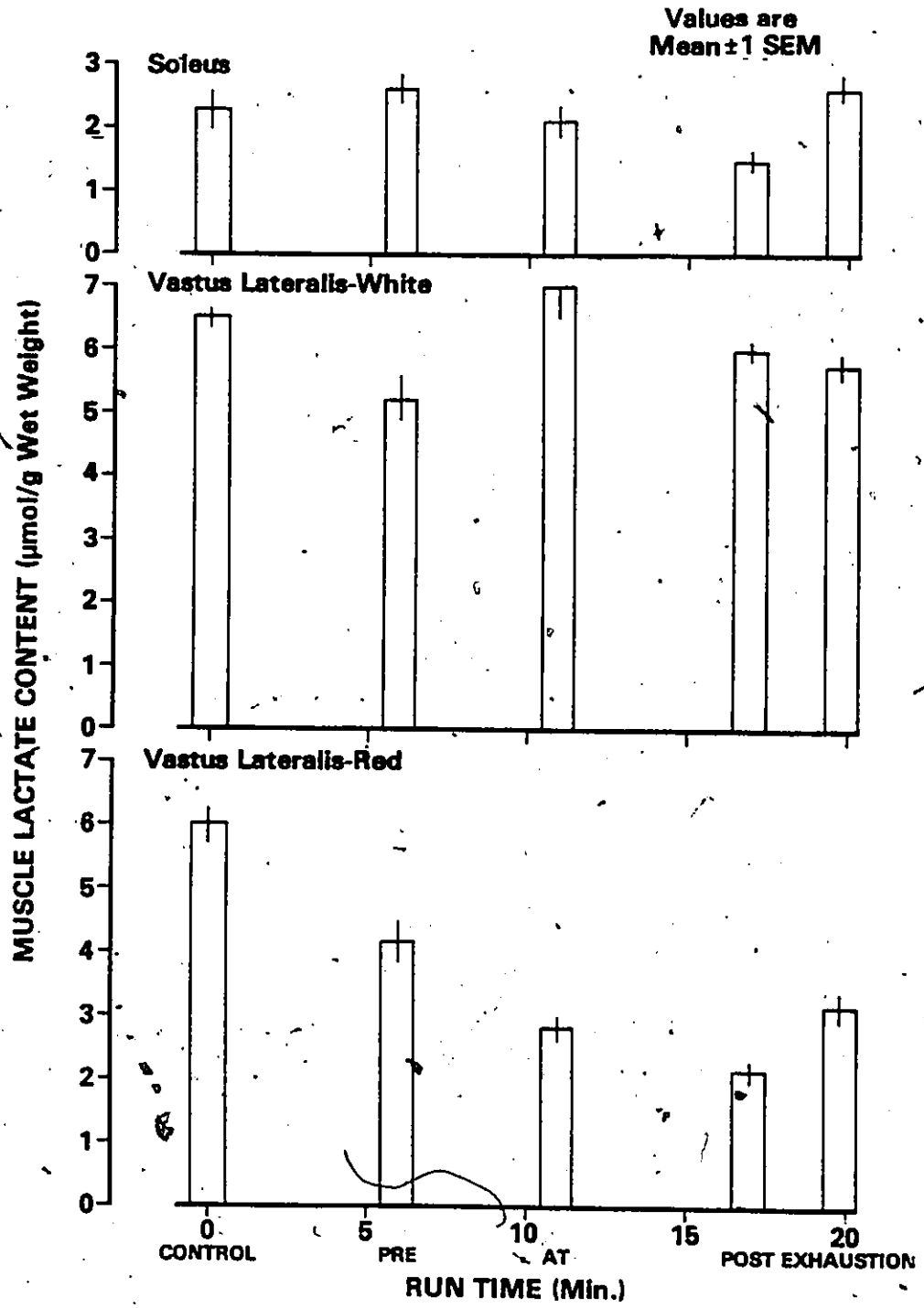
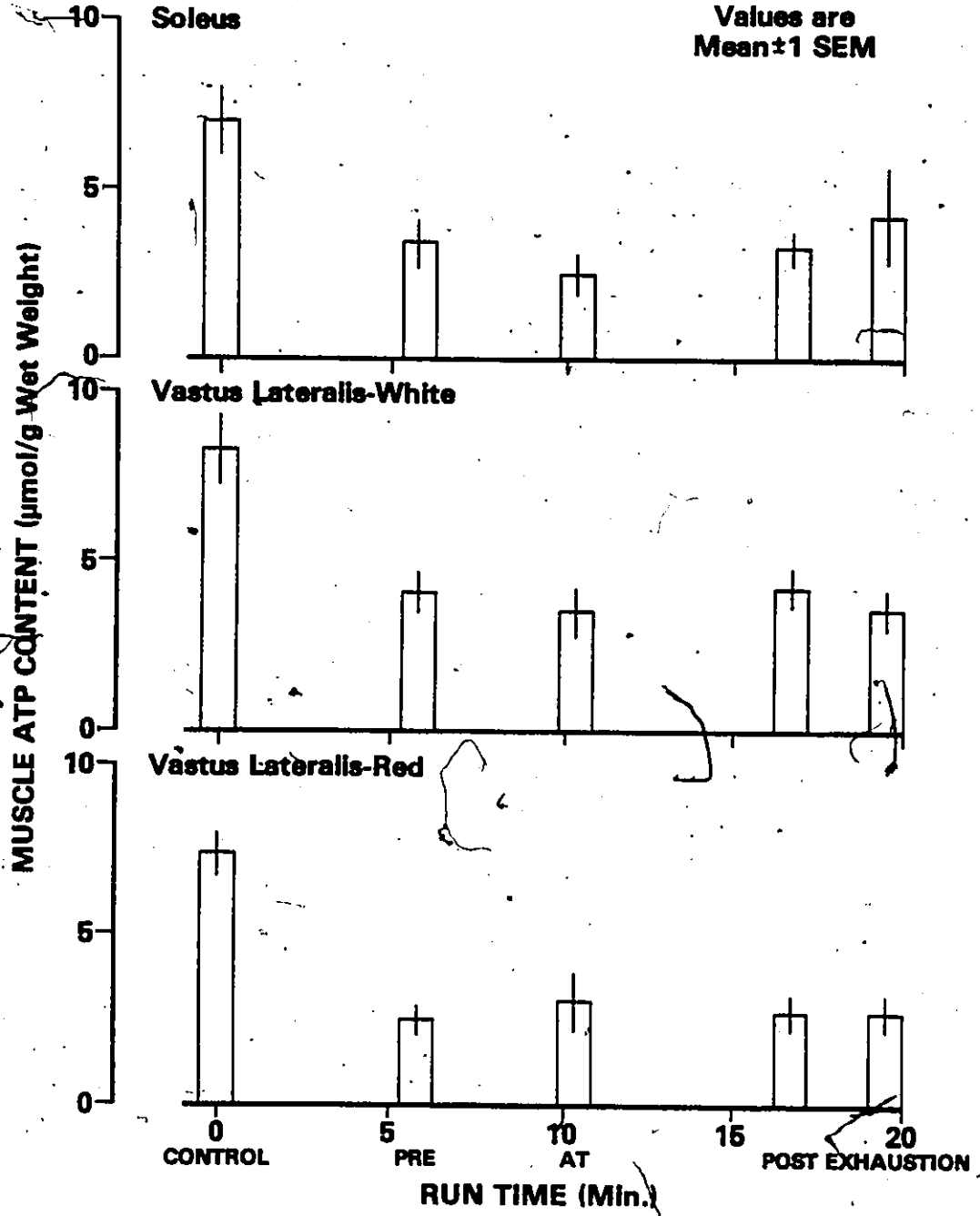


FIGURE 29. EXPERIMENT 3. Concentrations of Adenosine Triphosphate (ATP) in the Soleus, Vastus Lateralis White and Vastus Lateralis Red of animals run to exhaustion. Samples were obtained from the animals described in Figure 24.

MUSCLE ATP CONCENTRATIONS DURING EXHAUSTIVE EXERCISE



Blood energy substrate concentrations.

During exercise plasma glucose concentrations were maintained until the Late and Exhaustion samples when plasma glucose concentarions decreased slightly (Figure 30; Table 12). However this change was not significant (F=2.17).

Plasma lactate concentrations increased rogressively throughtout exercise (Figure 31; Table 12).

TABLE 12. EXPERIMENT 3. Effects of exhaustive exercise on the plasma concentrations of glucose and lactate.

CONCENTRATION OF PLASMA GLUCOSE AND LACTATE
(mM)

GROUP	CONTROL	PRE	AT	POST	EXHAUSTION
TIME	0.0	5.9	10.5	16.8	19.6
GLUCOSE	8.9+0.7	8.5+0.8	8.7+0.3	6.9+0.2	6.9+0.4
LACTATE	3.8+0.6	7.8+1.2*	12.2+1.4**	13.6+1.0**	19.7+1.8**

*p<0.05
**p<0.01

FIGURE 30. EXPERIMENT 3. Plasma glucose concentrations during exhaustive exercise.
Samples were obtained from the animals described in Figure 24.

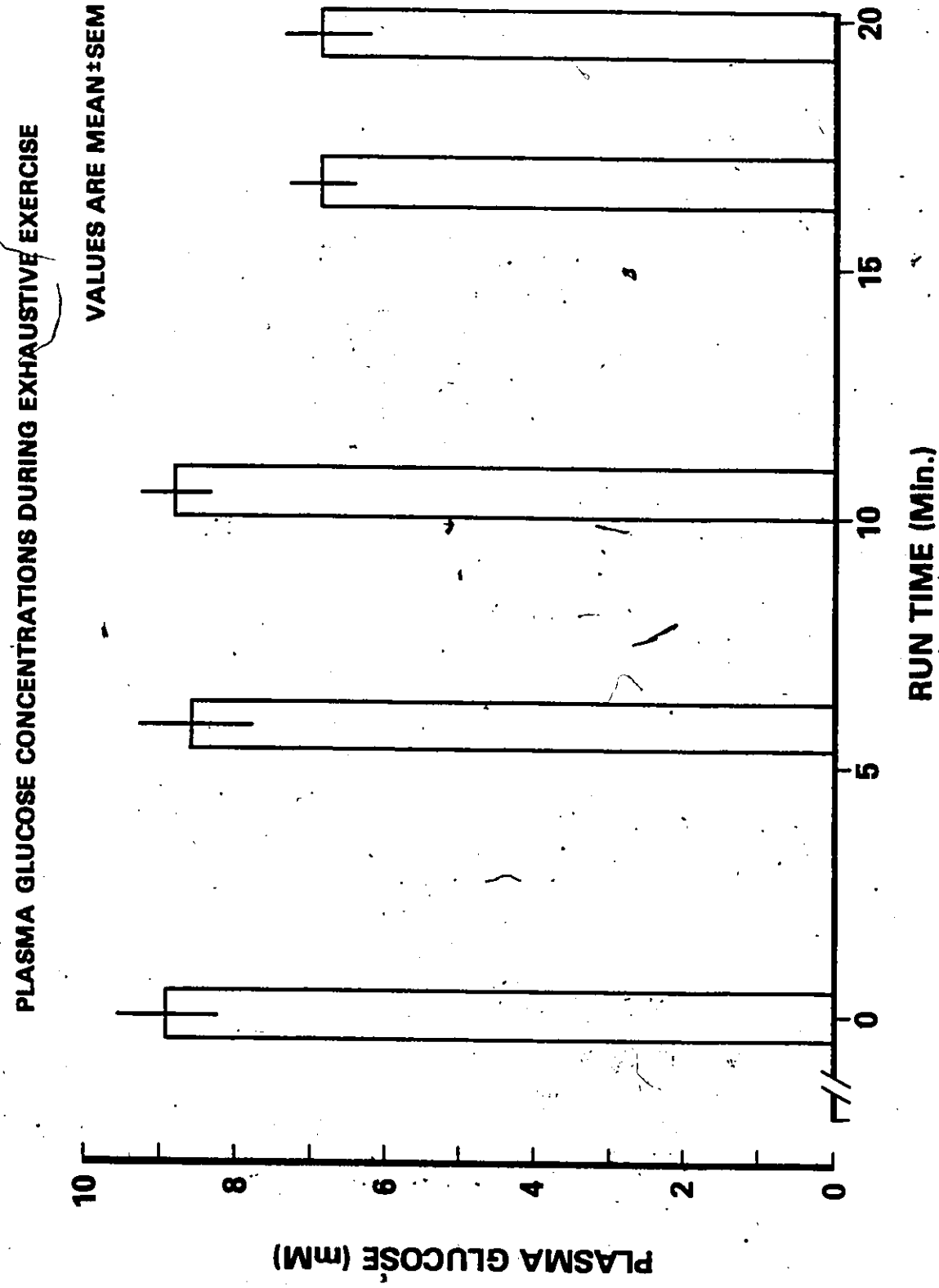
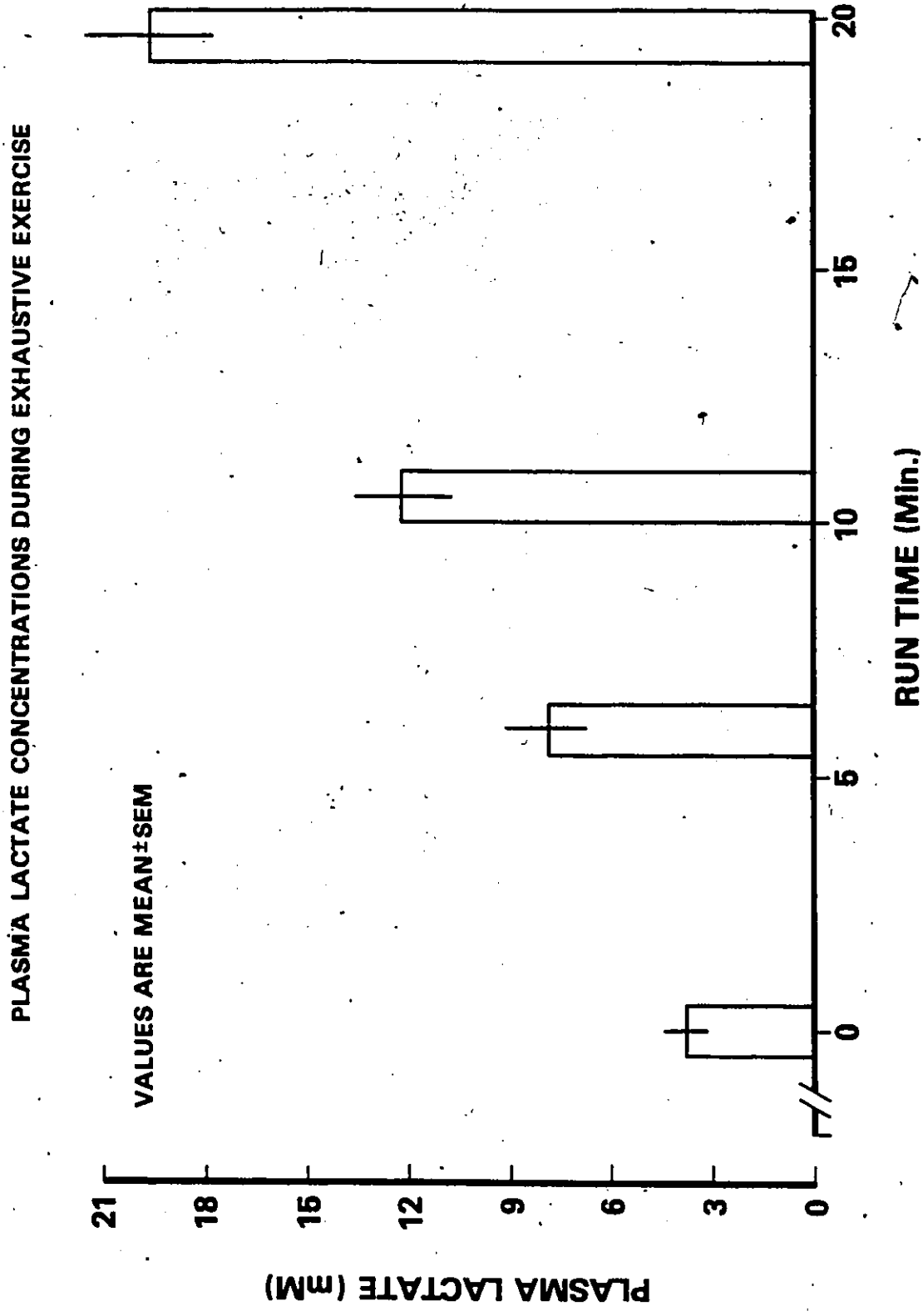

PLASMA GLUCOSE CONCENTRATIONS DURING EXHAUSTIVE EXERCISE
VALUES ARE MEAN±SEM
PLASMA GLUCOSE (mM)
0
2
4
6
8
10
0
5
10
15
20
RUN TIME (Min.)

FIGURE 31. EXPERIMENT 3. Plasma lactate concentration during exhaustive exercise.

Samples were obtained from the animals described in Figure 24.


PLASMA LACTATE CONCENTRATIONS DURING EXHAUSTIVE EXERCISE
VALUES ARE MEAN±SEM
PLASMA LACTATE (mM)
21
18
15
12
9
6
3
0
0
5
10
15
20
RUN TIME (Min.)

DISCUSSION.

CHAPTER 1.

OBJECTIVES.

The performance of physical exercises such as running and swimming requires rapid and accurate exchange of information between different brain regions, skeletal muscles and sense organs. This exchange of information serves to initite and coordinate muscle contractions and relaxations, to monitor and modify movement to ensure it corresponds to requirements and to evoke and control the physiological changes neccessary to maintain the supply of energy substrates and oxygen to working muscles.

Exercise performance cannot be maintained indefinately. A maximum voluntary contraction can be sustained for only short periods of time after which the force generated declines progressively (fatigue) until no force is generated (exhaustion). In the case of repeated muscle contractions, such as those required for running, work output can be maintained steadily only for a definite period before the animal or individual stops (exhausted). While the actual exercise is being performed, the individual or animal must increase its 'effort' (Cafarelli 1977) as muscle contractions become more difficult to perform (fatigue).

The underlying mechanisms responsible for fatigue and exhaustion are not known. However, depletions of muscle energy substrates such as glycogen, impairments of excitation contraction coupling have been considered (Edwards 1978; Lancet 1981). Evidence cited in the Introduction suggests that events within the central nervous system, (that is, proximal to the alph motor neuron), may be involved. No mechanisms has been identified for this 'central fatigue'.

It is clear that deficits in the efficient transfer of information across dopaminergic synapses result in significant disruptions in motor performance. For example, the rigidity, tremor and slowness of movement characteristic of Parkinson's disease results from the degeneration of the dopaminergic nigrostriatal pathway in these patients (Tera Vainen and Calne 1979; Marsden 1982). Less severe lossess of striatal dopaminergic activity accompany senescence (see Pradham 1981) and may be responsible for the deficits in motor function which develop with age (Marshall and Berios 1979). In normal animals, drugs antagonistic to the effects of dopamine also result in significant motor abnormalities (Laverty and Taylor 1970). On the other hand, drugs which act as dopamine agonists including apomorphine, L-Dopa and amphetamine improve movement and exercise performance to a small but significant extent (Gerald 1978; Marshall and

Berios 1979; Laites and Weiss 1981). I have therefore raised the question: Is a limiting factor to the performance of running exercise, the ability and capacity of striatal dopaminergic neurons to maintain the transfer of information?

Dopaminergic synapses possess a number of feedback systems which atempt to maintain an adequate supply of available dopamine for release into the cleft. These include increases in the enzymatic activity of tyrosine hydroxylase (Figure 4) and transfer of dopamine from the large storage pool to the functional pool (Figure 5). It is not clear whether these systems maintain the supply of dopamine in the functional pool such that the transfer of information across these synapses 'fatigues'. To test this question, I have set-up the following hypothesis:

A. Exercise increases the turnover rate of intracerebral dopamine in the neural pathways involved in the control of muscular exercise.

B. The demand for dopamine release exceeds the amount stored in the terminal and capacity for synthesis, resulting in a reduced availability or amount of dopamine released into the cleft and an inadequate degree of receptor stimulation.

C. This defficiency of dopaminergic function limits exercise performance.

There are four consequencies and predictions of this hypothesis.

1. If brain dopamine levels were reduced before exercsie, subsequent exercise performance should be decreased. Exercise performance should be restored in dopamine-depleted animals by dopamine receptor agonists.

2. If exercise performance was being limited in normal animals by the amount of dopamine receptor stimulation, exercise performance should be improved in normals by dopamine receptor agonists.

3. In an animal performing exercise the turnover rate of dopamie shoud be increased as indicated by increases in the regional concentrations of DOPAC and HVA.

4. If exercise performance was being limited by an inadequacy of dopamine release from the terminal due to a depletion of dopamine stores, an exhausted animal would have concentrations of dopamine in the brain which are significantly less than animals which have not exercised.

The purose of this thesis has been to test these prediction and to consider possible mechanisms responsible for a failure of information transfer across such synapses. The type of motor activity chosen was running exercise and the capacity to perform it was quantified as the time taken for an rat running on the treadmill to become 'exhausted'. Appendix I describes the properties of this particular system and the reason for chosing it.

CHAPTER 2.

EFFECTS OF INCREASES AND DECREASES IN INTRACEREBRAL DOPAMINERGIC ACTIVITY ON EXERCISE CAPACITY.

The availability of striatal dopamine can limit exercise capacity.

The first prediction of the hypothesis was that if the availability of intracerebral dopamine was reduced (by an intracerebroventricular injection of 6-OHDA) exercise capacity should be compromised.

Experiment 1 shows that depletion of striatal dopamine limits exercise capacity. The time of exhaustion in catecholamine-depleted rats was significantly reduced compared to control animals (Figures 16, 19 and 22). Failure to demonstrate this phonomena would render the hypothesis untenable. Catecholamine-depleted rats also differed from control animals during the actual exercise performance. The 6-OHDA-treated rats began to increase their photoelectric cell occlusion frequency to values greater than 3 occlusions/15s sooner than Sham rats (Figure 17, 3.5 min vs 11.5 min, $P<0.01$; Figure 20, 4.6 min vs 7.5 min, $p<0.05$). Also, the slope of the occlusion frequency curve above 3 occlusions/15 s was steeper in 6-OHDA rats the cantorl (Figures 17 and 20). It is important to note however that during the initial period of the run there was no difference

in the occlusion frequency between catecholamine-depleted rats and control rats. Furthermore no discernable difference in the style of running Sham and 6-OHDA rats could be identified by five independant observers who were unaware of the nature of the study. Therefore, catecholamine-depleted rats were quite able to perform the stepping motions necessay for running and avoiding the grid. What was different was their capacity to do so.

There was a tendency for 6-OHDA animals to decrease their body weights to a greater extent than Sham animals during the four day following their operation (Tables 6, 7 and 8). This may be because of the anorexic effects of 6-OHDA (Evetts, Uretsky, Iversen and Iversen 1970). It is possible therefore that the detrimental effects of 6-OHDA on exercise performance resulted from decreases in muscle energy reserves because of a decrease in food intake. Against this hypothesis is the fact that exercise performance was be restored in 6-OHDA animals with apomorphine given just one hour before their exhaustion run (Figures 19 and 20). This indicates that the effects of 6-OHDA on exercise are more probably dependant on a deficiency in the degree of central dopamine receptor stimulation during the actual exercise run rather than events which had occured in the 4 days following 6-OHDA injection (other than catecholamine depletion).

The injection of 6-OHDA also resulted in a significant depletion of norepinephrine from the striatum and hypothalamus (Table 4). The effects of 6-OHDA could therefore be the result of decreases in noradrenergic activity instead of or in addition to striatal dopamine depletion. Apomorphine alone (Figure 19) but not clonidine alone (Figure 22) was able to restore exercise performance in rats after both dopamine and norepinephrine depletion. This observation indicates that it was the depletion of striatal dopamine rather than the depletion of norepinephrine that was responsible for the effects of 6-OHDA.

Increases in dopaminergic activity increase exercise capacity.

The hypothesis predicted that increases in dopaminergic activity by the injection of apomorphine would increase exercise capacity. The results of Experiment 1C (Figure 19) and Experiment 2 (Figure 22) shows that apomorphine increased exercise capacity in both normal and 6-OHDA rats. A dose of apomorphine at 1.0 mg/Kg was effective in improving exercise performance in 6-OHDA animals whereas a dose of 2.0 mg/Kg was required to increase exercise capacity in normal animals. This may be evidence that increases in the number of post synaptic dopamine receptors that follows 6-OHDA treatment (Mishra, Marshall and Varmuza 1980) increased the responsiveness to

apomorphine in 6-OHDA animals compared to normals. If this is the case it is of note that receptor 'supersensitivity' requires 10 days to become detectable with in vitro methods. Perhaps a subtle change in dopamine receptor sensitivity has occured, which is undetectable by the in vitro methods currently used. However, caution should be exercised in comparing the effects of apomorphine in dopamine-depleted animals compared to normal animals. Apomorphine can also stimulate the autoreceptors for dopamine located on the presynaptic terminal. When activated these receptor depress the release of dopamine from the terminal (Hoffman and Cubeddu 1982a; 1982b). Furthermore, there are also dopamine receptors located on the cell body of the neuron in the substantia nigra which when activated by apomorphine reduces the firing rate of the neuron. In normal animals therefore stimulation of these autoreceptors may result in a relative inhibtion of the presynaptic dopaminergic neurons. Because 6-OHDA has the effect of damaging presynaptic dopaminergic synapses, the presynaptic effects of apomorphine would be less in these rats than in normal rats, because the endogenous release of dopamine is reduced.

The intracerebral sites of action of apomorphine were not investigated in the present studies. Apomorphine has been shown to increase glucose consumption in the caudate nucleus, ventrolateral thalamic nucleus,

subthalaminc nucleus, lateral habenular nucleus, septal nucleus, globus pallidus, and the sensorimotor cortex indicating that this drug is able to influence the neuronal activity in these brain areas (Brown and Wolfson 1978; McCulloch, Svaki and Sokoloff 1980; Grome and McCulloch 1983). The striatum is clearly a major candidate for the effects of apomorphine on exercise performance in the present studies.

Fatigue has also been reported in patients with Parkinson's disease although the term is used loosely and includes slowness of movement and the deterioration of writing and speech (Schwab, England and Petersen 1959; Marsden, Parkes and Quinn 1981). Schwab et al (1959) found reductions in the strength of the dorsal interosseus muscle in patients with parkinson's disease. During this fatigue strength could be restored by increase effort by the patients. Schwab et al also showed that stumulation of the muscle by surface electrodes also increased force of contraction. Although this increased fatiguability in Parkinson's patients may be secondary to to a reduced muscle work capacity through lack of use, the clinical observations are comparable to the present studies where rats and humans with dopamine depletion have the ability to perform motor tasks but with a reduced capacity.

CHAPTER 3.

EFFECTS OF EXHAUSTIVE EXERCISE ON MUSCLE ENERGY SUBSTRATE METABOLISM.

The mechanisms responsible for the limitation of exercise performance due to a defficiency in dopaminergic activity were investigated in Experiment 3. Here rats were run to varying degrees of exhaustion and measures of dopamine, DOPAC and HVA concentrations were made for evidence of a failure of dopaminergic transmission. Possible mechanisms for this include depletion of dopamine stores and a reduction in the degree of activity of the terminals. These changes will be discussed in the next chapter. In these animals the concentrations of energy substrates were also measured in representative muscles and the blood: factors which are traditionally thought more likely factors limiting exercise performance.

Muscle energy substrate metabolism.

Before considering muscle energy metabolism during exhaustive exercise I will discuss the values of muscle energy substrate concentrations before the exhaustion run was begun.

Muscle energy substrates in 'Control' rats.

The concentrations of glycogen, glucose-6-phosphate, lactate and creatine phosphate in soleus vastus laterlis

white (VLW) and vastus lateralis red (VLR) in samples taken immediately prior to the exhaustion run (Control; Figures 27 and 28, Table 11) were different from those predicted from a review of the literature (Bagby, Green, Katsuta and Gollnick, 1978; Hickson, Rennie, Conlee, Winder and Holloszy 1978; Dietz, Chiasson, Soderling and Exton 1980; Meyer, Dudley and Terjung 1980; Harris, Hultman and Sahlin 1981; Richter, Galbo and Christensen 1981). Rats in the present studies were given a 30 second warm-up run and then rested for 5 minutes before muscle samples were collected (Figure 13) whereas rats in the above studies were scarificed quickly without a warm-up run. Muscle glycogen levels were much lower and glucose-6-phosphate levels were much higher in Control muscles. However, the total muscle carbohydrate concentration (glycogen + glucose-6-phosphate + glucose) were similar to that of previous studies. It would appear therefore that there had been a significant breakdown of glycogen in Control rats presumably due to the activation of glycogen phosphorylase. It has been shown that epinephrine causes a prompt increase in glycogen phosphorylase activity in muscle and increases in muscle glucose-6-phosphate concentrations (Dietz et al 1980) via a beta-receptor and cAMP-dependant mechanism. Sonne and Galbo (1980) have shown that when rats are placed on a treadmill, (without running), plasma epinephrine concentrations increase. In Experiment 3

it is probable that decreases in muscle glycogen levels and increases in glucose-6-phosphate concentrations resulted from increases in circulating epinephrine induced by the protocol used. Circulating epinephrine also plays an important role in muscle glycogenolysis during running (Richter, Galbo, Sonne, Holst and Christensen 1980; Richter, Sonne and Christensen 1981; Richter Galbo and Christensen 1981).

Muscle lactate concentrations were increased in control muscles particularly in VLW and VLR muscles with a high glycolytic capacity. It would appear therefore, that there had been an increase in substrate flux through the glycolytic pathway to pyruvate. In order for there to be an increase in the flux of fructose-6-phosphate through to fructose-1,6-diphosphate, an increase in phosphfructokinase (PFK) activity must have occured (Newsholm and Crabtree 1979), perhaps by the cAMP produced by epinephrine stimulation of muscle beta-receptors. (This increase must have only been small, however, because there was a marked accumulation of glucose-6-phosphate). Presumably lactate is produced as a result of an increase in the $NADH+H^+$:NAD+ ratio, indicating at least through the following reaction catalysed by glyceraldehyde-3-phosphate dehydrogenase:

Glyceraldehyde-3-phosphate + Pi + NAD^+ ⟶

⟶ 1,3-diphosphoglycerate + NADH + H^+

The production of lactic acid would tend to buffer the protons released by the above reaction. However, lactic acid has a pKa value of 3.9 indicating it is almost completely ionized in the muscle with the concommitant release of protons. The protons would be buffered in part by the physicochemical buffer systems (proteins, bicarbonate, phosphate and carnosine) and certain chemical reactions including the conversion of ATP inosinate, the synthesis of NH_4^+ from amino acids and the hydroysis of creatinine phosphate (Hultman and Sahlin 1980).

It is of note that creatine phosphate levels were depleted in the Control rats of Experiment 3 although muscle ATP concentrations were normal (Table 11; Figure 28). Because the concentrations of other glycolytic intermediaries were not measured in the present studies it is not known where the high energy phosphate group from creatine phosphate was transferred to. If the phosphate group has been absorbed into the glycolytic pathway, there must have been increases in the concentrations of glycolytic substrates between fructose-1,6-biphosphate to 1,3-di-phosphoglycerate. It is of note that glyceraldehyde-3-phosphate dehydrogenase is an enzyme whose activity is decreased accumulation of protons

(Spies-Karotkin, and Constantinides 1978). Perhaps a decrease in the activity of this enzyme has occured, because of lactate accumulation, leading to an accumulation of fructose-1,6-diphosphate and glyceraldehyde-3-phosphate. In turn, a decrease in substrate flux through the glyceraldehyde-3-phosphate dehydrogenase step would limit the production of lactate.

Muscle energy metabolism during exhaustive exercise: No evidence for a peripheral biochemical mechanism for exhaustion.

Measurements of energy substrate profiles indicate that the point of exhausation was not correlated with a unique biochemical event in the periphery that could account for exhaustion.

Glycogen depletion.

Rats in the present study performed exercise at an intensity corresponding to approximatley 80% of their maximal oxygen consumption (Patch and Brooks 1980). The role of muscle carbohydrate stores in the limitation of exercise at this intensity has been controversial. In one study the point of exhaustion in human subjects exercised at 80% of their maximal oxygen consumption was correlated with depletion of muscle glycogen (Hermansen, Hultman and Saltin

1967). In contrast, Costill, Sparks, Gregor and Turner (1971) reported that the point of exhaustion in a group of human subjects run on a motor driven treadmill at the same work intensity was not associated with muscle glycogen depletion.

In the present studies muscle total carbohydrate reserves were depleted in all three leg muscles samples early on during the exercise performance (Figure 27). The rats continued to run during the remainder of the experiment with severely depleted carbohydrate stores. It is unlikely therefore that muscle carbohydrate availabilty was a factor in the limitation of exercise capacity.

The time of muscle carbohydrate depletion was the same as the time the occlusion frequencies began to increase (Figure 24). It is possible that the increase in the occlusion frequency and the change in running posture to a more sprawled style is due to the reduction in energy substrates within the muscle. It is of note that previous studies have defined the time of exhaustion as "... the point at which the animal could no longer keep pace with the treadmill speed and repeatedly fell back onto the shock grid ..." (Brooks and Gaesser 1980); and " ... the point when the animal seemed unable to maintain pace with and avoid the shock grid ..." (Hickson et al 1978). Probably the definition of the time of exhaustion in these two studies is

in fact the time required for the rats to begin to increase their occlusion frequencies in the present studies (Figures 17 and 20): a time that I do not define as exhaustion. Care must therefore be taken when making comparisons of studies where chemical and physiological correlates of exhaustion are made because the definitions of exhaustion may not be the same.

Accumulation of lactic acid: Role of intramuscular lactate acidosis in exhaustion.

Studies have indicated that accumulation of lactic acid during muscle contraction is associated with the development of an intramuscular acidosis and exhaustion (Karlsson, Bonde-Petersen, Henriksson and Knuttgen 1975). The mechanisms by which the lactacidosis limits muscular contraction are unclear but it has been suggested that the increase in H^+ concentration decreases in the catalytic activity of key regulatory enzymes of the glycogenolytic and glcolytic pathway (Sahlin 1978; Jones 1980; Hultman and Sahlin 1981) as is discussed below.

Glycogenolysis.

The activities of muscle phosphorylase b kinase, phosphorylase a and phosphorylase b are reduced by decreases in pH in vitro (Krebs, Love, Bratvold, Trayser,

Mayer and Fischer 1964; Kasvinsky and Meyer 1977) and cAMP production in muscle by epinephrine stimulation decreases with pH (Mawatari, Takagi and Rowland 1974). However, muscle glycogen was mobilized and metabolized by the rats in Experiment 3. Furthermore, muscle carbohydrate stores were depleted during the early phase of the exercise. Therefore, breakdown and metabolism of glycogen had occured and was not a limiting factor.

Glycolysis.

In vitro studies have indicated that the activity of PFK, the major regulatory enzyme of glycolysis, is reduced by decreases in pH (Danforth 1965; Ui 1966; Krzanowski and Matchinsky 1969; Lorensen and Mansour 1969; Lowry and Passonneau 1966; Mansour and Ahlfors 1968; Bock and Frieden 1976a, 1976b; Frieden, Gilbert and Bock 1976). Hultman and Sahlin (1981) have suggested that the accumulation of hexose monophosphates which has been observed after exhaustive isometric or dynamic bicycle exercise is evidence of inhibition of PFK (Ahlborg, Bergstrom, Ekelund, Guarnieri, Harris, Hultman and Nordesjo 1972; Bergstrom, Guarnieri and Hultman 1971). Such accumulation can be potentiated by inducing a metabolic acidosis in subjects before they exercised (Sutton, Jones and Toews 1981). Glyceraldehyde-3-phosphate dehydrogenase is another regulator enzyme of

glycolysys which is pH-sensitive (Spies-Karotkin and Constantinides 1978; Oguchi, Meriwether and Harting Park 1973; Oguch, Gerth, Fitzgerald and Harting 1973). Sutton et al (1981) observed an accumulation of fructose-1,6-bi-phosphate and dihydroxyacetone phosphate in subjects performing exhaustive bicycle exercise at 95% of their maximum oxygen consumption, which is consistant with decreased activity of this enzyme. Clearly, intramuscular acidosis may play an important role in the development of fatigue.

In the present studies lactate levels were high at the beginning of exercise and remained stable in soleus and vastus laterlis white throughout exercise and in vastus laterlis red actually decreased. Despite the high levels of lactate during the early phase of the exercise the rats were able to run and avoid the grid. Therefore, lactate accumulation appears unrelated to fatigue in Experiment 3.

Blood glucose concentrations.

Because of the tendency for muscle lactate levels to remain high during the run and the plasma lactate concentrations to steadily increase it is likely that carbohydrates were still a major substrate for energy metabolism during the late part of the run despite muscle carbohydrate depletion. The reduction in plasma glucose

concentrations was probably the result of an increase in the glucose uptake by the muscle. The resultant decrease in plasma glucose was not large and so it is unlikely that hypoglycemia was a limiting factor of exercise capacity.

Conclusion.

The measurements of muscle energy substrate profiles indicate that no unique biochemical event was correlated with the point of exhaustion.

CHAPTER 4

TURNOVER OF REGIONAL INTRACEREBRAL DOPAMINE DURING EXHAUSTIVE EXERCISE.

The lack of a peripheral factor which could explain the onset of exhaustion is consistant with there being a central mechanism. To test the hypothesis that this mechanism was based on a failure of information transfer across dopaminergic synapses, the concentrations of dopamine, DOPAC and HVA were measured in th[redacted] rats.

Background.

The striatum was chosen for these measurements because the striatum contains the highest concentrations of dopamine in the brain and the dopaminergic input to the striatum from the substantia nigra is clearly involved in the regulation of movement (Marsden 1982). The cell bodies and dendrites of the nigrostriatal pathway to the corpus striatum originate in the zona compacta of the substantia nigra itself located in the brain stem. It is of note that although the substantia nigra does not receive any dopaminergic input (synapses), this region neverthless synthesizes and stores dopamine (Bjorklund and Lindvall 1975; Hattori, McGeer and McGeer 1979). Dopamine is stored in the substantia nigra in in smooth endoplasmic reticulum (Hattori et al 1979). This dopamine is released (Korf,

Zielman and Westerink 1976) by direct electrical stimulation of the substantia nigra (Geffen, Jessell, Cuello and Iversen 1976) or neuroleptic drugs (Argiolas, Melis, Fadda and Gessa 1982; Argiolas, Melis, Fadda, Sera and Gessa 1982). Dopamine is metabolized to DOPAC and HVA in proportion to the intensity of stimulation and the rate of dopamine release (Roth et al 1976).

The relationship between the release of dopamine in the substantia nigra and the striatum is complex. For example, electrical stimulation of the forepaw of a cat has been reported to increase dopamine release in the ipsilateral nigra and the contralateral striatum but decrease dopamine release in the ipsilateral striatum and contralateral nigra (Nieoullon et al 1978). On the other hand, unilateral stimulation of the cerebral motor cortex (area 4) of the cat resulted in a bilateral increase in dopamine release in the striatum and an increase in dopamine release in the ipsilateral substantia nigra (Nieoullon, Cheramy and Glowinski 1978); the contralateral nigra was not investigated. Therefore, increases in nigral dopamine release are not always associated with increases in ipsilateral striatal dopamine release.

In the present studies the concentrations of DOPAC and HVA were used as indices of the rate of dopamine release in the brain stem and striatum.

Dopamine, DOPAC and HVA concentrations were also measured in the hypothalamus because the dopaminergic neurons in the hypothalamus are involved in the regulation of endocrine secreton, cardiovascular homeostasis and a number of other physiological systems which change during exercise.

Nigrostriatal dopaminergic activity.

Experiment 3 shows that in exhausted rats there was an increase in the turnover rate of dopamine in both the striatum and brain stem, indicating that the nigrostriatal tract is activated by running exercise. It is possible to gain an idea of how much dopamine is lost through metabolism (turnover) by measuring the increase in the concentrations of DOPAC and HVA. The half-life of DOPAC and HVA in the striatum are 13 and 15 minutes respectvly (Bacopoulos, Hattox and Roth 1979). Assuming steady state concentrations have been attained, the turnover of DOPAC and HVA has increased by 45% and 40% respectively in the striatum at exhaustion. However, it is not possible to calculate by how much the dopamine concentration in the cleft has increased because neither DOPAC nor HVA is produced specifically in the cleft. This parameter is important to know because it is the dopamine concentration in the cleft which represents the stimulatory capacity of dopamine on the post synaptic

receptor and neuron.

The concentrations of DOPAC and HVA cannot be used as a quantitative measure of how much dopamine has actually been released into the cleft. Located on the presynaptic terminal is a specific active transport mechanism which removes dopamine from the cleft back into the terminal for re-packaging into vesicles and subsequent re-use (Patton 1980). It is not known what proportion of the released dopamine follows this route in activated neurons. Recycling of released dopamine will considerably underestimate the rate of dopamine release.

A further factor which may reduce the apparent degree of activation of the nigrostriatal pathway is that only a fraction of the neurons had been activated. Ascending neurons from the substantia nigra terminate in the striatum with a distinct topography of medial-lateral, dorso-ventral and anterior-posterior projections (Fallon, Riley and Moore 1978; Redgrave and Mitchell 1982a). Recent studies demonstrate that stimulation of different regions of the striatum evoke different behavioural and locomotor responses which are dependant on the region being stimulated (Redgrave and Mitchell 1982b). Perhaps only selected neurons in the nigrostriatal pathway are activated during muscular exercise and calculations of the increase in the turnover of dopamine synapses is underestimated.

It is clear from Figure 25 that the increase in striatal DOPAC and HVA concentrations do not occur until late into the exercise performance, In the striatum this increase occurs only in exhausted rats although there is a trend for HVA concentrations to increase in Late samples. Brain stem DOPAC and HVA are also only increased late in the exercise (ie Late and Exhaustion samples). Why should the increase in dopamine turnover only begin late in the exercise performance?

Postulation for the delayed increase in nigrostriatal activation during exhaustive exercise.

The increased activity of the nigrostriatal pathway follows the point during exercise when rats show difficulty in avoiding the grid. Under these conditions peripheral feedback information from muscle and joint receptors would be informing the brain that the exercise is not being performed to the same degree of success as it was at the outset. In order to restore the force of contraction of fatiguing muscles to the desired output, two mechanisms are used: a) Motor units already activated are stimulated at a higher frequency. b) Additional motor units in the muscle are recriuted. The relative importance of these mechanisms vary from muscle to muscle (Viitasalo and Komi 1977; Maton 1980; Petrofsky 1981; Kukulka and Clamann 1981). The

additional motor units recruited, tend to be the motor neurons which have the largest cell bodies and have the highest threshold for stimulation in order for them to be recruited (Buchthal and Schmalbruch 1980). Both increases in motor unit stimulation frequency and recruitment of other motor units would require an increase in the frequency of stimulation of the alpha motor neuron by descending fibres from the brain: an increase in central neural drive. It is possible that the increase in turnover of dopamine in the later part of the exercise represents a neurochemical correlate of this increase in central neural drive in an attempt to increase the force of contraction of fatiguing motor units and recruit non-fatigued motor units. A further factor which may contribute to the increase in dopamine turnover may lie in the fact that as the muscles which are used during the first few minutes of exercise become fatigued other muscle groups are recruited to compensate for the decrease in the force of contraction of working muscles. This increase in the total muscle mass being activated would again require an increase in the activation of dopaminergic neurons. If this is the case, this increase in nigrostriatal activity may be a neurochemical correlate of 'effort'.

Another explanation for the delay in the increase in dopamine turnover may be due to a decrease in the responsiveness of the post synaptic neuron to a given degree

of dopamine release. If the sensitivity of the dopamine receptor were to decrease or a deficit in the mechanism which translates the degree of dopamine receptor binding into changes in the electrical activity of the post synaptic neuron were to develop, a greater release of dopamine must occur to bring the degree of activation of the post synaptic neuron back to the desired level.

It is possible that activation of nigrostriatal neurons is a consequence of the increased electric stimulation as the animal becomes fatigued and touches the grid more frequently. However, one study which has measured DOPAC and HVA levels in the striatum, and estimated dopamine synthesis rates in rats during electric shock have found evidence that the dopaminergic activity in the striatum was decreased by electric stimulation (Lane, Sands, Co, Cherek and Smith 1982). In the present study, it would be theoretically possible to evaluate the contribution of electric shock per se on nigrostriatal dopamine metabolism by exposing a group of rats to the same frequency and duration of electric stimulation, without running on the treadmill. However, in practical terms it is not possible to electrically stimulate an animal without there being a concomittant increase in locomotor output. Muscle relaxants could not be used to remove the movement aspect of electrical stimulation; although there may be no movement in

a parallyzed animals neverthless there will still be a central drive to the muscles. Anaethetized animals could not be used as control because the anaesthetic would remove the very stimulus that the experimant was designed to test.

The reason for the increase in dopaminergic activity of nigrostriatal dendrites in the brain stem before the striatum is unclear. It may be that both were activated at the same time, and the re-uptake systems were much more efficient in the striatum than the brain stem. It is also possible that another dopaminergic system, such as the mesocortical system was activated by exercise. Therefore, dopamine release was increased in the nigra but not the striatum.

CHAPTER 5.

POSSIBLE MECHANISMS FOR FATIGUE IN DOPAMINERGIC SYNAPSES.

There are a number of possible mechanisms that could be responsible for defficiences in information transfer across striatal dopaminergic synapses.

1. Depletion of dopamine stores.

Direct measurements of striatal dopamine concentrations show that despite increases in nigrostriatal dopamine turnover (Figures 25 and 26) no depletion of striatal dopamine was observed. Therefore, exercise performance was not limited because of a lack of dopamine for release.

2. Inhibition of dopamine binding to postsynaptic dopamine receptors.

Both DOPAC and HVA have chemical structures which are similar to dopamine. Perhaps one or both of these compounds can bind to dopamine receptors and act as a dopamine receptor antagonist. As described in Appenxix 3 there was no consistant evidence that DOPAC or HVA could bind to striatal dopamine receptors *in vitro* or that DOPAC could inhibit the spontaneous secretion of prolactin from isolated anterior pituitary glands *in vitro* or affect the inhibition of spontaneous prolactin secretion by dopamine

itself. These results suggest that neither DOPAC or HVA have dopamine receptor ligand properties, although this remains a possibility.

3. Inability of postsynaptic neuron to respond to increases in dopaminergic activity

If exercise was dependant on a progressive increase in dopamine release (to compensate for muscular fatigue: see above) exhaustion would occur when the postsynaptic dopamine receptor became saturated. Against this hypothesis is the fact that apomorphine increased exercise capacity in normal rats, an effect not expected if all postsynaptic dopamine receptors were already occupieed by dopamine (assuming the responsiveness of dopamine receptors was the same for apomorphine as it is for dopamine).

One question that arises from the effects of apomorphine is how can a drug which activates dopamine receptors in a tonic way be effective in improving movement which is repetative and would be expected to require phasic actions of relevant nerves? The traditional perception of neurotransmitters has been expanded recently with the concept of 'neuromodulators' which act to modulate the effects of other neurotransmitters. It is possible that the effects of apomorphine are due to its ability to mimic the effects of dopamine in synapses where dopamine is acting as

a 'neuromodulator' rather than a 'neurotransmitter'. In this respect, it is of note that Parkinson's disease is treated with the tonic application of dopaminergic drugs such as L-Dopa and bromocriptine. Perhaps the role of dopamine is to alter the 'tone' of striatal neurons on which other neurotransmitters act. Perhaps the label of 'neuromessenger' is a more appropriate one for dopamine.

4. Activation of mechanisms which suppress release of dopamine.

Although there was an increase in dopamine turnover in exhausted rats it is possible that this increase was insufficient to activate postsynaptic neurons to a sufficient degree to maintain movement. In exhausted rats I propose there is a relative defficiency in the degree of activation of postsynaptic dopamine receptors, which can be compensated for by apomorphine.

The next chapter will discuss a potential mechanism for this hypothesis.

CHAPTER 6.

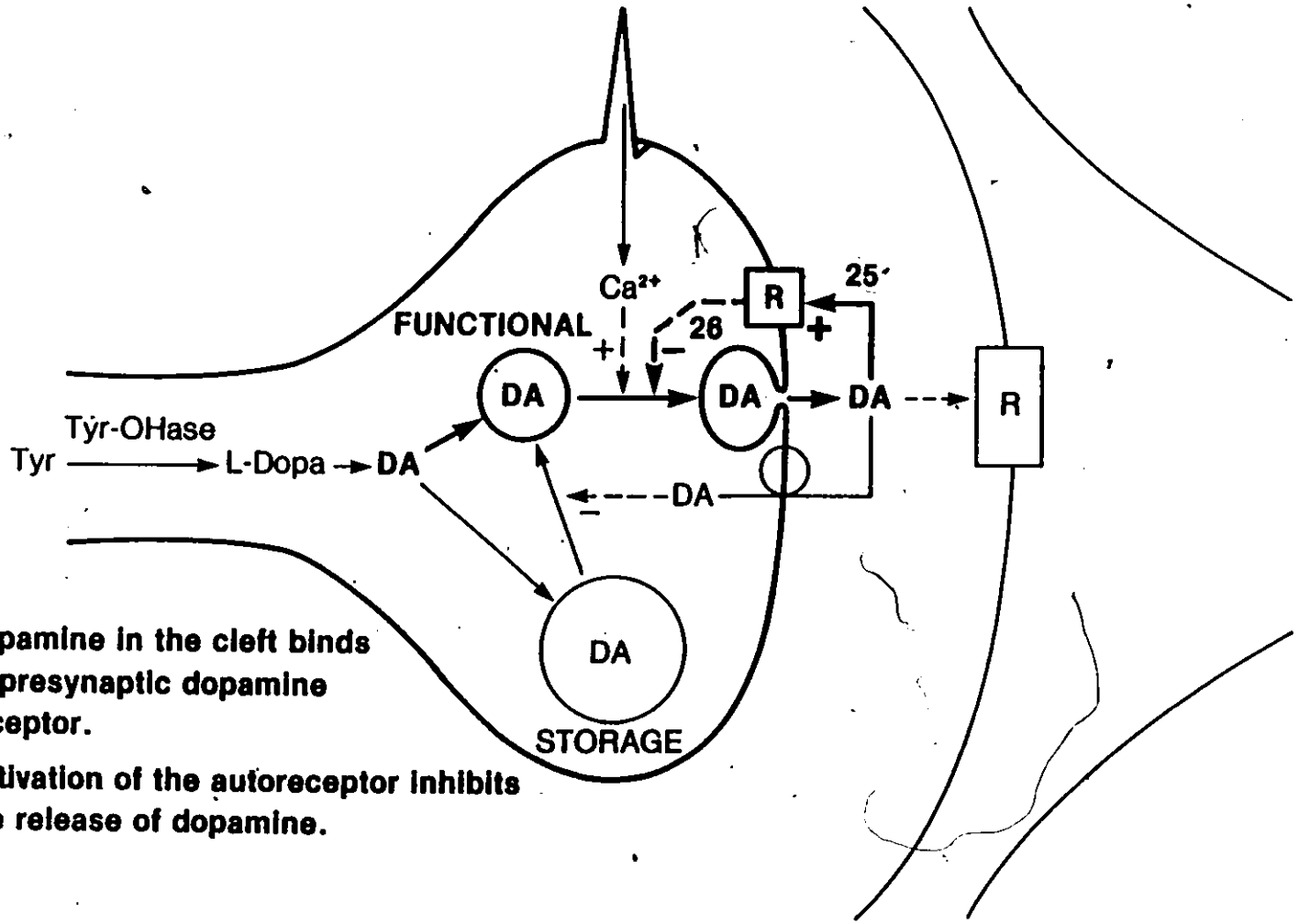
MECHANISM FOR THE LIMITATION OF DOPAMINE RELEASE.

Located on presynaptic terminals are dopamine receptors (Figure 32) that when activated by dopamine suppress the relase of dopamine from the terminal (Starke, Reimann, Zumstein and Hertting 1978; Reimann, Zumstein, Jackish, Starke and Hertting 1979; Hoffman, Naylor and Cubeddu 1980; Jackisch, Zumstein, Hertting and Starke 1980; Dubocovich and Weiner 1981; langer 1981; Kamal, Arbilla and Langer 1981; Cubeddu and Hoffmann 1982, 1983; Hoffmann and Cubeddu 1982a, 1982b). It is possible that in rats run to exhaustion this feedback mechanism was activated and the amount of dopamine released from the terminal was insufficient to accurately transfer information to the postsynaptic neuron.

FIGURE 32. Regulation of dopamine release.

A major factor which has been shown to regulate the release of dopamine is the dopamine autoreceptor located on the presynaptic membrane. The binding of dopamine in the cleft to this receptor (25) inhibits dopamine release (26). Antagonists of this receptor, such as haloperidol, increase neurogenic release of dopamine.

REGULATION OF DOPAMINE RELEASE



25 Dopamine in the cleft binds to presynaptic dopamine receptor.

26 Activation of the autoreceptor inhibits the release of dopamine.

Chemical significance of limitation of dopamine release.

Previous studies have proved how resistant intracerebral dopamine is to depletion. Stone (1975) has summarized the effects of 'stress' on the turnover of intracerebral dopamine and its concentration. Almost without exception, stresses which increased the turnover of dopamine were without effect on the concentrations of dopamine. These observations have encouraged statements such as that of Costa and Trabucchi (1975) that intracerebral dopaminergic synapses have the: "...ability ... to maintain the concentration of their transmitter at steady state in the face of continuous demand...". Similarly, in discussions on the effects of stress on behaviour (for example Weiss, Goodman, Losito, Corrigan, Charry and Bailey 1981) little attention is paid to potential involvement of dopamine bacause dopamine concentrations do not change.

I propose that it is the function of these dopamine autoreceptors to limit the release of dopamine so that the concentration of dopamine in the functional pool is maintained.

The reason why dopaminergic synapses are protected against dopamine depletion, even at the expense of deficits in information transfer across the synapse, is unclear particularly when the terminal contains a so-called 'storage pool'. This next section will discuss a further property of

dopaminergic terminals with resect to the regulation of dopamine release and the maintainence of the functional pool, namely the transfer of dopamine from the storage pool to the functional pool. Figures 5 and 33 summarizes the model of the dopaminergic synapse that will be used in this discussion. This figure is based on information derived from the papers of Shore and co-workers (Shore et al 1978; McMillen et al 1980).

Maintainence of the functional pool: Role of the storage pool.

Shore and co-workers (Shore et al 1978; McMillen et al 1980) have reasoned that dopamine in the storage pool can be transferred to the functional pool because inhibition of tyrosine hydroxylaase is without any behavioural effects until dopamine depletion is severe (>50%). The transfer of dopamine between the two pools is regulated by the concentration of free dopamnie in the cytoplasm of the terminal (see Figure 33 and Introduction for a description of this evidence). This transfer is not rapid however because the behavioural effects of the dopamine receptor antagonist haloperidol can be potentiated by inhibition of dopamine synthesis at a time when dopamine depletion is only 20% (Shore and Doris 1975). This observation suggests that at higher rates of dopamine release a large fraction of

dopamine within the terminals is relatively unavailable for release (Shore et al 1978; McMillen et al 1980).

Role of dopamine autoreceptors and regulation of transfer of dopamine between terminal pools.

The above discussion suggests that the amount of dopamine in the storage pool is maintained by the prevention of dopamine transfer to the functional pool by the concentration of free dopamine in the cytoplasm. In addition, the concentration of dopamine in the functional pool is maintained by the inhibition of excess dopamine release by dopamine autoreceptors. If these proposals are correct, activation of the synapse should deplete dopamine from the terminals if the effects of these feedback systems was prevented.

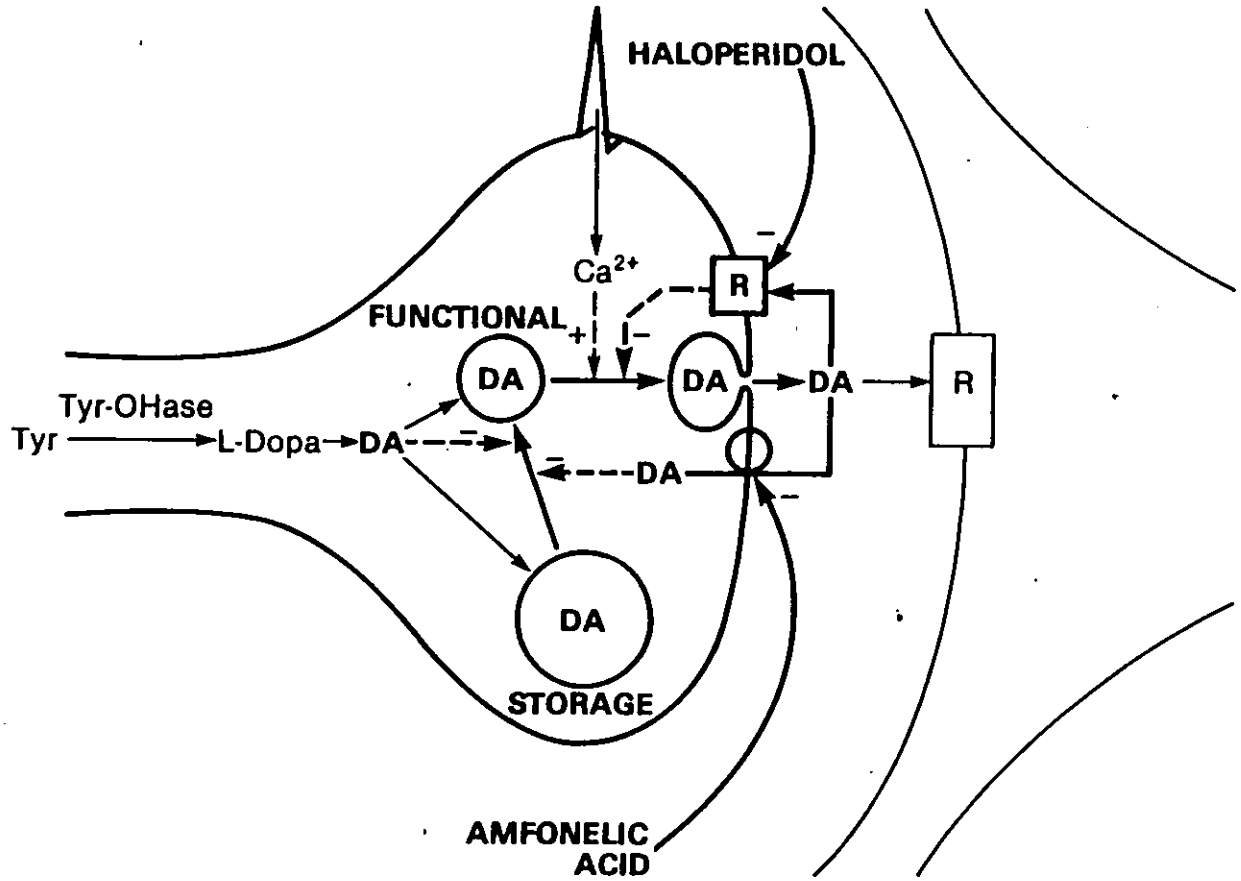
An experiment to test this hypothesis has already been performed by Shore (1976).

Pre-synaptic dopamine autoreceptors can be blocked by haloperidol to remove the feedback inhibition that this receptor has on dopamine release. Free dopamine concentrations in the terminal cytoplasm can be reduced by the inhibition of dopamine re-uptake into the terminal by amfonelic acid to remove the inhibition of dopamine transfer from the storage pool to the functional pool for release. Figure 33 summarizes the proposed effects of these drugs.

FIGURE 33. Blockade of presynapstic dopamine receptor by haloperidol and inhibition of dopamine re-uptake into the terminal by amfonelic acid.

Blockade of presynaptic autoreceptor by haloperidol removes the inhibitory effect that this receptor has on the release of dopamine. The inhibition of dopamine re-uptake by amfonelic acid prevents the increase in the concentration of dopamine in the cytoplasm of the terminal which occurs when the terminal is activated. This results in an increase in the transfer of dopamine from the storage pool to the functional pool. Each of these drugs administered alone result in an increase in the production of DOPAC and HVA, but on their own have no effect on the concentration of dopamine in the terminal. However, if both drugs are administered simultaneously, the increase in dopamine turnover is potentiated and the terminal begins to become depleted of dopamine. It would appear that at high rates of dopamine release, the activity of tyrosine hydroxylase cannot be increased sufficiency to fully compensate for the loss of dopamine to DOPAC and HVA. I propose that the presynaptic autoreceptor and the intraneuronal system to regulate the transfer of dopamine from the storage pool to the functional pool operates to prevent the excess o release of dopamine in excess of the maximum capacity for dopamine resynthesis.

REGULATION OF DOPAMINE RELEASE:
Effects of Haloperidol and Amfonelic Acid



Haloperidol and AFA when injected alone both increased the rate of dopamine turnover (DOPAC and HVA production) in the striatum by about 120% (Shore 1976). In both situations the concentration of dopamine remained unchanged (13.4 µg/g and 14.6 µg/g respectively compared to a control value of 13.6 µg/g). However, both drugs given together resulted in an increase in dopamine turnover (800%) which was much greater than that produced by the sum of each drug alone. It is of note that the concentrations of dopamine were decreased from 13.6 µg/g to 8.7 µg/g after 90 minutes. These observations indicate that removal of the feedback systems which regulate the release of dopamine and the intra-cellular transfer of dopamine between the two pools results in a depletion of dopamine. The results are consistant with the suggestion that the feedback systems operate in vivo to prevent the release of dopamine to levels higher than the ability of the terminal to synthesize dopamine and take dopamine back up into the terminal.

Westerink and Wirix (1983) have also observed the effects of haloperidol on the concentration of striatal dopamine. They observed that haloperidol alone decreased dopamine concentrations in the striatum by 20% after 90 minutes. The only difference between this study and that of Shore (1976) is that Westerink and Wirix (1983) injected haloperidol intravenously whereas Shore (1976) injected the

same dose of haloperidol intraperitonealy. It is probable that the concentrations of haloperidol in the brain were higher when given directly into the blood. Perhaps a higher dose of haloperidol given intraperitonealy would be successful in depleting dopamine.

Applying these ideas to the present work, it is possible that the apparent defficiency of dopamine receptor stimulation which was in part responsible for the limitation of exercise performance may have been the result of the feedback systems limiting the release of dopamine from the terminal. Although dopamine release was increased, it may have been less that the amount required to have been released in order to transfer information across the synapse.

CONCLUSION

The data I have obtained suggest that one of the factors that limits exercise capacity is an inability of striatal dopaminergic synapses to maintain the transfer of information.

APPENDIX 1

SUMMARY

The treadmill exercise system used to run rats to exhaustion is described. The system is a variable speed treadmill with an electric grid at the distal end of the treadmill belt. Photoelectric cells are placed over the grid to obtain a permanent chart record of the position of the rat on the apparatus. The time of exhaustion is defined as the time required for the running rat to stop running and rest on the grid for 10 seconds. The variability in the time of exhaustion can be reduced by running the rats for short periods over an 8-day period and rejecting rats which appear unwilling to run.

INTRODUCTION

Numerous methods are currently available to induce an animal to perform physical exercise. These methods include running on motor driven treadmills (Bedford, Tipton, Wilson, Opliger and Gisolfi 1979; Brooks and White 1978; Flaim, Minteer, Clark and Zelis 1979; Gleeson, Mullin and Baldwin 1983; Sonne and Galbo 1980; Gruner and Altman 1980; Rubin and Minkle 1982) and swimming in water (Lin, Chen and Baker 1979; Flaim et al 1979; Grunner and Altman 1980). The

most commonly used measure of the 'capacity' of an animal to perform exercise is the time taken for the animal to become 'exhausted' (Bagby et al 1978; Gerald 1978; Hickson et al 1978; Clark and Conlee 1979; Flaim et al 1979; Francesconi and Mager 1979; Baldwin, Hooker, Herrick and Schrader 1980; Brooks and Gaeser 1980). The method of measuring exercise capacity by running rats to exhaustion on a motor driven treadmill was chosen for the present study for the following reasons.

Swimming exercise was considered unsuitable to induce exhaustion because the animal may chose to 'rest' by floating or sinking (Porsolt, Anton, Blanet and Jalfre 1978; Hawkins, Phillips, Moore, Gilliland, Dunbar and Hicks 1980), rendering the work output neither continuous nor constant. Obviously the point of exhaustion may vary considerably as a consequence of the time spent immobile.

Treadmill running, on the other hand, has the advantage that the work output is continuous and can be controlled by the treadmill speed and running grade (Sheperd and Gollnick 1976; Bedford et al 1979; Patch and Brooks 1980; Sonne and Galbo 1980; Brooks and White 1978). The physiological responses to running are now well doccumented and are appropriate for the increase in muscular energy expenditure, namely increase in cardiac output, arterial blood pressure, muscle blood flow and the mobilization of

energy substrates from fat tissue and the liver (Bagby et al 1978; Flaim et al 1979; Gleeson et al 1983; Sonne and Galbo 1980). In contrast, swimming exercise appears to evoke more of a diving reflex without increases in cardiac output and muscle blood flow for example (Flaim et al 1979). Treadmill running was therefore chosen for the present studies.

The purpose of this appendix is to describe the development of the treadmill system, to explain the reasoning behind the protocol design and to describe the important characteristics of the system in particular the definition of exhaustion.

METHODS

The apparatus used was a modified variable speed Quinton treadmill with an electric grid system placed at the distal end of the running belt. A series of photoelectric cells were placed over the grid area in order to obtain a permanent record of the position of the animal on the apparatus (Figure 6).

In order to familirize the animals to running on the belt, a 'learning schedule' was designed which would do this without having any significant physiological training effect. It was decided to run the rats during their nocturnal period rather than their daytime period, as is usually done in by previous workers, because rats are

nocturnal animals and normally sleep during the day. The learning schedule protocol is described in Table 1.

EXPERIMENTS PERFORMED

Definition of Exhaustion

Currently there is no single criteria which has been consistantly used to define the onset of exhaustion. The criteria which have been used include: '... the point at which the rat could no longer keep pace with the treadmill speed and repeatedly fell back on the shock grid and could no longer right itself when placed on its back' (Brooks and Gaesser 1980); '... the point when the animal could no longer right itself when placed on its back' (Clark and Conlee 1979); '... until exhaustion was apparent' (Flaim et al 1979); '... the point when the animal seemed unable to maintain pace and avoid the shock grid' (Hickson et al 1978). In some studies the criteria used to define exhaustion are not stated (Gerald 1978; Bagby et al 1978).

In order to judge the individual merits of these criteria a group of six rats exposed to the learning schedule were run on the treadmill until they were unable to run any longer and lay immobile.

When the animals were unable to run and were removed from the treadmill five of the rats exhibited the following characteristics:

1) failed to exhibit a righting relex when dropped dorsal side downwards from a height of 12 cm or when placed on their backs.

2) remained immobile when placed on their feet on the floor.

3) failed to struggle or move when held under water for 10 seconds.

These animals had run well for the first 10 minutes of the run after which their posture had become increasingly sprawled and the number of times the animal touched the grid increased progressively. These animals would therefore appear to be 'exhausted'.

The remaining animal had stopped running when its running posture was unchanged from the style seen at the beginning of the run. When removed from the treadmill this animal:

1) displayed an efficient righting reflex.

2) attempted to escape when placed on the floor and struggled strongly when held.

This animal was clearly not 'exhausted'.

It was noted in subsequent trial runs that the learning scores of the animals which were 'exhausted' were different from those animals which displayed the characteristics of the last animal discussed above. The former group of animals was characterized by learing scores of 3 or 4 points during the learning schedule. In contrast, animals which were judged not to be completely 'exhausted' appeared to be reluctant to run as indicated by learning scores of less than 3 on one or more days of the learning schedule. These animals also tended to stop running during the early part of the exhaustion run and either turned over onto their backs, appeared to be looking around for escape or literally stood on the grid and refused to move. There were very few exceptions to these two characteristics (between 2-5%). It was therefore decided to exclude from further study and inclusion into the final experimental data animals with one or more of the following characteristics:

1) Animals with learning scores which were less than 3 during days 2 to 5 of the learning schedule, (by this time the learning scores had become typical of the score for the remaining learning days and predicted animals were the poor runners).

2) Animals which stopped running more than three times during the first ten minutes of the exhaustion run for longer than four seconds.

Other workers have also noted that certain animals are reluctant or unwilling to run on their treadmill systems (Bedford et al 1979; Brooks and Gaesser 1980; Gleeson et al 1983). These authors also eliminated such animals from their results.

As a consequence of these preliminary observations and the development of criteria with which to identify animals which were reluctant to running on the treadmill the following definition for exhaustion was used:

> The time required for a running animal to stop running and elect to rest on the grid for longer than ten seconds.

Exhaustion is an inability to continue performing work.

Influence of time of day and learning schedule on times of exhaustion.

Studies were designed to evaluate the influence that running animals at various times of the day and the effects the learning schedule had on the times of exhaustion.

Two group of animal were exposed to the 9 day learning schedule (Experienced) and on day 9 run to exhaustion at a speed of 37.0 m/min. One group was run beginning two hours after lights off (Nocturanl) while the other group was run beginning two hours after lights on (Daytime). A third Nocturnal group of rats was not exposed to the learning schedule (Naive) and run to exhaustion. The times of exhaustion of these animals are presented in Table 1-1.

Experienced Nocturnal animals ran for a time that was not significantly different from that of Naive Nocturnal animals (21.9 min vs 19.9 min). However, the Experienced rats had much less variablility than Naive animals in their exhaustion times as indicated by a smaller standard deviation (16.9% vs 44.7% of group mean exhaustion times respectively). In Experienced animals, the times of exhaustion were significantly higher in the Nocturnal group than in the Daytime group (21.9 min vs 15.0 min) with a slightly lower standard deviation (16.9 % vs 22% of group mean exhaustion times).

Previous studies have also included familiarization protocols in their studies (Sheperd and Gollnick 1976; Bagby et al 1978; Brooks and White 1978; Hickson et al 1978; Baldwin et al 1980; Brooks and Gaesser 1980). In some protocols the duration and intensity of the work loads were

high and likely to have had significant training effects (Sheperd and Gollnick 1976; Bagby et al 1978; Brooks and White 1978; Hickson et al 1978). The present protocol is unlikely to have had any training effect as indicated by similar times of exhaustion in Experienced and Naive animals. The lower standard deviation in Experienced animals indicates the effectiveness of the learning schedule in reducing the variability in the times of exhaustion.

Animals also ran with a greater capacity and with less varibility when run during their Nocturnal activity period. Clark and Conlee (1979) have reported that the exercise capacity of swimming rats was maximal when the animals were exercised towards the end of the dark cycle. The majority of studies reported in the literature have exercised their animals during the light phase of the light-dark cycle Brooks and White 1978; Brooks and Gaesser 1980; Patch and Brooks 1980); in some reports the time of day the animals were run is not stated (Sheperd and Gollnick 1976; Gerald 1978; Hickson et al 1979; Bedford et al 1979; Flaim et al 1979; Francesconi and Mager 1979; Lin et al 1979; Baldwin et al 1980; Sonne and Galbo 1980; Gleeson et al 1983). The time of day is clearly an important consideration in the determination of exercise capacity.

These two studies indicated that the variability in the times of exhaustion could be appreciably reduced by:

1) Exposing the rats to the 9 Day learning schedule and excluding and excluding those animals which scored less than 3 points.

2) Eliminating animals from the data which stopped running more than twice for longer than four seconds during the first ten minutes of the exhaustion run.

3) Exercising the rats during the Nocturnal period of the light-dark cycle.

Relationship between time of exhaustion and treadmill running speed.

The effects of treadmill speed on the times of exhaustion was evaluated by running four groups of experienced nocturnal animals to exhaustion at speeds ranging from 30.0 m/min to 40.0 m/min. The times of exhaustion (+1SD) were: 30.0 m/min, 33.5+3.6 min; 35.0 m/min, 30.1+4.8 min; 37.0 m/min, 21.9+3.7 min; 40.0 m/min, 20.9+3.3 min. Individual data are plotted in Figure 1-1. The correlation coefficient between running speed and the times of exhaustion was 0.77 ($p<0.001$).

Photoelectric cell occlusion frequency during exhaustive exercise.

The photoelectric cell occlusion frequency during the exhaustion runs of Figure 1-2 at speeds of between 30.0

m/min to 40.0 m/min are presented in Figure 1-2. During the first 15%, 30%, 30% and 50 % of the total run time at 40.0 m/min, 37.0 m/min, 35.0 m/min and 30.0 m/min respectively, the animals ran at the proximal end of the treadmill and had photoelectric cell occlusion frequencies of less than 3 occlusions/15 s. During the remainder of the exercise the animals running posture became increasingly sprawled in style and the photoelectric cell occlusion frequency increased progressively, with the maximum frequency occuring during the last 5% of the run.

This study represents the first attempt to record the position of the animal on a treadmill during the performance of an exhaustion run. Previous studies have used the time taken for the animal to begin to have difficulty in keeping pace with the treadmill and avoiding the grid as the time of exhaustion. If the point where the photoelectric cell occlusion frequency begins to increase represents this time, it is clear from the present studies that the animal can still maintain exercise output albeit at a reduced efficiency in performing the work (ie avoid the grid). The physiological mechanisms responsible for the difficulty in avoiding the grid are unclear. But there is evidence in Experiment 1 and 3 that the problem may result from either depletion of intramuscular carbohydrate stores or defficiencies in the transfer of information across striatal

dopaminergic synapses. However, the rats are still able to maintain a certain degree of work output despite this depletion. Perhaps the blood provides glucose for metabolism, as indicated by the decrease in plasma glucose concentrations Intramuscular lipid stores may also be an important source of energy substrates at this time.

Effects of Sham operation on exercise performance.

Because the Sham operation required traumatic surgery and damage to cortical areas it was possible that this may have rendered rats unable to run on the treadmill. An initial study indicated that rats were able to run. Figure 1-3 plots the times of exhaustion of all the Intact and Sham animals that were run during the present studies. Clearly, the Sham operation had no effects on exercise performance.

CONCLUSION

This model has refined previous systems for treadmill exercise in rats to define a point of exhaustion with a minimized variability in the times of exhaustion. The addition of a photoelectric cell system allows quantitation of the degree of fatigue during the actual run.

TABLE 1-1. Influence of the Learning Schedule and the Light-Dark cycle on the times of exhaustion.

GROUP	PERIOD OF RUNNING	TIME OF EXHAUSTION	STANDARD DEVIATION	n
Naive	Nocturnal	19.9	8.9	5
Experienced	Nocturnal	20.9	3.3	5
	Daytime	15.0*	3.3	5

* $P<0.05$ compared to Experinced animals run during their Nocturnal activity period. Running speed: 37 m/Min.

n = Number of animals.

Naive: Not exposed to the Learning Schedule.
Experienced: Exposed to the 8-Day Learning Schedule.
Daytime: Animals run during the Lights-on period.
Nocturnal: Animals run during the lights-off period.

This experiment indicated that varibility in the times of exhaustion could be reduced by running animals exposed to the 8-day familiarization protocol (Table 1) and running the animals during their Nocturnal activity period.

FIGURE 1-1. The relationship between the time of exhaustion and the treadmill speed. All animals were exposed to the 8-day learning schedule. On day 9 the animals were given a 0.5 min warm-up run and then run to exhaustion at speeds of between 30 m/min to 40 m/min.

RELATIONSHIP BETWEEN EXHAUSTION TIME AND RUNNING SPEED

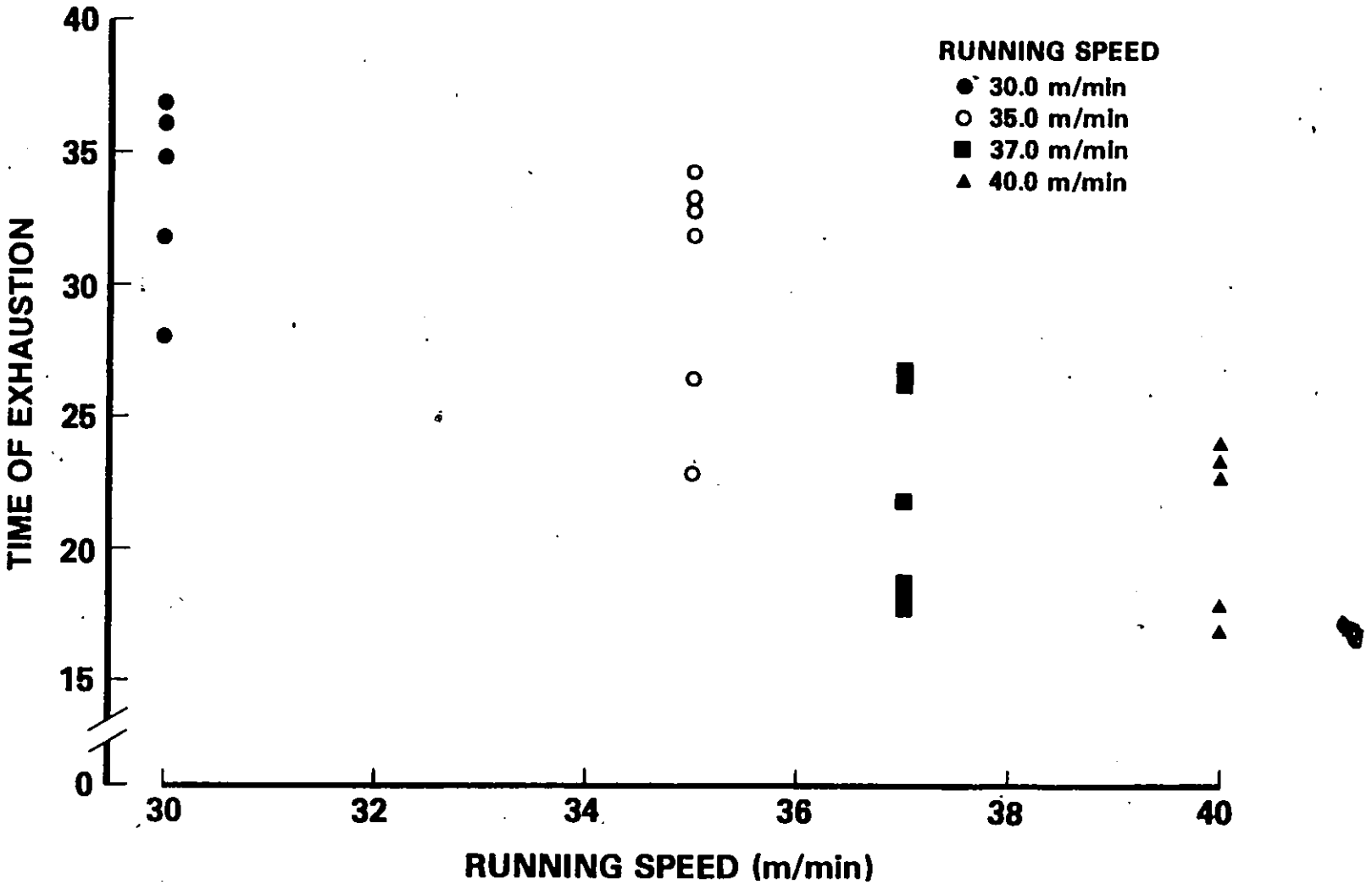


FIGURE 1-2. Photoelectric cell occlusion frequencies in animals run to exhaustion at speeds of 30 m/min, 35 m/min, 37 m/min and 40 m/min. These values were obtained from the animals described in Figure 1-1.

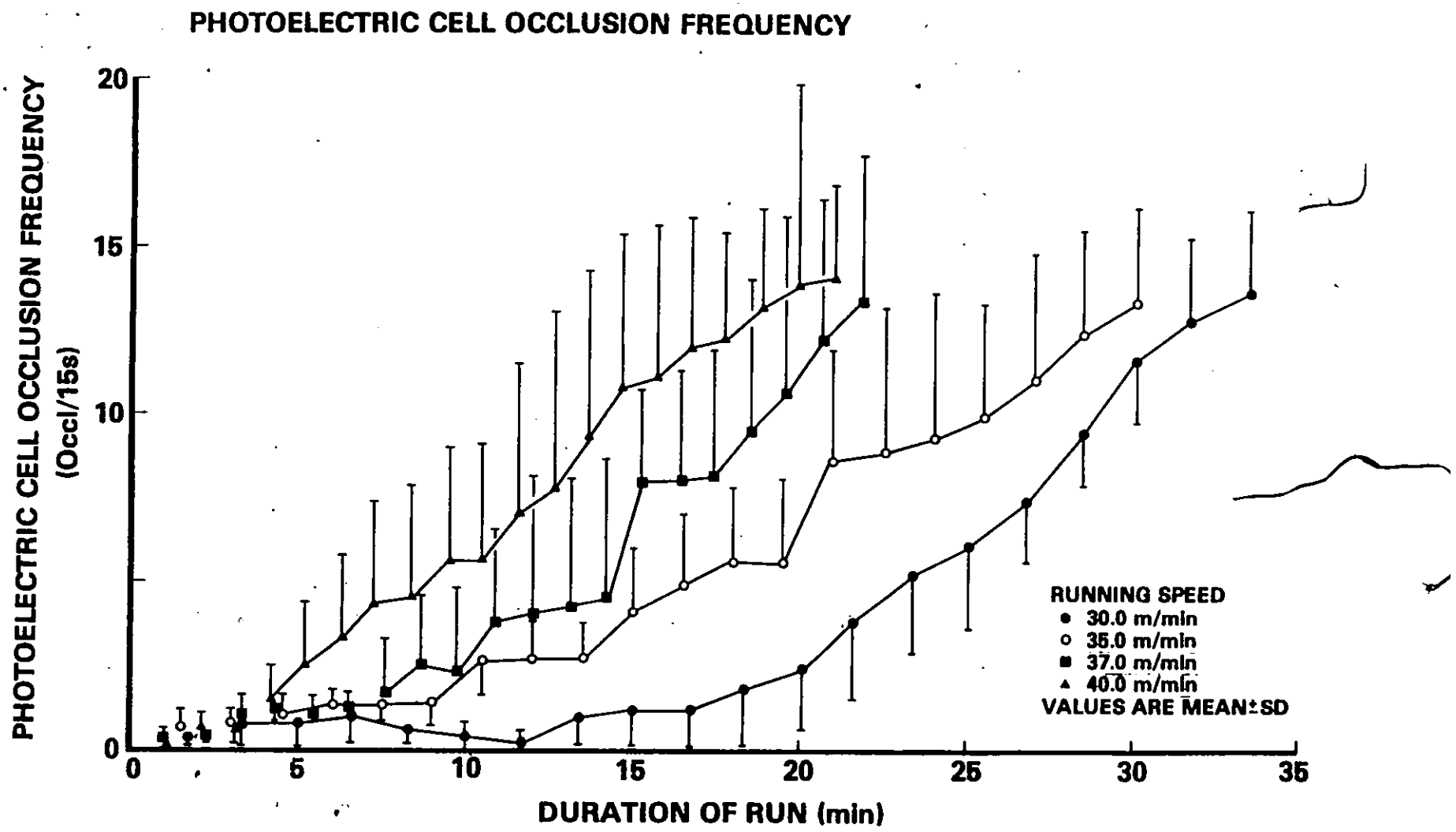
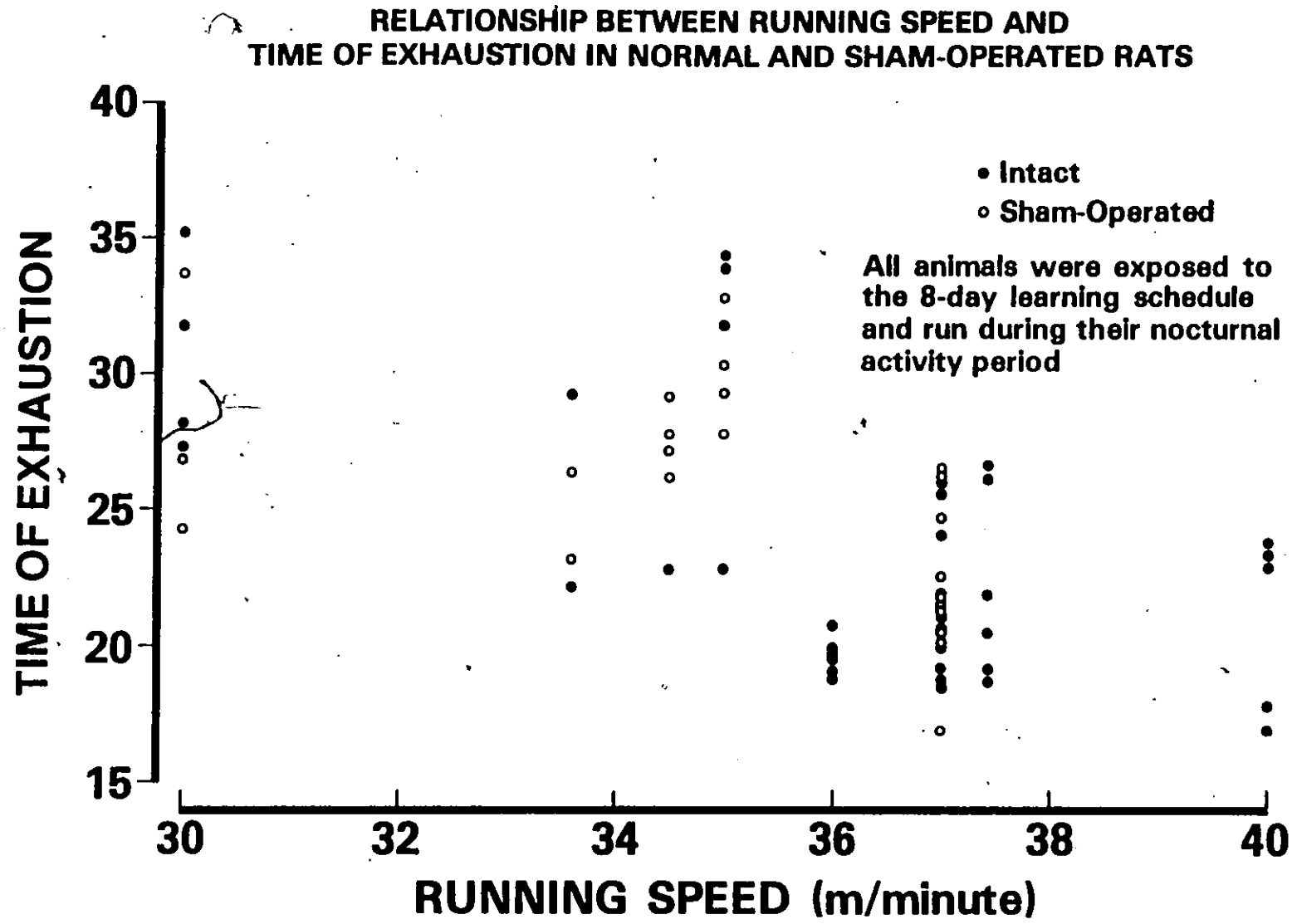

PHOTOELECTRIC CELL OCCLUSION FREQUENCY
PHOTOELECTRIC CELL OCCLUSION FREQUENCY (Occl/15s)
20
15
10
0
0
5
10
15
20
25
30
35
DURATION OF RUN (min)
RUNNING SPEED
30.0 m/min
35.0 m/min
37.0 m/min
40.0 m/min
VALUES ARE MEAN±SD

FIGURE 1-3 . Relationship between the time of exhaustion and the treadmill speed in Intact and Sham animals. All animals were exposed to the 8-day learning schedule. On day 5 the Sham animals received an injection of 20 ul of thorium dioxide whereas the Intact animals were left in their home cage. On day 9 the animals were given a warm-up run of 0.5 min and after 5 minutes run to exhaustion at speeds of between 30 m/min to 40 m/min.

There was no significant difference between the times of exhaustion between the Intact and Sham animals at any speed.


RELATIONSHIP BETWEEN RUNNING SPEED AND
TIME OF EXHAUSTION IN NORMAL AND SHAM-OPERATED RATS
TIME OF EXHAUSTION
40
35
30
25
20
15
• Intact
○ Sham-Operated
All animals were exposed to the 8-day learning schedule and run during their nocturnal activity period
30
32
34
36
38
40
RUNNING SPEED (m/minute)

APPENDIX 2

SUMMARY

This Appendix describes the radiographic technique used to identify correctly located cannula in the ventricular system of the rat brain without the need for histological examination. A suspension of thorium dioxide was infused through the cannula placed in the left lateral cerebral ventricle under stereotaxic guidance. Correctly located injections were identified by the presence of a distinct thorium shadow on lateral x-ray view of the in situ brain. When 6-hydroxydopamine was co-injected with thorium the resultant depletion of striatal dopamine, dihydroxyphenylacetic acid and homovanillic acid were correlated with the degree of spreading throughout the ventricular system. In addition the degree of the spreading of thorium was correlated with the changes in the ability of the animals to run on the treadmill.

INTRODUCTION

For Experiment 1 it was necessary to deplete intracerebral catecholamines by giving an injection of the neurotoxin 6-OHDA direct into the ventricular system of the brain. Because the experimental protocol required a number

of time-consuming proceedures after the injection, it was desirable to identify animals which did not have a correctly located injection so that they could be excluded from further study, without having to wait for post-mortem confirmation. To identify animals with a correctly injection into the cerebral venrtricles, the radio-opaque contrast medium thorium dioxide was used as carrier and the animals x-rayed in lateral view to visualize the presence or absence of a shadow of the ventricular system. This technique obviated the need for histological examination of post-mortem tissue.

This appendix describes the characteristics of the x-ray appearance of correctly and incorrectly injected animals. This description is based on the results of a pilot study consisting of 23 animals.

METHODS

Animals were anaesthetized and placed in a stereotaxic frame (Figure 7). The animals were then given an injection of 20 µl of a 25% suspension of thorium dioxide according to the following co-ordinates: A = 0.0 mm, L = 1.6 mm, D-V = 3.2 to 3.6 mm. Then the intact animals or isolated brains were x-rayed in lateral and anterio-posterial (A-P) views at a magnification of X3.

RESULTS AND DISCUSSION

When the infusion cannula had penetrated the lateral ventricle a thorium shadow was seen in lateral x-ray view of the in situ brain. Figures 2-1 and 2-2 show the extremes of appearance of these shadows (x-ray scores of 4 and 1 respectively). No shadows were visible in A-P view of the in situ brain. In three rats tested, the thorium shadow remained visible for five days.

In isolated brains, the lateral views further clarified the shape of the shadow (Figure 2-3) and in A-P views allowed visualization of the degree of spreading throughout the ventricular system. The extent of the spreading varied form complete, with filling of the lateral, III and IVth ventricles (Figure 2-4), to incomplete (Figure 2-5) and was occasionally laterally biased and even unilateral (Figure 2-6).

The presence of a distinct thorium shadow in the lateral view of an intact animal was unequivcably associated with the penetration of the infusion cannula into the lateral ventricle. No animal was observed with a cannula tract into the lateral ventricle which did not display a discernable ventricular shadow in the lateral view of the intact animal.

Although other radio-contrast media could be used, thorium has several advantages. Thorium has 4.9 times the

x-ray stopping power as iodine (Johns and Cunningham 1971). Thorium dioxide suspensions da not have the disadvantage of a high osmotic pressure and do not cause inflamation of the ependyma. These properties improve post-operative survival. Of 55 animals so injected, all but one survived. In addition, thoroum dioxide is only slowly cleared from the intracerebroventricular system in contrast to water soluble media such as iophendylate.

CONCLUSION.

This simple x-ray technique allows the rapid identification of animals correctly injected into the lateral cerebral ventricles of the rat. The technique has the advantage that animals which had not received a correctly located injection could be eliminated from further, time-consuming, study.

FIGURE 2-1. Lateral x-ray view of rat with 20 µl of thorium dioxide injected into the left lateral cerebral ventricle. The shadow of the ventricle is enclosed within the box.

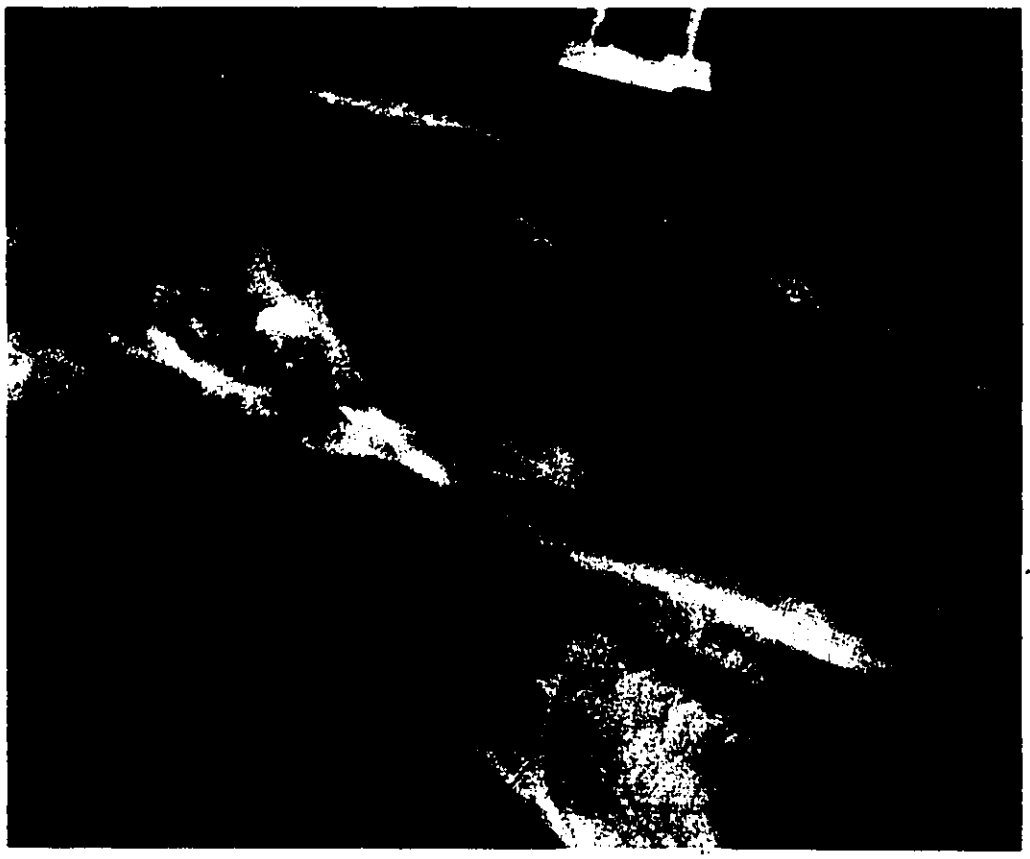


FIGURE 2-2. Lateral x-ray view showing a rat with an x-ray score of 1. Although there is no evidence of spreading of thorium through the ventricular system, histological examination nevertheless indicated that the infusion cannula was correctly located in the lateral ventricle.

FIGURE 2-3. Lateral x-ray view of the excised brain of the rat shown in Figure 2-1.

FIGURE 2-4. A-P view of the excised brain of the rat shown in Figures 2-1 and 2-3.

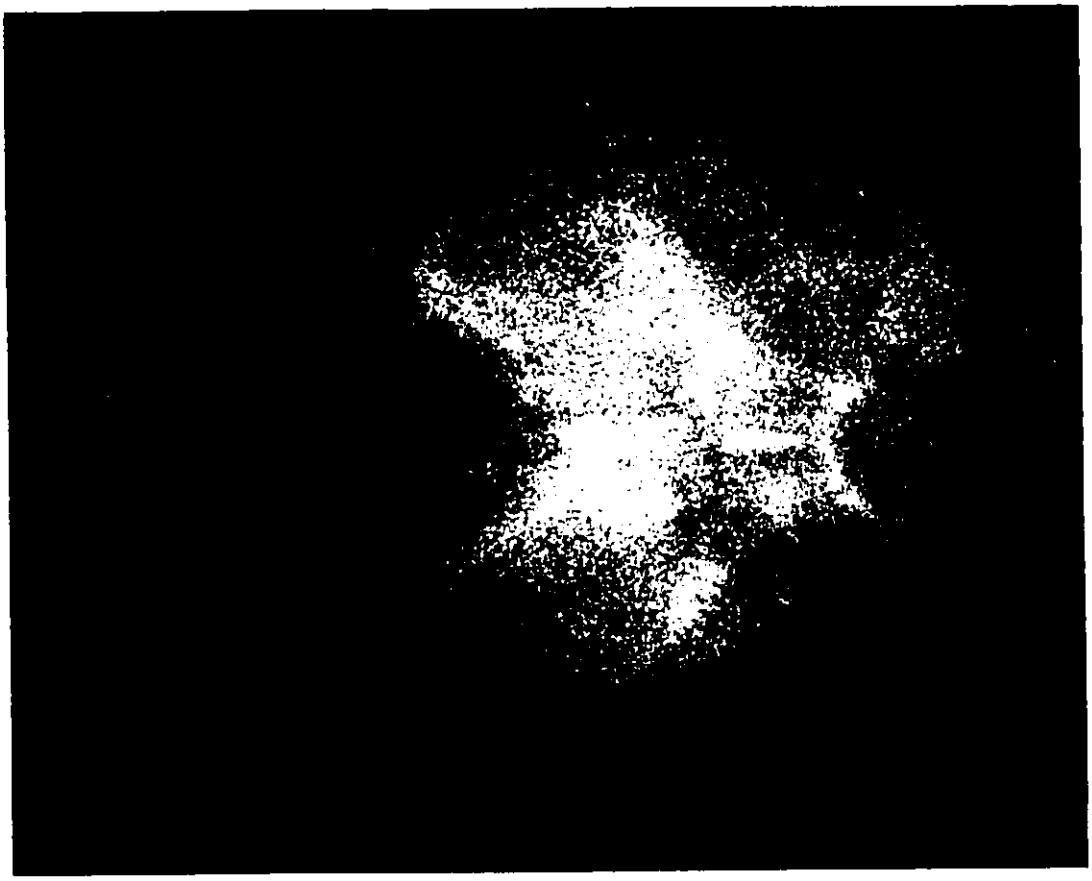


FIGURE 2-5. A-P view of a rat with an x-ray score of 2. Note that the spreading of thorium is not complete throughout the ventricular system.

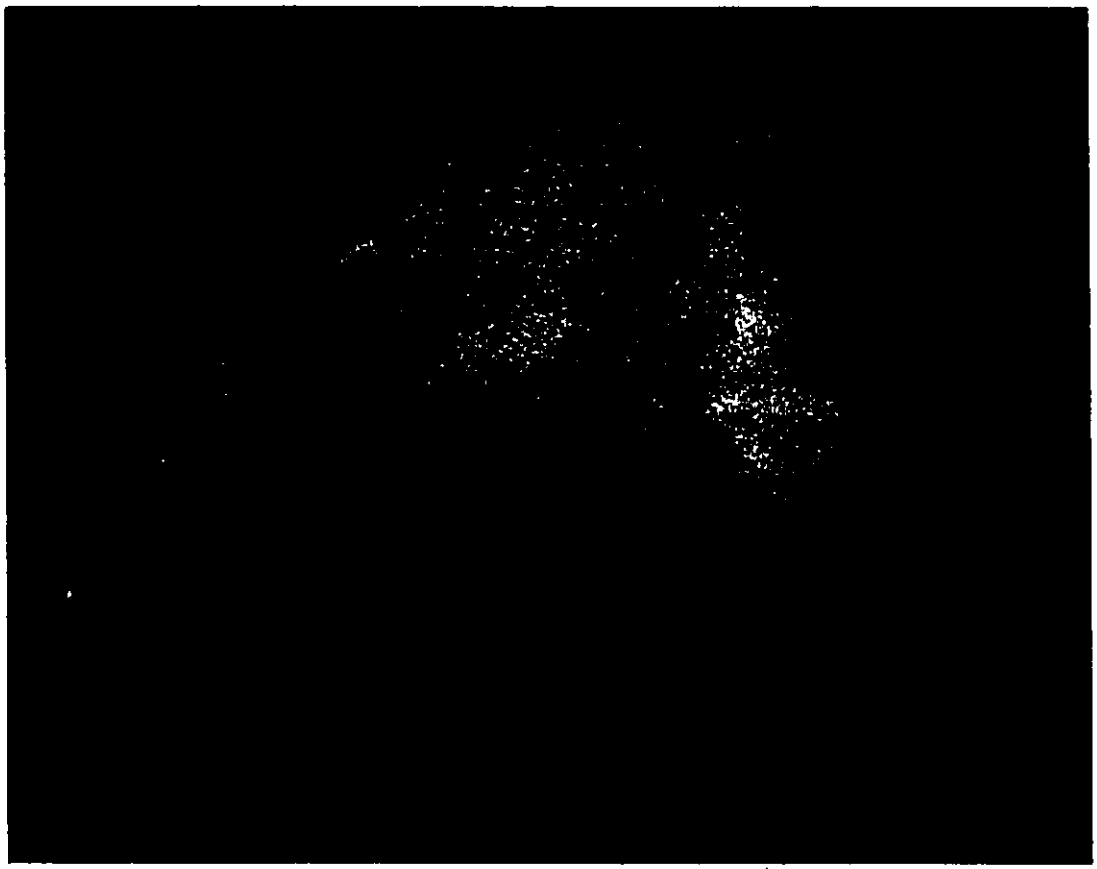
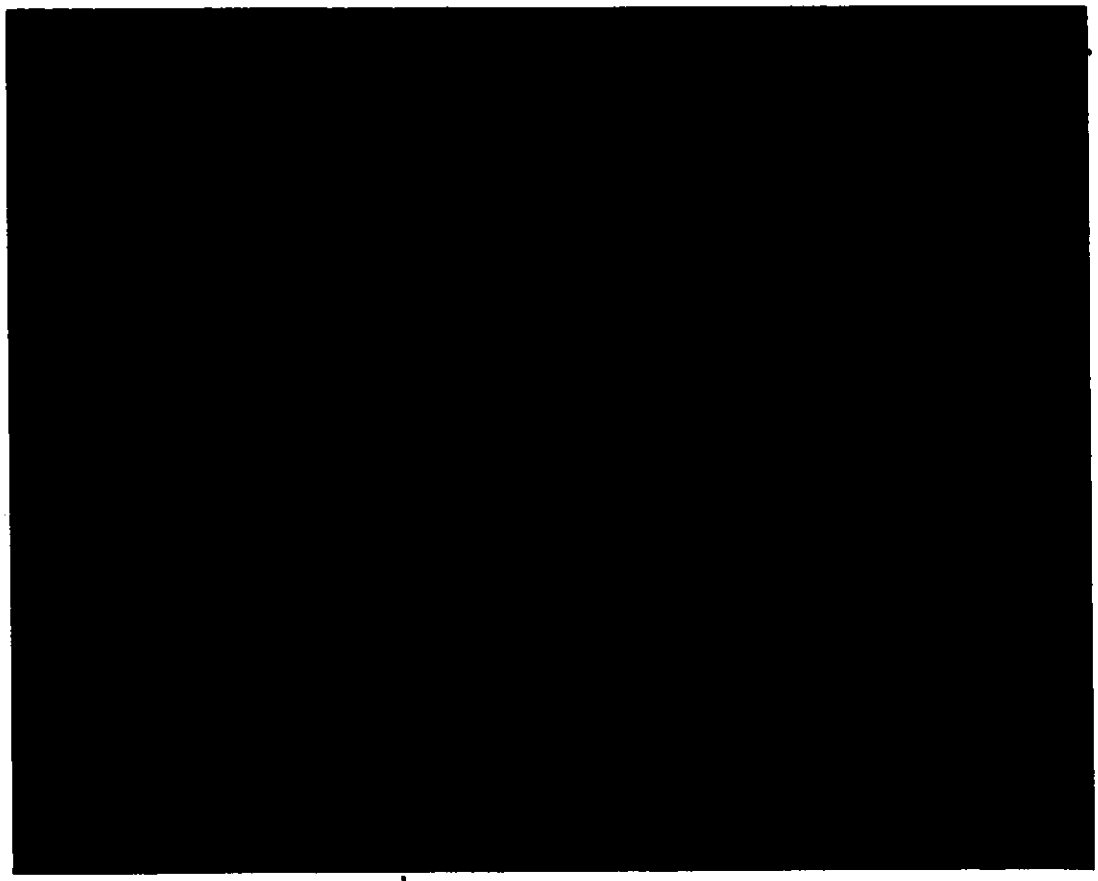


FIGURE-2-6. A-P view of an excised brain from a rat with an x-ray scose of 2. Note that the spreading of thorium is laterally biased.



APPENDIX 3.

SUMMARY.

The hypothesis that DOPAC and/or HVA bind to dopamine receptors and act as antagonists of dopamine was investigate *in vitro*. Neither DOPAC nor HVA bound to a purified extract of striatal dopamine receptors. DOPAC had nor effects of the secretion of prolactin by isolated hemipituitary glands or had any effect on the inhibitory effect of dopamine on prolactin secretion. These studies are evidence against DOPAC or HVA being dopamine receptor antagonists.

INTRODUCTION.

The observation that the concentrations of DOPAC and HVA were increased in the striatum and brain stem of exhausted animals raised the possibility that the apparent defficiency in post synaptic dopamine receptor stimulation may be due to the interferance of dopamine binding to its receptor by DOPAC and/or HVA. A literature search did not indicate whether DOPAC and HVA were dopamine receptor liganda (Seeman 1980). This Appendix reports experiments designed to obtain pharmacological and functional evidence to establish whether DOPAC or HVA binds to dopamine

receptors. Two approaches were used. First, DOPAC and HVA were added to a preparation of striatal dopamine receptors to see whether they interfered with the binding of spiroperidol (which binds specifically to dopamine receptors) in a radioreceptor binding assay (Varmuza and Mishra 1981). Second, DOPAC was added to preparations of prolactin-secreting anterior pituitary glands to see whether it could decrease the secretion of prolactin itself or interfere with the inhibition of prolactin secretion by dopamine itself.

METHODS.

Experiment 1. Do DOPAC and HVA bind to striatal dopamine receptors?

A preparation of striatal dopamine receptors was obtained by homogenization of striata in a buffer containing: 20 mM EGTA, 75 mM Tris maleate (pH 7.4) and stired at 0°C in the presence of: 48 mM Tris maleate (pH 7.4), 10 mM Magnesium sulphate, 11 mM Sodium cholate 38%, and 31% saturated ammonium sulphate. The crude extract of dopamine receptors was isolated by centrifugation at 150,000 to 200,000 Xg and further addition of saturated ammonium sulphate. The extract was resuspended in a buffer containing: 100 mM Tris maleate (pH 7.4) and 2 mM EGTA (20 to 40 mg protein/ml) and stored at -20°C.

For the assay, the extract was diluted to 1 to 3 mg protein/ml with a buffer contaning: 50 mN Tris, 10 µM pargylene, 0.01% ascorbic acid, 120 mM sodium chloride, 5 mM potassium chloride, 2 mM calcium chloride and 1 mM magnesium chloride and 0.25 ml placed into glass tubes containing 25 ul of 12 nM ^{3}H-Spiroperidol (NEN 24 Ci/mM) dissolved in 0.01% ascorbic acid. Then 25 µl of solutions containing dopamine, DOPAC, HVA or butaclamol were added dissolved in 0.01% ascorbic acid were added to make the final concentrations listed in Table 3-1. The tubes were then left at 21^{o}C for one hour for equilibration of the receptor-ligand complex.

After one hour the receptor-ligand complexes were isolated by filtration through Whatman GF/C glass fibre microfilters (2.4 cm in diameter) under suction by a 1225 Sample manifold (Millipore Bedford Mass.) and washed with 6 ml of buffer containing: 15 mM Tris HCl (pH 7.6), 5 mM EDTA, and 0.01% ascorbic acid. The ^{3}H content of the filters was then measured using liquid scintilation counting (Beckman LS-230 counter) in PCS liquid scintilation fluid (Amersham, Arlington Heights, Ill).

Non-specific binding (0%) was defined as the binding of ^{3}H-Spiroperidol to the extract in the presence of 1 uM butaclamol. Specific binding (100%) was defined as the binding of 12mM ^{3}H-spiroperidol in the absence of any

ligand minus the non-specific binding.

Experiment 2. Can DOPAC and HVA affect prolactin secretion from the anterior pituitary in vitro?

Anterior pituitary glands were obtained from rats immediately after decapitation, split in half and each sample placed into glass vials containing 3 ml of Medium 199. The vials were placed in a shaking water bath at 37°C. The medium was gassed with 95%O_2 and 5%CO_2.

In the first part of the experiment DOPAC was dissolved Medium 199 to give concentraions indicated in Table 3-2.,

In the second part of the experiment, varying concentrations of DOPAC or buffer alone were added to pituitary glands incubated with mM dopamine. This concentration of dopamine was chosen because it produced approximately 50% inhibition of prolactin secretion in this preparation. In both studies samples of buffer were taken after 45 minutes and 90 minutes for prolactin determinations.

Prolactin concentrations were measured by radioimmunoassay.

RESULTS.

Experiment 1.

The effects of DOPAC, HVA and dopamine on the binding of ^{3}H-spiroperidol are presented in Figure 3-1. Dopamine resulted in an inhibition of ^{3}H-spiroperidol binding with an IC 50 value of uM. Neither DOPAC nor HVA had any significant effect on the binding of H-spiroperidol, except in one study. In one experiment, DOPAC resulted in a significant inhibition of ^{3}H-spiroperidol binding having an IC50 value of 0.76 uM and a Hill coefficient of -0.688. This observation raised the possibility that DOPAC was indeed a dopamine receptor ligand although it is posssible that this observation was artifactual.

Experiment 2.

The effects of DOPAC on the secretion of prolactin from the anterior pituitary are presented in Figure 3-2. At no concentration did DOPAC inhibit the secretion of prolactin. Dopamine alone was effective in decreasing prolactin secretion bye approximately 50%. DOPAC did not have any significant effect on the inhibition of prolactin secretion by dopamine (Table 3-3).

DISCUSSION.

These experiments were desigened to test the hypothesis that DOPAC and/or HVA can bind to dopamine receptors or in some way alter the binding properties of the dopamine receptor. If one of these compounds was a dopamine recptor ligand there would have been a detectable change in the binding of spiroperidol to the preparation of striatal dopamine receptors. Except for one experiment where binding of DOPAC was detected, this did not appear to be the case. This assay has been used to identify with success compounds which are able to bind to dopamine receptors such as bromocriptine, haloperidol etc (Varmuza and Mishra 1981), although such binding does not predict whether the compound is an agonist or antagonist of dopamine. To identify a compound as a one or the other a functional study must be performed which can compare the effect of a receptor ligand on a paradigm of dopamine action.

The in vitro anterior pituitary preparation is one such system that can distinguish dopamine receptor ligands as either agonist or antagonist. Dopamine receptor agonists results in a decrease in the secretion of prolactin. DOPAC was tested in this preparation because of the one observation where binding appeared to occur. No effects of DOPAC on prolactin secretion or interaction with the action of dopamine itself were observed.

CONCLUSION.

Collectively these studies indicated that neither DOPAC nor HVA are dopamine receptor ligands.

TABLE 3-1. Dopamine, DOPAC and HVA binding to striatal dopamine receptors.

CONCENTRATION	% BINDING DOPAMINE	DOPAC	HVA
10^{-9}	101+1	90+2	98+9
10^{-8}	109+6	96+5	87+9
10^{-7}	99+2	97+9	82+9
10^{-6}	93+3	97+5	94+6
10^{-5}	80+8	99+6	99+4
10^{-4}	43+8	99+6	112+9
10^{-3}	15+3	90+5	97+8

All values are Mean+SEM of 4 to 6 experiments.
All samples in each experiment were done in triplicate.
Neither DOPAC or HVA showed any significant binding to dopamine receptors.

TABLE 3-2. Effects of DOPAC on prolactin secretion in vitro.

CONC OF DOPAC	0.0	10^{-4}	10^{-5}	10^{-6}	10^{-7}
TIME					
45	85+15	56+11	81+12	86+30	57+8
90	111+21	77+12	103+ 9	82+ 8	108+13

Values are prolactin concentrations in the incubation medium.
DOPAC was without effect on prolactin secretion.
Values are Mean+SEM.

TABLE 3-3. Effects of DOPAC on inhibition of prolactin secretion by dopamine in vitro.

CONC OF DA	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}
CONC OF DOPAC	0.0	10^{-4}	10^{-5}	10^{-6}	10^{-7}
TIME					
45	28+8	33+9	21+3	31+9	15+9
90	39+9	48+6	34+6	48+9	28+6

Values are mean+SEM of prolactin concentrations of the incubation medium.
DOPAC was without significant effect on the the inhibition of prolactin secretion by dopamine.

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