# A pharmacological study of Renshaw cell field potentials



by

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# LITERATURE REVIEW

## Cholinergic Transmission in the Spinal Cord: Renshaw Cell Pharmacology

In the peripheral nervous system, acetylcholine (ACh) is established as the transmitter released at the neuromuscular junction and at autonomic ganglia (for discussion, see Cooper, Bloom and Roth, 1978). Indications that spinal cholinergic neurotransmission exists came from early studies showing that intravenous or intra-arterial injection of nicotine or ACh (in the presence of anticholinesterases) depressed spinal reflexes (Schweitzer and Wright, 1937a,b; Bülbring and Burn, 1941). The exact mechanism of this depression was, however, obscure.

In 1946, Renshaw observed high frequency repetitive discharges of 30 - 50 msec duration in response to antidromic stimulation of the seventh lumbar ventral roots of cat and rabbit spinal cords. He postulated that these responses were due to the activation of interneurons within the spinal ventral horn, as described by Cajal (1909). In an earlier study, Renshaw (1941) demonstrated "antidromic inhibition" whereby antidromic impulses in motor axons inhibited motoneurons for a duration of 50 msec. Though this phenomenon was also investigated by Lloyd (1946, 1951), Eccles, Fatt and Koketsu (1954) established the relationship between Renshaw's postulated interneurons and the recurrent inhibitory post synaptic potentials (IPSPs) recorded intracellularly from motoneurons. This was done by recording from the interneurons, and from motoneurons, and demonstrating the parallel effects of varying antidromic stimulus parameters and pharmacological specificities. Shortening or prolongation of the Renshaw cell discharge by dihydro-beta-erythroidine (dHBE) or physostigmine respectively, produced

corresponding changes in the recurrent IPSPs.

The inhibitory interneurons were designated Renshaw cells by Eccles' group[1] who concluded that they are responsible for recurrent inhibition by showing them to be activated by antidromic impulses from ventral root stimulation which spread up to motor axon collaterals to terminals that synapse with Renshaw cells. In turn, the Renshaw cells synapse directly onto motoneurons. In addition to this monosynaptic activation of Renshaw cells, polysynaptic activation, via stimulation of dorsal root afferent fibers synapsing directly onto motoneurons of synergistic muscle groups (see Eccles, 1969), was shown to occur following excitation of appropriate dorsal roots (Renshaw, 1946; Eccles et al., 1954; Curtis et al., 1961), resulting again in recurrent inhibition of motoneurons.

The initial synchronous discharge of Renshaw cells generates a characteristic oscillatory field potential (Eccles et al., 1954). Recording from the seventh lumbar segment of the cat spinal cord, Eccles et al. (1954) noted that as a microelectrode moves ventrally through the cord, the polarity of these potentials reverses from dorso-lateral positivity to ventromedial negativity, with maximum negativity lying in the ventral horn where motor axons leave the gray matter, i.e. Rexed's (1952) lamina VII. Willis, Skinner and Wier (1969) confirmed the observations of Eccles et al. (1954)

[1] "...In addition to confirming almost all of Renshaw's findings, a detailed study of the interneuronal discharge has established that these interneurones [sic] form a specialized group mediating the inhibitory path from motor axons. They may appropriately be given the distinguishing title 'Renshaw cells' " (Eccles et al., 1954).

and determined the major sink area (i.e. where current enters the cell) for the Renshaw cell field was in the ventral region of lamina VII, while a source (where current leaves the cell) was found in the middle of lamina IX. Maximum negativity produced by antidromic action potentials of alpha-motoneurons centered in the ventral part of lamina IX. From this electrophysiological data, the Renshaw cell population is placed medial to the motor nucleus, with their axons projecting dorso-laterally into the motor nucleus.

The existence of Renshaw cells was however questioned by several workers (for reviews, see Willis, 1971 and Schiebel and Schiebel, 1971). Schiebel and Schiebel (1971) suggested that Renshaw cells were in fact the dendrites of motoneurons, supporting this hypothesis with their previous failure (Schiebel and Schiebel, 1966) to detect any short axoned interneurons in Golgi stained preparations of cat spinal cords. The suggestion that Renshaw cells are Golgi type II neurons (i.e. short axoned) derived from the fact that greatest recurrent effects tend to be between adjacent motoneurons (for refererences, see Willis, 1971). However, Jankowska and Smith (1973) demonstrated that it was possible to excite axons of single Renshaw cells at distances of more than 12mm away from their somas. These results supported those of Ryall, Piercey and Polosa (1971) who obtained indirect evidence for Renshaw cell axonal lengths of at least 5mm. Jankowska and Smith (1973) concluded that recurrent effects on motoneurons are mediated by short motor axon collaterals terminating on Renshaw cells with long axons.

Weight (1968) proposed that motor axon collateral terminals gave rise to the high frequency repetitive responses attributed to Renshaw cells, and

Erulkar et al. (1968) supported this hypothesis when the structures they observed following attempts to mark Renshaw cells intracellularly resembled synaptic boutons rather than neuronal cell bodies. However, the experiments of Erulkar et al. (1968) were criticized on the basis that it was unclear whether their electrodes remained intracellular during the passage of large currents required to eject dye (see Willis, 1971 for references).

Electrophysiological with morphological evidence for the existence of Renshaw cells was provided by Jankowska and Lindström (1971). These workers recorded intracellularly from Renshaw cells identified by their typical high frequency discharge to ventral root stimulation and subsequently stained these cells by ejection of procion yellow dye. The stained cells were shown to have a diameter of 10 - 15μM and were located at the ventro-medial border of the motor nuclei. More recently, Lagerbäck and Ronnevi (1982a,b) and Lagerbäck (1983) have provided an ultrastructural study of neurons (suggested to be Renshaw cells), lying ventro-medial to motor nuclei in lamina VII, which contact synaptic boutons of alpha motoneurons intracellularly labelled with horseradish peroxidase.

The cholinergic nature of transmission between motor axon collaterals and Renshaw cells was determined by Eccles et al. (1954). Dale (1934) postulated that identification of the chemical transmitter released by a neuron at peripheral sites may furnish a hint as to the transmitter released by the same neuron at central synapses. This postulate led Eccles' group to the hypothesis that because ACh is released at the peripheral nerve terminals of motoneurons (to activate muscle fibers), then it is also released from central motor axon collaterals (to activate Renshaw cells).

Acetylcholine, injected intra-arterially into the spinal cord, was shown to readily excite Renshaw cells and this effect was enhanced by prior intravenous injection of eserine, an anticholinesterase. In the absence of exogenous ACh, eserine and other anticholinesterases prolonged the duration of Renshaw cell discharges evoked by antidromic stimulation (at a rate of 1/3.5 seconds) of ventral roots, but suppressed the response to successive volleys in a repetitive series. Though these workers also noted that the field potentials of the Renshaw cells were similarly suppressed by 8 and 27Hz stimulation in the presence of the anticholinesterase tetraethylpyrophosphate (TEPP), Renshaw cell field potential pharmacology has largely been ignored in the literature. However, the pharmacology of Renshaw cell unit potentials (extracellular recordings made very close to the cell) has been extensively studied and is reviewed below.

Longo, Martin and Unna (1960), in agreement with Eccles et al. (1954), indicated that the nature of the acetylcholine receptor on the Renshaw cell was nicotinic since dHBE (a nicotinic antagonist) reduced Renshaw cell discharge duration. Further support for nicotinic activation came from Ueki, Koketsu and Domino (1961) who demonstrated that the nicotinic blocking drug mecamylamine reduced the terminal portions of the Renshaw cell discharge. Though atropine (a muscarinic antagonist) also depressed Renshaw cell response to antidromic volleys, the effect was weak even at high doses (Eccles et al., 1954). With the application of the microiontophoretic technique (Curtis and Eccles, 1958; Curtis, 1964), Curtis and Ryall (1966a,b) examined the effects of muscarinic (muscarine, acetyl-B-methylcholine) and nicotonic (nicotine, carbamylcholine) cholinomimetics, as well as ACh, on Renshaw cell discharges.

These investigations indicate both nicotinic and muscarinic receptor sites on the Renshaw cell. In addition, Curtis and Ryall (1966b) suggested a third type of receptor with which cholinomimetics may interact, in the presence of dHBE and atropine to produce depression and subsequent excitation of Renshaw cells. Atropine was also shown to reduce the non-specific excitatory effect of the amino acid dl-homocysteic acid on Renshaw cell discharge. However, this effect was probably due to non-specific depression of electrical excitability of the Renshaw cell membrane by atropine (Curtis and Phillis, 1960).

The probability that ACh is the transmitter substance acting between motor axon collaterals and the Renshaw cell is evidenced by the above investigations. However, not all criteria for the identification of a substance as a transmitter have been met. These criteria may be summarized as follows (see Shepherd, 1979):

(i) Anatomical: the substance is contained within presynaptic elements in appropriate amounts.

(ii) Biochemical: the presence of synthetic and degradative enzymes in the presynaptic neuron and synapse, respectively.

(iii) Physiological: physiological stimulation causes release of the substance and iontophoretic application of the substance mimics the physiological response.

(iv) Pharmacological: drugs affecting different enzymatic or biophysical steps have their expected effects on synthesis, storage, release, action, inactivation and reuptake of the substance.

Identification of a central transmitter substance poses more problems than identifying transmitters at peripheral sites such as the skeletal neuromuscular junction, which are more readily accessible. However, in an attempt to provide further evidence that ACh is indeed the transmitter substance exciting Renshaw cells, Kuno and Rudomin (1966) measured the ACh content of the effluent from the perfused lumbosacral cord of the cat. Antidromic stimulation of deafferented peripheral motoneurons significantly increased the amount of ACh released into the effluent. In addition, ACh release was enhanced following a short tetanic stimulation and was suggested to follow the post tetanic time course of the Renshaw cell discharge. Thus, the authors suggested that the ACh collected in the effluent was released from motor axon collateral terminals. They further supported this suggestion with the finding that the response of Renshaw cells to excitation of motor axon collaterals was depressed at stimulus frequencies of 20-100Hz at the same rate as an accompanied reduction in the amount of ACh released into the effluent.

Further biochemical evidence was shown by Gwyn, Wolstencroft and Silver (1972) who estimated choline acetyltransferase activity in selected regions of the cat spinal cord. The activity of this enzyme, which catalyzes the synthesis of ACh from choline and acetate (see Cooper et al., 1978), was expressed as μg ACh produced (as assayed on frog rectus abdominis or leech dorsal muscle) by each gram of tissue per hour of incubation in a choline rich medium. High activity was found in the region of the motor nuclei, indicating that motoneurons have the synthetic enzyme required for the synthesis of ACh in the central nervous system.

Applied microiontophoretically, hemicholinium (HC-3), which interferes

with the high affinity transport of choline into cholinergic nerve terminals (Guyenet et al., 1973; Yamamura and Snyder, 1973), produces a delay of the first spike of Renshaw cell discharges and a reduction in number, or total blocking of, spikes following ventral root stimulation (Quastel and Curtis, 1965). These results again imply that ACh is presynaptically released to activate Renshaw cells.

Following ACh release, Curtis and Ryall (1966c) outlined the postsynaptic events to consist of: an early firing (latency 0.5 - 0.7 msec, duration up to 50 msec) as a result of ACh combining with nicotinic receptors, since it could be depressed by dHBE and mecamylamine; a pause, lasting several hundred milliseconds, which followed the early discharge; and finally, a weak and delayed excitation following the pause. This delayed excitation could be suppressed by intravenous or microiontophoretic application of atropine (Curtis and Ryall, 1966c; King and Ryall, 1981), and was attributed to ACh combining with muscarinic receptors.

Curtis and Ryall (1966c) considered the late muscarinic discharge to be unphysiological, but Haas and Ryall (1975) determined that more physiological activation of peripheral receptors by stretching of the Achilles tendon produced a Renshaw cell discharge that could be reduced or abolished by atropine but not mecamylamine. King and Ryall (1981) supported these observations and further suggested that the nicotinic receptors of feline Renshaw cells are non-selective in their activation by agonists and antagonists and are unlike the nicotinic receptors found at peripheral sites. In addition, α-bungarotoxin (which binds to neuromuscular nicotinic receptors, see Taylor, 1980a), fails to block both the action of microiontophoretically applied ACh on cat Renshaw cells, and synaptic activation

by ventral root stimulation (Duggan, Hall and Lee, 1976), lending support to this hypothesis. Headley et al.(1975) also found that in the rat spinal cord, Renshaw cell responses to synaptic activation following ventral root stimulation and to iontophoretically applied ACh could be entirely blocked by both atropine and dHBE. Furthermore, dHBE could also block the action of acetyl-B-methylcholine. These results led the authors to suggest that the ACh receptors on rat Renshaw cells cannot be categorized into traditional muscarinic or nicotinic receptors. However, while Headley et al. (1975) failed to observe the late muscarinic response (Curtis and Ryall, 1966c) in their rats, this phenomenon was observed by Biscoe, Duggan and Lodge (1973). It was suggested (King and Ryall, 1981) that the muscarinic receptors in rats are less readily activated than those in cats.

In addition to activation by excitation of motor axon collaterals, Renshaw cells are subject to inhibition. Ryall (1970) and Ryall et al. (1971) demonstrated that antidromic volleys in deafferented motor nerve fibers sometimes failed to cause excitation but instead led to inhibition of Renshaw cell discharge. The latency of this inhibition (mean 2.2msec) was consistent with that of a disynaptic pathway, and the similarity in time course of the inhibition and that of the early discharge of Renshaw cells, together with the fact that dHBE blocked the response, led the authors to suggest that other Renshaw cells were responsible for this inhibition.

This 'mutual inhibition', along with the Renshaw cell's ability to inhibit group Ia excited interneurons (Hultborn, Jankowska and Lindström, 1968a; Ryall and Piercey, 1971), which mediate reciprocal Ia inhibition of motoneurons (Hultborn et al., 1968a,b,c), offers an explanation of the re-

current facilitation of motoneurons observed by Wilson, Talbot and Diecke (1960) and Wilson and Burgess (1962). The mutual inhibition has also been postulated (Ryall, 1970) as being responsible for the pause following the initial early firing of Renshaw cells. In addition to mutual inhibition, supraspinal inhibition of Renshaw cells by excitation of the contralateral reticular formation (Haase and van der Meulen, 1961) or by excitation of the capsula interna (Koehler et al., 1978) has been demonstrated.

From the discussion so far, it is apparent that there are three sites available for study of the pharmacology of inhibition by Renshaw cells: motoneurons (recurrent inhibition); group Ia excited inhibitory interneurons (which mediate reciprocal inhibition); and Renshaw cells (mutual inhibition). Recurrent inhibition of motoneurons is blocked by intravenous or microiontophoretic administration of strychnine (Eccles et al. 1954; Curtis, 1962; Curtis et al., 1976) an antagonist of the putative inhibitory transmitter glycine (Curtis, Duggan and Johnston, 1971). While the inhibition of group Ia excited inhibitory interneurons is also blocked by strychnine (Belcher, Davies and Ryall, 1976; Lodge, Curtis and Brand, 1977), mutual inhibition was found to be resistant to microiontophoretic or intravenous administration of this drug (Ryall, Piercey and Polosa, 1972), unless given in high concentrations which result in non-specific blocking of ACh and $\delta$-amino butyric acid (GABA) as well as glycine (Davidoff, Aprison and Werman, 1969; Curtis et al., 1971; Ryall et al., 1972).

The strychnine resistant mutual inhibition led Ryall et al. (1972) to conclude that either glycine receptors of Renshaw cells are resistant to strychnine (and therefore unlike those proposed to exist on motoneurons and group Ia excited inhibitory interneurons), or that glycine is not the

transmitter mediating mutual inhibition. To test this latter hypothesis, Piercey, Goldfarb and Ryall (1973) investigated the effects of picrotoxin and bicuculline (GABA antagonists) on mutual inhibition, choosing these substances because Kellerth (1968) observed that recurrent IPSPs of motoneurons which were occasionally insensitive to strychnine could be depressed by picrotoxin. Piercey et al. (1973) found that while bicuculline had no effect, picrotoxin depressed mutual inhibition of Renshaw cells. However, this effect was accompanied by reduction in excitation of Renshaw cells by motor axon collaterals, and it was postulated that picrotoxin interferes with the cholinergic transmission between motor axon collaterals rather than transmission between Renshaw cells. These workers therefore concluded that Renshaw cells do not release GABA to mediate mutual inhibition.

In a more recent study however, Cullheim and Kellerth (1981) recorded intracellularly from motoneurons excited by antidromic shock of various muscle nerves and found that strychnine did not completely abolish the recurrent IPSPs, but the strychnine resistant component was sensitive to picrotoxin and bicuculline. Similarly, a picrotoxin/bicuculline resistant component was blocked by strychnine, and combined administration of strychnine and picrotoxin/bicuculline virtually abolished the recurrent inhibition. The authors suggested that two populations of Renshaw cells exist, each mediating recurrent inhibition by release of GABA or glycine.

The nature of the Renshaw cell receptors which mediate mutual inhibition, therefore, remains unclear, and the pharmacology of Renshaw cell inhibition and excitation may be further complicated by the involvement of substance P (Belcher and Ryall, 1977; Krnjevic and Lekic, 1977; Ryall and

Belcher, 1977). Applied microiontophoretically, substance P predominantly depresses Renshaw cell excitation by antidromic ventral root volleys. While Substance P was shown to inhibit excitation evoked by microiontophoretically applied ACh, it did not inhibit excitatory responses to dl-homocysteic acid or to acetyl-B-methylcholine. Furthermore, substance P caused excitation in a few cells which was blocked by dHBE. This evidence led the authors to suggest that substance P, unlike GABA and glycine, does not alter postsynaptic membrane conductance directly, but exerts its effect by selectively preventing excitation of nicotinic receptors by ACh. Inhibition of Renshaw cell firing by substance P was thus attributed to interaction with an allosteric site on the nicotinic receptor, which either increases desensitization to ACh or selectively blocks a conductance mechanism not shared by other excitants. On the other hand, excitation of Renshaw cells by substance P was attributed to a presynaptic mechanism whereby substance P enhances ACh release, as occurs in the cerebral cortex (Pepeu, 1974).

Related to this phenomenon, Duggan and Curtis (1972) and Davies (1976) found that morphine excited Renshaw cells of cat and rats, respectively. As well as being blocked by the specific opiate antagonist naloxone (Davies, 1976) morphine excitation of Renshaw cells was antagonized by dHBE and substance P (Belcher and Ryall, 1977) and was therefore attributed to involve nicotinic receptors. This may be a presynaptic effect, however, since morphine has been shown to increase ACh release from the frog spinal cord (Nistri, 1975, 1976).

While Curtis et al. (1961) failed to detect any change in Renshaw cell activity following microiontophoretic application of norepinephrine or

epinephrine, other workers have demonstrated norepinephrine induced depression of Renshaw cell firing (Biscoe and Curtis, 1966; Weight and Salmoiraghi, 1966), though excitation was also occasionally observed (Weight and Salmoiraghi, 1966). The mechanism of this depression or occasional excitation is, however, unclear and requires further investigation before conclusions can be made regarding adrenergic involvement in Renshaw cell activity.

In summary, cholinergic transmission within the spinal cord has been discussed with reference to the Renshaw cell. Pharmacological studies on Renshaw cells show them to possess at least two types of ACh receptors which may differ from peripheral muscarinic and nicotinic receptors. In addition, Renshaw cells are sensitive to other putative neurotransmitters, including, GABA, glycine and substance P.

## Cholinergic Transmission and Anticholinesterase Drugs

The enzyme acetylcholinesterase (AChE) is widely distributed in the central and peripheral nervous systems, as well as in muscular tissue (for review, see Koelle, 1963a). It plays an important role in cholinergic transmission by hydrolyzing ACh and terminating its action at post junctional sites. Inhibition of AChE, therefore, leads to persistance of ACh resulting in excessive cholinergic muscarinic and nicotinic stimulation. The accepted mechanism by which carbamate and organophosphate (OP) compounds exert their toxic effects is through binding to a serine hydroxyl group at the esteratic site of AChE (for review, see Taylor, 1980a). Though such anticholinesterases have been known and used for centuries (for historical

review, see Karczmar, 1970a) and their biochemistry, pharmacology and toxicology the subject of many extensive reviews (see Holmstedt, 1959; Koelle, 1963b; Karczmar, 1970b; Taylor, 1980a), they are still of interest in the study of cholinergic pharmacology. This review will briefly describe the anticholinesterase activity of such compounds and examine effects independent of their anticholinesterase activity.

Physostigmine (eserine) derived from the calabar ("ordeal") bean (see Karczmar, 1970a), may be considered as the prototype carbamate anticholinesterase. The carbamylated enzyme, unlike the acetylated enzyme, undergoes hydrolysis slowly, the half life for regeneration of the free enzyme being in the order of one hour in contrast to microseconds for the acetylated enzyme (see Taylor, 1980a). On the other hand, the more potent OPs (e.g. paraoxon, di-isopropylfluorophosphate, soman, sarin) phosphorylate the enzyme and regeneration may take days or may not occur at all if the enzyme "ages"[1]. Thus, the carbamates are considered "reversible" and the OPs "irreversible" inhibitors of AChE.

The symptoms of OP poisoning are predicted from a knowledge of the actions of ACh at its various effector sites. The severity of symptoms depends on the drug, dose and route of administration, but may include: miosis, diarrhea, vomiting, salivation, urination, ataxia, tremors, paralysis and death from respiratory center depression. Effective treatment of OP poisoning requires administration of atropine (to block muscarinic exci-

[1]The phosphorylated enzyme loses one alkyl group as a carbonium ion, enhancing the stability of the enzyme complex which then defies dephosphorylation (see Taylor, 1980a).

tation) and an oxime reactivator, such as pyridine-2-aldoxime methiodide (2-PAM) which displaces the phosphyl residue from the active site to restore enzyme activity (see Usdin, 1970; Wills, 1970). However, once the enzyme has aged it cannot be reactivated. This and several other problems regarding oxime therapy have recently been reviewed by Ellin (1982) and are summarized below .

Unlike the OPs, 2-PAM is a highly charged molecule which does not cross the blood brain barrier. It, therefore, exhibits a degree of selectivity for activity at peripheral sites. Even though non-quaternary compounds have been developed (e.g. Pro-PAM, a tertiary compound which crosses the blood brain barrier and converts to 2-PAM), their overall efficacy does not exceed that of 2-PAM. The reason for this is obscure. In addition, the oximes may react with OP compounds to form phosphorylated derivatives which are more potent than are the original OPs. Thus, the protective effect of oxime therapy is limited (see Ellin, 1982 for references).

Several other approaches to protection against poisoning by OP compounds have been taken. Koster (1946) and Koelle (1946) used physostigmine to prevent poisoning by di-isopropylfluorophosphate (DFP). Since both compounds inhibit AChE, an additive effect would be expected. Indeed, this is so when the carbamate is administered after the OP (Koster, 1946), but not when the carbamate is administered prior to the OP. Harris, Stitcher and Heyl (1980) also suggested that non-quaternary carbamates, such as physostigmine, were superior to quaternary carbamates (neostigmine) in offering protection against OPs, presumably due to the former carbamate's ability to cross the blood brain barrier. In addition, a pretreatment consisting of physostigmine, atropine and mecamylamine was shown by Harris et

al. (1980) to offer complete protection in rats given several times the $LD_{50}$ dose of soman.

Green (1983) recently proposed that kinetic factors alone could account for the protective action of carbamates against OP poisoning. In this theory, Green (1983) suggests that carbamylation provides a pool of enzyme protected from OPs, and decarbamylation furnishes enough enzyme to maintain normal cholinergic transmission, until the OP is metabolized or excreted. Thus, conservation of normal cholinergic transmission is dependent upon the "minimum essential cholinesterase" (MEC) activity required for survival, and the "enzyme conservation index" (ECI) - the ratio of enzyme decarbamylation to enzyme phosphorylation. Thus, low MEC and high ECI values offer most protection. However, while carbamate prophylaxis may be desirable in circumstances where the potential for OP poisoning is great, it is clearly of no value when poisoning has already occurred, or when the toxicity of the carbamate itself is a greater risk than than OP exposure.

In response to the latter objection to carbamate prophylaxis, Ashani et al. (1983) developed a relatively non-toxic (i.e. 170 times less toxic than analogous carbamates) OP anticholinesterase, O-[3-(trimethylammonio)-phenyl]-1,3,2-dioxaphosphorinane-2-oxide iodide (TDPI). TDPI pretreatment conferred protection to mice receiving several times the $LD_{50}$ dose of paraoxon or soman. In addition, Wolthius, Benschop and Berends (1981) studied an OP structurally similar to soman, but devoid of anticholinesterase activity. The soman-simulator (som-sim) enhanced survival of soman poisoned rats ($6 \times LD_{50}$) when administered prophylactically, but neither som-sim nor TDPI are of therapeutic use subsequent to OP exposure.

In another approach to treatment of OP poisoning, Harris, Stitcher and

Heyl (1982) preadministered HC-3 (an inhibitor of ACh synthesis) to soman intoxicated rabbits. The protective ratio (i.e. "protected" $LD_{50}$ soman/ "unprotected" $LD_{50}$ soman) was increased by intracerebroventricular administration of HC-3. Grieve and VanMeter (1983) have also shown that HC-3 antagonizes depression of tetanic contractions of rat isolated phrenic nerve-diaphragm preparations following DFP in vitro or soman in vivo. HC-3 treatment may therefore be effective subsequent to OP exposure, and use of a tertiary hemicholinium may also increase survival.

Sodium phenobarbital pretreatment also increases soman $LD_{50}$ values in mice (Clement, 1983). While there was no increase in soman metabolism to account for this effect, liver and serum aliesterase levels were both elevated following phenobarbital treatment. Clement (1983) therefore suggested that phenobarbital induces these enzymes which provide non-specific binding and detoxifying sites for soman.

Tolerance to repeated sub-lethal injections of OPs to a total exposure of several times the $LD_{50}$ has been demonstrated in rats (with soman, Sterri, Lyngaas and Fonnum, 1980) and cats (with DFP, Sikora-VanMeter et al., 1983). The mechanism of this tolerance is not fully understood, but spontaneous reactivation of the cholinesterase enzyme is not a contributing factor since tolerance occurs in the presence of the completely irreversible inhibitor, soman (Coult, Marsh and Reed, 1966). Sterri et al. (1980) suggested the existence of a soman depot, and tolerance linked to storage of the compound in adipose tissue, or binding to liver or plasma proteins, which would concur with the results of Clement (1983) described above.

Jović (1974) hypothesized that "adaptation of synapses" to the abnormal situation (i.e. inhibited AChE) was responsible for the recovery of

animals from the toxic effects of soman. Russel et al. (1981) investigated the possibility of end product (ACh) inhibition of ACh synthesis. ACh levels in DFP treated rats were elevated and remained elevated during the period when tolerance developed. In addition, the rate of synthesis of $[^2H_4]$ ACh from $[^2H_4]$ choline administered prior to death (by microwave irradiation) was not changed after DFP treatment. These results, therefore, indicate that end product inhibition of ACh synthesis is not responsible for tolerance.

Grazit, Silman and Dudai (1979) found that the OP Tetram caused a reduction in binding of $[^3H]$ quinuclindinyl benzilate ($[^3H]$QNB, a specific muscarinic antagonist) to various regions of the rat brain. Thus, it was suggested that the OP causes a decrease in muscarinic receptor levels as a mechanism for tolerance. Dawson and Jarrott (1981) investigated the effects of chronic administration of physostigmine, pyridostigmine (a quaternary carbamate) and DFP on $[^3H]$QNB binding in guinea pig brain. These workers found no changes in the concentration of $[^3H]$QNB-bound receptors following each drug treatment. Though drug and species differences (Andersen et al, 1977) may explain these conflicting results, it is apparent that more investigation of OP induced changes in receptor density is required before this approach to the mechanism of tolerance is resolved.

Organophosphate compounds also produce effects unrelated to their anticholinesterase action. Jović et al. (1971) showed that, in vivo, lethal doses of soman and DFP depressed rat cerebral cortex succinate dehydrogenase and aldolase activities. This effect was considered to be relatively specific since hexokinase activity was unaffected. Changes in carbohydrate metabolism may account for the increase in glycogen granules observed in the

motoneurons of DFP treated cats (Sikora-VanMeter et al. 1983).

Apart from metabolic effects, AChE inhibitors have direct effects at cholinergic receptors. Kuba and Albuquerque (1973) demonstrated that in the presence of DFP inhibited cholinesterase, end plate currents were shortened and decreased in amplitude. When excess DFP was washed out of the preparation (but enzyme activity not restored), end plate current amplitude returned to 90% of control. Almost full recovery occurred after 2-PAM exposure to restore enzyme activity. The authors suggested that DFP reversibly interacts with the ACh receptor, modifying ionic conductance through conformational change of the "ionic conductance modulator", the molecular entities involved in ionic conduction regulation. VanMeter, Karczmar and Fiscus (1978) also demonstrated that repeated administration of DFP induced EEG siezures in rabbits even though no decrease in brain cholinesterase activity was observed following the initial DFP administration. These results indicate a direct and independent (of AChE inhibition or excess ACh) effect of DFP within the central nervous system.

More recently, Fossier, Baux and Tauc (1983) found that in the presence of various OPs and the carbamate prostigmine, post synaptic responses in a cholinergic synapse of Aplysia to presynaptic excitation or microiontophoretically applied ACh or carbachol (a nicotinic agonist resistant to hydrolysis by AChE) were increased. These authors suggest that the AChE inhibitors act directly on the ACh receptor, either by a reversible curare-like effect, or by causing a change in receptor affinity for agonist. Alternatively, they suggest that AChE and the ACh receptor are related molecularly and the state of AChE activity modulates receptor sensitivity to ACh. However, this does not explain why direct effects are still

observed after maximum enzyme inhibition, as reported by VanMeter et al. (1978).

In addition to effects on metabolism and receptor sites, some OPs also produce delayed neurotoxicity. This is a persistent effect characterized by degeneration of axons and myelin of long fibers and large diameter nerves. Clinical signs (flaccid paresis developing distally in legs) begin 6 - 14 days after exposure to a single dose of OP (for review, see Abou-Donia, 1981), though morphological evidence of neurotoxicity has been observed as early as 5 days after chronic treatment with DFP (Sikora-VanMeter et al., 1983). The effect is independent of AChE inhibition, since not all OPs (e.g. parathion; Soliman, Farmer and Curley, 1982) produce delayed neurotoxicity. However, the initial event in OP induced delayed neurotoxicity is believed to be phosphorylation and aging of a protein within the central nervous system (see Abou-Donia, 1981). The most likely candidate for this protein is neurotoxic esterase (Johnson, 1969).

Anticholinesterase agents are therefore of interest in the study of cholinergic transmission, not only with reference to prevention of ACh hydrolysis, but also to the direct and cholinesterase independent effects described above. Since problems do exist with therapy following OP poisoning (see Ellin, 1982), models for testing of putative antidotes are useful. Such a system is the already described cholinergic synapse between motoneurons and Renshaw cells which will be utilized in the present investigation of the effects of anticholinesterase drugs within the central nervous system.

## ABSTRACT

The cholinergic pharmacology of Renshaw cell field potentials (RFP), evoked by antidromic stimulation of spinal L7 ventral roots of DIAL anesthetized cats, was investigated using reversible (physostigmine) and irreversible (di-isopropylfluorophosphate, soman) cholinesterase inhibitors in addition to antinicotinic (mecamylamine) and antimuscarinic (atropine sulfate) drugs. Responses were observed during control stimulation (2Hz, 2x threshold) and in the presence of excess ACh generated by repetitive (20Hz) stimulation for 60 seconds.

RFP were depressed during 20Hz stimulation and RFP frequency, number and duration were reduced up to three minutes after 20Hz stimulation. Physostigmine and di-isopropylfluorophosphate (DFP) facilitated these responses by dose-dependently decreasing RFP frequency, number and duration after 20Hz stimulation. A time-dependent recovery of RFP from DFP facilitation of 20Hz stimulation was observed. The recovery of RFP was not accompanied by a concomitant recovery of cholinesterase activity of blood, spinal cord and caudate nucleus which remained inhibited by 79 - 100%. Soman, which reduced cholinesterase activity by 36 - 50% did not facilitate RFP response to 20Hz stimulation.

Mecamylamine reduced RFP number and duration during 2Hz stimulation and antagonized the percent decrease in RFP number and duration after 20Hz stimulation. Atropine sulfate also decreased RFP number and duration during 2Hz stimulation. The first two potentials of the RFP were unaffected by drug treatment.

Amphetamine, given to assess cholinergic and adrenergic interaction in

this system, fails to enhance physostigmine facilitation of 20Hz stimulation. However, in the absence of physostigmine, this drug facilitated the recovery of RFP from 20Hz stimulation.

The data suggest that RFP adapt to reduced cholinesterase activity and show cholinergic nicotinic and muscarinic sensitivity. In addition, a non-cholinergic component of RFP is indicated. The results are discussed with reference to Renshaw cell unit potential activity.

# INTRODUCTION

The electrophysiology and cholinergic pharmacology of single Renshaw cell units have been extensively studied as an example of chemical transmission in the mammalian spinal cord (c.f. Literature Review). A measure of the activity of a population of Renshaw cells may be obtained from the amplitude of the monosynaptic reflex volley recorded fron ventral motoneurons in response to orthodromic stimulation of dorsal spinal roots, since the number of discharges in a single Renshaw cell burst is directly proportional to this amplitude (Ross, Cleveland and Haas, 1972). Alternatively, the activity of a population of Renshaw cells may be studied from their field potentials which represent activity of Renshaw cell unit potentials (Eccles et al., 1954).

Renshaw cell field potentials (RFP), though readily recorded from cat spinal cords (Eccles et al., 1954), have received little attention in the literature. The effects of single Renshaw cells on recurrent inhibition is weak and insignificant compared to the recurrent IPSPs generated by a population of cells (Van Keulen, 1981). Therefore, a study of RFP, which provide a means of looking at the averaged summed responses of a defined population of Renshaw cells, is worthwhile. In addition, Renshaw cell unit activity can rarely be recorded for periods beyond one hour (Ueki, Koketsu and Domino, 1961). RFP on the other hand, are stable for hours and, therefore, time-dependency of drug action may be monitored.

This thesis provides a study of RFP pharmacology using cholinergic muscarinic and nicotinic receptor blocking drugs (atropine and mecamylamine, respectively). In addition, particular attention is paid to RFP responses

in the presence of anticholinesterase compounds (physostigmine, DFP and soman) which modify cholinergic transmission by preventing ACh hydrolysis in addition to direct effects on metabolism and at cholinergic receptor sites (c.f. Literature Review). Adrenergic and cholinergic interaction observed at other central sites (VanMeter and Karczmar, 1971; VanMeter, 1977) are also evaluated in this system.

Some of the results have been previously reported (Willetts and VanMeter, 1982).

# METHODS AND MATERIALS

Adult male or spayed female mongrel cats weighing 2.0 - 3.0kg were conditioned for three weeks prior to experiments. Animals were taken off food 24 hours prior to experiments, but allowed water ad libitum. Cats were anesthetized with 80mgkg$^{-1}$ DIAL compound (diallylbarbituric acid and urethane), supplemented with sodium pentabarbital (15 - 25mg i.v.) if required. Penicillin G (120,000 units i.m.) was routinely administered since experiments exceeded 12 hours in duration.

Blood pressure was monitored via a carotid cannula and supported above 60 torr by i.v. infusion of 5% dextrose in 50% lactated Ringer solution. Following cannulation of the left external jugular vein, a tracheostomy was performed. The spinal cord was exposed by laminectomy extending from sacral segment 1 (S1) to lumbar segment 1 (L1), the dura opened, and the cord transected at T13/L1 following local administration of 0.2cm$^3$ 2% lidocaine. Ipsilateral dorsal and ventral roots of spinal segments S1, L7 and L6 were ligated and transected at their entry through the dura. After surgical preparation and recovery (1 - 2 hours), the cats were placed in a David Kopf stereotaxic unit and the cord covered with a pool of light mineral oil retained by skin flaps. Body temperature was maintained above 35$^{o}$C by heating pads. Expired carbon dioxide was monitored throughout by a Beckman LB2 Medical gas analyzer and recorded with blood pressure on a Grass polygraph.

Renshaw cell field potentials, evoked by bipolar stimulation of L7 ventral root (2Hz, 2x threshold) were recorded from L7 ventral horn by conventional means via 2.7M NaCl-filled glass micropipettes with tip diameters

1.5 - 2.0μM, resistance 2.0 - 4.0Mohm. See Appendix A for detailed description of stimulating and recording equipment.

The effects of excess ACh produced by the failure of postsynaptic hydrolysis of this compound in the presence of inhibited cholinesterase was mimicked by repetitive stimulation (20Hz) of L7 ventral roots to increase presynaptic release of ACh. At this stimulation frequency, Renshaw cell discharge frequency is proportional to motoneuron discharge rate (Ross, Cleveland and Haase, 1976). Following control responses to 2Hz and 20Hz stimulation, atropine methyl nitrate ($0.3mgkg^{-1}$ i.v.) was administered to all cats to protect against untoward peripheral effects of anticholinesterases. Dose-response relationships to selected drugs during 2Hz and 20Hz stimulation were then investigated.

Physostigmine sulfate ($10 - 100\mu gkg^{-1}$ i.v.) was administered alone and in combination with amphetamine sulfate ($0.5 - 8.5mgkg^{-1}$ i.v.) to investigate effects of a reversible carbamate anticholinesterase and its possible potentiation by amphetamine. DFP ($0.1 - 2.0mgkg^{-1}$ i.v.) and soman ($1.0 - 10.0\mu gkg^{-1}$) were used to investigate irreversible organophosphate effects on the RFP. Atropine sulfate ($0.2 - 2.0mgkg^{-1}$) and mecamylamine hydrochloride ($0.2 - 2.0mgkg^{-1}$) were administered to study antimuscarinic and antinicotinic activity on the RFP, respectively.

Blood and/or spinal cord and caudate nucleus samples were taken from control and anticholinesterase-treated animals, with or without electrophysiological experiments for determination of cholinesterase activity by the pH stat method described by Augustinsson (1971). See Appendix B for details.

Cats were terminated by magnesium sulfate or sodium pentabarbital

overdose.

For the analysis of results, the frequency, number and duration of the field potentials were measured during 2Hz stimulation before and after 20Hz stimulation in the presence and absence of the selected drugs. These parameters, and the percent change in these parameters before and after 20Hz stimulation, for control and drug-induced responses were compared using student's t-test, and a probability level of 0.01 was chosen as significant. While no attempt was made to measure the mean amplitude of RFP, it has been noted in the results section if this parameter changed in response to drugs or to 20Hz stimulation. Where such a change has been noted, the response was observed in at least three different preparations.

## RESULTS

RFP were identified on the dorso-lateral surface of L7 spinal segment by their characteristic oscillatory response to antidromic ventral root stimulation. Phase-reversal of the RFP was observed as the microelectrode was advanced into the spinal cord (see Eccles et al., 1954, their Fig. 9).

RFP were antagonized during 20Hz stimulation (STM) and frequency, duration and number of potentials were reduced following 20Hz stimulation (postSTM, Table 1). RFP amplitude was also decreased postSTM (see "Control" responses, Figs. 1 and 2). Atropine methyl nitrate ($0.3 mg kg^{-1}$, i.v.) did not change any characteristics of the RFP before (preSTM) or after 20Hz stimulation (Table 1).

Table 1: RFP frequency, duration and number in the presence and absence of atropine methyl nitrate prior to and post 20Hz stimulation

| | | CONTROL (mean ± sem) | Atropine Methyl Nitrate (mean ± sem) |
|---|---|---|---|
| Frequency (Hz) | PreSTM | 821 ± 23.9 | 846 ± 40.8 |
| | PostSTM[a] | 700 ± 26.5 | 687 ± 22.6 |
| | % change | 14.8 ± 1.36* | 18.3 ± 1.64* |
| Duration (msec) | PreSTM | 9.03 ± 0.49 | 8.81 ± 0.59 |
| | PostSTM[a] | 7.35 ± 0.56 | 7.57 ± 0.45 |
| | % change | 18.0 ± 5.01* | 12.6 ± 4.27* |
| Number | PreSTM | 6.5 ± 0.37 | 6.2 ± 0.35 |
| | PostSTM[a] | 4.6 ± 0.33 | 4.7 ± 0.33 |
| | % change | 29.7 ± 4.20* | 23.7 ± 4.67* |

[a] Observations were made 30 seconds after 20Hz stimulation.

*$p<0.01$ for significant difference pre- and postSTM (n=20).

Physostigmine (10 - 100$\mu gkg^{-1}$, i.v.) did not alter RFP frequency, duration or number preSTM (Table 2), but reduced the time taken to depress RFP during, and prolonged recovery from, 20Hz stimulation (Figs. 1 and 2). RFP frequency, duration and number were dose-dependently reduced postSTM in the presence of physostigmine and percent changes in these parameters are shown in Figs. 3 and 4. At a dose of 100$\mu gkg^{-1}$, i.v., physostigmine also decreased RFP amplitude during 2Hz stimulation (Fig. 2).

Table 2: Effect of physostigmine on RFP frequency, duration and number, preSTM

| Physostigmine dose ($\mu gkg^{-1}$) | (n) | FREQUENCY (Hz) (mean ± sem) | DURATION (msec) (mean ± sem) | NUMBER (mean ± sem) |
|---|---|---|---|---|
| 0 | (6) | 1023 ± 63.01 | 9.11 ± 0.75 | 7.50 ± 0.43 |
| 10 | (4) | 929 ± 124.9 | 7.94 ± 0.88 | 6.33 ± 0.33 |
| 20 | (5) | 1024 ± 87.00 | 8.44 ± 1.19 | 6.60 ± 0.30 |
| 50 | (4) | 869 ± 84.00 | 12.19 ± 1.43 | 7.67 ± 1.67 |
| 100 | (4) | 862 ± 172.0 | 8.79 ± 1.51 | 7.33 ± 1.85 |

While amphetamine failed to enhance RFP response to low doses of physostigmine (Fig. 5), 20Hz stimulation-induced depression of RFP was antagonized by, and recovery facilitated, in the presence of amphetamine (Fig. 6). RFP frequency was not altered by amphetamine, but percent change in RFP duration and number following 20Hz stimulation was dose-dependently decreased by amphetamine (Fig. 7).

In the presence of DFP (0.1 - 2.0$mgkg^{-1}$, i.v.), RFP frequency was reduced preSTM (Fig. 8). The percent change in this parameter was decreased over control values at a dose of 1.0$mgkg^{-1}$ DFP, but increased over control values by 2.0$mgkg^{-1}$ DFP (Fig. 8). RFP duration was not altered in the

Fig. 1: RFP response to 20Hz stimulation in the presence and absence of physostigmine (Eserine, 50$\mu gkg^{-1}$, i.v.)

The top row (Control) shows RFP during 2Hz stimulation prior to 20Hz stimulation (preSTM), the response at 30 seconds of 20Hz stimulation (STM 20Hz), and the time-dependent recovery of RFP following 20Hz stimulation during 2Hz stimulation (postSTM, 2Hz). Physostigmine, at 7, 15, 30 and 45 minutes post injection time, enhances the effects of 20Hz stimulation. Note the complete antagonism of RFP at 30 seconds of 20Hz stimulation 15 minutes post physostigmine injection, and the slower recovery after stimulation. At 45 minutes post injection of physostigmine, partial recovery of RFP to control responses is observed. Full recovery occurs within 90 minutes of injection (not shown).

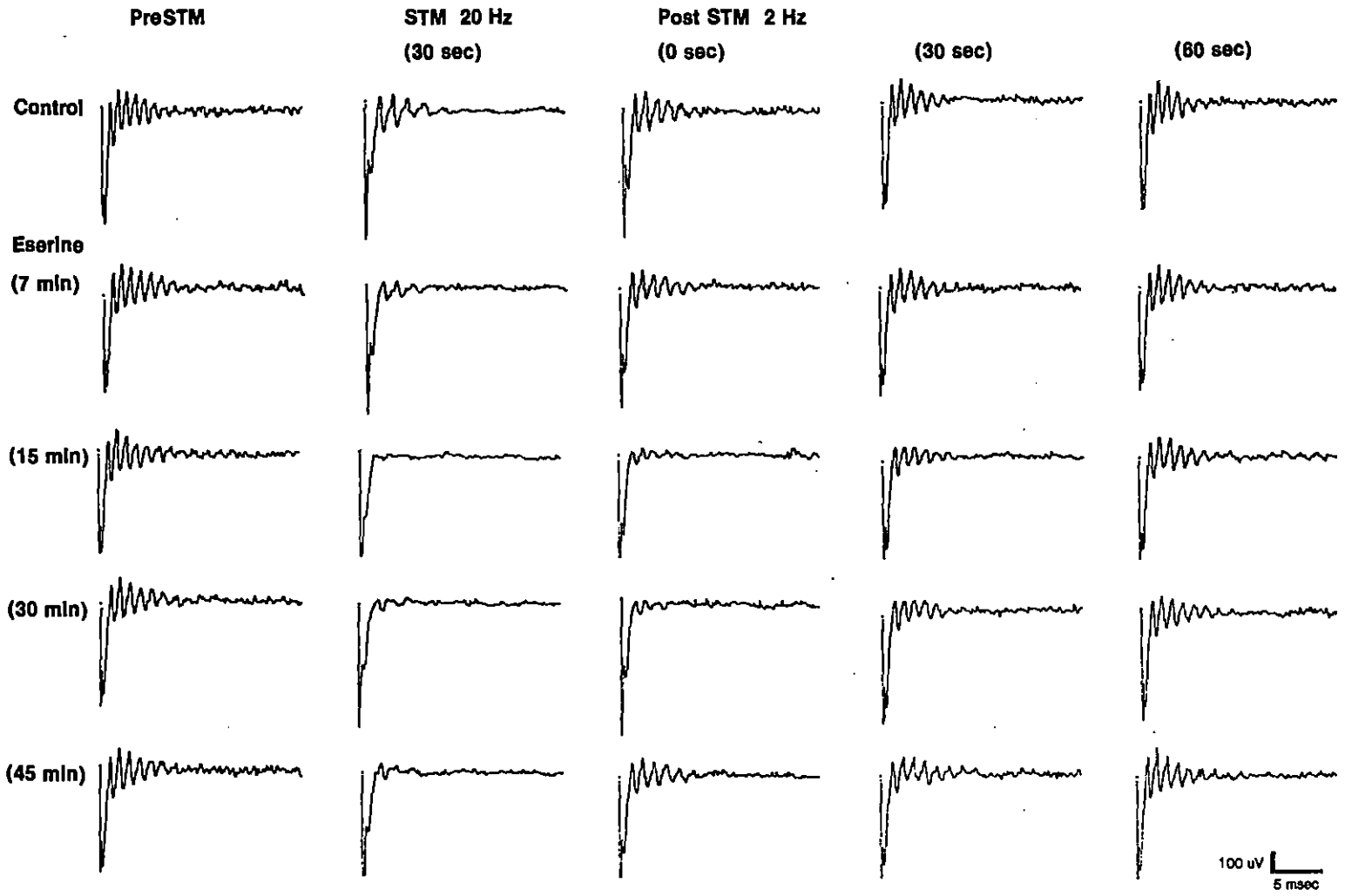
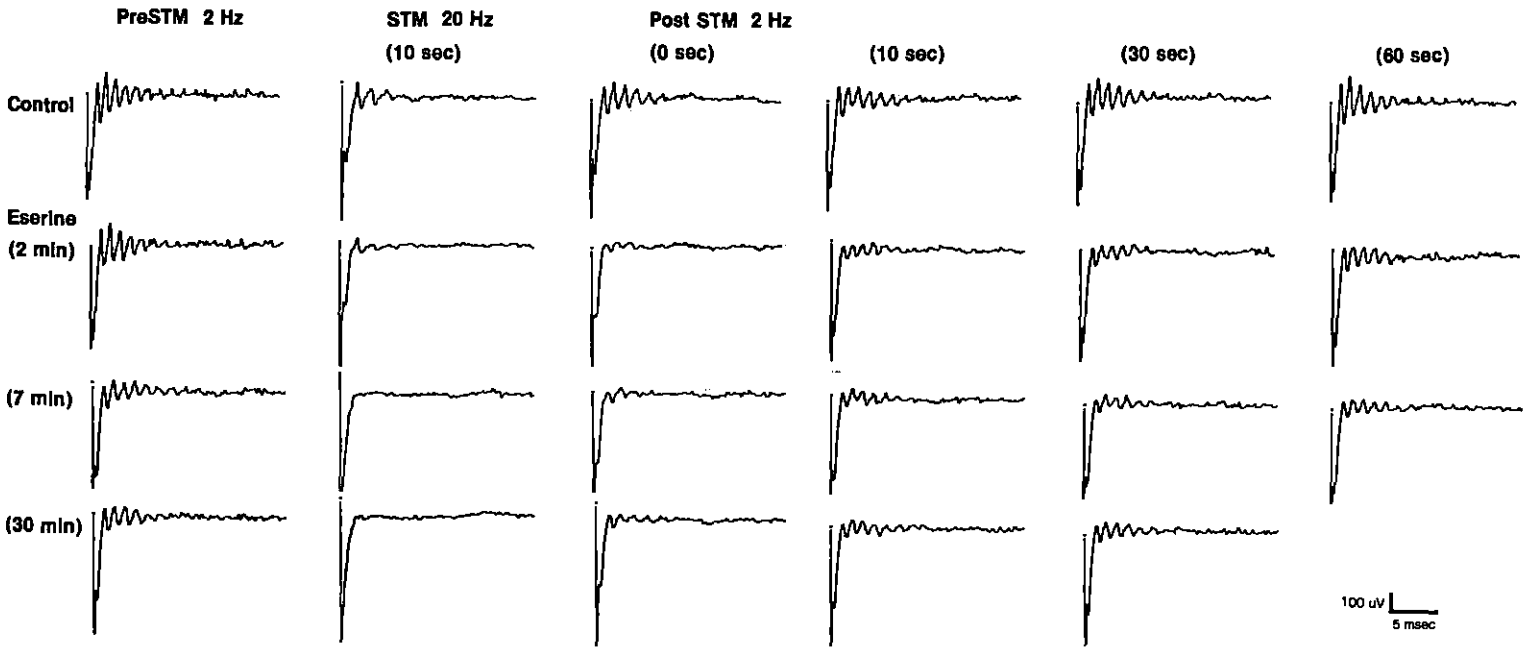

PreSTM
STM 20 Hz
(30 sec)
Post STM 2 Hz
(0 sec)
(30 sec)
(60 sec)
Control
Eserine
(7 min)
(15 min)
(30 min)
(45 min)
100 uV
5 msec

Fig. 2: RFP response to 20Hz stimulation in the presence and absence of physostigmine (Eserine, 100$\mu gkg^{-1}$, i.v.)

The top row shows RFP response to 20Hz stimulation and time-dependent recovery as described in Fig. 1. Note that RFP are completely inhibited at 10 seconds of 20Hz stimulation (STM 20Hz) 7 minutes after physostigmine administration, an effect which persists at 30 minutes post injection. Recovery of the RFP following 20Hz stimulation (postSTM, 2Hz) is also antagonized in the presence of physostigmine. Note also that RFP amplitude is reduced prior to 20Hz stimulation (preSTM, 2Hz) in the presence of 100$ugkg^{-1}$ physostigmine.


PreSTM 2 Hz
STM 20 Hz
(10 sec)
Post STM 2 Hz
(0 sec)
(10 sec)
(30 sec)
(60 sec)
Control
Eserine
(2 min)
(7 min)
(30 min)
100 uV
5 msec

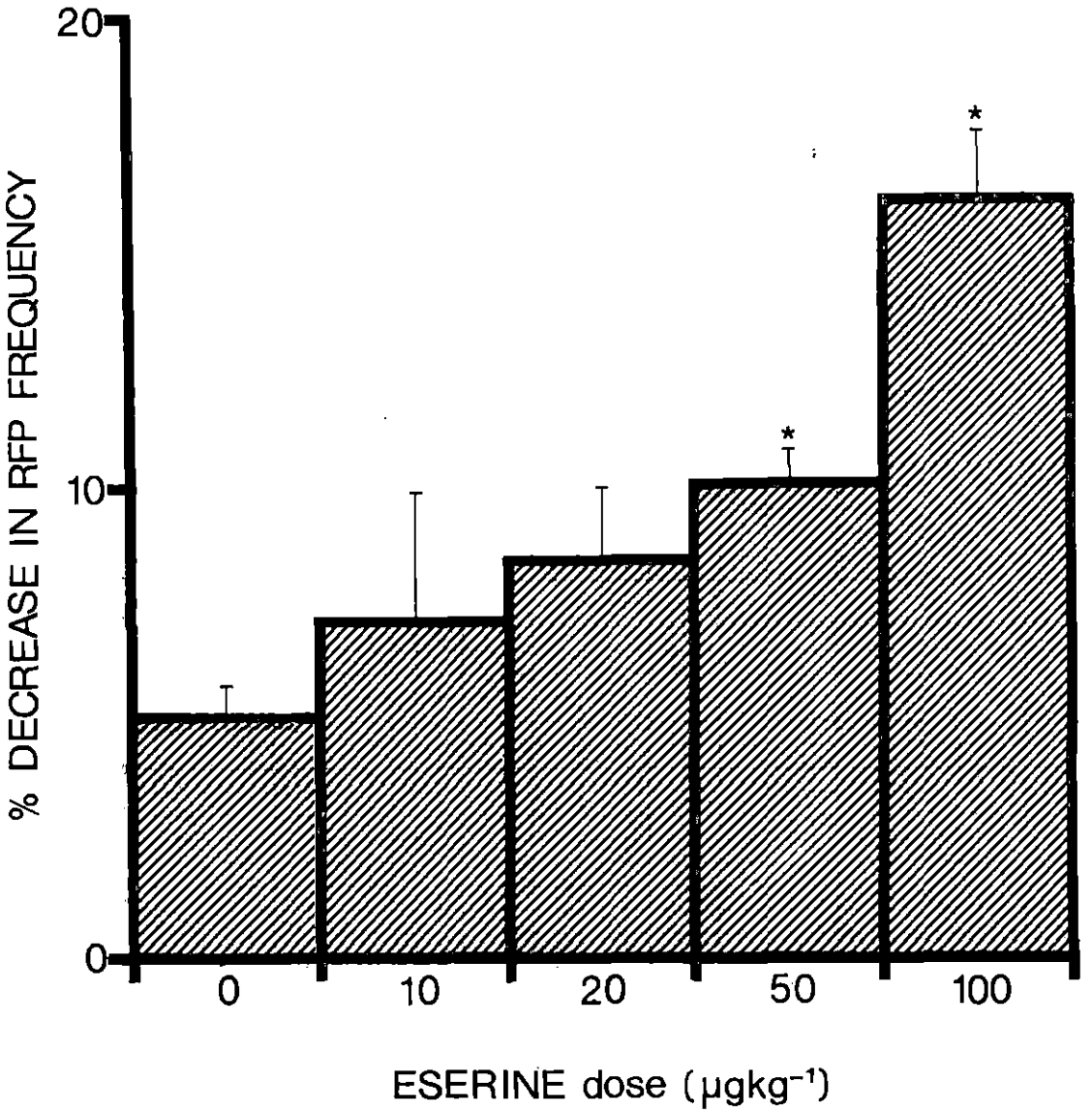



Fig. 3: Percent change in RFP frequency following 20Hz stimulation

RFP frequency is decreased 30 seconds after 20Hz stimulation and physostigmine (eserine, 10 - 100$\mu gkg^{-1}$, i.v.) dose-dependently facilitates this decrease. (Mean + sem, *$p<0.01$.)

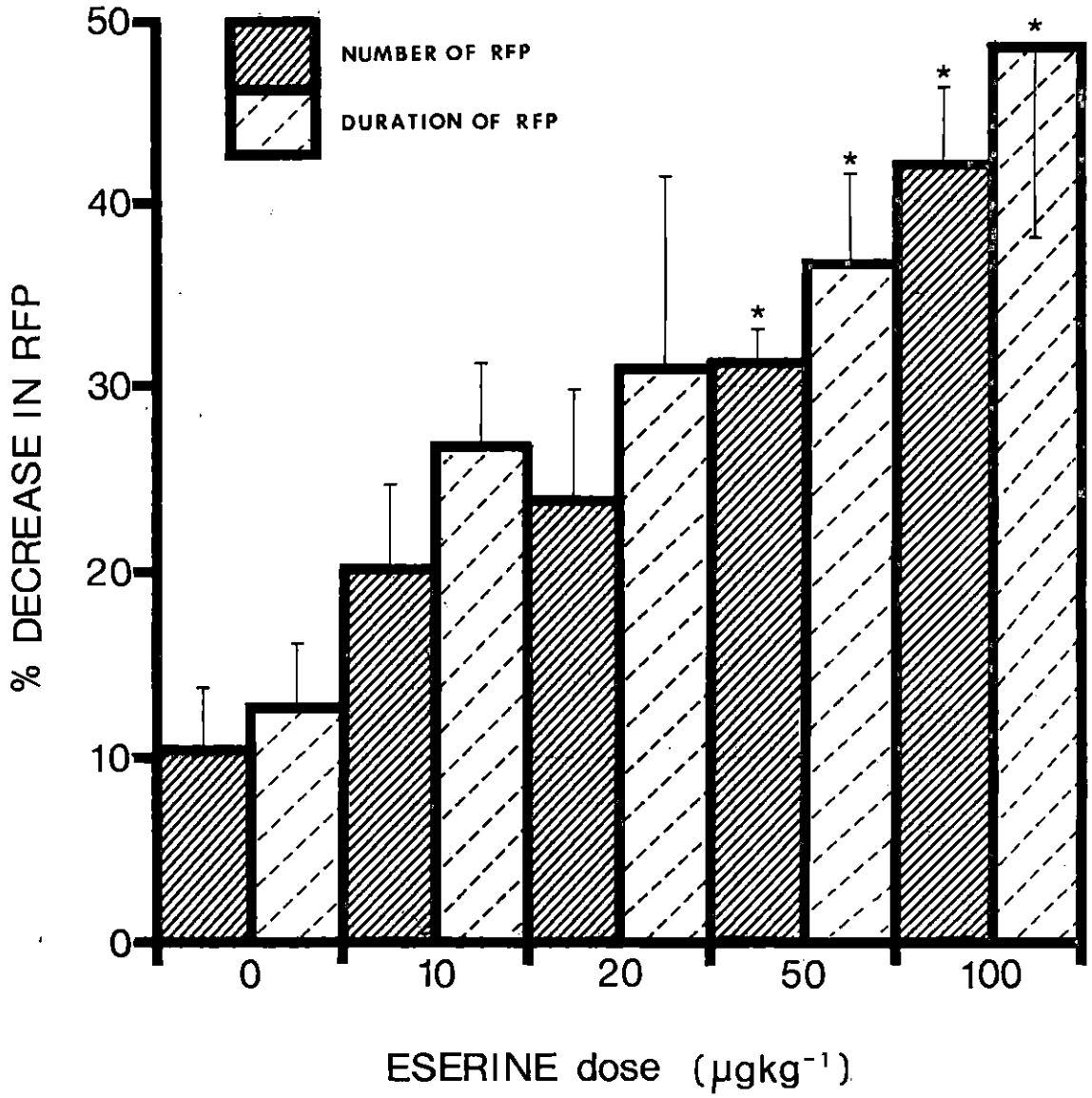



Fig. 4: Percent change in RFP number (solid stripes) and duration (broken stripes) following 20Hz stimulation

RFP number and duration are reduced 30 seconds after 20Hz stimulation and physostigmine (eserine, 10 - 100$\mu gkg^{-1}$, i.v.) dose-dependently facilitates this response. (Mean $\pm$ sem, *$p<0.01$.)

Fig. 5: RFP response to low dose physostigmine administration (Eserine, 20ugkg$^{-1}$) in the presence and absence of amphetamine (1.0mgkg$^{-1}$, i.v.)

Control response (top row) shows RFP before (preSTM, 2Hz) and at 30 and 60 seconds of 20Hz stimulation (STM 20Hz). Recovery of the RFP after 20Hz stimulation is time-dependent (c.f. Figs. 1 and 2). Note physostigmine facilitation of 20Hz stimulation (middle row). Amphetamine fails to enhance the effects of physostigmine (bottom row), but instead facilitates recovery of the RFP following 20Hz stimulation (c.f. Fig. 6).

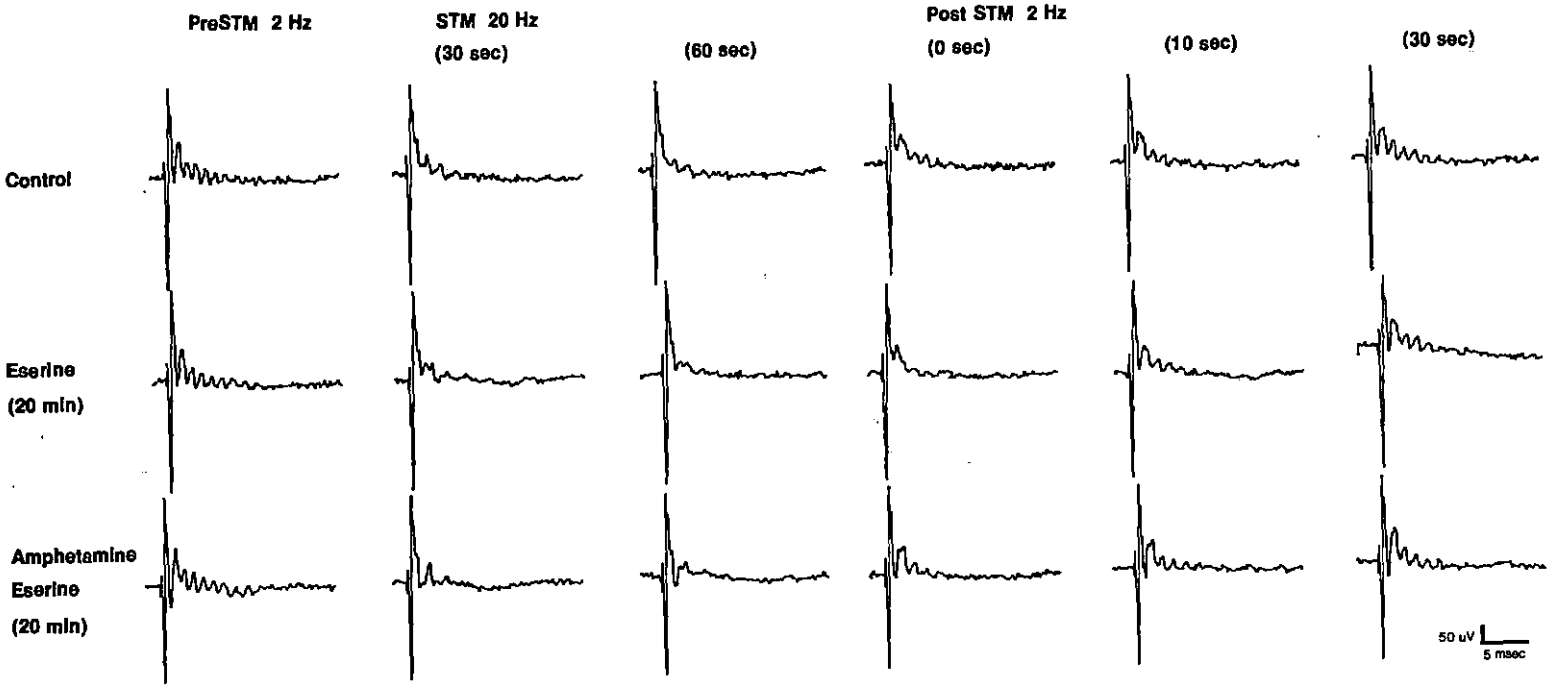
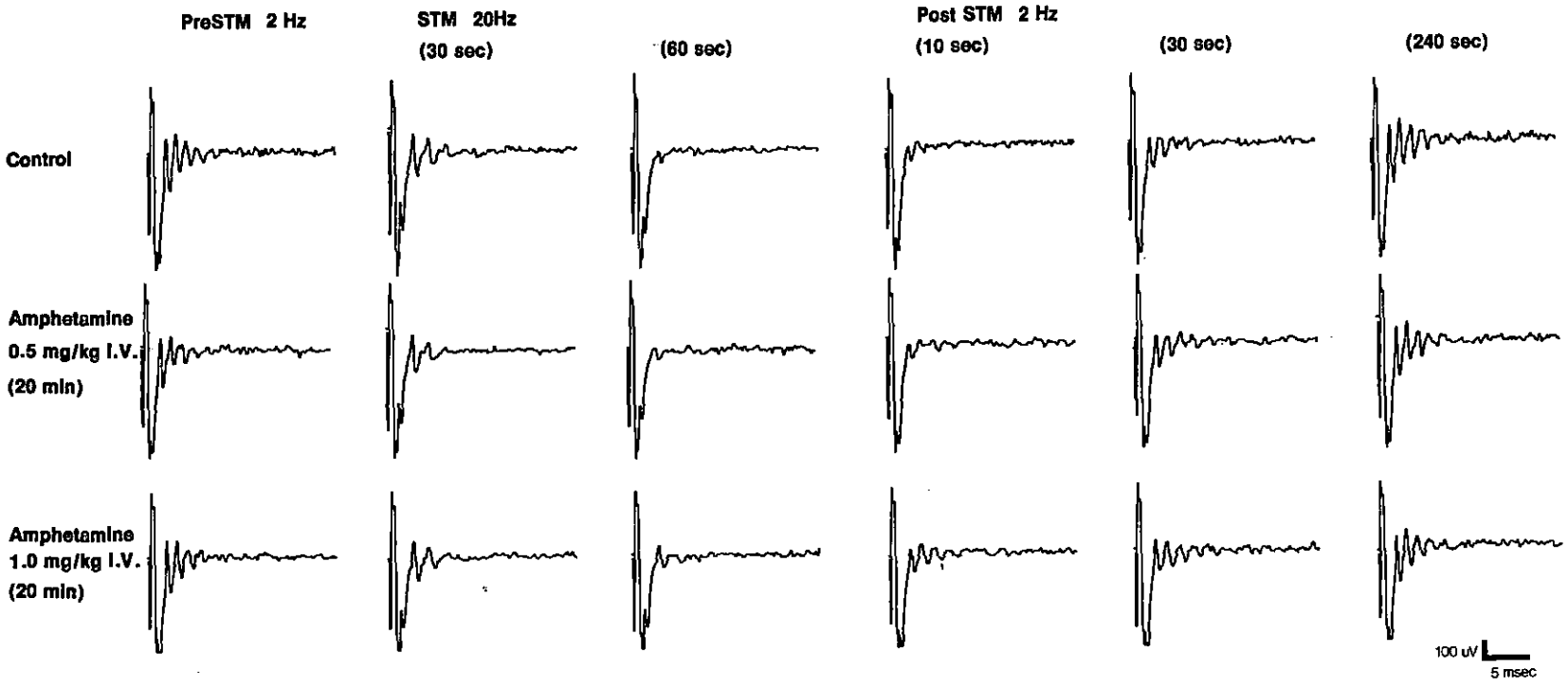

PreSTM 2 Hz
STM 20 Hz
(30 sec)
(60 sec)
Post STM 2 Hz
(0 sec)
(10 sec)
(30 sec)
Control
Eserine
(20 min)
Amphetamine
Eserine
(20 min)
50 uV
5 msec

Fig. 6: Amphetamine antagonism of 20Hz stimulation

The top row shows the control recording and RFP are antagonized by 60 seconds of 20Hz stimulation (STM 20Hz) with slight recovery occurring at 10 seconds after 20Hz stimulation (postSTM). Amphetamine (middle and bottom rows) dose-dependently antagonizes 20Hz stimulation (note potentials at 60 seconds, STM 20Hz) and facilitates RFP recovery after 20Hz stimulation (note potentials at 10 seconds, postSTM).


PreSTM 2 Hz
STM 20Hz
(30 sec)
(60 sec)
Post STM 2 Hz
(10 sec)
(30 sec)
(240 sec)
Control
Amphetamine
0.5 mg/kg I.V.
(20 min)
Amphetamine
1.0 mg/kg I.V.
(20 min)
100 uV
5 msec

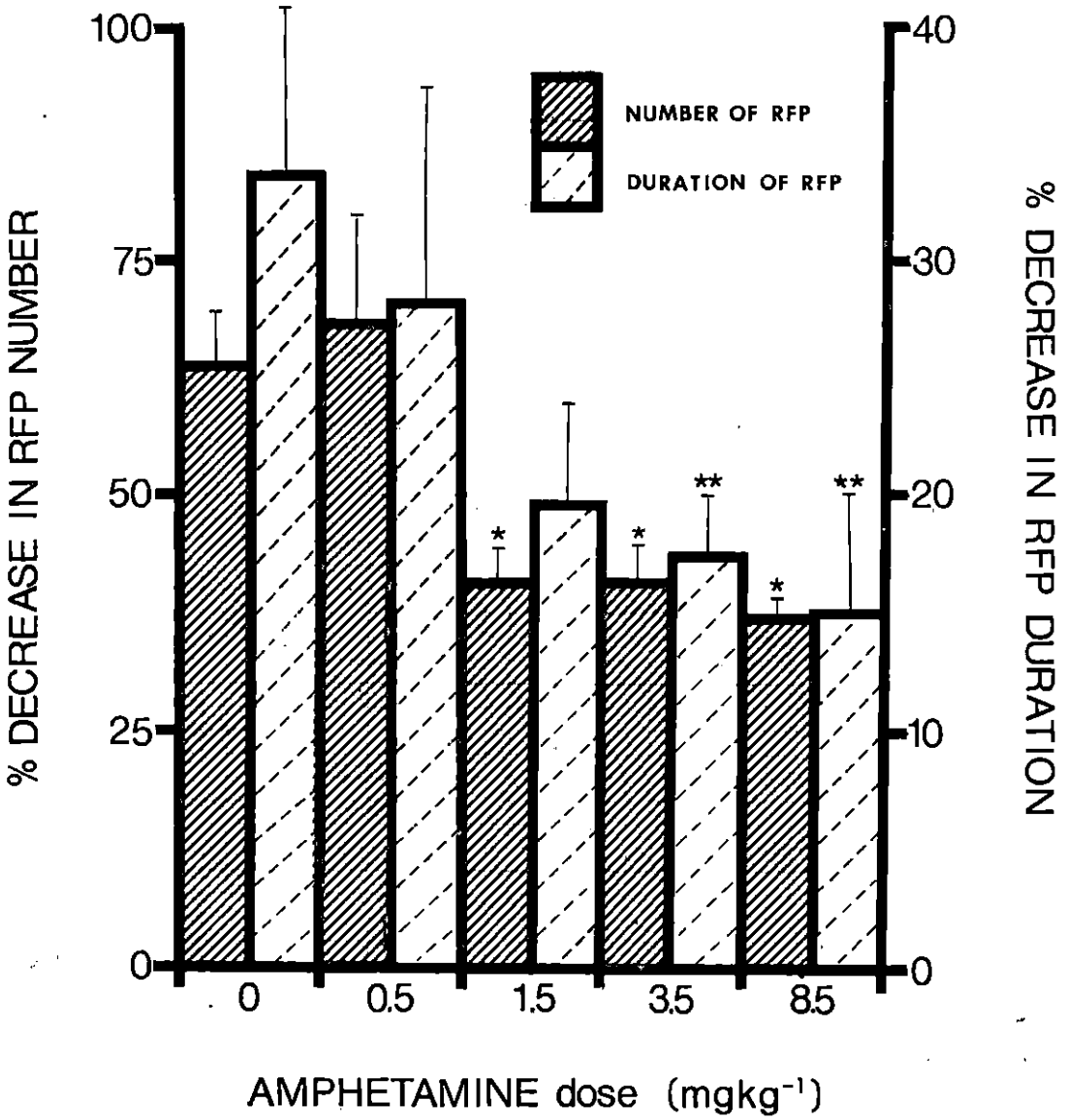



Fig. 7: Percent decrease in RFP number (solid stripes) and duration (broken stripes) immediately following 20Hz stimulation in the presence and absence of amphetamine (0.5 - 8.5mgkg$^{-1}$, i.v.)

Amphetamine dose-dependently antagonizes the percent decrease in RFP number and duration induced by 20Hz stimulation. (Mean + sem, *$p<0.01$, **$p<0.05$.)

presence of DFP preSTM, but was dose-dependently reduced postSTM. This is shown along with percent change in this parameter in Fig. 9. Similarly, RFP number was reduced postSTM in the presence of DFP, and at a dose of $2.0 mgkg^{-1}$, DFP also reduced RFP number preSTM (Fig. 10).

Recovery of RFP from the described effects of DFP was observed at two hours after the last injection of DFP (cumulative dose $2.0 mgkg^{-1}$, i.v., Fig. 11), and the parameters measured are shown in Figs. 12, 13 and 14. The possibility that RFP recovery was due to recovery of cholinesterase activity was investigated. However, blood, spinal cord and caudate nucleus samples taken from DFP-treated cats at 30, 60 and 120 minutes after DFP injection show a significant reduction in cholinesterase activity, with no recovery occurring (Table 3).

Table 3: Cholinesterase activity[a] of blood, spinal cord and caudate nucleus samples from DFP[b]-treated cats

| TREATMENT | BLOOD (mean ± sem) | (n) | SPINAL CORD (mean ± sem) | CAUDATE NUCLEUS (mean ± sem) | (n)[c] |
|---|---|---|---|---|---|
| NONE | 36.75 ± 5.64 | (6) | 2.63 ± 0.23 | 22.37 ± 1.17 | (3) |
| DFP + 30min | 1.35 ± 1.33* | (6) | 0.80 ± 0.05* | 1.18 ± 0.20* | (3) |
| DFP + 60min | 0.00 ± 0.00* | (4) | 0.70 ± 0.35* | 1.83 ± 0.52* | (3) |
| DFP + 120min | 0.00 ± 0.00* | (2) | 0.58 ± 0.29* | 2.00 ± 0.65* | (3) |

[a]Measured as $\mu M$ acetate $min^{-1}g^{-1}$ for tissue and $\mu M$ acetate $min^{-1}cm^{-3}$ for blood.

[b]Final cumulative dose of DFP is $2.0 mgkg^{-1}$.

[c]n is the same for spinal cord and caudate nucleus samples.

*$p<0.01$ compared to respective controls, not significant compared to other values in respective groups.

Fig. 8: RFP frequency, and percent change in frequency 30 seconds after 20Hz stimulation, in the presence and absence of DFP (0.1 - 2.0mgkg$^{-1}$, i.v.)

Upper graph: RFP frequency during 2Hz stimulation before 20Hz stimulation (open bars) is reduced in the presence of DFP. In addition, RFP frequency is reduced after 20Hz stimulation (dotted bars). (Mean + sem, *$p<0.01$.)

Lower graph: While RFP frequency is reduced before and after 20Hz stimulation in the presence of DFP (see upper graph), the percent decrease in RFP frequency at 30 seconds after 20Hz stimulation is unchanged over control until a cumulative dose of 1.0mgkg$^{-1}$ DFP is attained. At this dose, DFP reduces the decrease in frequency. However, in the presence of 2.0mgkg$^{-1}$ DFP, 20Hz stimulation-induced depression of RFP frequency is facilitated. (Mean + sem, *$p<0.01$.)

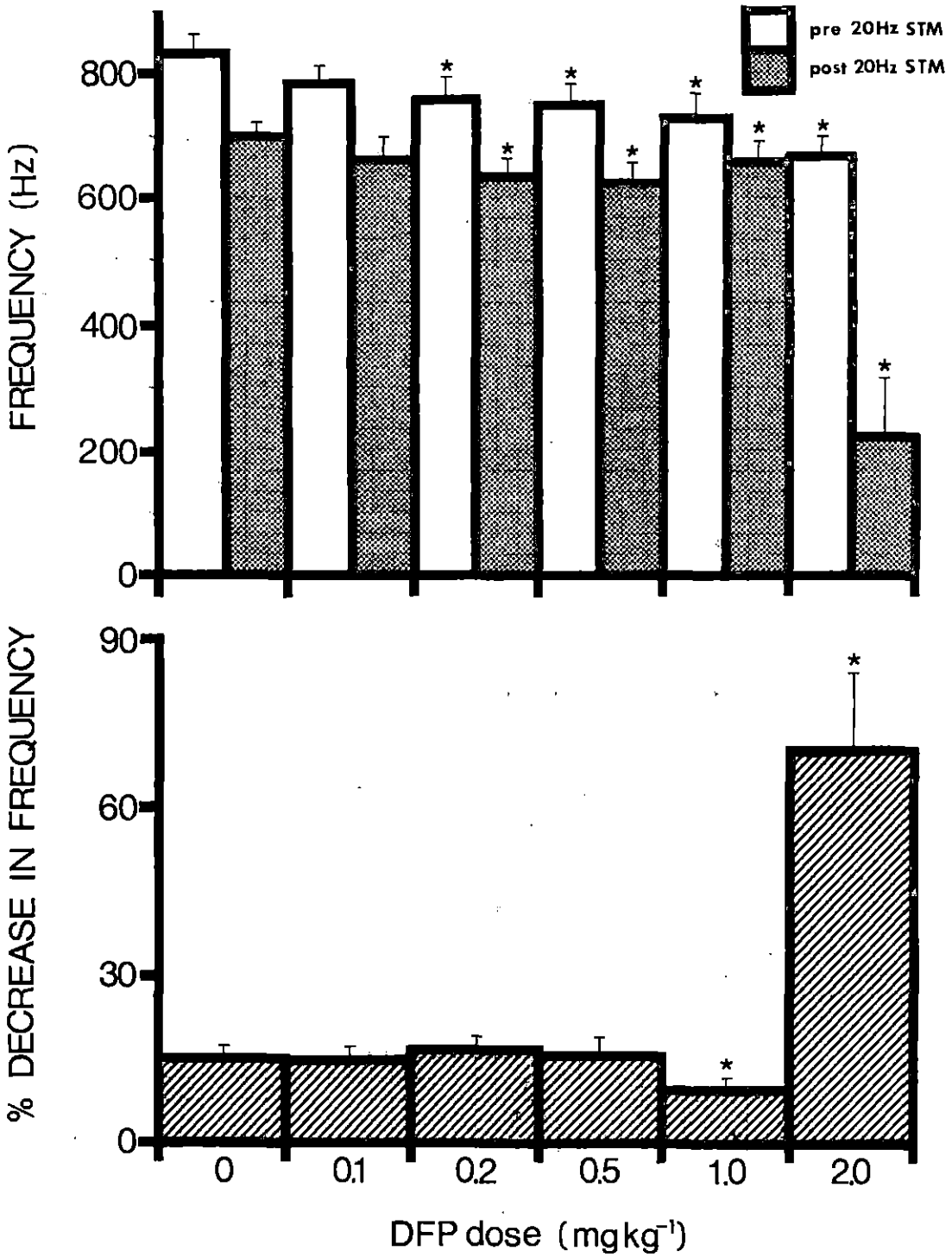

pre 20Hz STM
post 20Hz STM
FREQUENCY (Hz)
800
600
400
200
0
% DECREASE IN FREQUENCY
90
60
30
0
0
0.1
0.2
0.5
1.0
2.0
DFP dose (mg kg⁻¹)

Fig. 9: RFP duration, and percent change in duration 30 seconds after 20Hz stimulation, in the presence and absence of DFP (0.1 - 2.0mgkg$^{-1}$, i.v.)

Upper graph: DFP does not affect RFP duration during 2Hz stimulation prior to 20Hz stimulation (open bars). However, RFP duration at 30 seconds post 20Hz stimulation (dotted bars) is reduced and DFP facilitates this reduction. (Mean + sem, *$p<0.01$.)

Lower graph: The decrease in RFP duration following 20Hz stimulation (upper graph, dotted bars) is reflected in a greater percent decrease in duration in the presence of DFP. (Mean + sem, *$p<0.01$,)

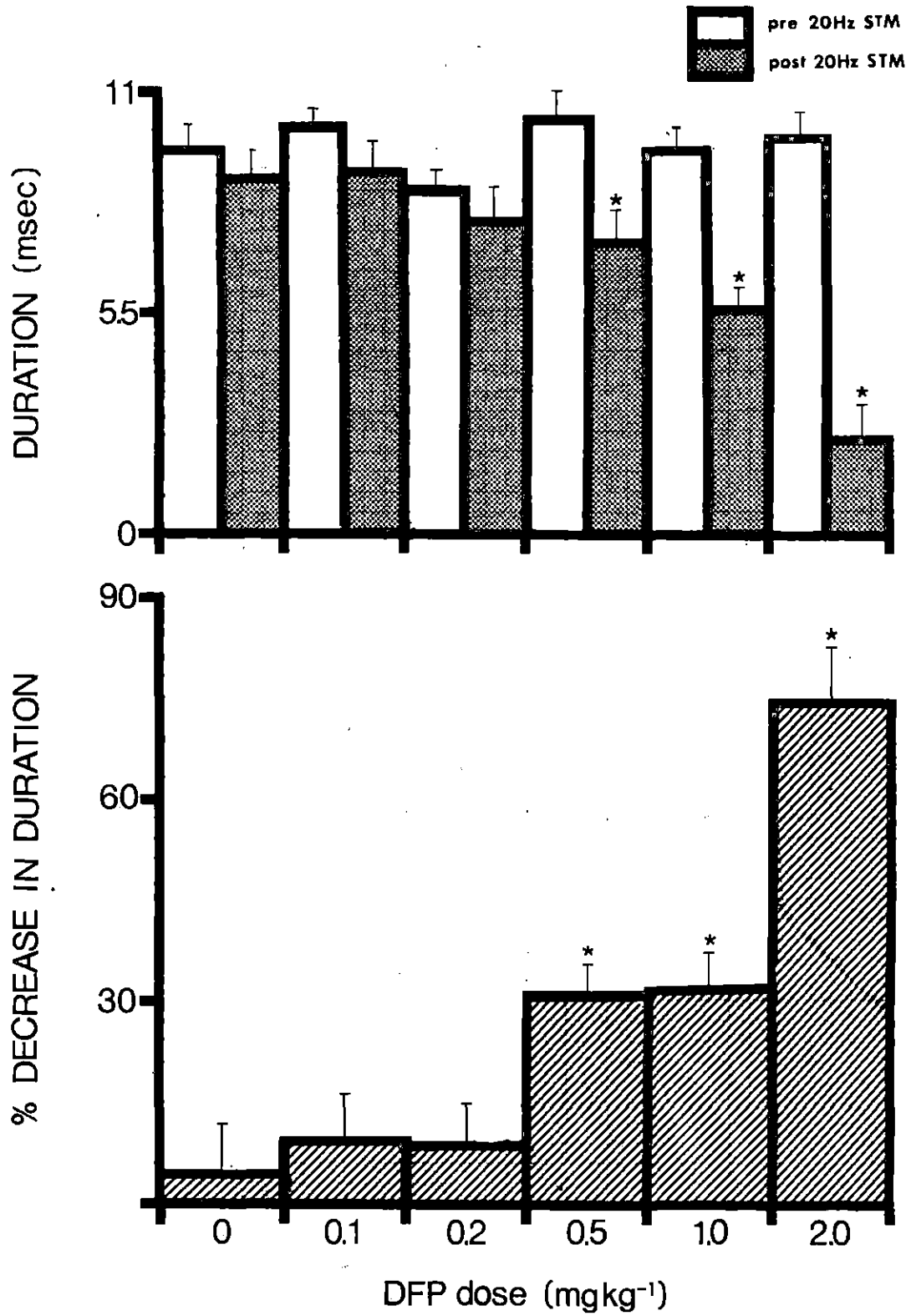

pre 20Hz STM
post 20Hz STM
DURATION (msec)
11
5.5
0
% DECREASE IN DURATION
90
60
30
0
0.1
0.2
0.5
1.0
2.0
DFP dose (mgkg⁻¹)

Fig. 10: RFP number, and percent change in number 30 seconds after 20Hz stimulation, in the presence and absence of DFP (0.1 - 2.0mgkg$^{-1}$)

Upper graph: At a dose of 2.0mgkg$^{-1}$, DFP reduces the number of RFP during 2Hz stimulation prior to 20Hz stimulation (open bars). Following 20Hz stimulation (dotted bars), reduction in RFP number is enhanced in the presence of DFP. (Mean + sem, *$p<0.01$.)

Lower graph: Percent decrease in RFP frequency following 20Hz stimulation is enhanced in the presence of DFP. (Mean + sem, *$p<0.01$.)

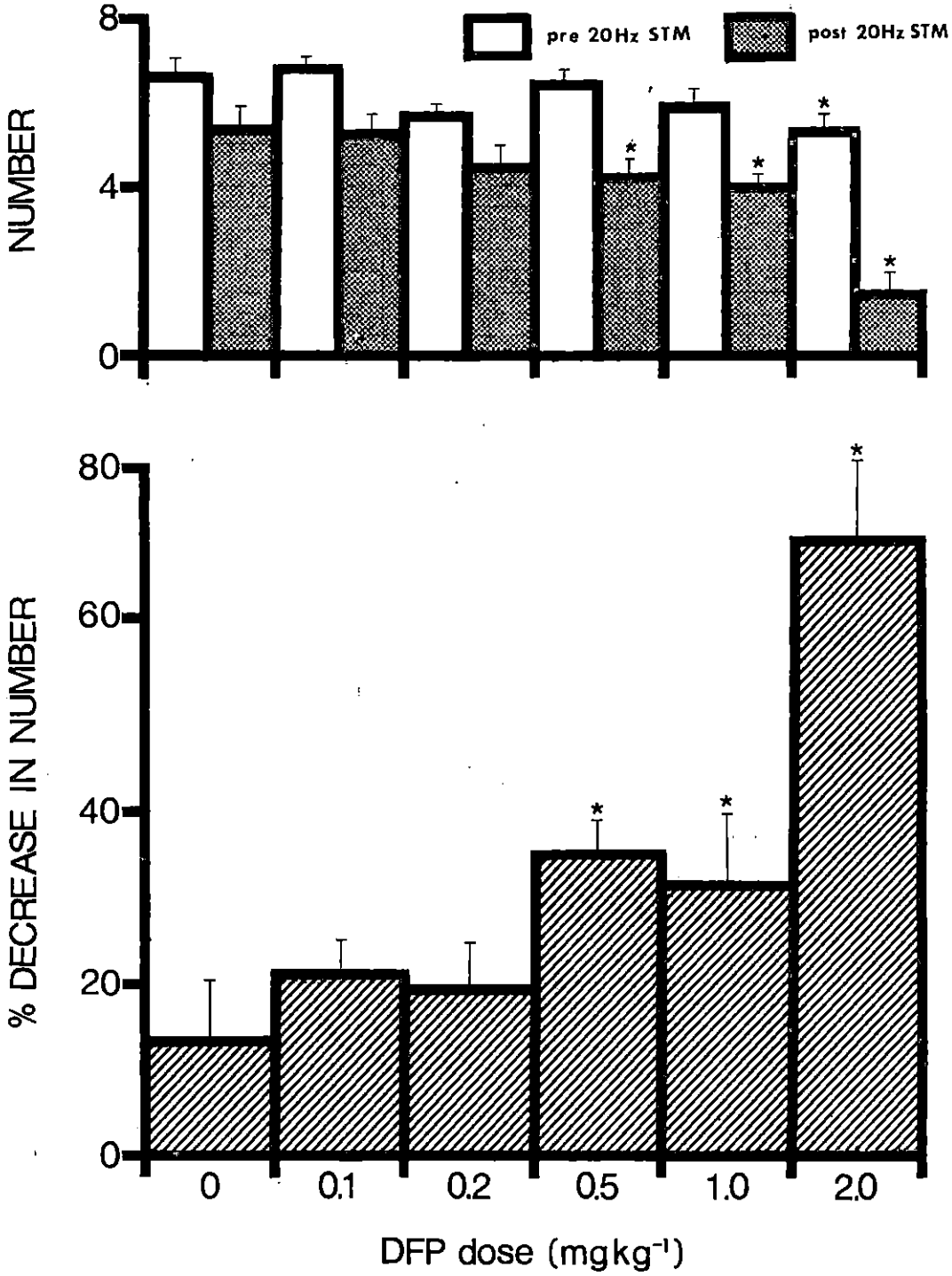

pre 20Hz STM
post 20Hz STM
NUMBER
8
4
0
% DECREASE IN NUMBER
80
60
40
20
0
0
0.1
0.2
0.5
1.0
2.0
DFP dose (mgkg⁻¹)

Fig. 11: Time-dependent recovery of RFP from DFP facilitation of 20Hz stimulation-induced RFP depression

The top row shows typical control RFP response during 2Hz stimulation (preSTM), at 30 seconds of 20Hz stimulation (STM 20Hz), and recovery following 20Hz stimulation (postSTM). DFP, at 0.5mgkg$^{-1}$ (second row) has little effect on RFP. At 30 minutes post injection of DFP (2.0mgkg$^{-1}$, i.v., cumulative dose, third row), RFP are depressed after cessation of 20Hz stimulation (postSTM, 0 sec, 30 sec). However, at two hours after DFP injection (2.0mgkg$^{-1}$, i.v., cumulative dose, fourth row), RFP response to 20Hz stimulation recovers to control (note potentials at STM 20Hz and postSTM, 0 sec, 30 sec).

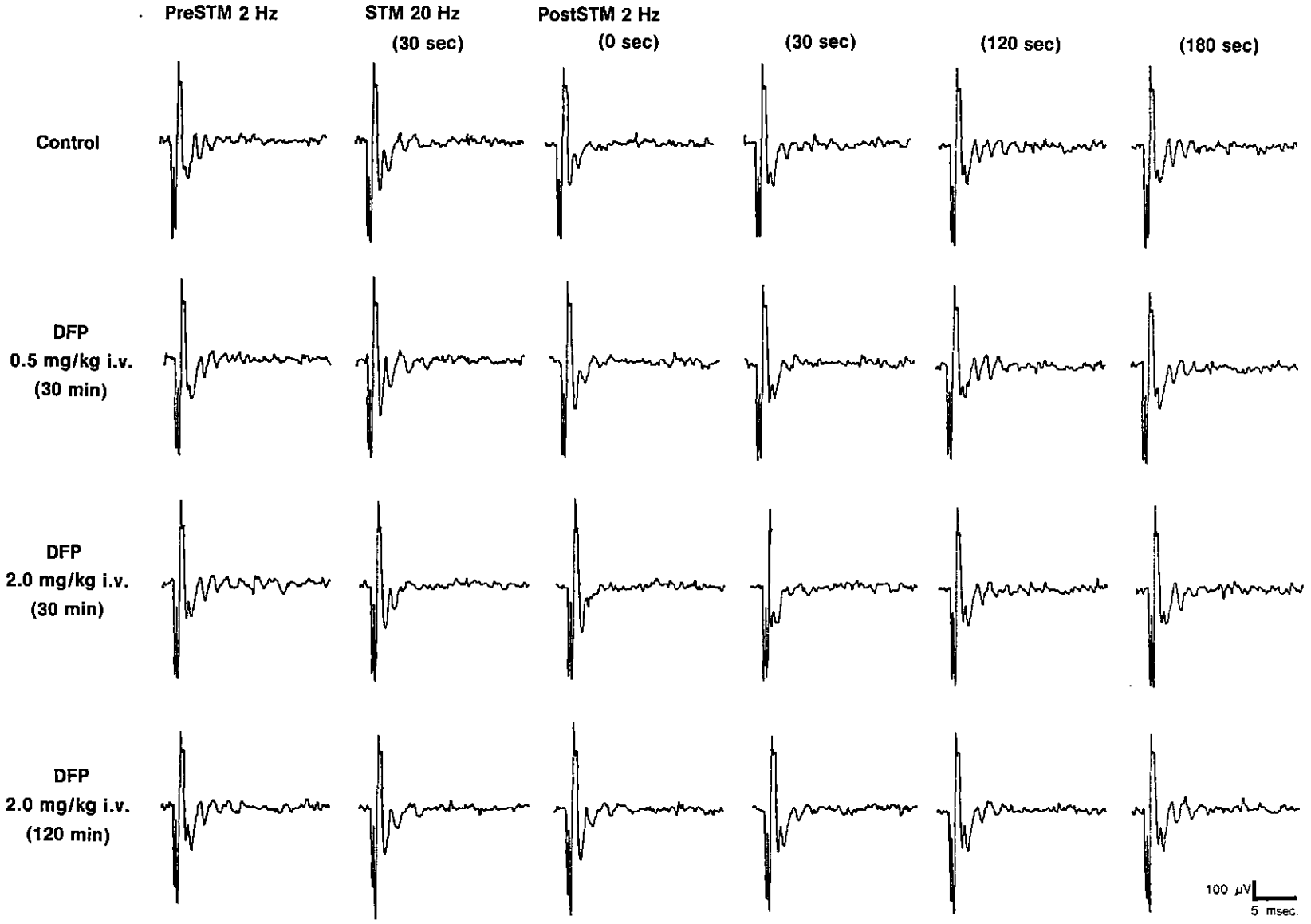

PreSTM 2 Hz
STM 20 Hz
(30 sec)
PostSTM 2 Hz
(0 sec)
(30 sec)
(120 sec)
(180 sec)
Control
DFP
0.5 mg/kg i.v.
(30 min)
DFP
2.0 mg/kg i.v.
(30 min)
DFP
2.0 mg/kg i.v.
(120 min)
100 μV
5 msec.

Fig. 12: Time-dependent recovery of RFP from 2.0mgkg$^{-1}$ DFP facilitation of 20Hz stimulation-induced RFP depression

Upper graph: During 2Hz stimulation prior to 20Hz stimulation (open bars), DFP depression of RFP frequency is maintained up to two hours after injection of DFP (2.0 mgkg$^{-1}$, cumulative dose). However, the DFP facilitation of 20Hz stimulation- induced depression of RFP frequency (dotted bars) observed at 30 minutes after DFP injection recovers with time, and at two hours post DFP injection, RFP frequency following 20Hz stimulation returns to control. (Mean + sem, *$p<0.01$, compared to control, @$p<0.01$ compared to response at 30 minutes after DFP injection, but n.s. compared to control.)

Lower graph: The 20Hz stimulation-induced percent decrease in RFP frequency is enhanced by DFP 30 minutes after injection, but recovers with time so that at two hours after DFP injection, the response has recovered to control. (Mean + sem, *$p<0.01$ compared to control, @$p<0.01$ compared to response at 30 minutes after DFP injection, but n.s. compared to control.)

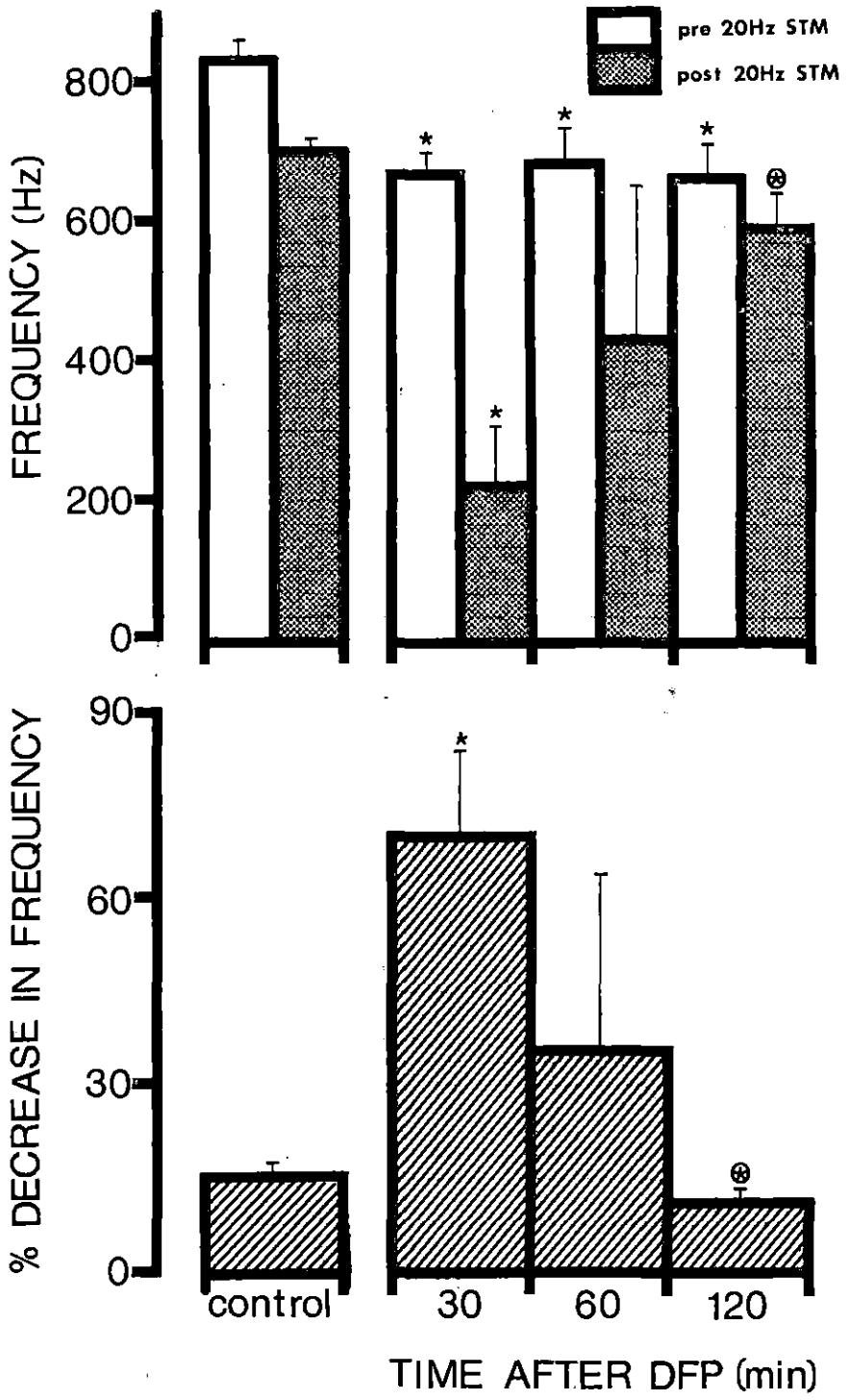

pre 20Hz STM
post 20Hz STM
FREQUENCY (Hz)
800
600
400
200
0
% DECREASE IN FREQUENCY
90
60
30
0
control
30
60
120
TIME AFTER DFP (min)

Fig. 13: Time-dependent recovery of RFP from 2.0mgkg$^{-1}$ DFP facilitation of 20Hz stimulation-induced RFP depression

Upper graph: RFP duration was unchanged by DFP prior to 20Hz stimulation (open bars). However, RFP duration was decreased following 20Hz stimulation (dotted bars) 30 minutes after 2.0mgkg$^{-1}$ DFP, but recovered two hours after DFP injection. (Mean + sem, *$p < 0.01$ compared to control, @$p < 0.01$ compared to response 30 minutes after DFP injection, but n.s. compared to control.)

Lower graph: The percent decrease in RFP duration is enhanced by 2.0mgkg$^{-1}$ DFP 30 minutes after injection, but recovers to control after two hours in the presence of DFP. (Mean + sem, *$p < 0.01$ compared to control, @$p < 0.01$ compared to response 30 minutes after DFP injection, but n.s. compared to control.)

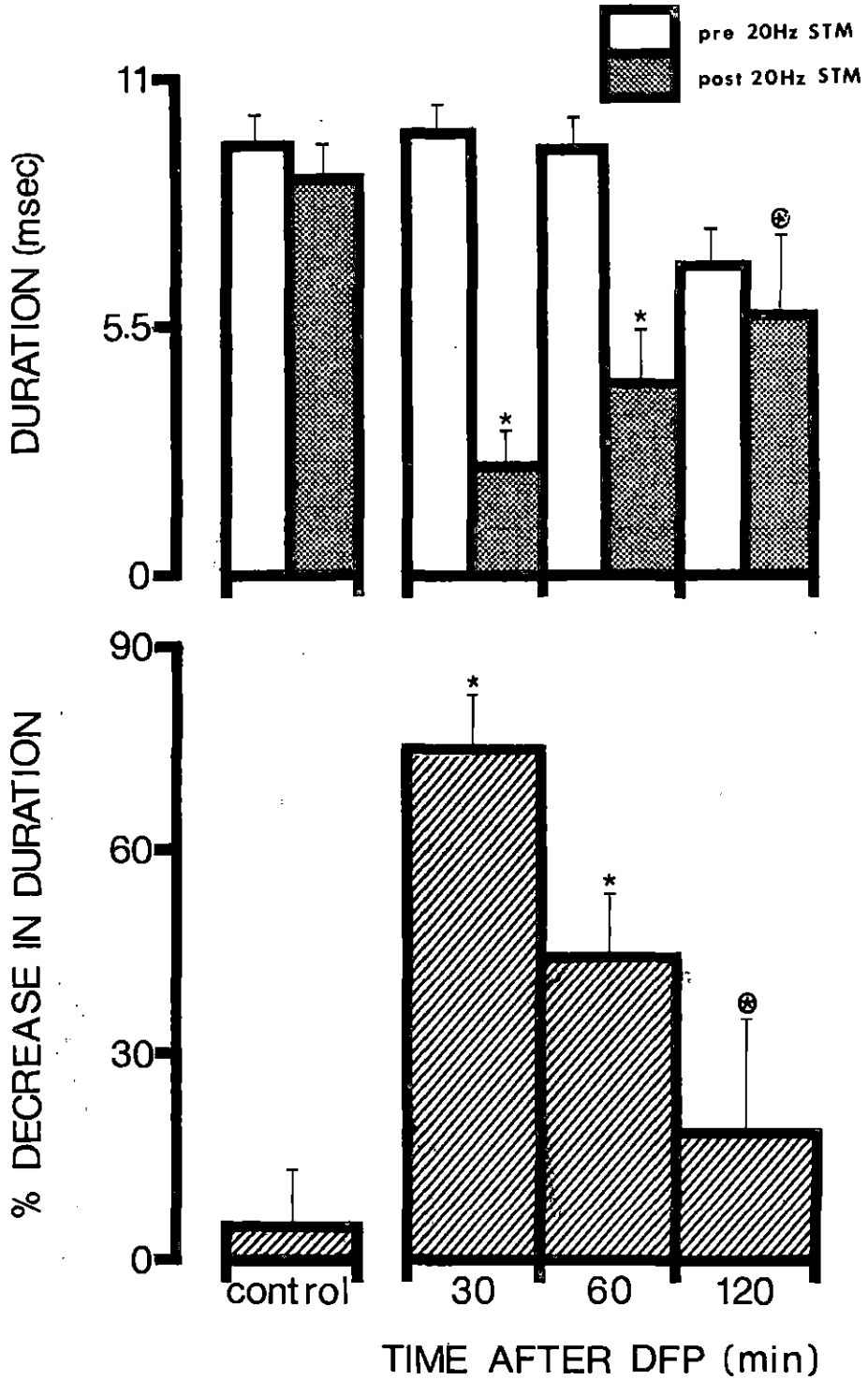
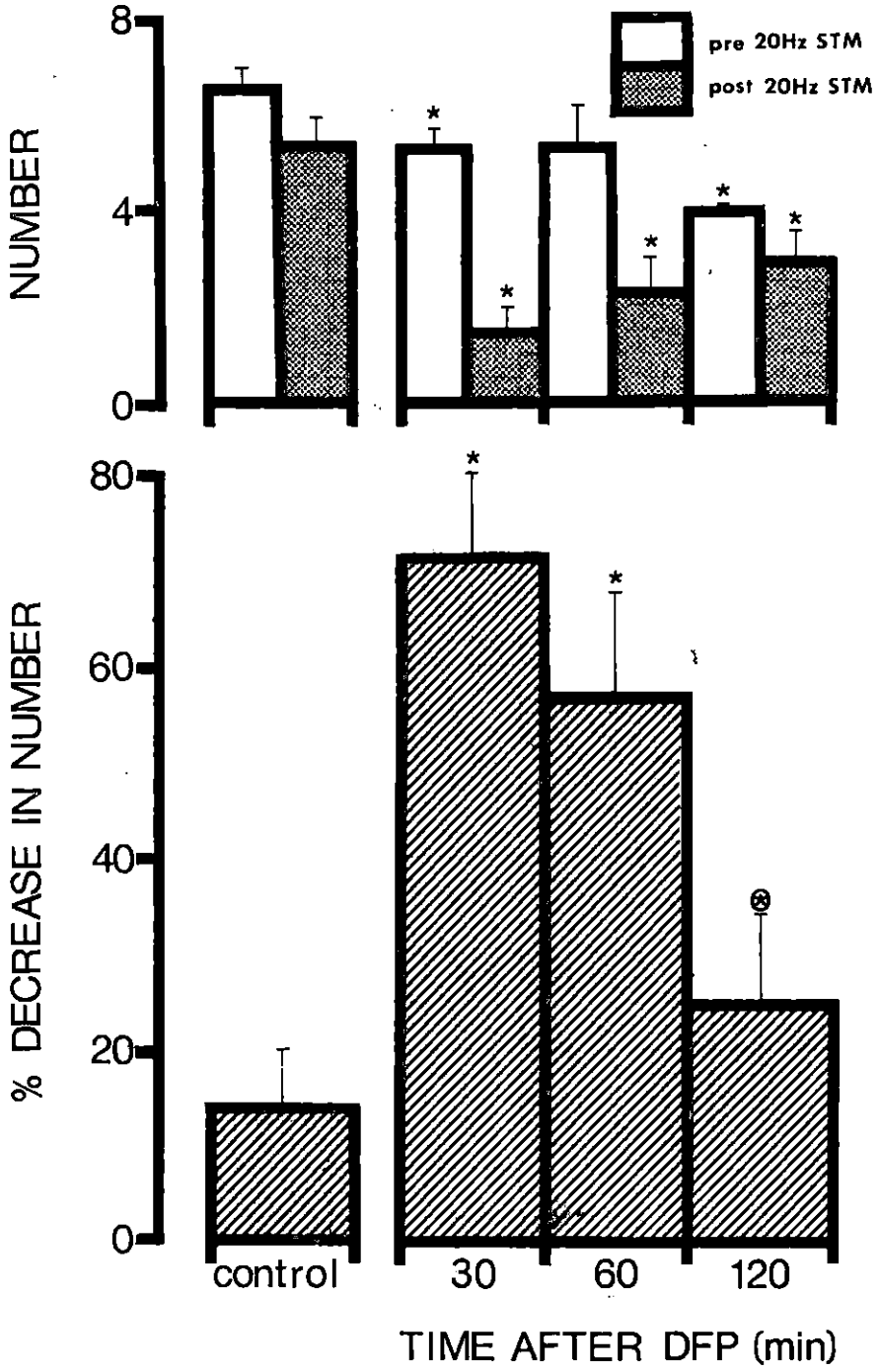

pre 20Hz STM
post 20Hz STM
DURATION (msec)
11
5.5
0
% DECREASE IN DURATION
90
60
30
0
control
30
60
120
TIME AFTER DFP (min)

Fig. 14: Time-dependent effect of 2.0mgkg$^{-1}$ DFP on RFP number, and percent decrease in number following 20Hz stimulation

Upper graph: RFP number during 2Hz stimulation prior to 20Hz stimulation (open bars) was reduced 30 and 120 minutes after injection of DFP. RFP number following 20Hz stimulation (dotted bars) was also significantly decreased over control values in the presence of DFP at all times. (Mean + sem, *$p<0.01$ compared to control.)

Lower graph: While RFP number is depressed by 2.0mgkg$^{-1}$ DFP at all times after injection (upper graph, dotted bars), the percent decrease in RFP number following 20Hz stimulation is greatest 30 minutes after DFP injection. Recovery of the percent decrease to control occurs two hours after DFP injection. (Mean + sem, *$p<0.01$ compared to control, @ p 0.01 compared to response at 30 minutes after DFP injection, but n.s. compared to control.)


8
4
0
NUMBER
pre 20Hz STM
post 20Hz STM
80
60
40
20
0
% DECREASE IN NUMBER
control
30
60
120
TIME AFTER DFP (min)

At a dose of 10$\mu gkg^{-1}$, soman reduced RFP frequency pre- and postSTM, but percent change in this parameter was not altered in the presence of soman (Table 4). In addition, soman (1.0 - 10.0$\mu gkg^{-1}$, i.v.) did not alter RFP duration (Table 5) or number (Table 6) pre- or postSTM. Cholinesterase activity of blood, spinal cord and caudate nucleus samples taken from soman treated cats (10$\mu gkg^{-1}$, i.v.) were reduced (Table 7), but this reduction was not as great as in cats receiving 2.0$mgkg^{-1}$ DFP (c.f. Tables 3 and 7).

Table 4: Effect of soman on RFP frequency before and 30 seconds after 20Hz stimulation

| Soman dose ($\mu gkg^{-1}$) | (n) | FREQUENCY (Hz) preSTM (mean ± sem) | FREQUENCY (Hz) postSTM (mean ± sem) | % change (mean ± sem) |
|---|---|---|---|---|
| 0 | (5) | 790 ± 16.9 | 683 ± 16.3 | 13.56 ± 1.99 |
| 1.0 | (4) | 765 ± 20.6 | 659 ± 20.3 | 13.72 ± 0.75 |
| 2.0 | (4) | 738 ± 16.1 | 614 ± 29.2 | 16.99 ± 2.37 |
| 5.0 | (5) | 741 ± 24.9 | 593 ± 32.1 | 18.93 ± 6.10 |
| 10.0- 30min | (3) | 701 ± 20.2* | 585 ± 31.0 | 16.18 ± 4.73 |
| - 60min | (3) | 659 ± 0.3* | 536 ± 5.2* | 18.74 ± 4.02 |
| -120min | (3) | 651 ± 8.7* | 573 ± 20.7* | 12.20 ± 3.67 |
| -240min | (3) | 660 ± 15.6* | 578 ± 28.0 | 12.54 ± 2.53 |

*$p<0.01$ for significant difference compared to control.

Mecamylamine (0.2 - 2.0$mgkg^{-1}$, i.v.) did not alter RFP frequency pre- or postSTM (Table 8). However, despite the small sample size (n=3) and high variability, it was observed that the duration and number of RFP were reduced preSTM and percent changes in these parameters following 20Hz stimulation were decreased over control values in the presence of mecamylamine

Table 5: RFP duration pre- and 30 seconds post 20Hz stimulation in the presence and absence of soman

| Soman dose ($\mu gkg^{-1}$) | (n) | DURATION (msec) preSTM (mean ± sem) | postSTM (mean ± sem) | % change (mean ± sem) |
|---|---|---|---|---|
| 0 | (5) | 11.01 ± 1.79 | 9.52 ± 1.83 | 13.29 ± 4.66 |
| 1.0 | (4) | 12.39 ± 4.22 | 10.27 ± 2.98 | 13.60 ± 5.77 |
| 2.0 | (4) | 12.34 ± 4.18 | 9.55 ± 3.26 | 22.58 ± 5.11 |
| 5.0 | (5) | 9.50 ± 1.72 | 7.74 ± 1.74 | 22.69 ± 5.27 |
| 10.0- 30min | (5) | 7.67 ± 0.78 | 5.82 ± 0.72 | 23.66 ± 5.27 |
| - 60min | (3) | 9.39 ± 1.13 | 6.00 ± 1.28 | 31.60 ± 20.0 |
| -120min | (3) | 9.14 ± 0.49 | 7.45 ± 0.11 | 18.11 ± 3.77 |
| -240min | (3) | 8.79 ± 1.22 | 7.03 ± 0.85 | 15.67 ± 17.1 |

Table 6: Effect of soman on RFP number pre- and 30 seconds post 20Hz stimulation

| Soman dose ($\mu gkg^{-1}$) | (n) | NUMBER preSTM (mean ± sem) | postSTM (mean ± sem) | % change (mean ± sem) |
|---|---|---|---|---|
| 0 | (5) | 7.2 ± 1.24 | 5.6 ± 0.87 | 21.38 ± 2.35 |
| 1.0 | (4) | 8.0 ± 2.34 | 3.8 ± 1.81 | 25.83 ± 4.38 |
| 2.0 | (4) | 7.0 ± 1.68 | 5.0 ± 1.35 | 29.58 ± 3.01 |
| 5.0 | (5) | 6.0 ± 1.09 | 4.2 ± 1.04 | 32.66 ±10.89 |
| 10.0- 30min | (5) | 4.6 ± 0.40 | 3.2 ± 0.38 | 30.67 ± 5.28 |
| - 60min | (3) | 5.0 ± 0.58 | 3.3 ± 0.88 | 30.56 ±19.45 |
| -120min | (3) | 5.7 ± 0.58 | 4.0 ± 0.00 | 28.89 ± 4.45 |
| -240min | (3) | 5.0 ± 0.81 | 4.0 ± 0.81 | 16.67 ±16.66 |

Table 7: Cholinesterase activity[a] of blood, spinal cord and caudate nucleus samples from soman[b]-treated cats

| TREATMENT | BLOOD (mean ± sem) | (n) | SPINAL CORD (mean ± sem) | CAUDATE NUCLEUS (mean ± sem) | (n)[c] |
|---|---|---|---|---|---|
| NONE | 57.30 ± 0.52 | (3) | 3.28 ± 0.16 | 22.72 ± 1.18 | (3) |
| Soman/30min | 0.00 ± 0.00* | (3) | 1.45 ± 0.05* | 12.18 ± 3.16** | (3) |
| Soman/240min | - | - | 2.08 ± 0.42** | 11.53 ± 1.44* | (4) |

[a]Measured as μM acetate $min^{-1}g^{-1}$ for tissue, and μM acetate $min^{-1}cm^{-3}$ for blood.

[b]Final cumulative dose of soman is $10.0\mu gkg^{-1}$.

[c]n is the same for spinal cord and caudate nucleus samples.

*$p<0.01$, **$p<0.05$, compared to respective controls, not significant compared to other values in respective groups.

Table 8: RFP frequency pre- and 30 seconds post 20Hz stimulation in the presence and absence of mecamylamine

| Mecamylamine dose ($mgkg^{-1}$) | (n) | FREQUENCY (Hz) preSTM (mean ± sem) | postSTM (mean ± sem) | % change (mean ± sem) |
|---|---|---|---|---|
| 0 | (3) | 767 ± 81.5 | 673 ± 78.3 | 12.3 ± 6.13 |
| 0.2 | (3) | 737 ± 55.2 | 670 ± 81.4 | 9.64 ± 4.84 |
| 0.5 | (3) | 745 ± 48.0 | 720 ± 32.3 | 2.95 ± 3.81 |
| 1.0 | (3) | 726 ± 55.0 | 734 ± 45.6 | -1.39 ± 3.68 |
| 2.0 | (3) | 730 ± 21.0 | 709 ± 21.0 | 2.80 ± 2.79 |

(Figs. 15 and 16). In the presence of 2.0mgkg$^{-1}$ DFP, mecamylamine still reduced the duration and number of RFP. However, two potentials remained preSTM and quickly recovered after 20Hz stimulation (Fig. 17).

As with mecamylamine, atropine sulfate failed to alter RFP frequency or percent change in frequency, however, duration and number of RFP were decreased preSTM (Fig. 18), though the percent change in these parameters was not significantly different from control values. Unlike mecamylamine, atropine sulfate did not antagonize the time-dependent recovery of RFP following 2.0mgkg$^{-1}$ DFP (Fig. 19).

Fig. 15: Mecamylamine antagonism of 20Hz stimulation induced depression of RFP duration

Upper graph: During 2Hz stimulation prior to 20Hz stimulation (open bars), mecamylamine reduces RFP duration. However, following 20Hz stimulation (dotted bars), mecamyalmine did not alter RFP duration until a dose of 2.0mgkg$^{-1}$ was attained. (Mean + sem, *$p<0.01$, **$p<0.05$.)

Lower graph: The antagonistic effect of mecamylamine on 20Hz stimulation-induced depression of RFP duration is illustrated by the reversal of the percent decrease in RFP duration following 20Hz stimulation to a percent increase (as denoted by a negative percent decrease) in the presence of mecamyalmine. (Mean ± sem, **$p<0.05$.)

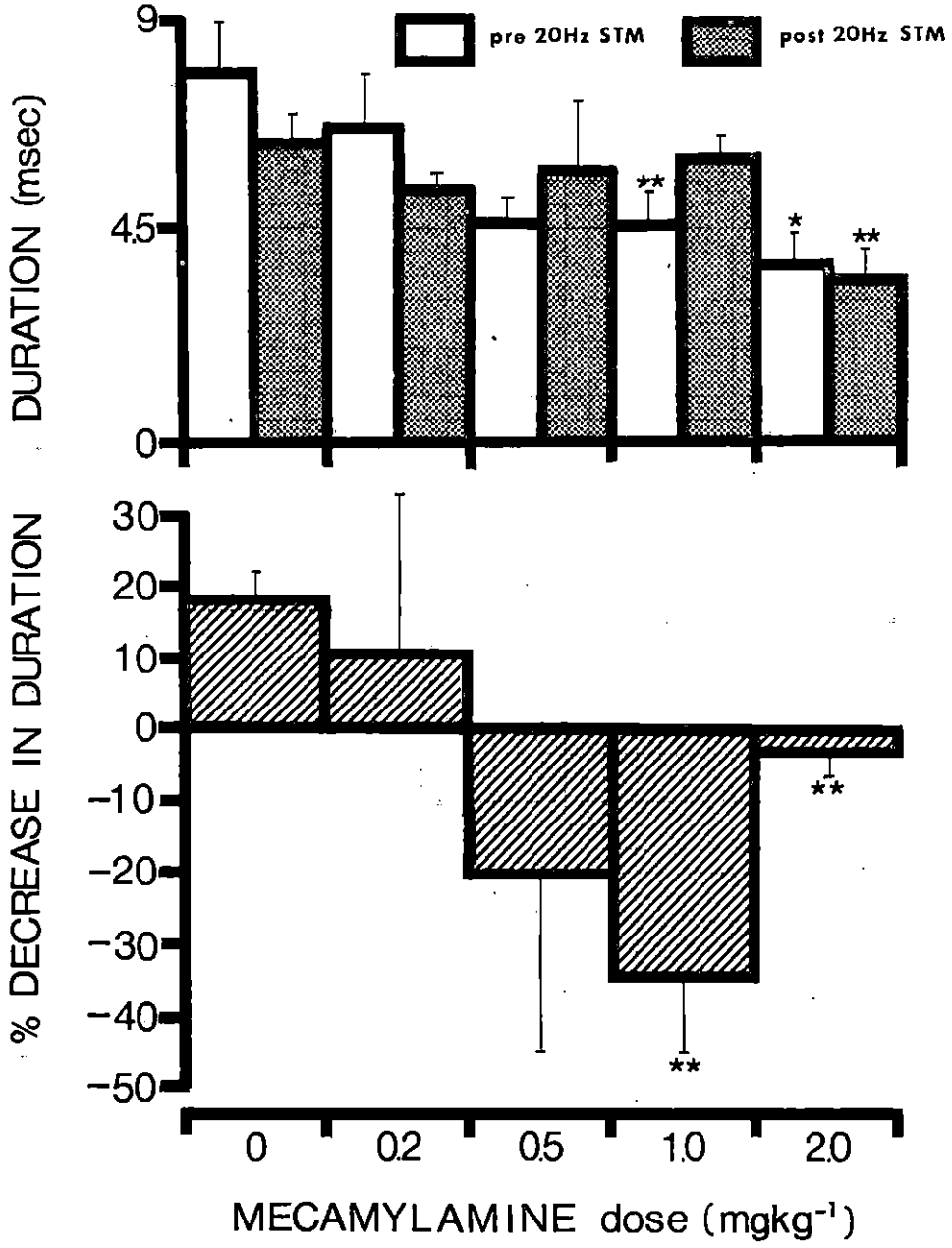

pre 20Hz STM
post 20Hz STM
DURATION (msec)
9
4.5
0
**
*
**
% DECREASE IN DURATION
30
20
10
0
−10
−20
−30
−40
−50
**
0
0.2
0.5
1.0
2.0
MECAMYLAMINE dose (mgkg⁻¹)

Fig. 16: Mecamylamine antagonism of 20Hz stimulation-induced depression of RFP number

Upper graph: The number of RFP during 2Hz stimulation prior to 20Hz stimulation (open bars) was significantly reduced by 2.0mgkg$^{-1}$ mecamylamine, as was RFP number after 20Hz stimulation (dotted bars). (Mean + sem, *p<0.01, **p<0.05,)

Lower graph: The antagonistic effect of mecamylamine on 20Hz stimulation-induced RFP depression is shown by the failure of 20Hz stimulation to cause a percent decrease in RFP number in the presence of 1.0 and 2.0mgkg$^{-1}$ mecamylamine. (Mean ± sem, *p<0.05.)

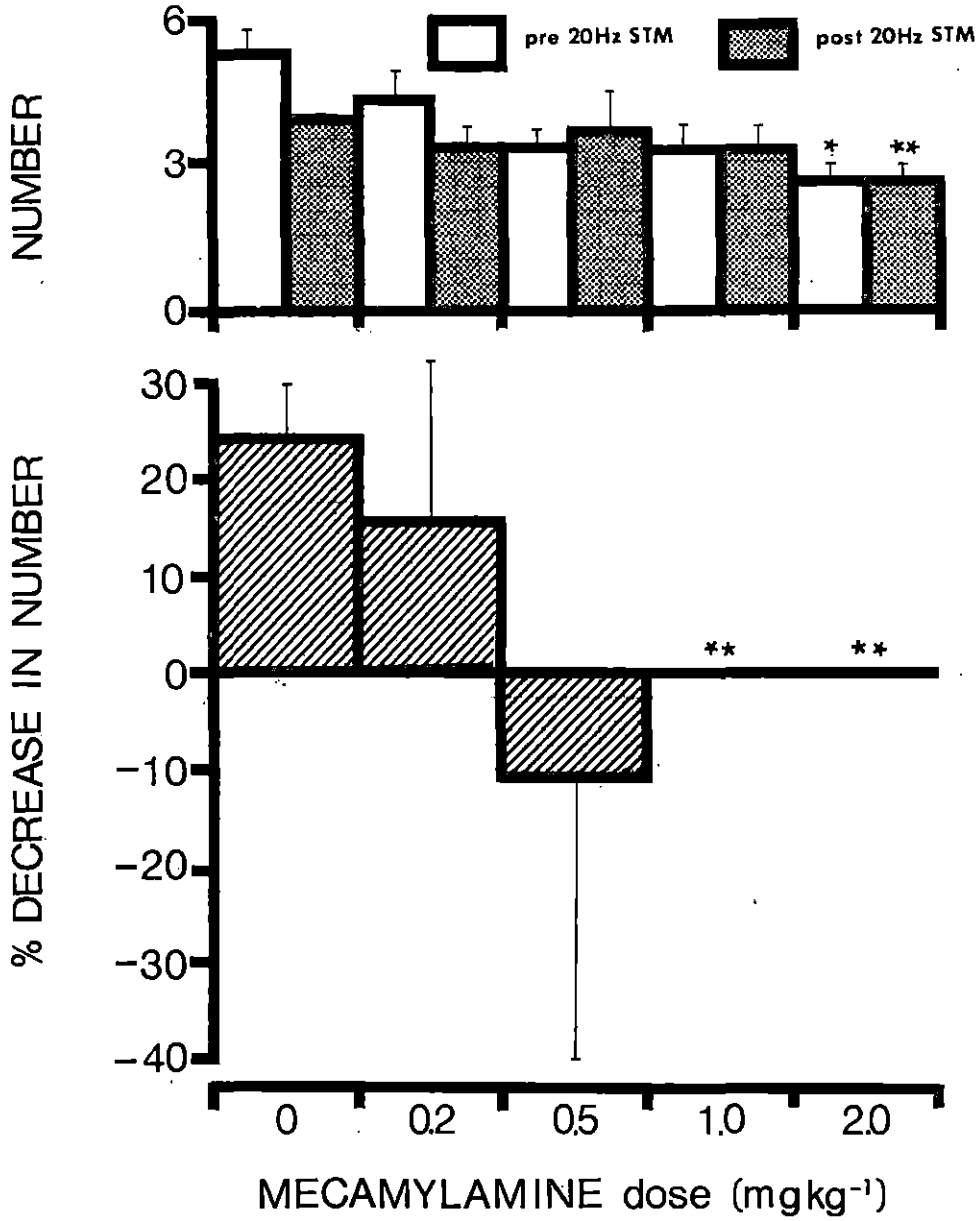

pre 20Hz STM
post 20Hz STM
NUMBER
6
3
0
*
**
% DECREASE IN NUMBER
30
20
10
0
−10
−20
−30
−40
**
**
0
0.2
0.5
1.0
2.0
MECAMYLAMINE dose (mgkg⁻¹)

Fig. 17: Effect of atropine sulfate (0.2 - 2.0mgkg$^{-1}$, i.v.) on RFP duration and number

Upper graph: Atropine caused a significant decrease in duration of RFP during 2Hz stimulation prior to 20Hz stimulation (open bars), but did not affect 20Hz stimulation-induced depression of RFP duration (dotted bars). (Mean + sem, *$p<0.01$, **$p<0.05$.)

Lower graph: Atropine also reduces RFP number during 2Hz stimulation prior to 20Hz stimulation (open bars), but did not affect this parameter after 20Hz stimulation (dotted bars). (Mean + sem, *$p<0.01$.)

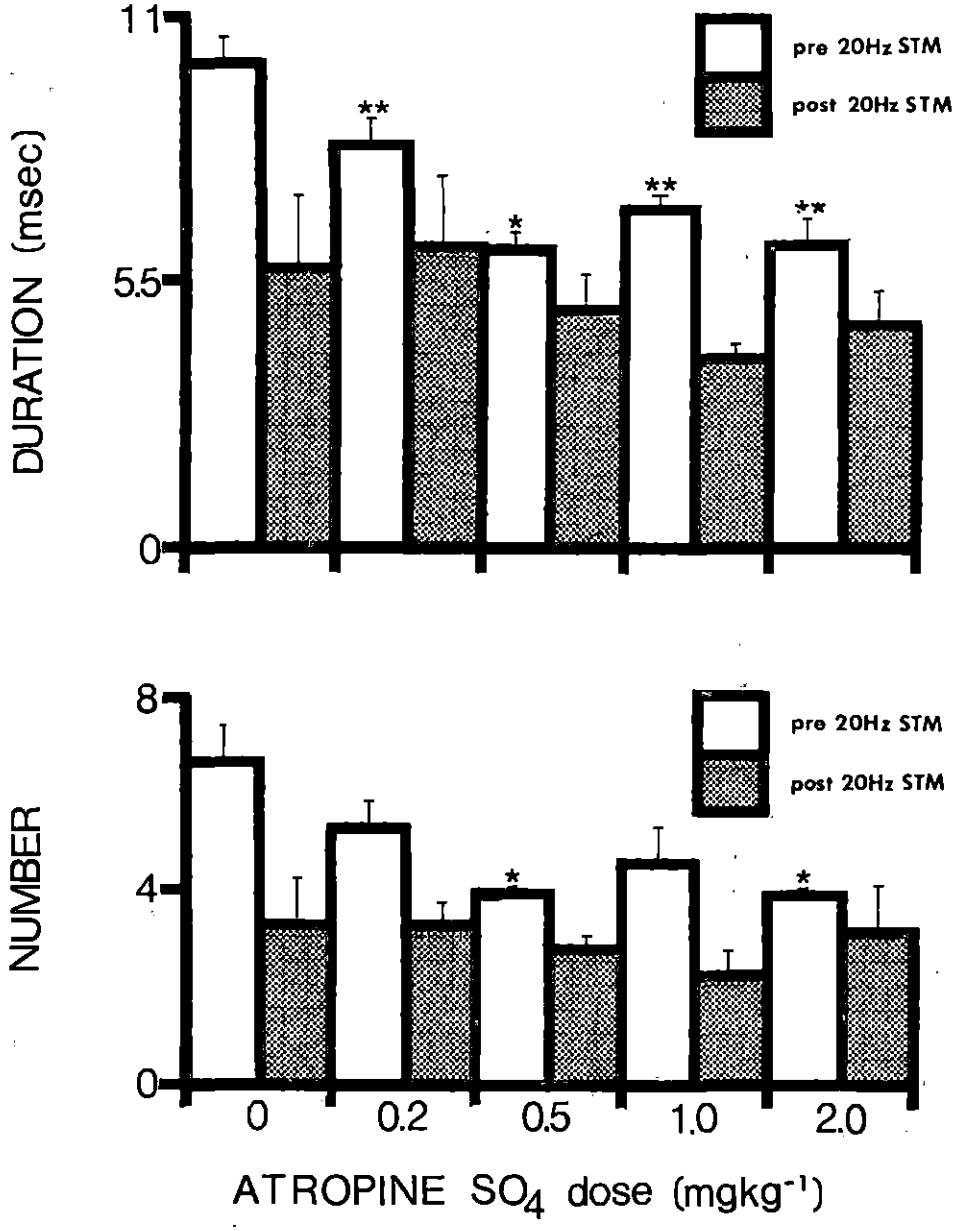

DURATION (msec)
11
5.5
0
pre 20Hz STM
post 20Hz STM
**
*
**
**
NUMBER
8
4
0
pre 20Hz STM
post 20Hz STM
*
*
0
0.2
0.5
1.0
2.0
ATROPINE SO4 dose (mgkg-1)

Fig. 18: Effect of mecamylamine on 20Hz stimulation-induced depression of RFP in the presence of DFP (2.0mgkg$^{-1}$, i.v.)

The top row shows the control recording of RFP during 2Hz stimulation (preSTM 20Hz), at 30 seconds of 20Hz stimulation (STM 20Hz) and recovery from 20Hz stimulation (postSTM 2Hz, 0sec, 30sec, 60sec). 30 minutes after administration of DFP (cumulative dose 2.0mgkg$^{-1}$, i.v., second row), RFP are inhibited at 30 seconds of 20Hz stimulation and do not recover up to 60 seconds after 20Hz stimulation. At 120 minutes after DFP administration in the presence of 0.5mgkg$^{-1}$, i.v., mecamylamine (third row), the number of RFP are reduced prior to 20Hz stimulation and two potentials recover quickly after 20Hz stimulation (note postSTM, 30sec). Increasing the dose of mecamylamine to 2.0mgkg$^{-1}$, i.v. (fourth row) has little further effect on RFP at 220 minutes after DFP injection. The time-dependent recovery of RFP from DFP facilitation of 20Hz stimulation observed in Fig. 11 is absent.

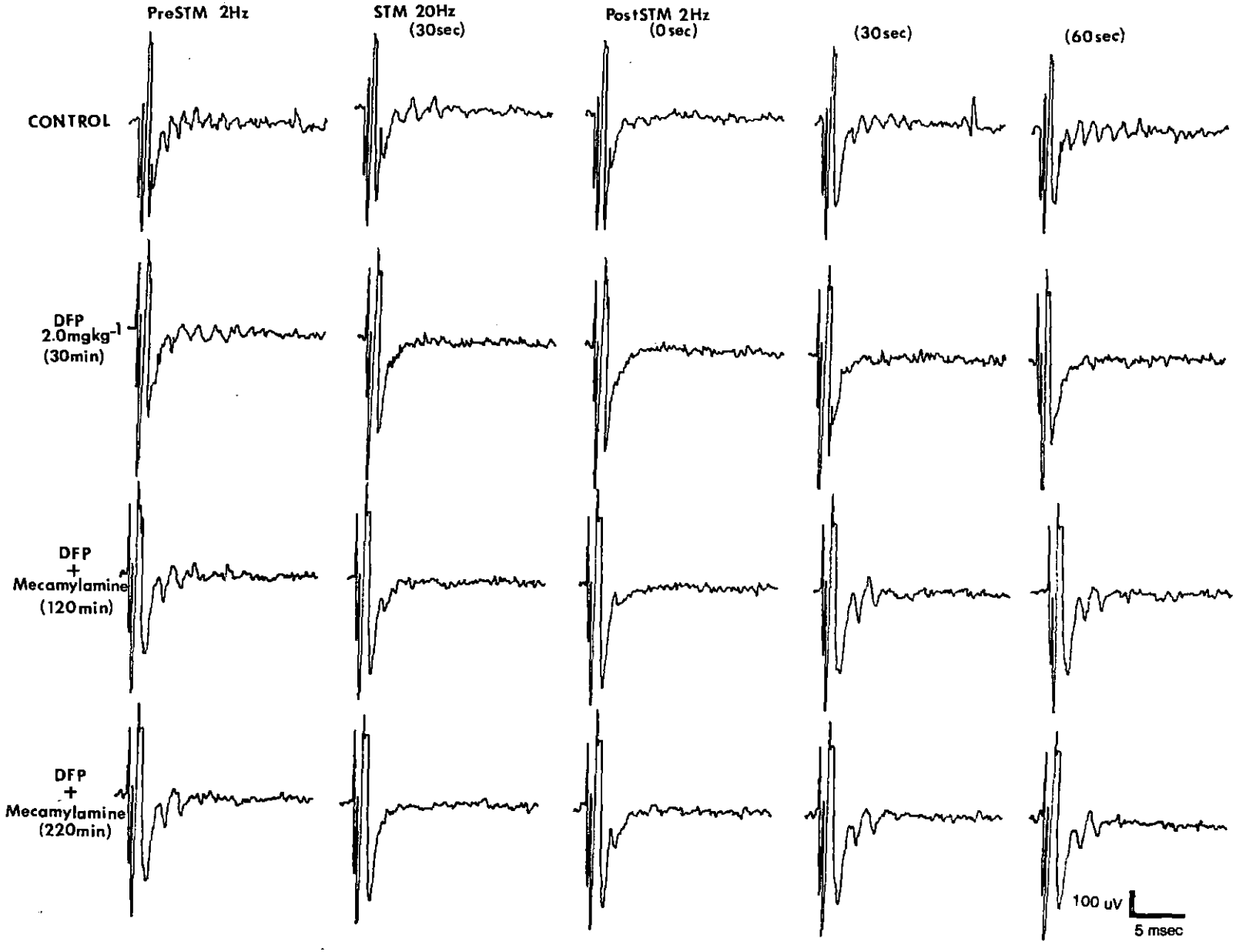
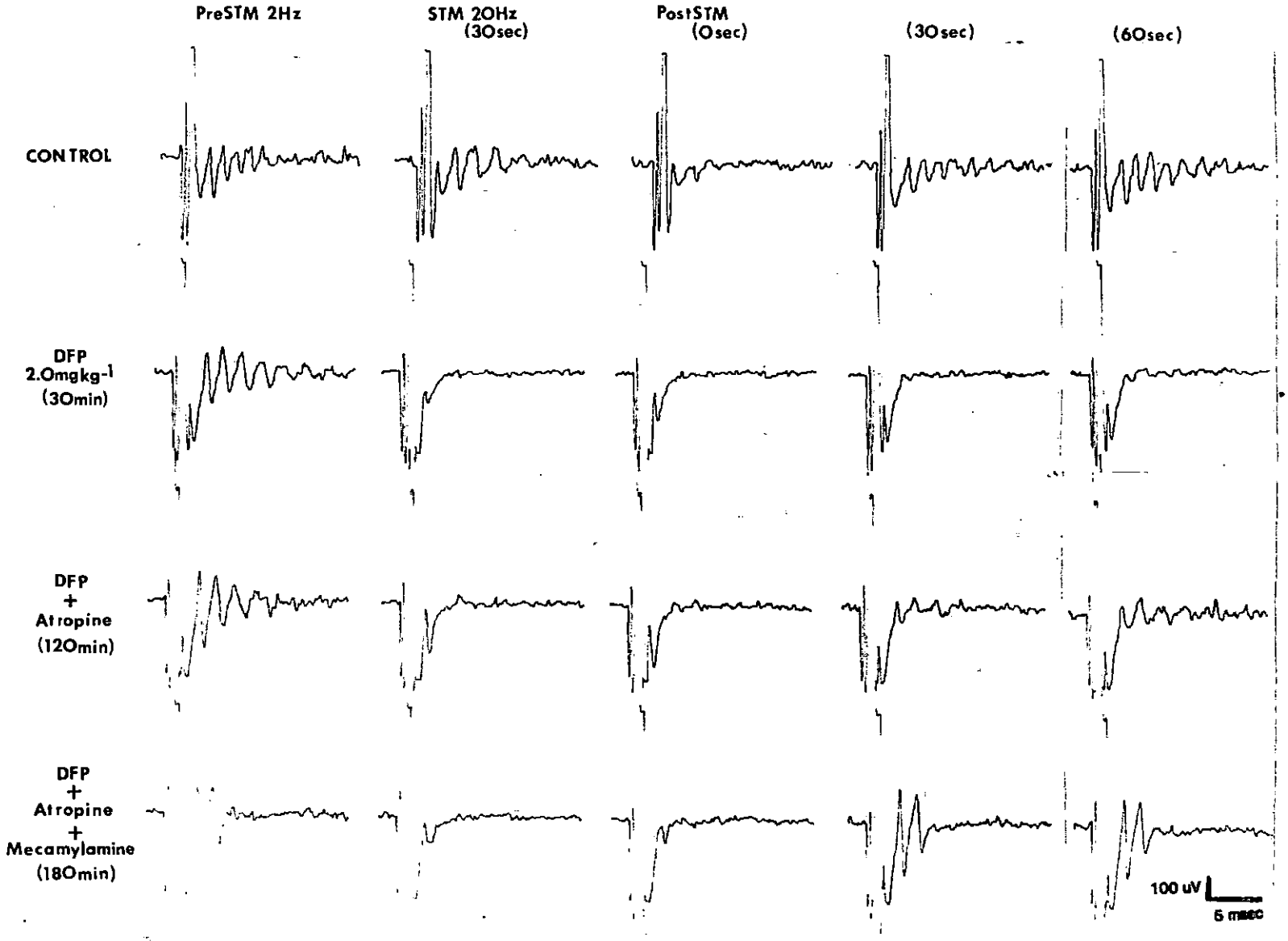

PreSTM 2Hz
STM 20Hz
(30sec)
PostSTM 2Hz
(0sec)
(30sec)
(60sec)
CONTROL
DFP
2.0mgkg-1
(30min)
DFP
+
Mecamylamine
(120min)
DFP
+
Mecamylamine
(220min)
100 uV
5 msec

Fig. 19: Effect of atropine sulfate alone and in combination with mecamylamine on 20Hz stimulation-induced depression of RFP in the presence of DFP ($2.0 mgkg^{-1}$, i.v.)

The top row shows the control recording of RFP during 2Hz stimulation prior to 20Hz stimulation (preSTM, 2Hz), at 30 seconds of 20Hz stimulation (STM 20Hz) and recovery from 20Hz stimulation (postSTM, 2Hz, 0sec, 30sec and 60sec). DFP ($2.0 mgkg^{-1}$, i.v.) facilitation of 20Hz stimulation-induced depression of RFP at 30 minutes post injection time (second row, c.f. Figs. 11 and 18) is observed. In the presence of atropine sulfate ($1.0 mgkg^{-1}$, i.v., third row), some recovery of RFP from DFP facilitation of 20Hz stimulation is still observed (c.f. Fig. 11) at two hours post injection of DFP. On the addition of mecamylamine ($1.0 mgkg^{-1}$, i.v., fourth row) however, the number and duration of RFP are reduced during 2Hz stimulation, but the remaining potentials recover quickly after 20Hz stimulation (c.f. Fig. 18).


PreSTM 2Hz
STM 20Hz (30sec)
PostSTM (0sec)
(30sec)
(60sec)
CONTROL
DFP 2.0mgkg-1 (30min)
DFP + Atropine (120min)
DFP + Atropine + Mecamylamine (180min)
100 uV
5 msec

# DISCUSSION

RFP are generated by the initial synchronous discharge of a relatively homogenous population of Renshaw cells (Eccles et al., 1954; Willis et al., 1969). In an attempt to relate the electrophysiological characteristics of Renshaw cell unit potentials (RUP) and RFP, VanMeter (1983a) demonstrated that in the cat lumbar spinal cord, RFP amplitude (and to a lesser extent, duration) increased as antidromic stimulus intensity increased, while RUP responded to increasing stimulus intensity by an increase in duration only. Thus, RFP amplitude and RUP duration are both functions of stimulus intensity, and while it may be hypothesized that RFP amplitude is therefore a function of RUP duration, the recruitment of higher threshold, or longer latency (Ross et al., 1973) Renshaw cells in response to increasing stimulus intensity may also contribute to increases in RFP amplitude.

That RFP mirror the activity of RUP is demonstrated by the fact that RUP and RFP respond to repetitive stimulation in the same manner (VanMeter, 1983a; Willetts and VanMeter, 1982). This response consists of depression during repetitive stimulation, and post-tetanic depression which may or may not be followed by post-tetanic potentiation. Similar effects of 20Hz stimulation were observed in this study (see Fig. 6, "Control"). The mechanism of this depression may be hypothesized as changes in receptor sensitivity in the presence of excess ACh. At the motor end plate, prolonged exposure to ACh causes receptor desensitization with failure of response and no conductance through ion-specific channels (see Thesleff, 1975). Alternatively, ACh accumulation at the synaptic cleft may decrease ACh release from the presynaptic terminal (see Szerb, 1975). This may reflect facilitation of

synaptic transmission resulting in enhanced recurrent IPSPs in motoneuron terminals.

Anticholinesterase agents such as physostigmine prolong the early discharge (i.e. before the pause) of Renshaw cells (Eccles et al., 1954; Longo et al., 1960; Curtis et al., 1961). In this study, RFP duration was not increased by physostigmine (Table 2), DFP (Fig. 9) or soman (Table 5), and the only measured effect of any of these compounds during 2Hz stimulation was the reduction in RFP frequency and number in the presence of DFP (Figs. 8 and 10). The cause of these reductions is obscure, but may represent some asynchronicity of the early discharge of RUP. One explanation for this phenomenon may be facilitation of synaptic transmission in the presence of excess ACh induced by DFP, resulting in more efficient recurrent inhibition of motoneurons and therefore raising the stimulus intensity required for generation of an action potential. Alternatively, these effects may be independent of cholinesterase inhibition (and therefore ACh accumulation) since physostigmine and soman did not produce this type of response. Such independent effects of DFP have been demonstrated at the motor end plate (Kuba and Albuquerque, 1973).

At a dose of $100 ug kg^{-1}$, physostigmine reduced the amplitude of the RFP (Fig. 2). Antagonism of RUP has been demonstrated with higher doses of physostigmine (VanMeter, 1983b). The reduced RFP amplitude may reflect reduced numbers of physostigmine-sensitive RUP contributing to the discharge.

In agreement with Eccles et al. (1954) who observed that in the presence of TEPP, RFP were inhibited during 8 and 27Hz stimulation, in this study, physostigmine and DFP enhanced the effects of 20Hz stimulation (Figs. 1, 2 and 11). This enhancement consisted of a decrease in RFP

number and duration following 20Hz stimulation in the presence of physostigmine (Fig. 4) and DFP (Figs 9 and 10). However, the effect of DFP on RFP frequency (Fig. 8) differed from that of physostigmine (Fig.3). At a dose of $1.0 mgkg^{-1}$ DFP, the percent decrease in RFP frequency following 20Hz stimulation was lower than in control recordings (Fig. 8), suggesting that DFP protected the RFP against 20Hz stimulation at this dose. Again, this may reflect some cholinesterase-independent effect of DFP, but as percent decreases in RFP number and duration were not similarly affected, this result is difficult to reconcile in terms of cholinesterase dependent or independent effects.

At the neuromuscular junction, it has been observed that two thirds of cholinesterase activity must be inhibited before any effects on muscle contraction are observed (Barstad, 1960). If this result is also applicable to spinal cholinergic transmission, it may explain why soman did not have significant effects on RFP (Tables 4, 5 and 6), since cholinesterase activity in spinal cord and caudate nucleus samples were reduced to only approximately 50% of control values (Table 7).

The recovery of RFP from DFP facilitation of 20Hz stimulation-induced RFP depression could not be explained in terms of recovery of cholinesterase activity (Table 3). This is in agreement with Jovič (1974) who showed there was a close relationship between signs of toxicity and cholinesterase inhibition during the first two hours post injection of soman in rats. However, from 2 - 24 hours after soman injection, the correlation between signs of toxicity and cholinesterase inhibition disappeared, there being a gradual decrease in severity of symptoms while cholinesterase activity was not restored. Jovič proposed that some "adaptation of synapses" was re-

sponsible for the recovery he observed, and this theory is upheld by the results presented here. While prolonged exposure to a depolarizing agent reduces the sensitivity of motor end plates to that depolarizing agent (see Thesleff, 1975), the exact mechanism of this response is not fully understood. It may be postulated that changes in nicotinic and muscarinic receptors of Renshaw cells (see Literature Review for references) could account for the recovery of the RFP. After repeated exposure to DFP, [$^3$H]QNB binding in the guinea pig brain is decreased (Grazit et al. 1979; Yamada, Isogai, Okudaira and Hayashi, 1983) and in the presence of disulfoton (an anticholinesterase), binding of [$^3$H]nicotine in the rat brain is also decreased (Costa and Murphy, 1983). These results indicate that certain anticholinesterases can reduce muscarinic and nicotinic receptor density in the CNS. However, the rapidity of the recovery of the RFP from DFP facilitation of 20Hz stimulation-induced RFP depression probably excludes reduction in receptor density as a mechanism of the observed adaptation.

Mechanisms of transmitter actions at receptor sites have recently been reviewed (McBurney, 1983; Brown, 1983; Siegelbaum and Tsien, 1983; see also Tuček, 1979). Much of the work on elucidating the mechanism of action of ACh has been performed at motor end plates, or using the patch clamp technique (see McBurney, 1983). Results of such studies indicate that at least two ACh molecules combine with one nicotinic receptor to induce channel opening and increase sodium and potassium permeability (Selyanko and Skok, 1979). Closure of channels, rather than falling transmitter concentration was deemed to be responsible for the decay of synaptic currents and it was also suggested that channels may exist in three states: open and "activable" closed but activable, and closed and non-activable (i.e. desensitized, see

McBurney, 1983 for references).

Amphetamine potentiates the antagonism of thalamocortical recruitment by sub-effective doses of physostigmine (VanMeter, 1977). Since thalamocortical recruitment may be mediated through a cholinergic system (VanMeter and Karczmar, 1971; VanMeter et al., 1978), it was suggested that a similar action of amphetamine might be observed on RFP. However, while amphetamine did not enhance the effects of physostigmine in this system (Fig. 5), amphetamine did enhance the recovery of RFP from 20Hz stimulation, in a dose-dependent manner (Figs. 6 and 7).

Amphetamine has been reported to evoke release of ACh from phrenic nerve terminals and reduce postsynaptic sensitivity to endogenously released ACh in rat phrenic nerve diaphragm preparations (Snider and Gerald, 1982). Cholinergic independent effects have also been reported at this site (Meldrum, Snider and Gerald, 1982) since, in the presence of $\alpha$- and $\beta$-bungarotoxins and (+) tubocurarine, amphetamine produces a biphasic response consisting of enhanced muscle contraction at low doses, while higher doses produce a concentration-dependent blockade of directly stimulated muscle contraction. These results were not altered in the presence of tyramine (an indirectly acting amine which has a similar mechanism of action to amphetamine in causing release of endogenous norepinephrine), phentolamine (an $\alpha$- adrenergic antagonist) or propanolol (a $\beta$- adrenergic antagonist), suggesting the effect is also independent of adrenergic mechanisms.

However, Meldrum et al. (1982) did observe that changes in ionic concentrations of the bathing medium or pretreatment with drugs affecting sodium movement across excitable membranes (e.g. local anesthetics, vera-

atridine) altered the muscle's response to amphetamine. Precisely how amphetamine exerts these cholinergic and adrenergic-independent effects at this muscle requires further investigation, but it seems probable that it acts via interaction with membrane ionic channels. Elucidation of this mechanism may help explain the action of amphetamine on RFP.

The early discharge of Renshaw cells is due to nicotinic receptor activation (see Literature Review for references). RFP also demonstrate a nicotinic component, since mecamylamine reduced RFP number and duration during 2Hz stimulation. In addition, mecamylamine offered some protection from 20Hz stimulation to the RFP by reversing the percent decrease in RFP duration and number following 20Hz stimulation (Figs. 16 and 17). However, mecamylamine did not completely abolish RFP (Figs. 16, 17 and 18). This is in agreement with the results obtained on RUP where mecamylamine (VanMeter, 1983b) and dHBE (Eccles et al., 1954) failed to antagonize the two initial spikes of the Renshaw cell burst. The two remaining potentials are not muscarinic in nature since they occur in the presence of atropine sulfate (Fig. 19). These results indicate a non-cholinergic component to the RFP and possible peptidergic involvement in cholinergic transmission at this site.

Substance P immunofluorescent fiber networks are found in close vicinity to motoneurons in cat ventral horns (for review, see Pernow, 1983). However, microiontophoretic application of substance P to Renshaw cells predominantly depresses Renshaw cell firing (see Literature Review for references). Thus, substance P may not contribute to the non-cholinergic component of RFP, but coexistence of ACh and several other peptides (e.g. vasoactive intestinal polypeptide, luteinizing hormone-releasing hormone)

has been demonstrated (for review, see Lundberg and Hökfelt, 1983). However, confirmation of a peptide role in Renshaw cell excitation awaits development of specific peptide antagonists.

Some sensitivity of the RFP to atropine sulfate was also observed (Fig. 19), in agreement with a muscarinic component to RUP (see Literature Review for references). However, as RFP represent the initial synchronous discharges of Renshaw cells (Eccles et al., 1954), and the muscarinic component of RUP is a late discharge (Curtis and Ryall, 1966c; King and Ryall, 1981), it is unclear whether atropine sensitivity of RFP represents the atropine sensitivity of RUP. Atropine also exhibits local anesthetic-like actions on Renshaw cells (Curtis and Phillis, 1960) which may explain these results. Alternatively, presynaptic muscarinic effects of atropine at motoneuron terminals might also be speculated as a causative factor.

In summary, RFP have been demonstrated to be similar to RUP in their sensitivity to repetitive stimulation and to drugs which modify cholinergic transmission. That RFP display muscarinic and nicotinic sensitivity is demonstrated by their response to atropine sulfate and mecamylamine. In addition, RFP demonstrate a non-cholinergic (atropine and mecamylamine resistant, amphetamine sensitive) component. An advantage of studying RFP rather than RUP was demonstrated by the recovery of RFP from DFP facilitation of 20Hz stimulation-induced RFP depression. It is unlikely that such a recovery could be demonstrated on single Renshaw cell units where recording periods are comparatively brief.

# REFERENCES

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# APPENDIX A

Stimulation and Recording Equipment

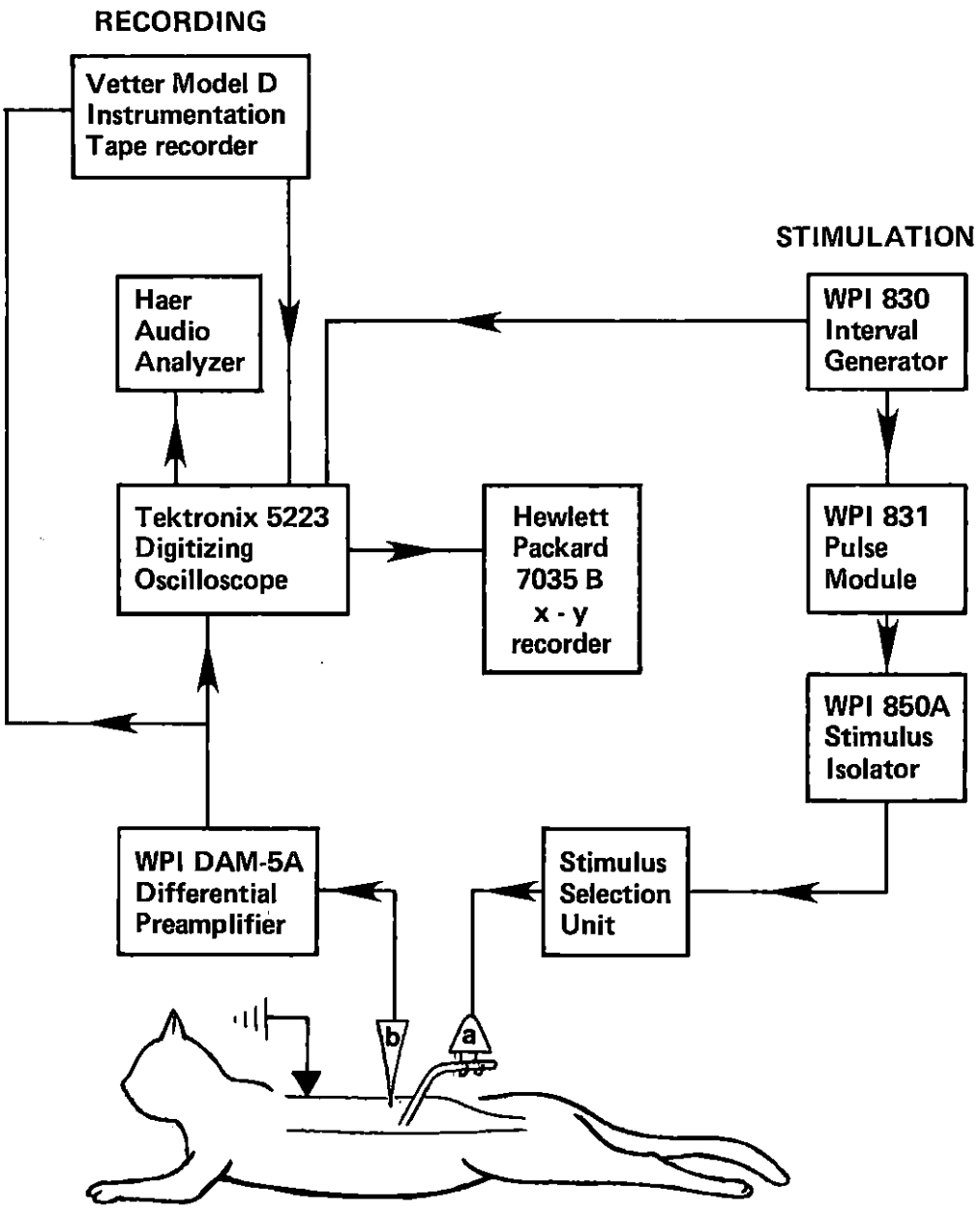


Fig. A-1: Block diagram showing equipment used for stimulation and recording of RFP.

a - bipolar stimulating electrode
b - glass recording microelectrode.

# APPENDIX B

## Determination of Cholinesterase Activity by pH Stat Method

### Apparatus

Radiometer Titrator TTT2

Radiometer Autoburette ABU11 (0.25$cm^3$ capacity)

Radiometer Titrigraph Pen Drive REA 300

Radiometer Servograph REC 51

pH electrodes

Radiometer micro-glass electrode G22c

Radiometer micro-calomel electrode K4112

Micro-reaction chamber (0.5 - 3.0$cm^3$ capacity) with magnetic stirrer

Water circulator at 30°C

Sonifier cell disrupter

### Reagents

NaCl (0.1M and 1.0M for tissue and blood samples respectively)

Medium (0.1M NaCl, 0.02M $CaCl_2$)

Titrant ($CO_2$-free 0.01M or 0.0193M NaOH for tissue and blood samples, respectively. Concentration is checked against potassium hydrogen phthalate standard)

Substrate (AChCl, 1.0M or 0.11M for tissue and blood samples, respectively)

Saponin (1% solution, to lyse blood cells)

Procedure

Blood is drawn into heparinized syringes and refrigerated overnight prior to assay. Tissue samples are quickly removed into liquid nitrogen and kept frozen until assay (usually 1 - 2 days).

Prior to assay, the electrodes are calibrated with pH 7.00 and pH 4.01 buffers and end point is set at pH 7.6. A continuous stream of nitrogen is begun over the reaction chamber.

Tissue samples are weighed to the nearest mg, placed in 10 volumes 0.1M NaCl and homogenized by ultrasonic cell disruption. Homogenized samples are kept on ice until assayed. $0.01cm^3$ tissue homogenate is added to $2.5cm^3$ medium (at $30^oC$) in a reaction vessel and the pH of the mixture brought up to pH 7.6 by manual addition of the titrant (the contents of the reaction vessel are stirred constantly). The apparatus is then set on automatic titration. Aliquots (0.1 - 0.3ul) of titrant are automatically added to the reaction vessel in response to a fall in pH below 7.6, and each addition is recorded. After recording spontaneous acid liberation for 3 - 4 minutes (or until linearity is obtained), $0.1cm^3$ substrate (ACh) is added. Acetic acid liberated by cholinesterase-catalyzed ACh hydrolysis is titrated against NaOH added to the reaction vessel in response to the fall in pH.

For blood samples, $4.5cm^3$ saponin is added to $0.5cm^3$ whole blood, mixed, and allowed to stand at room temperature for 10 minutes. $0.25\ cm^3$ NaCl is added, mixed, and $2.0cm^3$ of the solution is transferred to a reaction vessel. Titration proceeds as for tissue samples, but little spontaneous acid liberation is seen with blood samples.

From the slopes of the recorded lines, the rate of NaOH addition is

determined before and after ACh addition. The difference between the two rates is due to cholinesterase activity.

For tissue, cholinesterase activity is determined by the following equation:

$$\frac{\mu l\ NaOH\ min^{-1}\ (a - b)\ (\mu M\ NaOH\ ul^{-1})}{(vol.\ x\ conc.\ tissue\ homogenate\ used)} = \mu M\ NaOH\ min^{-1}\ g^{-1}\ tissue\ (_{weight}^{wet})$$

where: a is the rate of NaOH addition after ACh

b is the rate of NaOH addition before ACh

vol is $0.1cm^3$

conc is usually $100mgcm^{-3}$.

Since 1.0M NaOH is required to neutralize 1.0M acetic acid liberated by ACh hydrolysis,

$$\text{Cholinesterase Activity} = \mu M\ \text{acetate liberated}\ min^{-1}\ g^{-1}\ \text{tissue}\ (_{weight}^{wet})$$

Similarly for blood, cholinesterase activity is determined:

$$\frac{\mu l\ NaOH\ min^{-1}\ (a - b)\ (\mu M\ NaOH\ ul^{-1})}{(vol.\ blood)} = \mu M\ acetate\ min^{-1}\ cm^{-3}\ blood.$$