1-1-1983

The effects of the [alpha]-adrenergic agonist clonidine on the flexor reflex in intact and spinalized rats.

John Herr Kehne
University of Massachusetts Amherst

THE EFFECTS OF THE α-ADRENERGIC AGONIST CLONIDINE ON THE FLEXOR REFLEX IN INTACT AND SPINALIZED RATS

A Dissertation Presented

by

John Herr Kehne

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

February 1983

Psychology

THE EFFECTS OF THE α-ADRENERGIC AGONIST CLONIDINE ON THE FLEXOR REFLEX IN INTACT AND SPINALIZED RATS

A Dissertation Presented

By

JOHN HERR KEHNE

Approved as to style and content by:

Jerrold S. Meyer
Jerrold Meyer, Chairperson

Dorothy W. Gallager
Dorothy W. Gallager

Robert S. Feldman
Robert S. Feldman

Michael Davis
Michael Davis

Charles A. Sorenson
Charles A. Sorenson

Bonnie Strickland
Bonnie Strickland, Department Head
Psychology

ACKNOWLEDGEMENT

I would like to thank the members of my committee, Jerrold Meyer, Robert Feldman, Al Sorenson, Dorothy Gallager, and Michael Davis, for their input and patience during the course of this dissertation. I would especially like to thank Michael Davis, for more-than-generous financial support, and for a constant flow of ideas, one of which provided the impetus for this study. Finally, thanks to Randy Commissaris for "Asteroids", to the Loctite Company for its miracle adhesive, and to Susan Dolan for her patience and encouragement throughout the course of this work.

ABSTRACT

The effects of the α-adrenergic agonist clonidine on the flexor reflex in intact and spinalized rats

John Herr Kehne, B.A., Amherst College

M.A., University of Massachusetts, Ph.D., University of Massachusetts

Directed by: Professor Jerrold Meyer

A series of experiments investigated the effects of the α-adrenergic agonist clonidine on the flexor reflex (FR) in spinalized and intact rats. Clonidine produced a dose-dependent facilitation of the FR in spinalized rats, whereas it produced a dose-dependent inhibition in intact rats (Expt. 1). The facilitatory effect was completely blocked by pretreatment with the α_1-adrenergic antagonist prazosin, but not by the α_2-adrenergic antagonist piperoxane whereas the inhibitory effect was blocked by piperoxane but not by prazosin (Expt. 2). Intraventricular, but not intrathecal, administration of the α_2-adrenergic agonist oxymetazoline produced a dose-dependent depression of the FR in intact rats (Expt. 3) that was reversed by piperoxane (Expt. 4). Two hypotheses that would explain the shift in the effects of clonidine from α_2-adrenergically mediated inhibition in intact rats to α_1-adrenergically mediated excitation in spinalized rats were tested. An increase in the number of α_1-adrenergic receptor sites (Expt. 6) and a delayed onset of the excitatory effect of clonidine following transection (Expt. 5) were consistent with the rapid development of α_1-adrenergic supersensitivity. However, halothane was shown to have residual effects which interfered with the expression of

the excitatory effect of clonidine (Expt. 8) and intrathecal administration of the α_1-adrenergic agonist phenylephrine failed to produce an enhanced FR response in spinalized rats relative to intact rats (Expt. 7). Blockade of spinal impulse flow with intrathecal procaine (Expt. 9) or by spinal ligation (Expt. 10) (in which the residual effects of halothane were ruled out) was found to produce an immediate shift of the FR from inhibition to excitation. The magnitude of clonidine's facilitatory effect was similar fifteen minutes or two hours following ligation (Expt. 11); furthermore, no change in α_1-adrenergic receptor binding was seen at these two times (Expt. 12). These data indicate that the shift in clonidine's effects from inhibition in the normal rat to excitation in the spinalized rat is attributable to removal of a supraspinal α_2-adrenergic inhibitory system which, when activated in the intact rat, prevents the expression of the excitatory effect of α_1-adrenergic stimulation. Finally, clonidine still depressed the FR in decerebrate rats (Expt. 13), suggesting that the supraspinal site mediating clonidine's inhibitory action is located in the caudal regions of the brainstem.

TABLE OF CONTENTS

APPROVAL i

ACKNOWLEDGEMENT iii

ABSTRACT iv

LIST OF TABLES ix

LIST OF FIGURES x

CHAPTER
I. NEUROCHEMICAL MODULATION OF REFLEX BEHAVIOR 1

Descending Serotonergic Neurons 3
Anatomy 3
Modulation of Spinal Reflexes 4
Whole Limb Extensor Reflexes 4
Whole Limb Flexor Reflexes 4
Individual Flexor Muscles 6
Stretch Reflexes 8
Modulation of Other Behaviors 10
Post-decapitation Reflex 10
Spontaneous Activity 11
Modulation of Sensory Transmission (Pain) 12
Supersensitivity 13
Transection 13
Neurotoxins 14
Neurophysiology (Evoked Potentials) 15
Monosynaptic Reflexes 15
Recurrent Inhibition of the Extensor MSR 17
Presynaptic Inhibition of the Extensor MSR 18
Bulbospinal Inhibition of the MSR 18
Polysynaptic Reflexes 20
Single Cell Activity 20
Dorsal Horn 22
Summary 23

Descending Noradrenergic Neurons 24
Anatomy 24
Whole Limb Flexor Reflexes 25
Individual Flexor Muscles 26
Stretch Reflexes 27
Post-decapitation Reflex 29
Spinal Stepping 29
Modulation of Sensory Transmission (Pain) 30
Supersensitivity 30
Neurophysiology 31
Monosynaptic Reflexes 31

Polysynaptic Reflexes 32
Single Cell Activity 32
Summary 33

Descending Dopaminergic Neurons 34
Anatomy 34
Modulation of Spinal Reflexes 35
Whole Limb Flexor Reflexes 35
Single Muscle Flexor Reflex 35
Stretch Reflexes 35
Modulation of Sensory Information (Pain) 36
Supersensitivity 36
Modulation of Other Behaviors 37
Spontaneous Activity 37
Neurophysiology 37
Monosynaptic Reflexes 37
Single Cell Activity 38
Summary 38

Spinal GABAergic Neurons 39
Anatomy 39
Modulation of Spinal Reflexes 39
Whole Limb Flexor Reflexes 39
Single Muscle Flexor Reflexes 39
Stretch Reflexes 40

Spinal Glycinergic Neurons 40
Anatomy 40
Modulation of Spinal Reflexes 41
Flexor Reflexes 41
Stretch Reflexes 41
Summary (GABA and Glycine) 41

Other Neurochemical Systems 42
Cholinergic Neurons 42
Peptides: Substance P and Thyrotropin Releasing Hormone . 42

Spinal Neurochemical Modulation of the Acoustic Startle Reflex 44
Serotonin 45
Dopamine 46
GABA 46
Glycine 46
Norepinephrine 47
Spinal Neurochemical Modulation: A Comparison of Flexor and Startle Reflexes 48

II. THE EFFECTS OF THE α-ADRENERGIC AGONIST CLONIDINE ON THE FLEXOR REFLEX IN INTACT AND SPINALIZED RATS 52

Experiment 1: Effects of clonidine on the flexor reflex in intact and spinally-transected rats 52

Experiment 2: Piperoxane and prazosin antagonism of clonidine's effects on the flexor reflex in intact and spinalized rats 58

Experiment 3: Effects of intrathecal or intraventricular oxymetazoline on the flexor reflex in intact rats 63

Experiment 4: Piperoxane antagonism of oxymetazoline's effect on the flexor reflex 64

Experiment 5: Effects of clonidine on the flexor reflex at different times following spinalization . . . 67

Experiment 6: ^{3}H-prazosin and ^{3}H-clonidine receptor binding in spinalized vs. intact rats 69

Experiment 7: Effects of intrathecal phenylephrine on the flexor reflex in intact and spinalized rats . 75

Experiment 8: Effects of re-exposure to halothane anesthetic on the clonidine induced facilitation of the flexor reflex in spinalized rats 78

Experiment 9: Intrathecal procaine-induced reversal of effects of clonidine 80

Experiment 10: Spinal ligation-induced reversal of effects of clonidine on the flexor reflex in intact rats. 87

Experiment 11: Effects of clonidine on the flexor reflex 15 minutes or 2 hours following spinal ligation . 86

Experiment 12: ^{3}H-prazosin and ^{3}H-clonidine binding at 15 minutes or 2 hours following spinal ligation . 87

Experiment 13: Effects of clonidine on the flexor reflex in acutely-decerebrated rats 89

III. GENERAL DISCUSSON 92

BIBLIOGRAPHY 105

APPENDIX 114

LIST OF TABLES

1. A Comparison of the Effects of Drugs on the Flexor and Startle Reflexes 123

2. Effects of Intraventricular Administration on Rats Pretreated With Piperoxane 125

LIST OF FIGURES

1. Effects of Clonidine on the Flexor Reflex in Spinalized and Intact Rats 126

2. Dose-Dependent Effects of Clonidine on the Flexor Reflex in Intact and Spinalized Rats (Change Scores). 128

3. Dose-Dependent Effects of Clonidine on the Flexor Reflex in Intact and Spinalized Rats (Absolute Scores). 130

4. Effects of Clonidine on the Flexor Reflex in Rats Pretreated With Piperoxane or Prazosin (Absolute Scores) 132

5. Effects of Clonidine on the Flexor Reflex in Rats Pretreated With Piperoxane or Prazosin (Change Scores) 134

6. Effects of Intraventricular and Intrathecal Infusion of Oxymetazoline on the Flexor Reflex in Intact Rats 136

7. Effects of Clonidine on the Flexor Reflex at Different Times Following Spinalization 138

8. Scatchard Analysis of (^{3}H)-Prazosin Binding in Spinalized Spinalized and Intact Rats 140

9. Scatchard Analysis of (^{3}H)-Clonidine Binding in Spinalized Spinalized and Intact Rats 142

10. Scatchard Analysis of (^{3}H)-Prazosin Binding in Low-Spinalized and Intact Rats 144

11. Effects of Intrathecal Phenylephrine in Spinalized and Intact Rats 146

12. Comparison of Intrathecal Phenylephrine and Intraperitoneal Clonidine on the Flexor Reflex in Spinalized Rats 148

13. Effects of Intrathecal Procaine on the Flexor Reflex in Rats Pretreated With Clonidine 150

14. Effects of Spinal Ligation on the Flexor Reflex in Rats Pretreated With Clonidine 152

15. (^{3}H)-Prazosin and (^{3}H)-Clonidine Binding 15 Minutes or or 2 Hours Following Spinal Ligation 154

16. Effects of Clonidine on the Flexor Reflex in Decerebrate Rats .156

CHAPTER I

NEUROCHEMICAL MODULATION OF REFLEX BEHAVIOR

The science of psychopharmacology is concerned with analyzing the effects of drugs on behavior. Drugs are utilized as tools for manipulating the activities of specific neurotransmitter systems. The resultant behavioral changes that accompany drug administration can thus give a clue as to how neurotransmitter systems modulate behavior.

In the course of its rather brief development, psychopharmacology has focused on the brain mechanisms that underlie drug induced changes in behavior. With the refinement of anatomical and biochemical techniques for identifying discrete neurotransmitter pathways in the central nervous system (CNS) has come a realization that many of the identified transmitter systems that project throughout the brain also project into the spinal cord.

Norepinephrine (NE), serotonin (5-HT), and even dopamine (DA)-containing neurons have specific spinal projections from their cell bodies in the brainstem (Ungerstedt, 1972; Nygren and Olson, 1977; Bowker et al., 1981). In addition, other neurotransmitters, such as the inhibitory transmitters λ-aminobutyric acid (GABA) and glycine, are known to be released by spinal interneurons (Barber and McLaughlin, 1980). Therefore, it is quite reasonable to think that the spinal cord might be an important site where neural modulation by various neurochemical systems takes place.

In fact, physiologists and pharmacologists have been the first to recognize that a great deal of neurochemical modulation takes place at

the level of the spinal cord (see Fed. Proc. Symposium, 40, 13). Spinal reflexes have been extensively utilized for evaluating the effects of drugs, primarily because they provide relatively simple stimulus-response systems (e.g. Jurna, 1981). The neural machinery for the spinal reflex, the basic unit of motor organization in the CNS, is present at the segmental level and can be analyzed pharmacologically, electrophysiologically, and functionally. Importantly, the spinal cord can be analyzed in the absence of 'higher' neural influences from the brain using the spinally-transected preparation. By using drugs as tools to alter transmission in neurochemical systems that are either extrinsic (i.e. that descend from the brain) or intrinsic to the spinal cord, one can determine how specific neural systems modulate reflex transmission at the spinal level.

The information provided by this type of analysis may be of general significance in providing information relevant to the study of complex behaviors that require sensorimotor integration at the supraspinal level. Since the spinal cord is the final output for most behavior, it is reasonable to think that neurochemical modulation that occurs at spinal levels could exert important, general influences on behavior. For example, learning an avoidance task may require conditioning that takes place in the brain, yet because the performance of the actual response requires activation of α-motoneurons in the spinal cord, the <u>expression</u> of the response may be altered by activity in a <u>spinal</u> neurochemical system.

The purpose of this chapter is to review behavioral, anatomical, pharmacological and electrophysiological studies which have

investigated the spinal function of the major identified neurochemical systems (norepinephrine, dopamine, serotonin, GABA, acetylcholine, glycine). Though the focus will be predominantly on spinal reflex behavior, mention will be made of studies that have implicated these neurochemical systems in the modulation of other types of spinally-mediated motor activity and in sensory processing (pain). It is hoped that this approach will acquaint the reader with the complexity and potential importance of neurochemical modulation in the spinal cord.

Descending Serotonergic Neurons

Anatomy. The spinally-projecting 5-HT pathways have been classically associated with the more caudally-located medullary raphe nuclei (B_1-B_3) (using the terminology of Dahlstrom and Fuxe, 1965). The raphe magnus projects via the dorsolateral funiculus to the substantia gelatinosa (an area shown to be critical in the modulation and transmission of sensory information--especially pain signals--to the brain). 5-HT axons from the raphe pallidus and raphe obscurus descend in the lateral and ventral funiculi to innervate the ventral horn, as well as the sympathetic lateral column (cf. Gilbert et al., 1982). Recent work using combined horseradish peroxidase and 5-HT immunocytochemistry has shown a heretofore unknown descending projection originating from more rostrally located 5-HT nuclei in the midbrain reticular formation (Bowker et al., 1981). Interestingly, this projection appears to innervate only the cervical cord.

Measurements of 5-HT levels following transection of the spinal

cord at the cervical level have shown that most, if not all, of the intrinsic spinal 5-HT is contained within descending fibers (Carlsson et al., 1963). However, a recent report has indicated that a small number of 5-HT neurons may be intrinsic to the spinal cord, at least in monkeys (La Motte et al., 1981).

Modulation of spinal reflexes.

Whole limb extensor reflexes. The early work of Anden and his colleagues suggested that increasing spinal 5-HT transmission resulted in an enhancement of hindlimb extensor reflexes. Hindlimb extension was semi-quantitatively estimated by observing the reflex response that resulted from pinching the base of the tail of the rat. Using this measure, it was found that administration of the 5-HT precursor 5-hydroxytryptophan (in combination with the monoamine oxidase inhibitor nialimide) produced a dose-dependent increase in the extensor reflex. Other drugs purported to have a 5-HT agonist or mixed-agonist properties, i.e. LSD, N, N-dimethyltryptamine (DMT; 10-50 mg/kg), and psilocybin (1-50 mg/kg) also produced an enhancement of extensor reflexes (Anden et al., 1967; Anden et al., 1971). The effect of 5-HTP was blocked by pretreatment with a decarboxylase inhibitor, whereas pretreatment with the α-noradrenergic receptor blocker phenoxybenzamine, or the dopamine receptor blocker haloperidol had no effect. More recently, the 5-HT agonist 5-methoxy-dimethyltryptamine (5-MeODMT) has also been found to increase the extensor reflex using this model (Fuxe et al., 1972; Fuxe et al., 1974).

Whole limb flexor reflexes. In their studies on the hindlimb extension reflex, Anden and his colleagues commented that spinal 5-HT exerted little or no influence on flexor reflexes. Using a semi-quantitative evaluation scale (observer estimation of the magnitude and force of toe-pinch induced flexor withdrawal), it was reported tht 5-HTP (plus the peripheral decarboxylase inhibitor nialimide), LSD, and methysergide all failed to produce a clear increase of the flexor reflex. Certainly, any effects seen were little in comparison to the activation seen following norepinephrine stimulation (see NE section).

More recently, using different techniques of measurement and different means for eliciting the flexor reflex, it has been reported that 5-HT activation facilitates this reflex. Nozaki et al. (1977) measured whole limb contraction by attaching the hindlimb to a force transducer. Flexor contraction was elicited by presenting electrical stimuli through electrodes implanted subcutaneously in the plantar region of the hindpaw of the rat. LSD (10 ug/kg) increased flexor contraction (Nozaki et al., 1977); similar findings were earlier reported in spinal cats (Little et al., 1957). This facilitation was blocked by pretreatment with the 5-HT antagonist cyproheptadine, which by itself has no effect on the reflex (Nozaki et al., 1977). Interestingly, 5-HTP produced a facilitation in rats that had been chronically, but not acutely transected, a finding attributed to the development of denervation supersensitivity in the chronically transected preparation (see section on supersensitivity). Tryptamine was also found to activate the whole limb flexor reflex, and this effect was reversed by cyproheptadine.

Individual flexor muscles. Maj and his coworkers have carried out numerous studies utilizing a single flexor muscle in the hindleg (the tibialis anterior) to study 5-HT modulation of the flexor reflex. The single muscle was dissected free from the limb by cutting the tendon, and tied to a force transducer for measurement of the contraction. The eliciting stimulus was a short duration (5 msec) electrical pulse to the plantar region of the hindpaw. Using this preparation, it has been shown that increasing 5-HT transmission in the spinal cord results in a facilitation of flexor reflex activity.

Administration of LSD (2 - 10 ug/kg) increased the contraction of the tibialis anterior flexor reflex, and this increase was blocked by cyproheptadine or metergoline (Maj et al., 1976). This activation was not attenuated by treatment with the α-noradrenergic receptor blocker phenoxybenzamine, nor the dopamine receptor blocker haloperidol (Maj et al., 1976). Surprisingly, however, the "atypical" neuroleptic clozapine and the potent DA antagonist pimozide were effective in blocking the LSD effect, leading the authors to conclude that these agents possessed some 5-HT antagonist properties.

LSD facilitation of the flexor reflex has also been reported using a different flexor muscle, the semitendinosus (cf. unpublished findings, Maj et al., 1976). Thus, LSD produced qualitatively similar responses on both single muscle and whole limb flexor reflexes, with the exception of the studies using the toe-pinch induced reflex.

Other 5-HT agonists have been reported to facilitate the single muscle flexor reflex. Mescaline was found to produce a rapid onset, long duration (60 - 90 minutes) increase in the tibialis anterior

response (Maj et al., 1977) as did quipazine (Palider and Rawlow, 1977). Both of these effects were blocked by cyproheptadine. Furthermore, using electromyographic (EMG) discharge in the psoas flexor muscle as their measure of flexor activation, Austin et al. (1976) found that the 5-HT agonist 5-MeODMT produced a short-lasting, rapid onset facilitation.

Using a similar preparation in the cat as that used by Maj et al. in the rat, Marley and Vane (1967) reported that 5-HT (0.5 - 2.0 mg/kg), DMT (2.0 mg/kg) and tryptamine produced increases in the tibialis flexor reflex. The higher doses of tryptamine and 5-HT produced a biphasic effect on the reflex, that is, an initial increase followed by depression and then recovery. The depression was not attributable to actions of metabolites, since infusions of various metabolites were without effect. The tryptamine effect was blocked by methysergide (but not phenoxybenzamine) and increased by nialimide.

Drugs purported to enhance the terminal release of 5-HT, e.g. para-chloroamphetamine (PCA) and fenfluramine, were also found to produce an increase in the amplitude of the tibialis anterior flexor reflex (Maj et al., 1976; Maj et al., 1977), and these responses were cyproheptadine or metergoline reversible. Again, however, complete specificity was not found, in that phenoxybenzamine and clozapine (but not haloperidol) blocked the excitatory effects of the two drugs. Clozapine has been reported to have some α-adrenergic antagonist actions (Bartholini, 1973), a property in common with phenoxybenzamine that might explain their abilities to block the effects of PCA and fenfluramine. In support of this idea, pimozide (which has both

dopamine and norepinephrine antagonist properties), but not haloperidol, blocked the effect of fenfluramine.

Finally, adminstration of 5-HT precursors 5-HTP and l-tryptophan were found to increase the tibialis anteror flexor reflex (Maj et al., 1976), and these effects were blocked by cyproheptadine. The increase following 5-HTP administration is in contrast to the negative results reported using the whole limb flexor reflex (Nozaki et al., 1977). Both preparations were acutely spinalized, suggesting that time after transection was not a factor. The different effects obtained may represent an instance in which a drug effect on the single muscle and whole limb response is not qualitatively similar.

Stretch reflexes. The previous sections have described the effects of increasing 5-HT activity on flexion and extension reflexes that are elicited by cutaneous stimuli (pressure, electrical stimulation of skin receptors). These reflexes are mediated through polysynaptic circuits. Reflex activation of muscles can also be achieved through stretch-induced activation of the "λ-loop". This loop involves a simple two-neuron, monosynaptic pathway from the primary (Ia) afferent to the λ-motoneuron. Stretching a muscle results in compensatory contraction of the muscle through a feedback loop from the muscle spindle to the α-motoneuron. Importantly, the sensitivity of the stretch reflex can be altered by changing the sensitivity of the muscle spindle through activation of the fusimotor fiber system ("λ-efferent" system). Activation of λ-motoneurons in the ventral horn activates the fusimotor fibers of the muscle spindle, changing the sensitivity of the spindle

such that, when the muscle is stretched, there is a greater discharge in the Ia afferent fibers and (via a monosynaptic activation of α-motoneurons) a greater contraction of the muscle. The λ-motoneurons can be activated by neurons that descend from the brain, thereby changing the "bias" on the muscle spindle and increasing the excitability of the muscle without causing direct contraction of the muscle.

Although tryptamine was reported to be ineffective in increasing the tail-pinch induced extensor reflex (Anden et al., 1971), it has been found effective in increasing the patellar stretch reflex in man (Martin and Sloan, 1970). Similarly, the tendon jerk reflex in spinal cats has been reported to show an enhancement following administration of 10 ug/kg LSD (Martin and Sloan, 1970). One possible site of drug action is at the level of the α-motoneuron (5-HT modulation could directly, or indirectly through interneurons influence the activity of the α-motoneuron). Alternatively, 5-HT activation could cause a facilitation of λ-motoneuron activity which would activate the "λ-loop". As will be seen later, there is evidence for 5-HT modulation at both the level of the α- and λ-motoneuron.

Following transection of the spinal cord, there is a loss of the tonic stretch reflex in the extensor soleus muscle. Ahlman and Grillner (1971) have shown that administration of 5-HTP restores this reflex, and concomitantly produces a "resting discharge" in static λ-efferent motoneurons in the ventral horn of the cord. This activation is reversed by 5-HT antagonists.

Recently, Commissiong and Sedgwick (1979) have suggested that

spinal 5-HT exerts inhibitory effects on the stretch reflex of the extensor gastrocnemius-soleus complex, in that 5-HTP administration produced a potent, dose-dependent inhibitory effect on the stretch reflex. Importantly, this work was carried out in decerebrate animals, in contrast to the studies by Ahlman and Grillner, which were carried out in spinalized preparations. Furthermore, it was reported that l-DOPA produced a biphasic effect on the stretch reflex (activation followed by inhibition), and that prior treatment with 5,6-DHT abolished the late inhibitory, but not the early excitatory effect. This finding provided further support for the hypothesis that activation of the 5-HT system in the decerebrate animal resulted in a depression of the stretch reflex.

Whether the opposite functional effects of 5-HT transmission in the decerebrate and spinal animal are due to additional actions of 5-HTP not present in the spinal preparation, or whether they are due to a fundamental change in the mechanism of action of 5-HTP remains to be determined.

Modulation of other behaviors.

Post-decapitation reflex. The post-decapitation kicking response (PDR) is a series of rapid, alternating flexions and extensions of the hindlimbs that begin immediately following decapitation and last for approximately 20 seconds. The occurrence of the PDR has been attributed to two fundamentally different mechanisms. On the one hand, it has been suggested that the reflexive kicking results from a

transection-induced "release" from tonic supraspinal inhibition (Kamut and Sheth, 1971). Alternatively, the transection might serve to stimulate cerebro-spinal fiber tracts, causing a release of transmitter in the spinal cord that activates the neural circuitry for the PDR (Eichbaum et al., 1975). Currently, there is no evidence that would conclusively support one of these hypotheses.

Spinal 5-HT appears to exert a slight modulatory influence on the PDR in that prior depletion of 5-HT with 5,7-dihdroxytryptamine (5,7-DHT) or blockade of 5-HT synthesis with PCPA has been shown to reduce the strength of the PDR (Pappas et al., 1980). These authors suggested that this result was consistent with data suggesting 5-HT facilitation of extensor reflexes (Anden et al., 1968); that is, the effect of decreased 5-HT transmission was to reduce the hindlimb extension component of the PDR. In light of the considerable data indicating that 5-HT facilitates flexor responses as well, this suggests that a reduction of the PDR is due to the loss of a facilitation of the flexor component as well.

Spontaneous activity. Reports on the effects of agents which increase 5-HT transmission on spinal reflexes describe the appearance of spontaneous limb activity (e.g. athetoid movements, tremors) (Anden et al., 1968). Spinalization rules out any contribution of supraspinal influences to these observed effects. Bedard et al. (1979) recently reported that 5-HTP produced a marked enhancement of spontaneous EMG activity in both flexor and extensor muscles of the hindlimb in the chronically-spinalized rat, and that this increased activity was

selectively blocked by cyproheptadine (but not by phenoxybenzamine). Intraperitoneal injection of 5-HT did not mimic this effect, suggesting a central site of action for 5-HTP. Furthermore, administration of l-DOPA or the dopamine agonist apomorphine had no such effect, suggesting that the EMG activation (which correlated with spontaneous movements) was specific to 5-HT activation.

The spectrum of behaviors induced by 5-HT agonists in the intact cat or rat has been referred to as the "5-HT syndrome." This consists of "forepaw treading", head swaying and bobbing, and splayed hindlimbs (Sloviter et al., 1978). Another correlate of 5-HT activation is "limb flicking" seen most clearly in cats. Many of the components of the 5-HT syndrome can be seen in the pontine decerebrate preparation, suggesting that 5-HT in the lower brainstem and/or spinal cord is sufficient to mediate this activation (Jacobs and Klemfuss, 1975).

Modulation of sensory transmission (pain). Yaksh and Wilson (1979) have suggested that spinal 5-HT release in the dorsal horn blocks the transmission of stimuli that code for pain. Although these studies were not carried out in spinalized rats, local administration of drugs to the spinal cord was achieved by infusion through intrathecal catheters. Using this technique, the authors found that intrathecal 5-HT elevated response thresholds to aversive thermal stimuli, and this elevation was enhanced by treatment with 5-HT uptake blockers (fluoxetine, imipramine). Quipazine, but not 5-HTP or tryptamine, mimicked the effects of 5-HT. Quipazine and 5-HT were blocked with the 5-HT antagonist methysergide but not with the NE antagonist

phenoxybenzamine. Methysergide alone produced hyperalgesia, suggesting that 5-HT exert a slight, tonic inhibitory influence on pain transmission (Proudfit and Hammond, 1981).

Spinal 5-HT fibers appear to mediate some of the supraspinal actions of morphine, as well. Yaksh et al. (1980) found that behaviorally defined analgesia (an increase in tail flick latency) that was produced by morphine injected into the periaqueductal grey in the brainstem was partially blocked by intrathecally administered methysergide. However, such antagonism was not evident using the hot plate test, leading Yaksh to suggest that this "supraspinally organized" pain measure was not affected by descending 5-HT systems. It should be noted that the tail flick is a spinal reflex, and as such the effects of intrathecally administered drugs should be evaluated with caution. The need to evaluate possible contributions of motor deficits is clear.

Supersensitivity.

Transection. Several studies have provided evidence that reflex supersensitivity to 5-HT agonists develops following spinal transection. The 5-HT agonists 5-MeODMT (0.1 - 2.0 mg/kg) and quipazine (5 - 20 mg/kg) were shown to produce augmented spinal reflexes, beginning as soon as 1 - 2 days after transection (Sautter et al., 1981). Interestingly, enhancement of 5-HT receptor binding (indicated by an increase in the number of $[^3H]$5-HT binding sites, without a change in affinity) was seen beginning only a week after

transection. That is, functional supersensitivity preceded biochemical indices of supersensitivity. The authors interpreted this finding to mean that enhanced responsiveness seen less than a week after transection was due to alterations in presynaptic mechanisms.

Nozaki and his colleagues have also provided evidence for functional 5-HT supersensitivity following transection, using the whole limb flexion reflex. They found that chronic spinal rats (rats transected for two months or more) showed greater responses to 5-HTP, LSD, and tryptamine than did acute spinal rats (rats transected for two days or less).

Neurotoxins. Nygren et al. (1974) have provided functional evidence for supersensitivity of the hindlimb extensor reflex following intracisternal administration of 5,6-DHT. They found that 5-MeODMT or l-tryptophan + nialimide produced greater responses in neurotoxin treated rats *vs.* controls as soon as four days (5-MeODMT) or at a week (l-tryptophan).

As mentioned previously, 5-HTP induced increases in spontaneous EMG activity in hindlimb flexor and extensor muscles following transection (Bedard et al., 1979). Interestingly, these increases showed a progressive augmentation, such that, at 20 days following transection, a maximal response was achieved. At any given point, the 5-HTP induced activity was reversed by cyproheptadine. That this progressive increase is attributable to supersensitivity is supported by the finding that rats treated with 5,6-DHT and, twenty days later, transected, showed EMG responses to 5-HTP the day following transection

that were equal in magnitude to those seen twenty days after transection. Intrathecal depletion of 5-HT with 5,7-DHT has also been shown to enhance the antinociceptive effects of 5-HT and 5-HT agonists (Howe and Yaksh, 1981).

Neurophysiology (evoked potentials). Measures of monosynaptic (short latency) and polysynaptic (long latency) massed electrical reflex activity can be obtained by stimulating cut dorsal roots and recording the response in ventral roots in anesthetized or unanesthetized (decerebrate/spinal) preparations. Drug-induced changes in the parameters of the monosynaptic reflex (MSR) indicate direct or indirect modulation at the level of the motoneuron whereas changes in the polysynaptic reflex (PSR) suggests that modulation takes place at multiple sites of action along the interneuronal pathways. Recording or stimulating from nerve fibers from individual extensor or flexor muscles or muscle groups provides information that is more specific than that obtained from ventral or dorsal root fibers.

Monosynaptic reflexes. Studies in spinalized preparations have yielded results that are, in general, compatable with the excitatory effects of 5-HT activation on reflex behavior and spontaneous activity. Administration of the 5-HT precursor 5-HTP has been reported to increase the amplitude and decrease the latency of the MSR in the acute spinal cat (Taber and Anderson, 1973; Banna and Anderson, 1968). This facilitation was blocked by cinnaserin, cyproheptadine, methysergide and LSD (Banna and Anderson, 1968). The antagonist effect of LSD is in

contrast to its facilitatory effect on flexor reflexes (Nozaki et al., 1977; Maj et al., 1976).

Tryptamine has been reported to facilitate the MSR in cats, and this facilitation was blocked by cyproheptadine, but not phenoxybenzamine (Vaupel and Martin, 1976). Tryptamine was previously shown to enhance the flexor, but not the extensor, cutaneous reflexes. Finally, the 5-HT agonist quipazine similarly augments the MSR of the acute spinal cat, and this increase was blocked by cinnanserin (Goldstein and Anderson, 1973) but not by metergoline (Goldstein and Anderson, 1981).

Studies utilizing electrical stimulation of the raphe nuclei in decerebrate preparations have yielded conflicting results. Electrical stimulation of the median raphe has been reported to increase the amplitude of the MSR in the rat (Barasi and Roberts, 1973; 1974). This effect was potentiated by l-tryptophan, whereas treatment with methysergide, cinnanserin, or LSD blocked the effect. Clineschmidt and Anderson (1976), on the other hand, reported that raphe stimulation produced both excitatory and inhibitory effects on the MSR, suggesting the possible involvement of multiple pathways.

It should be recalled that Commissiong and his colleagues found that, in the decerebrate preparation, stimulation of 5-HT neurons produced an inhibition of extensor reflexes, in contrast to the excitatory effects seen in studies utilizing spinalized preparations. In the decerebrate preparation, activation of competing spinal and supraspinal 5-HT pathways may exert opposite modulatory influences on spinal reflex behavior.

In an elegant series of studies, Sastry and Sinclair have provided evidence for 5-HT involvement in the modulation of a number of inhibitory processes that affect the amplitude of the MSR. Their studies included analyses of both flexor (posterior biceps, semitendinosus complex) and extensor (quadriceps) muscle groups. The majority of the evidence supported a primary role in the modulation of extensor activity.

Recurrent inhibition of the extensor MSR. Activation of an α-motoneuron is known to produce a feedback inhibition (via a collateral synapsing onto inhibitory Renshaw cells) of itself and adjacent motoneurons. This inhibitory feedback serves the purpose of distributing activation of motoneurons evenly among the cells comprising a homonymous motoneuron pool. Recurrent inhibition of an extensor (quadriceps) MSR was reduced by agents which facilitate 5-HT transmission (5-HTP, the 5-HT uptake blockers fluoxetine and imipramine, and pargyline) (Sinclair and Sastry, 1974; Sastry and Sinclair, 1976). This antagonism was blocked by cyproheptadine. Furthermore, thoracic block of descending impulse flow (these studies were carried out in decerebrate cats) blocked the effects of fluoxetine and imipramine. Since the spinal block alone increased recurrent inhibition (and this increase was blocked by PCPA pretreatment), it was concluded that the 5-HT descending fibers exerted a tonic inhibitory influence on extensor recurrent inhibition. It should be noted that alpha-methyl-para-tyrosine (AMPT, a catecholamine synthesis inhibitor) produced results similar to those obtained with PCPA, suggesting

catecholamine involvement as well.

Presynaptic inhibition of the extensor MSR: Imipramine and fluoxetine blocked presynaptic inhibition of the quadriceps MSR that is elicited by stimulation of antagonist flexor Ia afferent fibers ("reciprocal inhibition") (Sastry and Sinclair, 1977). This blockade was prevented by cold block of the thoracic cord, or by pretreatment with cyproheptadine. Either of these treatments alone enhanced presynaptic inhibition, suggesting that 5-HT exerted a tonic inhibitory influence on this process. PCPA pretreatment prevented the imipramine reduction and the cold block enhancement of presynaptic inhibition, supporting a tonic facilitatory role of descending 5-HT neurons. Again, AMPT effectively antagonized the effect of imipramine, leading the authors to conclude that descending 5-HT and NE fibers were "serially arranged" and both served to antagonize presynaptic inhibition of the extensor MSR.

Bulbospinal inhibition of the MSR: Stimulation of the ventromedial bulbar reticular formation produces an inhibition of the MSR of both flexors and extensors (Llinas and Terzuolo, 1964). Using antidromic field potentials from the extensor quadriceps nerve as a measure of motoneuron activity and field potentials from the afferent nerve as a measure of presynaptic activity, Sastry and Sinclair (1976) reported that bulbospinal stimulation produced both postsynaptic inhibition (reduction of motoneuron field potentials) and presynaptic inhibition. These effects were antagonized by imipramine (Sastry and Sinclair,

1976; Sinclair and Sastry, 1974), and the imipramine effect was itself blocked by cyproheptadine or by prior treatment with PCPA, and not AMPT (Sastry and Sinclair, 1976).

Studies similar to those described above were carried out on a flexor (posterior biceps-semitendinosus) MSR as well. In marked contrast to the results obtained with the extensor, it was found that altering 5-HT transmission in the spinal cord had little effect on recurrent inhibition (Sastry and Sinclair, 1976; Sinclair and Sastry, 1974) and presynaptic inhibition (Sastry and Sinclair, 1977) of the flexor MSR. However, bulbospinal inhibition of the flexor MSR was antagonized (albeit weakly) by administration of imipramine.

The studies by Sastry and Sinclair illustrate subtle, yet important influences of the descending 5-HT systems on inhibitory processes affecting the monosynaptic reflexes. It is difficult to relate these findings to studies looking at the behavioral concomitants of spinal 5-HT activation. At this point, they are of importance in emphasizing that modulation of reflex behavior by descending pathways may be exerted at many different loci within the cord and through a number of different mechanisms. An excitatory effect on a reflex may be attributable to direct or indirect activation of motoneurons or interneurons or to removal of any of a number of inhibitory influences. These studies further illustrate important differences in the type of preparation used to study spinal reflexes: the decerebrate preparation in which the descending 5-HT systems were functionally intact revealed tonic 5-HT influences that would have gone undetected in the spinal preparation.

Polysynaptic reflexes. 5-HTP has been found to depress polysynaptic reflexes (Banna and Anderson, 1968), though the lack of reversibility by 5-HT antagonists led the authors to conclude that the effect was not serotonergically-mediated. 5-HTP was also found to depress dorsal root reflexes. Recently, it has been shown that quipazine, a 5-HT agonist, also depresses various indices of dorsal root activity (dorsal root potential, dorsal root discharge, dorsal root reflex) in acute spinal rats (Goldstein and Anderson, 1981). This effect was gradual in onset (taking about 30 minutes to occur), and was reversed by metergoline, but not by cinnanserin. It is interesting to compare this effect with the effect of quipazine on the MSR: quipazine produced a rapid onset facilitation which was blocked by cinnaserin but not by metergoline (Goldstein and Anderson, 1981).

On the other hand, tryptamine has been reported to facilitate polysynaptic reflexes (Vaupel and Martin, 1976; Bell and Martin, 1974) in a cyproheptadine-reversible fashion. Even more pronounced effects were obtained when long latency polysynaptic reflexes (elicited by preferential stimulation of high threshold, slowly-conducting C-fibers) were measured. Thus, administration of l-tryptophan (Bell et al., 1976) or tryptamine produced a marked increase in the long-latency PSR which was blocked by cyproheptadine. In contrast to its antagonist effect on the MSR, LSD produced a cyproheptadine reversible increase in polysynaptic reflexes (Bell and Martin, 1974). This effect was somewhat gradual in onset, and peaked an hour after injection.

Single cell activity. As previously mentioned, electrical stimulation

of the median raphe increased the amplitude of the MSR (Barasi and Roberts, 1973, 1974). Using multibarreled recording/iontophoresis, these authors showed that raphe stimulation increased the size of the evoked field potential in the ventral horn. This facilitatory effect could be mimicked by iontophoretic application of 5-HT onto motoneurons. Furthermore, the increases in field potentials could be antagonized by iontophoretic application of cinnanserin or methysergide onto motoneurons. White and Neuman (1980) similarly found that iontophoretic application of 5-HT onto motoneurons increased the excitability of these cells to iontophoretically-applied glutamate, or to antidromic stimulation from the ventral roots. This increase in excitability occurred without a direct increase in the firing of the motoneurons, consistent with a role of 5-HT as a neuromodulator in the spinal cord. Recently, similar findings have been made with the putative 5-HT agonist quipazine (Neuman and White, 1982).

Myslinski and Anderson (1978) carried out an elegant series of experiments investigating the effects of 5-HT precursors (5-HTP and l-tryptophan) on α- and λ-motoneuron fibers in dissected ventral root fibers. They found that l-tryptophan produced increases in both α- and λ-motoneuron activity. The increased α- motoneuronal activity was not attributable to activation of the λ-loop, since opening the loop (by cutting the dorsal root fibers) failed to abolish the α-motoneuron response. Thus, activation of the stretch reflex was not solely attributable to increased λ-motoneuron discharge.

Furthermore, by recording motor units in peripheral nerve filaments from both flexor (semitendinosus) and extensor

(gastrocnemius) muscles, they discovered that α- and λ-motoneuron activation occurred in both types of fibers. These effects were reversed by cyproheptadine and cinnanserin. These findings are consistent with the enhancement of EMG activity that occurred in both flexors and extensors following 5-HTP administration (Barbeau and Bedard, 1980). Furthermore, they clarify seemingly conflicting reports that facilitation of flexor and extensor reflexes occurs following drug-induced increases in 5-HT transmission.

Myslinski and Anderson acknowledged that they could not determine whether α-motoneuron activation was direct or through interneurons. However, in view of the findings of White and Neuman (1980), and in view of the activation of interneurons seen following 5-HTP administration (Phyllis et al., 1968), it is likely that the latter mechanism (activation indirectly through interneurons) is operative.

Dorsal horn: Consistent with behavioral studies suggesting that 5-HT release in the dorsal horn of the spinal cord produces analgesia, several studies have shown that electrical stimulation of the raphe magnus reduces the excitability of dorsal horn interneurons, and this effect is blocked by 5-HT antagonists (Duggan and Griersmith, 1979; Fields et al., 1977; Proudfit and Anderson, 1974). Responses of dorsal horn interneurons to noxious stimuli were more effectively blocked than was activity in large diameter fibers. Furthermore, iontophoretically applied 5-HT depressed the firing of neurons activated by noxious stimuli ("nociceptors" in the substantia gelatinosa), and this effect was blocked by methysergide (Belcher et al., 1978; Headley, et al.,

1978; Griersmith and Duggan, 1980).

Recently, two populations of spinothalamic neurons have been identified as having opposite responses (excitation and inhibition) to 5-HT (Jordan et al., 1979). There appeared to be a functional distinction between these two types of neurons, in that cells excited by 5-HT received their inputs from "deeply-situated" muscle afferents, whereas neurons depressed by 5-HT were more responsive to "exteroceptive" stimuli originating from hair movements, skin stimulation, and painful plantar stimulation. This report provides evidence that descending 5-HT neurons may modulate, in opposite ways, cells in the spinal cord that are responsible for transmitting sensory information to supraspinal levels.

Summary. There is strong anatomical and neurochemical evidence for the existence of descending serotonergic fiber systems. Furthermore, activation of these pathways in the spinal animal facilitates flexor and extensor spinal reflexes that are elicited by activation of cutaneous and muscle afferents. However, there is evidence that 5-HT may exert inhibitory influences on extensor stretch reflexes in non-spinalized, decerebrate animals, suggesting that multiple 5-HT pathways may exert opposite effects on the reflex. Facilitation of spinal cord 5-HT activity in spinalized animals can also produce spontaneous movements in flexors and extensors, and may be partly responsible for the occurrence of the post-decapitation reflex. Behaviorally-defined analgesia has been reported to accompany activation of spinal 5-HT neurons, probably those that terminate in the dorsal horn. Chronic

destruction of descending 5-HT fibers, either by transection or by use of specific 5-HT neurotoxins, produces signs of denervation supersensitivity. Thus, increases in the facilitatory effects of 5-HT agonists on spinal reflexes and on activity of muscles, as well as enhanced antinociceptive effects of these drugs, have been found. Neurophysiological studies provide evidence for many types of serotonergic modulation on spinal neural activity. Facilitatory modulation of spinal mono- and polysynaptic reflexes and single cell activity has been shown. Inhibitory influences have also been reported, especially in decerebrate animals, suggesting that 5-HT may exert opposite effects on spinal neurons through multiple (spinal and supraspinal) sites of action.

Descending Noradrenergic Neurons

Anatomy. Studies employing a variety of techniques have established that NE cell bodies in two primary areas of the brainstem (designated A1 - 3 and A6, according to the terminology of Dahlstrom and Fuxe, 1964) give rise to terminal projections in the dorsal and ventral horns along the entire length of the spinal cord (Dahlstrom and Fuxe, 1964; Carlson et al., 1964; Ungerstedt, 1974; Lindvall and Bjorklund, 1974; Nygren and Olson, 1977; Commissiong et al., 1978; Karoum et al., 1980). Nearly all of the norepinephrine found in the spinal cord is contained within descending fibers, since total disappearance of NE occurs within a week following transection (Anden et al., 1964). The pontine (A6) cells, located in the locus coeruleus and subcoeruleus, appear to

provide the major noradrenergic projection to the ventral horn and the ventral half of the dorsal horn of the spinal cord, whereas the medullary (A1-3) cell bodies contribute to the dorsal horn and the sympathetic lateral column (Nygren and Olsen, 1977; Commissiong et al., 1978; Guyener, 1980; Mason and Fibiger, 1979). Spinal noradrenergic fibers would thus appear to be anatomically well-suited to exert modulatory influences over spinal reflexes.

Whole limb flexor reflexes. The isomers of amphetamine are known to promote the release, and block the reuptake of catecholamines (see Groves and Rebec, 1976). d-Amphetamine has been found to potently facilitate the flexor reflex elicited by electrical stimulation of the plantar region of the hindpaw of spinalized rats (Nozaki et al.., 1977; Nozaki et al., 1980) or spinalized dogs (Martin and Eades, 1967). This excitation was attributed to action on NE neurons, since it was blocked by pretreatment with the α-NE antagonist phenoxybenzamine. Phenoxybenzamine alone was reported to have little or no effect on the baseline of the flexor reflex (Martin and Eades, 1967; Nozaki et al., 1980); furthermore, the activation by amphetamine was not blocked by a 5-HT antagonist (cyproheptadine), or by a β-NE antagonist (dichloroisoproterenol) (Nozaki et al., 1977; Martin and Eades, 1967). Finally, l-amphetamine has also been found to increase the amplitude of the toe-pinch elicited flexor reflex (Svensson et al., 1975).

Intravenous administration of the α_1-NE agonist methoxamine has been reported to facilitate the flexor reflex in spinalized rats (Nozaki et al., 1977, 1980) and dogs (Martin and Eades, 1967). As with

amphetamine, this stimulation appeared to be dependent on stimulation of α-noradrenergic receptors, since it was blocked by phenoxybenzamine but not by the β-NE antagonist dichloroisoproterenol (Martin and Eades, 1967).

The catecholamine precursor l-DOPA has been shown to facilitate the flexor reflex evoked by pinching the toes of spinalized rats (Anden et al., 1970) and this effect was blocked by phenoxybenzamine. The lack of attenuation by even high doses of the DA antagonist pimozide (20 - 25 mg/kg) supports the conclusion that the effect of l-DOPA is not mediated by increased activity at spinal DA receptors (see, however, section on DA involvement in spinal reflex behaviors).

Clonidine, a drug purported to have mixed α-agonist properties (Clough and Hatton, 1981), has been shown to produce a marked, l-DOPA-like facilitation of the hindlimb flexor reflex in spinalized rats (Anden et al., 1970; Anden et al., 1978; Nutt et al., 1980). This effect has been attributed to the activation of α_1-NE receptors, since it was blocked by the α_1-NE antagonists prazosin (Anden et al., 1978) and phenoxybenzamine (Anden et al., 1970; Anden et al., 1975). Pretreatment with α_2-antagonists yohimbine and piperoxane has been found to be less efficacious in antagonizing this effect (Anden et al., 1975).

Individual flexor muscles. Maj and his coworkers have utilized the tibialis anterior flexor reflex (described in the 5-HT section) to analyze the contribution of spinal NE neurons to the flexor reflex. Their findings support the results from the whole limb flexor studies,

i.e. that a facilitation of spinal noradrenergic activity increases the flexor reflex. Thus, clonidine and d-amphetamine both produced an increase in amplitude of the flexor reflex, and these effects were blocked by pretreatment with phenoxybenzamine (Maj et al., 1976). Haloperidol was later reported to block the increase produced by clonidine (Maj et al., 1978). The authors attributed this blockade to noradrenergic blocking properties of haloperidol, since they found that the DA antagonist pimozide did not attenuate the clonidine induced effect. Blockade of 5-HT receptors with several drugs (cyproheptadine, metergoline) failed to influence the effect of clonidine or amphetamine on the flexor reflex (Maj et al., 1976). Finally, clonidine has been reported to produce a phenoxybenzamine-reversible activation of the psoas flexor reflex (Austin et al., 1976), consistent with the results obtained with the tibialis anterior and whole limb flexor reflexes.

Stretch reflexes. The stretch reflex is absent in spinalized rats, though (as mentioned previously) it can be restored by the administration of l-DOPA (Anden et al., 1964). Because this effect was reversed by pretreatment with phenoxybenzamine, it was concluded that l-DOPA increased the availability of NE which then activated noradrenergic receptors in the ventral horn. Furthermore, it was concluded (on the basis of electrophysiological measurements) that NE increased the discharge in static λ-motoneurons, thereby restoring resting tension in the intrafusal fibers (and restoring the sensitivity of the muscle spindle afferent fibers to stretch). Recently, Commissiong has challenged this hypothesis, suggesting rather that the

effects of l-DOPA are attributable to activation of DA receptors. Commissiong found that l-DOPA increased the stretch reflex in the decerebrate rat, and that this increase was blocked completely by 0.3 mg/kg pimozide (Commissiong and Sedgwick, 1979). (See section on DA for other data.) Furthermore, Commissiong (1981) concluded that NE release in the spinal cord of the decerebrate rat was, in fact, inhibitory to the stretch reflex. He cited several experiments to support this conclusion: (1) DOPS (an analog of l-DOPA which is directly metabolized to NE) produced only a depression of the stretch reflex; and (2) administration of cyclobenzaprine, a muscle relaxant, increased discharge of the locus coeruleus (and increased turnover of NE in the ventral horn) and concomitantly depressed the stretch reflex in the decerebrate rat. However, Barnes et al., (1980) found just the opposite results with cyclobenzaprine (that is, the drug depressed locus coeruleus firing while at the same time depressing spinal monosynaptic responses). Finally, Maxwell (1975) suggested that the increase in extensor tone following intercollicular decerebration (so-called "λ-rigidity") might be due to enhanced noradrenergic activation, since drugs which blocked noradrenergic transmission ameliorated the condition. Clearly, more direct evidence needs to be gathered before definite conclusions regarding the role of NE in modulating tonic and phasic stretch reflexes can be made. Nonetheless, it is an intriguing possiblity that multiple noradrenergic pathways may exert both excitatory and inhibitory influences on stretch reflexes through actions at spinal and supraspinal levels, respectively. This "dual-action" could provide a parallel to that suggested for serotonergic

systems (see 5-HT section).

Post-decapitation reflex. The integrity of the post decapitation kicking response (PDR -- described earlier) is highly dependent upon noradrenergic transmission (Roberts et al., 1978, 1979; Pappas et al., 1979). Thus, attenuation of the response occurs following pretreatment with AMPT, the α_1-NE antagonist prazosin, chlorpromazine, or U14-624 (a DA-β-hydroxylase inhibitor) (Pappas et al., 1979). Lesions of the locus coeruleus (either electrolytic or 6-OHDA) also reduce the PDR. Trifluoperazine, a DA receptor blocker, did not affect the response; nor did lesions of the ascending fibers of the NE pathways (Roberts et al., 1978, 1979).

Spinal stepping. Elegant studies by Grillner and his colleagues have shown that the "pattern generator" for producing the alternation of flexors and extensors that comprises locomotion is found within the spinal cord. In fact, each limb appears to have its own "pattern generator" which (while capable of working independently) work in concert to produce locomotor movements. This conclusion has been derived from studies in which spinalized animals are placed on a moving treadmill. When all afferent input to the spinal cord is cut, either stimulation of the cut dorsal root, or intravenous injection of l-DOPA, can produce "treadmill stepping" movements. L-DOPA activates the "stepping generator" by increasing the availability of NE in the spinal cord, since its effects are blocked by pretreatment with the NE antagonist phenoxybenzamine. Electrophysiological studies have

indicated that the locomotor movements are produced by a co-activation of α- and λ-motoneurons. Thus, descending neurons of the locus coeruleus have been implicated in being a "command center" for activating a spinal locomotion generator.

Modulation of sensory transmission (pain). Descending NE neurons, like 5-HT, appear to modulate the transmission of sensory information in the dorsal horn of the spinal cord. Electrical stimulation of the nucleus reticularis gigantocellularis produces behaviorally defined analgesia (Mohrland and Gebhart, 1980) (Yaksh et al., 1981) that is blocked by intrathecal administration of the α-adrenergic antagonist phentolamine, but not the 5-HT antagonist methysergide (Yaksh et al., 1981). Furthermore, a variety of NE agonists produce analgesia when injected intrathecally (Reddy and Yaksh, 1980; Reddy et al., 1980; Yaksh and Wilson, 1979). These effects were antagonized selectively by α_1-NE receptor blockers. Finally, analgesia produced by morphine injected into the periaqueductal grey is attenuated by intrathecal phentolamine, suggesting that descending NE fibers play an important role as a "link" between the brainstem and spinal cord in the modulation of pain signals.

Supersensitivity. Nygren et al. (1976) found that acutely-spinalized rats depleted of spinal NE by prior pretreatment with the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) showed enhanced FR responses to clonidine. The authors suggested that the potentiated clonidine effect was indicative of the development of underlying noradrenergic receptor

supersensitivity. Recently, Howe and Yaksh (1982) demonstrated that depletion of spinal NE with intrathecal 6-OHDA produced an enhanced antinociceptive effect of NE and NE agonists, again interpeted to be an indicant of denervation supersensitivity.

Neurophysiology.

Monosynaptic reflexes. In spinalized cats, administration of the α_1-adrenergic agonist methoxamine has been found to increase the amplitude of the MSR in a dose-dependent manner (Vaupel and Martin, 1976), and this effect was blocked by phenoxybenzamine. Additionally, l-DOPA was found to increase the amplitude of the MSR (Baker and Anderson, 1970), and this effect was reversed by phenoxybenzamine and chlorpromazine. It should be recalled that Commissiong and his colleagues found that increasing noradrenergic transmission in decerebrated rats produced an inhibition of the extensor stretch reflex, in contrast to the excitatory effects seen in the transected preparation. Recently, it has been reported that electrical stimulation of the locus coeruleus markedly increased the amplitude of the lumbar MSR in decerebrate cats (Strahlendorf et al., 1980). This augmentation was reversed by phenoxybenzamine and chlorpromazine, but not haloperidol, supporting the conclusion that the stimulation-induced increase was mediated by noradrenergic neurons. This study does not support Commissiong's contention that noradrenergic neurons inhibit the stretch reflexes. (In addition, it was found that locus coeruleus stimulation produced a facilitation of both extensor and flexor MSRs,

suggesting that descending NE neurons exert general facilitatory influences on monosynaptic reflex activity).

Polysynaptic reflexes: l-DOPA has been shown to depress the dorsal root reflex and the PSR elicited by sural nerve stimulation (Baker and Anderson, 1970). The inhibitory effect of DOPA on the dorsal root reflex was shown to be blocked by phenoxybenzamine or chlorpromazine.

Single cell activity. Lundberg and his colleagues published a series of papers describing the effects of l-DOPA on interneuronal activity in the spinal cord. These interneurons, identifiable only in spinal animals and only after injection of l-DOPA, showed complex patterns of responses to peripheral activation that made them well-suited to participate in a "spinal generator" for the production of alternate extensions-flexions needed for basic movement (Jankowska et al., 1967; Kostyuk and Vasilenko, 1979).

Following spinal transection, there is a reduction in static λ-efferent activity, and increase in dynamic λ-efferent activity. l-DOPA increases static and decreases dynamic activity, leading to the conclusion that a NE pathway "sets the balance" between static and dynamic fusimotor activities (Grillner, 1969). Furthermore, this mechanism has been found to underlie the production of spinal stepping by l-DOPA in cats (see Grillner, 1975). Similar electrophysiological changes have been found in rats following d-amphetamine administration (Sorenson et al., 1978).

Until recently, studies employing iontophoretic application of NE

onto spinal motoneurons found weak, inconsistent effects (usually inhibitory) (Engberg and Ryall, 1966). However, recently it has been suggested that NE might not act as a classical transmitter in the spinal cord, but rather as a neuromodulator. Thus, iontophoretic application of NE onto motoneurons of the lumbar cord increases the excitatory effects of afferent input to those cells (White and Neuman, 1980). This increase occurred without the direct activation of motoneuronal firing, and was blocked by pretreatment with phenoxybenzamine.

Interestingly, Hodge et al (1981) recently reported that stimulation of the locus coeruleus produced inhibitory responses on dorsal horn neurons (lamina 4 and 5, areas purported to be involved in the relay of pain), whereas cells in the ventral horn (lamina 6) were actually activated. Thus, opposite effects of stimulation of the same descending system neurochemical system can exert opposite effects on neurons in the dorsal vs. ventral horn. These responses may help explain contradictory functional findings reported previously.

Summary. There are several descending noradrenergic pathways that could affect spinal reflex activity, most notably the pathway that arises from the nucleus locus coeruleus/subcoeruleus complex. Increasing spinal noradrenergic activity increases polysynaptic flexor reflexes and restores monosynaptic stretch reflexes in spinalized animals. However, there is some evidence that supraspinal noradrenergic neurons may exert opposite (inhibitory) influences on spinal reflexes. Spinal noradrenergic activity is critical for the

occurrence of the PDR. Furthermore, spinal noradrenergic activation produces "stepping movements" and results in behaviorally-defined analgesia.

Neurophysiological studies indicate that increasing noradrenergic transmission in the spinal cord facilitates mono- and polysynaptic reflexes, and activates the neural circuitry that produces stepping movements. Single unit and iontophoretic studies indicate that NE acts as a facilitatory neuromodulator in the ventral horn. However, cells in the dorsal horn that transmit nociceptive information to the brain have been found to be inhibited by increased spinal noradrenergic transmission.

Descending Dopaminergic Neurons

Anatomy. Until recently, it has been thought that the small quantities of DA measured in the spinal cord existed only as precursor to NE. However, with the development of refined techniques for tracing monoamine pathways, it has become evident that discrete descending dopaminergic pathways do in fact exist. Interestingly, the highest concentrations of DA are found in the dorsal horn. High levels are also found in the zona intermedia, suggesting an interaction with preganglionic sympathetic neurons (Commissiong, 1979). Discrete hypothalamo-spinal (Blessing and Chalmers, 1979) and nigro-spinal (cf. Commissiong, 1979) pathways have recently been identified.

Modulation of spinal reflexes.

Whole limb flexor reflexes. Administration of the DA agonist apomorphine (0.5 mg/kg) has been reported to produce an increase in the hindlimb flexor reflex in rats (Nygren and Olsen, 1976). However, this effect was only seen in rats pretreated with 6-OHDA; thus, action of the drug on supersensitive DA receptors might be necessary to "bring out" an excitatory effect of the drug. Commissiong and Neff (1979) have suggested that spinal DA receptor activation may mediate the facilitatory effects of l-DOPA on the flexor reflex, on the basis of neurochemical evidence that l-DOPA increases the synthesis of primarily DA. However, Anden et al. (1970) concluded that l-DOPA was not working through DA receptors, since even extremely high doses of the DA antagonist pimozide (20 - 25 mg/kg) failed to block the effects of l-DOPA on the hindlimb flexor reflex.

Single muscle flexor reflex. Maj and his coworkers have reported that spinal dopaminergic neurons play an insignificant role in the modulation of flexor reflexes, in that doses as high as 5 mg/kg (intravenously) failed to affect the amplitude of the tibialis anterior reflex (Maj et al., 1978).

Stretch reflexes. The catecholamine precursor l-DOPA can restore the stretch reflex which is otherwise absent in the spinalized preparation. This effect has been attributed to the stimulation of NE receptors, since it is blocked by phenoxybenzamine. However, Commissiong (1974)

suggested that DA might be involved, since the effect of l-DOPA on the stretch reflex in an extensor muscle was blocked by a low (.3 mg/kg) dose of pimozide. Furthermore, apomorphine (0.5 gm/kg) was found to produce a short (10-15 minute) activation of the stretch reflex (Commissiong and Sedgwick, 1979).

Modulation of sensory information (pain). Although there is currently no evidence that DA release in the spinal cord is involved in antinociception, there is an indication that a descending DA pathway from the substantia nigra might indirectly affect spinal pain transmission. Barnes et al. (1979) found that nigral stimulation produced a marked inhibition of lamina V cells that were normally excited by noxious stimuli. Similar effects were elicited by stimulation of the periaqueductal grey and the dorsal raphe. Since the nigral stimulation has been reported to be effective in decerebrates, and since it is blocked by dopamine antagonists (Barnes et al., 1979), the effect appeared to be attributable to a descending pathway. Furthermore, since the inhibition of lamina V activity by stimulation of all three sites (nigra, raphe, and periaqueductal grey) were blocked by 5-HT antagonists, it was tentatively concluded that the descending DA pathway (and the pathway from the periaqueductal grey) converged on the raphe and influenced spinal transmission through the raphe-spinal projection.

Supersensitivity. Gentleman et al. (1981) recently reported that a DA-activated adenylate cyclase (reversible by haloperidol) exists in the

spinal cord. This cyclase can also be activated by apomorphine. Furthermore, following transection of the spinal cord, a 5-10 fold increase in the sensitivity to DA was found, indicative of the development of denervation supersensitivity.

Modulation of other behaviors.

Spontaneous activity. Commissiong and Sedgwick (1979) reported that apomorphine (0.5 mg/kg) produced a marked increase in spontaneous EMG activity, which they attributed to increased λ-motoneuron activation. Since this data was gathered in the decerebrate preparation, it is possible that the drug indirectly affected motoneuronal activity by acting on supraspinal receptors.

Neurophysiology.

Monosynaptic reflexes. Electrical stimulation of the substantia nigra has been reported to facilitate the MSR in both flexor and extensor muscles (York, 1972). This facilitation appeared to be DA-mediated, since it was reversed by haloperidol. Furthermore, descending systems were involved, since the facilitation was still present in decerebrate rats.

Langer et al. (1979) recently reported that haloperidol administration (in doses up to 0.3 mg/kg, i.p.) reduced the MSR of both flexor and extensor α-motoneurons (though flexors were more sensitive). They suggested that neuroleptic induced catalepsy which is classically

associated with blockade of forebrain DA receptors, might involve a decrease in the efficacy of Ia muscle afferents to elicit activation of α-motoneurons.

Single cell activity. Barasi and Roberts (1977) investigated the effects of iontophoretically applied DA to α-motoneurons in the ventral horn of the lumbar cord of rats. DA was found to increase the excitability of motoneurons (indicated by an increase in the field potentials from antidromically activated cells). This facilitation was blocked by the DA receptor blocker α-flupenthixol, which by itself did not influence the excitability of the cells. α-fllupenthixol did not increase the excitability produced by 5-HT, though unexplicably, it enhanced the excitability induced by NE.

Summary. There is now evidence for the existence of discrete descending dopaminergic neurons, though the primary projection areas may be the dorsal horn. Activation of DA neurons in the spinalized preparation does not affect cutaneous flexor reflexes, though it has been reported to facilitate extensor stretch reflexes, as well as producing tonic, spontaneous muscle activity. Descending DA neurons from the substantia nigra may indirectly exert antinociceptive effects by influencing raphe-spinal stimulation. Biochemical evidence for DA supersensitivity in the spinal cord after transection has been found, though there are no reports of a functional correlate to this supersensitivity. Neurophysiological evidence suggests that a nigral-spinal descending projection can exert facilitatory effects on flexor

and extensor reflexes. Furthermore, there is some evidence at the single cell level for facilitatory effects on motoneurons.

Spinal GABAergic Neurons

Anatomy. GABA and its synthesizing enzyme GAD are localized in the greatest concentration in the dorsal horn or spinal cord, particularly the substantia gelatinosa (Barber and McLaughlin, 1980; Barber et al., 1978).

Modulation of spinal reflexes.

Whole limb flexor reflexes. Administration of the GABA antagonist bicuculline has been reported to produce a slight enhancement of the hindlimb flexor reflex (Nutt et al., 1980), suggesting that spinal GABA neurons exert a slight tonic inhibition on the reflex. Muscimol and THIP, putative GABA agonists (Johnston, 1978), and vinyl GABA, a GABA-transaminase inhibitor that elevated spinal GABA levels by 100%, all had little effect on the whole limb flexor reflex in spinalized rats. On the other hand diazepam, a benzodiazepine, produced a marked, dose-dependent depressant effect on the reflex (Nutt et al., 1980).

Single muscle flexor reflexes. In contrast to the slight excitatory effect on whole limb flexion, bicuculline has been reported to have no effect on the flexor reflex measured by an EMG in the biceps femoris muscle (MacDonald and Pearson, 1979). (This experiment was carried out

in non-spinalized rats, a condition which might account for the different results.) On the other hand, the GABA antagonist picrotoxin (2 mg/kg) was found to facilitate the tibialis anterior flexor reflex in acutely spinalized rats (Sypniewska, 1979; Georgiev and Rawlow, 1978). This finding supports the conclusion that spinal GABA neurons are tonically inhibitory to the flexor reflex. Baclofen, a putative GABA agonist (Johnston, 1978) was found to depress the reflex (Sypniewska, 1979); however, neither picrotoxin nor bicuculline antagonized this depression, suggesting that the drug was not working through a GABA mechanism. Interestingly, baclofen appeared to act more like a 5-HT antagonist, since it attenuated the effects of quipazine and LSD on the flexor reflex (Sypniewska, 1979).

Stretch reflexes. Diazepam has a marked muscle relaxant effect which has been attributed to its ability to depress λ-motoneuron discharge. This effect is achieved with very little effect on α-motoneuron activity (Takano and Student, 1978). Consistent with its inability to silence α-motoneuron activity, diazepam has been shown to have little effect on mono- or poly-synaptic reflexes, even at high doses (e.g. 10 mg/kg) (Polc et al., 1974). In fact, this preferential blockade may explain why diazepam is effective as a muscle relaxant in some preparations but not others (Andersen and Raines, 1972; Maxwell, 1975).

Spinal Glycinergic Neurons

Anatomy. The existence of glycine neurons in the spinal cord,

particularly the ventral horn, is well documented (cf. Johnston, 1978). The transmitter is organized in a rostral -caudal gradient in the central nervous system, with the greatest concentration of neurons and receptors found in the spinal cord. Glycine has been shown to mediate post-synaptic inhibition in both the spinal cord and brainstem (Curtis et al., 1968; Werman et al., 1968; Belcher et al., 1976).

Modulation of spinal reflexes.

Flexor reflexes. Like GABA, spinal glycine neurons appear to exert a tonic inhibitory influence on the flexor reflex, since administration of the glycine antagoist strychnine (Curtis et al., 1968) increased the amplitude of the flexor reflex in spinal rats (MacDonald and Pearson, 1979).

Stretch reflexes. Intravenous administration of glycine into decerebrate cats has been found to produce a marked, long-lasting depression of the stretch reflex of the extensor triceps surae muscle (Takano and Neumann, 1972). Strychnine reversibility was not tested.

Summary (GABA and glycine). There is clear evidence for the presence of spinal GABA and glycine neurons. Studies using putative GABA and glycine antagonists have shown that these drugs produce facilitatory effects on cutaneous flexor reflexes, probably through a blockade of tonic spinal inhibition. GABA agonists (there is not a good glycine agonist currently available) produced little or no effects on the

flexor reflexes, suggesting that the tonic inhibition is near maximal. Benzodiazepines, most notably diazepam, appear to selectively block α-motoneuron discharge, without directly acting on λ-motoneurons.

Other Neurochemical Systems

Cholinergic neurons. Recent work using receptor binding techniques has revealed sizeable concentrations of muscarinic cholinergic receptors throughout the grey matter of the spinal cord, the number of which are unaffected by transection (Kayaalp and Neff, 1980; although see Charlton et al., 1981 for report that receptor number is diminished following transection). However, very little work has been devoted to the systematic evaluation of spinal cholinergic systems to the control of reflex behavior. Martin and Eades (1967) studied the effects of cholinergic agonists and antagonists on the flexor reflex in chronically-spinalized dogs and found that physostigmine, a cholinesterase inhibitor, increased the amplitude of the reflex. The muscarinic cholinergic antagonist atropine blocked this increase, leading the authors to conclude that muscarinic receptor activation facilitated the flexor reflex.

Peptides: substance P and thyrotropin releasing hormone. Substance P and thyrotropin releasing hormone (TRH) have been shown to be co-localized in descending 5-HT terminals (Gilbert et al., 1982), particularly those terminals innervating the ventral and lateral grey matter of the spinal cord. These peptides do not appear to be found in

descending CA terminals, since intraventricular injection of 6-hydroxydopamine failed to affect spinal localization. On the other hand, intraventricular injection of 5,7-DHT resulted in a virtual disappearance of spinal 5-HT, TRH, and substance P; two other peptides, met-enkephalin and somatostatin, were unchanged. Interestingly, there was no depletion of substance P in the brain following 5,7-DHT treatment, suggesting that spinal 5-HT neurons may be unique in their peptide content. Gilbert et al. commented that 5-HT, TRH, and substance P-labelled fibers could be seen most strikingly localized around somatic motor neurons.

Krivoy et al. (1980) have recently provided electrophysiological evidence that substance P functions as a neuromodulatory transmitter in the spinal cord. They found that substance P applied iontophoretically to motoneurons of the cat spinal cord produced an increased sensitivity of the cells (at high currents) to afferent barrages from stimulation of the dorsal root.

Substance P has been suggested to have a role in the dorsal horn as a transmitter for sensory (pain) information, perhaps at the level of the primary afferent. Iontophoretic application of substance P produced a strong excitation of responses in laminae I - III to noxious stimuli (while depressing or having no effect on responses of neurons to light touch or pressure) (Randic and Miltic, 1977). Furthermore, intraspinal injection produced scratching, indicative of hyperalgesia (Piercey et al., 1981).

Spinal Neurochemical Modulation of the Acoustic Startle Reflex

The evidence presented thus far illustrates that a number of neurochemical systems that either descend from the brain (norepinephrine, serotonin, dopamine) or that are intrinsic to the spinal cord itself (GABA, glycine, acetylcholine) can modulate spinal reflex activity. One could now pose the question, do these spinal transmitter systems have relevance to behaviors whose neural circuitry are of supraspinal origin? Recent work in the laboratory of Dr. Michael Davis has indicated that spinal neurochemical systems play an important role in the modulation of the acoustic startle response. The acoustic startle reflex is a short-latency activation of the skeletal musculature that follows the presentation of an intense auditory stimulus. The neural circuitry underlying the startle reflex involves a relatively simple ear-brainstem-spinal cord-muscle reflex arc. Stimulation, lesion, and anatomical studies indicate that the transmission of the startle-eliciting neural impulses proceeds from the auditory nerve to the ventral cochlear nucleus to the nuclei of the lateral lemoriscus, to the nucleus reticularis pontis caudalis; and then down the reticulo-spinal tract where (either directly or through interneurons) activation of α-motoneurons (and subsequent muscle contraction) takes place.

In order to localize the site of action of a systemically-administered drug to the spinal cord, investigators studying spinal reflexes routinely use a spinal transection to eliminate the influence of supraspinal structures. Obviously, when studying a behavior such as

the acoustic startle reflex which requires the integrity of the brainstem-spinal cord connection, such a procedure cannot be used. However, Tony Yaksh has developed a simple technique for infusing drugs directly into the subarachnoid space of the lumbar spinal cord in unanesthetized, behaving animals (see Yaksh and Rudy, 1976). Using the "intrathecal technique", Davis and coworkers have found that intrathecal infusion of a variety of drugs that affect spinal neurochemical transmission can alter the amplitude of the acoustic startle response.

Serotonin. Using this technique, it has been shown that intrathecal infusion of drugs that increase 5-HT transmission in the spinal cord (5-HT + pargyline, 5-MeODMT, PCA) all produce increases in acoustic startle. Furthermore, these effects are blocked by pretreatment with 5-HT antagonists (Davis et al., 1980; Davis, 1980; Davis et al., 1979). Thus, on a qualitative level, the startle reflex would appear to respond similarly to treatments which enhance 5-HT transmission in the spinal cord as do the whole limb and single muscle flexor reflexes (see previous section).

In marked contrast to the facilitatory effects of intrathecal administration of 5-HT agonists on the startle reflex, intraventricular infusion of these drugs produces an opposite (inhibitory) effect on this behavior (Davis et al., 1980). The opposite effects of spinal vs. supraspinal infusions of these drugs has been interpreted as representing the activation of spinal excitatory and supraspinal inhibitory 5-HT systems. The ultimate behavioral outcome of systemic

injection would thus depend upon the net balance between these anatomically distinct modulatory systems.

Dopamine. Systemic adminstration of the DA agonist apomorphine produces a potent increase in acoustic startle (Davis and Aghajanian, 1976). However, this effect does not appear to be mediated in the spinal cord, since intrathecal administration of apomorphine, over a wide range of doses, failed to affect startle amplitude (Davis and Astrachan, unpublished observations).

GABA. Recent work indicates that the acoustic startle reflex is modulated by spinal GABA neurons. Intrathecal administration of the GABA antagonist picrotoxin has been shown to produce a dose-dependent increase in startle amplitude, in doses that are subconvulsive (Gallager et al., submitted). These data suggest that GABA neurons tonically inhibit the startle reflex pathway, perhaps through direct postsynaptic inhibition of α-motoneurons involved in the startle reflex pathway.

Glycine. Systemic administration of strychnine has been found to produce a potent facilitation of acoustic startle in subconvulsive doses (Kehne et al., 1981). This effect appears to be mediated at least in part by action of strychnine at the level of the spinal cord, since intrathecal administration of the drug produced a dose-dependent increase in startle. These data suggest that glycine neurons normally exert a tonic inhibitory influence on the acoustic startle pathway,

perhaps through direct, post-synaptic inhibition of the α-motoneurons involved in the response. This conclusion is supported by preliminary data which indicates that direct, intrathecal administration of glycine produces a slight depression of startle (unpublished data).

Norepinephrine. A variety of evidence supports the conclusion that increasing NE transmission in the spinal cord produces an increase in acoustic startle. Intrathecal administration of NE increased startle (Astrachan and Davis, 1981). This effect was blocked by the α_1-adrenergic antagonist WB-4101. The α_1-adrenergic agonist phenylephrine also increased startle when given intrathecally. The effect of phenylephrine has been shown to be blocked by WB-4101, but not by the β-adrenergic antagonist propanolol, the DA antagonist haloperidol, or the 5-HT antagonist cyproheptadine (Astrachan and Davis, 1980; Davis and Astrachan, 1981). Intrathecal administration of d-amphetamine also increased startle in a dose-related fashion, and this effect was blocked selectively by α_1-adrenergic antagonists.

Clonidine, a drug that has been reported to have mixed α-agonist properties (e.g. Clough and Hatton, 1978), produced a marked depressant effect on the acoustic startle reflex. This depression occurs either when the drug was given systemically or intrathecally (Davis et al., 1977; Davis and Astrachan, 1981). The depressant effects of clonidine were reversed by the α_2-adrenergic antagonist yohimbine (Davis and Astrachan, 1981) suggesting that clonidine depresses the startle reflex by stimulating α_2-adrenergic receptors. However, the depressant effect of clonidine does not appear to be simply due to its ability to

suppress firing of locus coeruleus neurons, since bilateral electrolytic lesions of this locus failed to abolish the depressant effect (Davis et al., 1977). However, recent evidence indicates that the adjacent "subcoeruleus" gives rise to descending noradrenergic fibers (Guyenet, 1980). Therefore, the depressant effect of clonidine might be due, at least in part, to a depression of descending noradrenergic neurons from the subcoeruleus.

Spinal Neurochemical Modulation: A Comparison of Flexor and Startle Reflexes

The studies just described using intrathecal drug administration have implicated a number of spinal neurochemical systems in the modulation of the acoustic startle response. This should hardly be surprising, since the startle reflex involves a widespread contraction of the flexor musculature (Landis and Hunt, 1939), and data cited earlier in this chapter showed the prominent effects of pharmacological agents on spinal reflexes. In fact, if the pharmacology of the startle reflex is compared with that of the flexor reflex (FR) (see Table 1), a number of striking parallels are seen. For example, agents which facilitate 5-HT transmission (5-HT itself; the putative 5-HT agonists LSD, 5-MeODMT; the 5-HT releasing agent PCA) increase the flexor and startle reflexes. An enhancement of spinal DA transmission (with the DA agonist apomorphine) fails to alter either reflex behavior. Furthermore, blockade of GABA transmission (with the GABA antagonist picrotoxin) or glycine transmission (with the antagonist strychnine)

enhances both startle and flexor reflexes. (Since these drugs are antagonists, an excitatory effect on the behavior implies that spinal GABA and glycine neurons normally exert a tonic inhibitory effect on the two reflexes.) Finally, agents which facilitate noradrenergic transmission, such as the releasing agent d-amphetamine, and the α_1-adrenergic agonists methoxamine and phenylephrine, produce excitatory effects on the startle and/or flexor reflexes. It should be emphasized that, in the flexor studies, the animals are spinalized, thereby ruling out supraspinal sites of action of the systematically adminisstered drugs. Furthermore, the intrathecal infusion of drugs in intact rats insures direct administration at the level of the lumbar cord. In conclusion, the striking correspondence between the excitatory effects of these drugs on the FR and the startle reflex suggests that common spinal sites of action are involved.

However, there is a notable exception to this generalization. Administration of the α-adrenergic agonist clonidine has been reported to facilitate the FR when given systemically (Anden et al., 1970, 1975, 1978; Austin et al., 1976; Nutt et al., 1980). On the other hand, the drug markedly depresses acoustic startle when given either systemically (Davis et al., 1977) or intrathecally (Davis and Astrachan, 1981). Clonidine has been reported to have 'mixed' α-adrenergic agonist properties; that is, it is thought to act on both α_1-adrenergic and α_2-adrenergic receptors (Clough and Hatton, 1981; Kobinger and Pichler, 1981). The clonidine induced vasopressor effect has been attributed to its peripheral α_1-adrenergic agonist properties (Kobinger, 1978), while centrally, clonidine acts as a potent α_2-agonist to depress the firing

of neurons in the locus coeruleus (Cedarbaum and Aghajanian, 1977; Svensson et al., 1975). The α_1- and α_2-agonist actions of the drug are blocked by specific antagonists, such as prazosin (α_1-adrenergic antagonist, Cavero and Roach, 1978) and piperoxane (α_2-adrenergic, Cedarbaum and Aghajanian, 1977; Svensson et al., 1975).

The clonidine-induced facilitation of the FR has been reported to be more readily blocked by α_1- than α_2-antagonists (Anden et al., 1970, 1975, 1978; Austin et al., 1976; Nutt et al., 1980), whereas the clonidine-induced depression of the startle reflex has been shown to be blocked by the α_2-adrenergic antagonist piperoxane, but not by the α_1-adrenergic antagonist phentolamine (Davis et al., 1977). Given the otherwise consistent pharmacology of the FR and the startle reflex, it is not readily apparent why clonidine should act more like an α_2-adrenergic agonist to depress startle while acting like an α_1-adrenergic agonist to facilitate the FR.

In fact, clonidine has been reported to exert α_2-adrenergically mediated depressant effects on a number of different behaviors, such as locomotor activity (Clineschmidt et al., 1980; Delini-Stula et al., 1979; Skolnick et al., 1978; Strombom and Svensson, 1980; Reinstein and Isaacson, 1977; Tilson et al., 1977), self-stimulation (Franklin and Herberg, 1977), conditioned avoidance (Laverty and Taylor, 1969), food-reinforced operant responding (Tilson et al., 1977), pain-elicited aggression (Laverty and Taylor, 1969), as well as exerting sedative effects (Kosman, 1975; Pettinger, 1975; Laverty and Taylor, 1977). Thus, across a wide range of behaviors, clonidine exerts depressant effects that are consistent with the effect seen on acoustic startle,

but not with the effect seen on the FR.

In conclusion, the FR is clearly different from other behavioral paradigms in revealing an α_1-adrenergic excitatory effect of clonidine. The reason for this discrepancy is not readily apparent, though it should be noted that the FR is routinely measured in spinally-transected ('spinalized') animals, whereas other behavioral paradigms involve the use of intact animals. Spinalization per se might result in conditions which would produce a shift in the properties of clonidine from an α_2- to an α_1-adrenergic agonist. If this were the case, then it might be predicted that clonidine would show an effect on the FR in "intact" (i.e. non-spinalized) rats that was more consistent with the effects of the drug on other behaviors. That is, clonidine might be expected to depress the FR in the intact rat through a stimulation of α_2-adrenergic receptors.

Consistent with this prediction, the following studies showed that clonidine produced an α_2-adrenergically mediated depression of the FR in intact rats, in contrast to its α_1-adrenergic excitatory effect in spinalized rats. Furthermore, it appears that this inhibitory effect is due to the activation of supraspinal α_2-adrenergic receptors, whereas the excitatory effects of clondine in the spinal rat are due to the activation of spinal α_1-adrenergic receptors. In the intact rat, the inhibitory effect of clonidine predominates over the excitatory effect, whereas, in the spinalized rat, the supraspinal α_2-adrenergic system is disengaged, allowing the α_1-excitatory effect to be expressed.

CHAPTER 2

EFFECTS OF THE α-ADRENERGIC AGONIST CLONIDINE ON THE FLEXOR REFLEX IN INTACT AND SPINALIZED RATS

Experiment 1: Effects of clonidine on the FR in intact and spinally-transected rats. The purpose of this experiment was to determine the effects of a range of doses of IP clonidine on the FR in spinalized and intact (non-spinalized) rats.

Subjects. Male, albino rats (350-450 g) were used in this and all subsequent experiments. The weight range was 350-450 g unless otherwise specified. Rats were housed in group cages (4 - 5 per cage) in a colony room with a reverse light-dark cycle (12h - 12h). Food and water were available ad libitum.

Spinal transection. Previous studies have demonstrated excitatory effects of clonidine on the FR in rats that have undergone low cervical (C8), high thoracic (T1 - T3), mid-thoracic (T4 - T5), or low thoracic (T10 - T12) spinal transections (Anden et al., 1970; Austin et al., 1976; Nozaki et al., 1980; Nozaki et al., 1977; Nutt et al., 1980; Nygren and Olson, 1977). Pilot studies similarly indicated that the precise level of transection was not critical for observing this effect. For consistency, all transections performed in the following studies were carried out at T1-T2 (first and second thoracic vertebrae). This high thoracic level was chosen for several reasons:

(1) The greater distance from the site of the FR circuitry (the lumbar spinal cord) was thought to minimize non-specific damage to the reflex pathway that might otherwise occur with lower transections. (2) It was easier to obtain surgical access to the spinal cord through the vertebral column at this level, since the column is more "flexible" than at higher levels (allowing for normal movement). (3) This level is marked by a prominent landmark, a vertically-projecting spinous process that allowed rapid and precise localization of the site of transection.

For the 'spinalized' group, rats were maintained under halothane anesthesia throughout the duration of the operation (10 - 15 minutes). Following initial anesthetization, a rat was placed in a Kopf stereotaxic instrument and a 6 cm incision was made through the skin on the back. Overlying thoracic muscles were displaced away from the midline with surgical retractors, and the ligaments and tissues surrounding the column at the site of the transection were cut away. A laminectomy was then performed using a pair of bone rongeurs. By removing both the dorsal and lateral components of the vertebrae, the spinal cord could be easily visualized. A curved suture needle held firmly with a pair of hemostats was then inserted underneath the cord, and the cord was gently lifted. A pair of surgical microscissors was used to completely sever the spinal cord. After bleeding had been controlled, the site of the transection was covered with Gelfoam and gauze to control further bleeding and the incision was sutured shut. The final step involved inserting a metal ring (made from a 19 gauge hypodermic needle) through the skin behind the base of the Achilles'

tendon. This ring served as the point of attachment of the hindlimb to the force transducer.

Following completion of the operation, the rat was removed from the stereotaxic instrument and was placed in a cage under a tensor lamp until the time of testing. Despite the apparent severe nature of the operation, mortality was rarely encountered. Rats recovered from the anesthesia within 5 - 10 minutes, and, despite the complete paralysis of their hindquarters, would often move themselves about the cage with their forelimbs.

For the 'intact' group, rats were anesthetized with halothane and placed in the stereotaxic instrument for 10 minutes and then allowed two hours for recovery.

Testing.

Apparatus. The apparatus used to quantify the flexor reflex was modified from descriptions from the literature (Austin et al., 1976; MacDonald and Pearson, 1979; Anden et al., 1978; Nygren and Olson, 1976), as were the various stimulation parameters. Prior to testing, rats were placed in a Bollman restraining cage that had been modified so that the hindlimbs of the rats hung down through two holes cut in the bottom of the cage. The left leg was taped to a support so that it remained stationary. The right leg was connected to a Grass force transducer by means of a metal ring inserted through the skin behind the base of the Achilles' tendon. The output of the force transducer was amplified through a Grass Model 7P1 A Low-Level D.C. pre-amplifier

and displayed on a multi-channel polygraph chart recorder. The sensitivity of the pre-amplifier was maintained at 2 mv/cm. The stimulus for eliciting the hindlimb flexor reflex was a 2 msec square wave pulse from a Grass S88 Stimultor fed through a Grass SIU5 Stimulus Isolation Unit. The stimulus was delivered through two insect pins (the ends of which were separated by 3 mm) implanted subcutaneously in the sole of the right hindpaw. The electrode was taped securely to the hindpaw to assure that it remained in a stationary position throughout the period of testing. The amplitude of the flexor reflex was quantified by measuring the length (in mm) of the response traced on the polygraph paper.

Procedure. Both 'spinalized' (n=27) and 'intact' (n=12) groups were tested under similar conditions 2 hours following termination of the anesthesia. Five minutes after being placed in the test apparatus, 30 eliciting stimuli were presented at a 20 second interstimulus interval (i.e. a 10 minute 'baseline' period). Three different stimulus intensities (15v, 30v, and 60v), presented in a quasi-randomized order, were used to evoke a range of flexor responses. The parameters were derived from previous reports (Austin et al., 1976; MacDonald and Pearson, 1979). Following the 10 minute 'baseline' period, rats were injected IP with saline, and 15 minutes later, were presented with a second group of 30 stimuli (20 second interstimulus interval; 'saline' period). At the end of the 'saline' period, rats were injected IP with either saline or a single dose of clonidine. Fifteen minutes after this second injection, a third test was carried out ('clonidine'

period). Thereafter, the rats were removed from the test apparatus. Each rat was tested only once.

Results and discussion. Fig. 1 shows the effects of saline injections alone (right panels) or saline injection followed by 0.5 mg/kg clonidine (left panels) in spinalized rats (upper panels) or intact rats (lower panels). In the groups that received saline injections only, the amplitude of the FR remained relatively stable across the three test periods. In the rats that received saline followed by clonidine, a marked depression occurred in the intact group following clonidine administration, whereas an excitation occurred in the spinalized group.

As seen in Fig. 2, clonidine produced a dose-dependent depression of the FR in 'intact' rats, whereas it produced a dose-dependent excitation in 'spinalized' rats. Each bar represents the change scores (mean response amplitude after clonidine adminstration minus the mean response amplitude for the test period prior to clonidine administration) at each dose of clonidine or following saline administration.

It is important to recognize the dramatic depression of baseline level of responding seen in the 'spinalized' group relative to the 'intact' group (see Figs. 1,2). Without exception, transected rats showed weak responses to the same intensity stimuli that produced vigorous responses in the non-spinalized rats. Since the change scores used to generate Fig. 3 described drug-induced changes in the FR relative to the predrug baseline, it might be argued that, in one

sense, the clonidine 'depression' of the FR is artificial. That is, the post-clonidine scores in 'intact' rats, though lower than the baseline scores, might be similar to the levels achieved in the 'spinalized' rats following clonidine injection (and thus revealing an underlying excitatory effect). Fig. 3 shows the absolute post-clonidine scores (averaged across the stimulus intensities) following the injection of the different doses of clonidine. Clearly, the absolute levels of responding are lower in the 'intact' rats relative to the 'spinalized' at the 0.5 and 2.0 mg/kg doses. Therefore, in both absolute and relative terms, clonidine depressed the FR in 'intact' rats and increased the FR in 'spinalized' rats.

A one-factor analysis of variance (ANOVA) of the change scores in the 'spinalized' group revealed a significant overall drug effect ($F = 11.8$; $df = 4,10$; $p<.001$) that was linearly related to the doses tested (0.125, 0.25, 0.5, 1.0, and 2.0 mg/kg) ($F_{1in} = 42.4$; $df = 1,10$; $p<.001$). A similar analysis of the change scores for the 'intact' group showed a significant overall drug effect ($F = 6.70$; $df = 4,16$; $p<.005$) that was lineraly related to the doses used (0.007, 0.03, 0.125, 0.5, and 2.0 mg/kg) ($F_{1in} = 25.3$; $df = 1,16$; $p<.001$). Cubic and quadratic trend analyses were not significant for either 'intact' or 'spinalized' groups.

The dose range for the excitatory effects of clonidine on the FR is similar to that reported by other investigators (Nutt et al., 1980; Anden et al., 1978; Austin et al., 1976). In the 'spinalized' group, a 0.125 mg/kg dose of clonidine produced a threshold FR activation. Two of the three rats tested at this dose showed no increases at any of the

three stimulus intensities used, and the third showed only a very slight augmentation at the highest intensity. In contrast, the depressant effects in the 'intact' rats were evident at a lower (0.03 mg/kg) dose.

Experiment 2: Piperoxane and prazosin antagonism of clonidine's effects on the FR in intact and spinalized rats. The purpose of the second experiment was to determine whether the observed effects of clonidine on the FR were attributable to an activation of α_1-adrenergic and/or α_2-adrenergic receptors. Prazosin and piperoxane have been shown to be relatively specific α_1-adrenergic and α_2-adrenergic antagonists, respectively, in electrophysiological, biochemical, and behavioral studies (Menkes et al., 1981; Cavero and Roach, 1980; Langer et al., 1980; Greengrass and Bremner, 1979; Hornung et al., 1979; Davis et al., 1977; Cedarbaum and Aghajanian, 1977; Cedarbaum and Aghajanian, 1976; Langer, 1974).

Methods. 'Spinalized' (n=9) and 'intact' (n=9) groups of rats were prepared as described previously. Rats were pretreated with either 10 mg/kg piperoxane, 1.0 mg/kg prazosin, or vehicle (distilled water) one-half hour before being placed into the test apparatus. Rats were tested using a different procedure from that used in the prior experiment (the procedure to be described will be employed in all subsequent experiments unless otherwise designated). Five minutes after the rats were secured in the apparatus, five eliciting stimuli (30 v, 2 msec duration) were presented at a one minute interstimulus

interval. This constituted the pre-drug 'baseline'. Immediately after the fifth stimulus, the rats were injected with either 0.5 mg/kg clonidine or vehicle (IP). The dose of clonidine was chosen since it produced near-maximal effects on the FR (as seen in Fig. 2). The rats were presented with an additional 30 stimuli, also at a 1 minute interstimulus interval (the 'post-injection' scores). Animals were tested only once.

Results and discussion. The bottom three panels of Fig. 4 show the effects of pretreatment with either vehicle (left panels), 10.0 mg/kg piperoxane (middle panels), or 1.0 mg/kg prazosin (right panels) on the FR in 'intact' rats before and after treatment with either 0.5 mg/kg clonidine (closed circles) or saline vehicle (open circles). Each point represents the mean amplitude FR across 5 stimulus presentations. In the vehicle and prazosin pretreated groups, clonidine treatment produced a marked depression of the FR, relative to both the pre-injection baselines, and to the post-vehicle treatment scores. There was no significant effect of the three pretreatments on post-vehicle treatment scores. In the piperoxane pretreated group, the clonidine depression appeared to be blunted (though not completely blocked). Fig. 5 shows the mean change scores (30 minutes post-injection minus 5 minutes pre-injection) following vehicle or clonidine treatment for the three pretreatment groups. This graph shows that the piperoxane pretreatment blunted the depressant effect of clonidine, while prazosin was without effect.

An overall ANOVA with 'pretreatment' (vehicle, piperoxane, and

prazosin) and 'treatment' (vehicle, clonidine) as factors revealed a non-significant pretreatment effect ($F = 2.73$; $df = 2,12$ n.s.), a significant treatment effect ($F = 88.2$; $df = 1,12$ $p<.001$) and a significant pretreatment x treatment interaction ($F = 6,96$; $df = 2,12$; $p<.01$). A subsequent two-factor ANOVA with 'treatment' (vehicle, clonidine) and 'pretreatment' (vehicle, piperoxane) as factors revealed a significant treatment effect ($F = 33.3$; $df = 1,8$; $p<.001$), a non-significant pretreatment effect ($F = 3.62$, $df = 1,8$; $.05<p<.1$), and a significant treatment x pretreatment interaction ($F = 8.5$; $df = 1,8$; $p<.025$). The significant interaction effects supports the conclusion that piperoxane blunted the depressant effect of clonidine. A similar analysis for the prazosin pretreated group revealed only a significant treatment effect ($F = 82.8$; $df = 1,12$; $p<.001$), supporting the conclusion that prazosin had no effect on the clonidine depression of the FR.

The top three panels of Fig. 4 show the effects of pretreatment with vehicle (left panel), 1.0 mg/kg prazosin (right panel), or 10 mg/kg piperoxane (middle panel) on the FR in 'spinalized' rats before and after treatment with vehicle or 0.5 mg/kg clonidine. In contrast to its lack of effect on clonidine inhibition in 'intact' rats, prazosin pretreatment completely prevented the excitatory effect of clonidine on the FR. Piperoxane appeared to partially block the effect of clonidine. These observations are further illustrated in Fig. 5, in which the mean change scores are expressed. The three pretreatments provided similar effects on the baseline, with response levels declining to near zero across the test session.

A two-factor ANOVA with 'pretreatment' (vehicle, prazosin) and 'treatment' (vehicle, clonidine) as factors revealed significant pretreatment ($F = 21.0$; $df = 1,8$; $p<.001$) and treatment ($F = 31.0$; $df = 1,8$; $p<.001$) effects and a significant pretreatment x treatment interaction effect ($F = 23.0$; $df = 1,8$; $p<.001$). A similar analysis for the piperoxane pretreated groups revealed significant pretreatment ($F = 6.0$; $df = 1,8$; $p<.05$) and treatment ($F = 60.1$; $df = 1,8$; $p<.001$) effects, and a significant pretreatment x treatment interaction ($F = 5.54$; $df = 1,8$; $p<.05$). The significant interactions in both pretreatment groups support the conclusion that both piperoxane and prazosin antagonized the clonidine response, though from the graphs it is apparent that the prazosin blockade was by far the greater effect.

The results from the first two experiments suggest that clonidine depresses the FR in intact rats, at least in part, through an α_2-adrenergic mechanism, whereas it increases the FR in spinalized rats through an activation of α_1-adrenergic receptors. Because the spinalization procedure effectively eliminates the effect of direct supraspinal neural influences on the FR circuitry, it is reasonable to conclude that the excitatory effects of clonidine are attributable to an activation of spinal α_1-adrenergic receptors. This conclusion is consistent with anatomical data showing the presence of noradrenergic nerve terminals in the spinal cord, the cell bodies of which originate in the pons (Nygren and Olsen, 1977; Commissiong, 1980; Commissiong et al., 1978a,b; Lindvall and Bjorklund, 1974). However, the first two experiments do not address the localization within the central nervous system of the α_2-adrenergic mediated inhibitory effects of clonidine.

One approach to determining where a drug acts within the CNS to produce a given effect is to use local drug infusions into the cerebrospinal fluid at the level of the forebrain (intraventricular administration), or at the level of the spinal cord (intrathecal administration). However, with highly lipophilic compounds (such as clonidine), these techniques must be used with caution, since, despite the local administration, the drug might gain rapid access to other sites of action (Marwaha et al., 1982).[1] Therefore, in the next experiment, the lipophobic α_2-adrenergic agonist oxymetazoline was administered directly into the spinal subarachnoid space (intrathecal administration) or into the lateral ventricle (intraventricular administration).

---

[1] There is a strong evidence that clonidine depresses unit activity of single cells in the locus coeruleus via stimulation of α_2-adrenergic "autoreceptors" located directly on the cell bodies (Cedarbaum and Aghajanian, 1976). Thus, a series of electrophysiological studies were carried out using the firing of single units in the locus coeruleus as a marker for supraspinal actions of clonidine (Marwaha et al., in prep.). Clonidine produced a rapid, α_2-adrenergic antagonist reversible depression of locus coeruleus neurons when the drug was administered intravenously, intrathecally or intraventricularly. Furthermore, the depression seen following intrathecal administration was still present in spinally-transected rats, ruling out the contribution of a 'feedback loop' from the spinal cord to the locus coeruleus. Finally, intraventricular, but not intrathecal or intravenous administration of the lipophobic α_2-agonist oxymetazoline produced an α_2-adrenergically mediated depression of locus coeruleus firing. Taken together, these findings suggested that intrathecal administration of clonidine, but not oxymetazoline, could gain rapid access to supraspinal sites.

Experiment 3: Effects of intrathecal or intraventricular oxymetazoline on the FR in intact rats.

Methods.

Catheterization. Rats were implanted with intrathecal or intraventricular catheters using procedures described in previous studies (Kehne et al., 1981). For intraventricular implants, a 26-gauge needle was inserted into a 4 cm length of PE-20 tubing and the tip of the needle was rounded off. Under halothane anesthesia, a burr hole was drilled through the skull 1.2 mm lateral from the intersection of bregma and the longitudinal suture. The PE-tubing was then lowered stereotaxically until the top just entered the brain. A 30-cm length of tubing filled with sterile saline was attached to the catheter and held in a vertical position, and the tip lowered into the brain until the fluid in the tubing flowed freely. At this point, the needle was judged to have entered the lateral ventricle. The catheter was then secured at this point with dental cement, which adhered to skull screws. The 26-gauge needle was then removed, and the rats were allowed to recover 3-4 days before testing.

For intrathecal implants, rats were anesthetized under halothane and placed in a Kopf stereotaxic instrument. The cisterna magna was exposed and the overlying membrane punctured. A 8.5-cm length of PE-10 polyethylene tubing fused to a 3-cm length of PE-20 tubing was inserted into the cistern and gently 'snaked' down over the spinal cord to terminate in the lumbar enlargment as described by Yaksh and Rudy

(1976). The PE-20 portion of the catheter was filled with sterile saline, and the rats were allowed to recover 3-5 days before testing.

Testing. On the day of testing, rats were placed in the test apparatus, and a 5 minute pre-infusion baseline was taken, after which the rats were infused with either 10 ul of vehicle, or a range of doses of oxymetazoline (intrathecal doses: 12.5 ug, 50 ug; intraventricular doses: 3.1 ug, 12.5 ug, 50 ug). (These doses were chosen on the basis of pilot studies.) Animals were then presented with an additional 30 stimuli (i.e. 30 minutes of testing) and then discarded.

Results and discussion. Fig. 6 shows the mean scores (30 minutes post infusion minus 5 minutes pre-infusion) for the intraventricular (left panel) and intrathecal (right panel) administration of oxymetazoline. Intrathecally-infused oxymetazoline did not differ from vehicle in its effects on the FR. However, intraventricular infusion of the drug produced a marked, dose-dependent depression of the reflex. For the intraventricular group, a one-way ANOVA with 'dose' as a factor revealed a significant overall depression of the FR ($F = 10.2$; $df = 3,9$; $p<.005$) that was linearly related to the dose used ($F_{1in} = 29.5$; $df = 1,9$; $p<.001$). The overall F for the intrathecal condition was not significant ($F = .18$).

Experiment 4: Piperoxane antagonism of oxymetazoline's effect on the FR. If the depressant effect of intraventricular oxymetazoline on the FR is attributable to a stimulation of α_2-adrenergic receptors, then

this depression should be reversed by an α_2-adrenergic antagonist. The purpose of this experiment was to investigate the effect of systemic piperoxane (which was shown to antagonize the effect of clonidine in intact rats) on the oxymetazoline-induced depression of the FR.

Methods. Rats were implanted with intraventricular catheters as described before. On the day of testing, rats were injected with either piperoxane (10 mg/kg) or vehicle (IP) and one-half hour later placed in the test apparatus. A 5 minute baseline was taken, after which either 10 ul vehicle or 3.25 ug oxymetazoline (in 10 ul vehicle) was infused, followed by a 30 minute test session.

Results and discussion. Table 2 shows the mean change scores (30 minutes post oxymetazoline minus 5 minutes pre-injection) for the four test groups. Pretreatment with piperoxane blocked the depressant effect of this low dose of oxymetazoline. A two-factor ANOVA with 'pretreatment' (vehicle, piperoxane) and 'treatment' (oxymetazoline, vehicle) as factors revealed a significant treatment effect ($F = 6.06$; $df = 1,8$; $p<.05$), a non-significant pretreatment effect ($F = .53$), and a significant pretreatment x treatment interaction ($F = 20.0$; $df = 1,8$; $p<.005$). The significant interaction supports the conclusion that piperoxane pretreatment blocked the effect of intraventricular oxymetazoline on the FR.

It should be noted that this dose of intraventricular oxymetazoline is just threshold for producing a depression of the FR (from previous experiment). Preliminary data suggest that higher

intraventricular oxymetazoline doses may be more resistant to blockade by systemic piperoxane pretreatment. Taken together with the data from the last experiment, these results nevertheless support the notion that a stimulation of supraspinal α_2-adrenergic receptors produces a depression of the FR in rats. Since the depressant effect of IP clonidine was also attenuated by piperoxane, these data indicate that the depressant effects of clonidine on the FR are, at least in part, attributable to a stimulation of supraspinal α_2-adrenergic receptors.

One might argue that the data presented thus far support a "defacilitation" hypothesis. That is, in the intact rat activation of supraspinal α_2-adrenergic receptors (such as those on locus coeruleus neurons) by clonidine would decrease impulse flow in noradrenergic neurons and therefore reduce the normal, tonic facilitation of the FR circuitry in the spinal cord. (Alternatively, clonidine might act by disinhibiting an inhibitory spinal interneuron, allowing it to now inhibit transmission in the FR circuit.) Consistent with both of these mechanisms, spinal transection _per se_ produces a profound depression of the reflex, as if a tonic facilitatory influence had been removed. Furthermore, the inhibitory effects of clonidine in the intact rat were evident at lower doses than those at which the excitatory effects became evident in the spinalized rat. In fact, the doses expected to affect presynaptic α_2-adrenergic receptors would be lower than those required to affect post-synaptic receptors (Cedarbaum and Aghajanian, 1976, 1977). However, there are two pieces of data that are inconsistent with this schema. First, pretreatment with the α_1-adrenergic antagonist prazosin alone failed to produce a profound

'transection-like' defacilitation of the FR. Second, even at higher doses, an excitatory effect of clonidine was not seen in the intact rat. (Recall that the absolute levels of response amplitude following clonidine administration were lower in the intact than in the spinal rats.) Clearly, there is some condition in the intact rat that prevents the α_1-adrenergic effect of clonidine from being expressed.

One possibility is that spinal transection induces a rapid increase in α_1-adrenergic sensitivity in the spinal cord. That is, abrupt cessation of impulse flow in descending noradrenergic neurons could produce "denervation supersensitivity", resulting in clonidine's having an α_1-adrenergic facilitatory effect on the FR. Another possible explanation is that spinal transection removes an α_2-mediated supraspinal inhibitory system that, in the intact rat, prevents or overrides the α_1-adrenergic spinal excitatory effect. In the intact rat, the α_2-mediated inhibition would predominate over the α_1-excitation, leading to a net inhibitory effect. Spinalization would prevent the α_2-adrenergic effect from influencing spinal activity and thus "unmask" α_1-mediated excitation. The purpose of the next series of experiments was to evaluate the relative merits of the "supersensitivity" vs. "unmasking" hypotheses.

Experiment 5: Effects of clonidine on the FR at different times following spinalization. There is reason to believe that rapid changes in α_1-adrenergic sensitivity may take place following transection-induced denervation of spinal α_1-adrenergic receptors. Using ^{3}H-Prazosin binding, Corr et al. (1981) have reported that ischemia can

produce a rapid (within 30 minutes) increase in α_1-adrenergic receptor number (without a change in affinity) in myocardium. Since ischemia can also produce an increase in the electrophysiological responsiveness of myocardium to α_1-adrenergic agonists (see Corr et al., 1981), these data indicated a rapid development of supersensitivity of peripheral α_1-adrenergic receptors.

The progressive development of supersensitivity following transection might be reflected by a progressive increase in the facilitatory effect of clonidine on the FR. The purpose of this experiment was to determine the magnitude of the clonidine response at various times following spinal transection.

Methods. Four groups of rats (n=3 in each group) were transected as previously described, and were then tested either immediately, 1/2, 2, or 6 hours following transection for their responses to 0.5 mg/kg clonidine (IP). The testing procedure was the same as that used in the antagonism experiment (Expt. 2). Briefly, 5 minutes after being placed in the cages, a 5 minute baseline was taken (5 stimuli, 30 v intensity, 1 minute interstimulus interval). At the end of the pre-injection baseline, 0.5 mg/kg clonidine was injected and an additional 30 stimuli (30 minutes of testing) given.

Results and discussion. Fig. 7 shows the effects of 0.5 mg/kg clonidine at various times after transection. Each point represents the mean change scores (last 20 minutes of testing minus pre-injection baseline). This figure shows a progressive increase in the effect of

clonidine, with a maximal effect occurring 2 hours following transection. When tested immediately following transection, there was no sign of clonidine-induced activation.

A one-factor ANOVA showed a significant overall time effect ($F = 13.6$; $df = 3,12$; $p<.001$) that was linearly related to the time after transection ($F_{lin} = 25.5$; $df = 1,12$; $p<.001$). There was also a significant quadratic trend ($F_{quad} = 7.9$; $df = 1,12$; $p<.025$), consistent with the slight downward turn in the curve at the last point.

The finding that, immediately following transection, no excitatory effect of clonidine was apparent is inconsistent with the notion that the spinalization "unmasks" an α_1-adrenergic effect that is normally held in check by supraspinal α_2-adrenergic activaton. The progressive increase that culminated at 2 hours following transection is consistent with the idea that a progressive (yet, nonetheless rapid) change in receptor sensitivity is occurring.

Experiment 6: ^{3}H-prazosin and ^{3}clonidine receptor binding in spinalized vs. intact rats. Receptor binding techniques have been used to evaluate biochemical correlates of receptor supersensitivity in denervated neural tissues. An increase in the number of α_1-adrenergic receptors has been found to correlate highly with increased responsiveness to α_1-adrenergic agonists in peripheral and central systems (cf. Schwartz et al., 1978). If an increase in α_1-adrenergic receptor sensitivity following transection is responsible for the emergence of clonidine's excitatory effect in the transected

preparation, then an increase in the number of α_1-adrenergic receptor sites might be seen at the time following transection that corresponds to the point of maximal clonidine facilitation.

Methods. Two groups of rats were prepared. One group ('transected', n=10) was transected under halothane, using the procedure previously described. The other group ('intact', n=10) received exposure to the halothane anesthesia for an equivalent amount of time. Two hours following termination of anesthesia, rats were sacrificed and their lumbar spinal cords were dissected out and frozen at -70°C. One day later, these tissues were analyzed for ^{3}H-prazosin and ^{3}H-clonidine binding using a Scatchard analysis as described below.

Receptor Binding Assays. A combined ^{3}H-prazosin/^{3}H-clonidine assay, modified from previously reported procedures (Hornung et al., 1979), was carried out to estimate dissociation constants (K_D) and receptor densities (B_{max}) in crude membrane homogenates of lumbar spinal cords of treatment and control groups. At specified times following spinal transection or sham treatment, rats were sacrificed and their lumbar cords (180 - 230 mg) were rapidly dissected out, weighed, and frozen on dry ice and stored at -70°C until assay. Binding assays were carried out 1-2 days following sacrifice. Binding parameters were not altered by storage for at least 3 weeks at -70°C.

At the time of assay, lumbar cords were pooled for each treatment condition (7 - 10 rats for each group) and homogenized (using a Brinkman Polytron, setting 8 for 15 seconds) in ice-cold 50 mM Tris HCl

buffer (pH 7.4) in a 1:40 weight to volume (w/v) ratio. The resulting homogenate was centrifuged 42,000 x g for 10 minutes, resuspended in Tris buffer and centrifuged two additional times. The washed membrane pellets were resuspended in cold 1:10 (w/v) 50 mM Tris HC1 buffer (pH 7.4). Half of this homogenate was removed and stored on ice for use in the ^{3}H-clonidine assay (see below). The other half was further diluted in Tris buffer (pH 8.0) to a final 1:40 (w/v) concentration. Aliquots (0.8 ml) of this homogenate were incubated in ascorbic acid (0.1%) in the presence of varying concentrations of ^{3}H-prazosin (0.05 to 1.0 nM; Amersham-Searle; specific activity 20.2 Ci/mMole) and 0.5 M Tris buffer (pH 8.0) to a 1 ml total volume. Non-specific binding was assessed by the addition of 10 uM WB-4101 (Ward-Blenkinsop Ltd.). Quadruplicate samples and duplicate non-specific binding values were obtained for each concentration of ^{3}H-prazosin. Incubations were carried out at room temperature for 30 minutes within which time steady state conditions were achieved. (^{3}H-clonidine assay): Aliquots (0.2 ml) of the homogenate were assayed for ^{3}H-clonidine binding in the presence of varying concentrations of ^{3}H-clonidine (0.2 - 5.0 mMole) (New England Nuclear; specific activity 23.8 Ci/mMole) in 0.50 M Tris HC1 buffer (pH 7.4) to a 10 ml total volume. Non-specific binding was assessed by the addition of 10 uM oxymetazoline. Quadruplicate samples and duplicate non-specific binding values were obtained for each concentration of ^{3}H-clonidine. Incubations were carried out at room temperature for 20 minutes, at which time steady state conditions were achieved. (Filtering): Following the end of the incubation period, samples were rapidly filtered through GF/B filters (Whatham, C., U.K.) under low

vacuum. The filters were washed with three, 6 ml aliquots of ice-cold 50 mM Tris bufer (pH 7.4) and placed in a liquid scintillation counter. Results of the Scatchard analysis are reported as specific prazosin (or clonidine) binding per mg protein, (estimated for each homogenate by the method of Lowry, et al. (1951)) representing total binding minus binding in the presence of 10 uM WB-4101 (^{3}H-prazosin assay) or 10 uM oxymetazoline (^{3}H-clonidine assay). Scatchard plots of binding of various concentrations of ^{3}H-prazosin (or ^{3}H-clonidine) were derived. Plotted lines, calculated by least squares regression, were used to estimate K_D and B_{max}.

Results and discussion. The Scatchard plot of ^{3}H-prazosin binding to rat lumbar spinal cord membranes for the 'spinalized' (closed circles) and 'control' groups is shown in Fig. 8. B/F is the ratio of specific bound ^{3}H-prazosin to free ^{3}H-prazosin. The negative inverse of the slope of each curve gives an estimate of the equilibrium dissociation constant (K_D at 25^{o}C) and the intersection with the abscissa gives an estimate of the total number of binding sites (B_{max}). The calculated values were: control: K_D = 1.09 nM; B_{max} = 61.6 fmol/mg protein; spinalized: K_D = .92 nM; B_{max} = 65.8 fmol/mg protein. Thus, relative to controls, there was a 15% decrease in K_D in the transected group of a 7% increase in the B_{max}. Figure 9 shows the Scatchard plot for ^{3}H-clonidine binding in the lumbar spinal cord. Linear regression revealed the following: control: K_D = 3.33 nM; B_{max} = 309.3 fmol/mg protein, transected: K_D = 3.85 nM; B_{max} = 281.4 fmol/mg protein. Thus, relative to controls, an increase in K_D (15.6%) and a decrease in

B_{max} (9%) was found.

It should be noted that a previous assay (in which ^{3}H-prazosin binding Scatchard analysis alone was carried out), revealed the following results: control: K_D = 2.22 nM; B_{max} = 256 fmol/mg protein; transected: K_D = 2.56 nM; B_{max} = 178.7 fmol/mg protein. Thus, relative to controls, there was a decrease in K_D (15%) and a marked increase in B_{max} (38%). However, the reported values for control B_{max} and K_D were 2-3 times higher than those found in other studies in which ^{3}H-prazosin binding was studied in the lumbar spinal cord (Astrachan, Davis, and Gallager, submitted). In these studies, a relatively high ligand concentration (5 nM) was included. Previous prazosin binding studies have reported more than one site apparent at high ligand concentrations (Horning et al., 1979). Thus the high K_D and B_{max} probably reflect the combination of both high and low affinity components of prazosin. Later studies used only relatively low (0.05 to 1 nM) concentrations of prazosin; Scatchard analysis of these data indicated single population of high affinity binding sites.

Although all the functional experiments reported in this research were carried out on rats that had received high thoracic (T_1-T_2) transections, facilitatory effects of clonidine on the FR have been similarly reported at mid- and low-thoracic levels (Anden et al., 1970; Austin et al., 1976; Nozaki et al., 1980; Nozaki et al., 1977). Pilot work confirmed that clonidine still facilitated the FR in low-thoracic transected rats (T_{10}-T_{11}). To determine if similar changes in α_1-adrenergic receptor sites were seen in low-thoracic transected animals, ^{3}H-prazosin binding was carried out in rats transected at

T_{10}-T_{11}). Scatchard analysis of these data is presented in Fig. 10. Calculated values were: <u>control</u>: K_D = .51 nM; B_{max} = 39.3 fmol/mg protein; <u>transected</u>: K_D = .44 nM; B_{max} = 43.9 fmol/mg protein. Again, relative to controls, a decrease in K_D (13.7%) and an increase in B_{max} (11.7%) was seen.

Thus, across the three experiments done, there was a consistent increase in the number of ^{3}H-prazosin labelled binding sites in the lumbar spinal cords of transected rats relative to controls. However, the magnitude of the increases were variable, and if the data from the original experiment is excluded (in which the control values were unexpectedly high), the increases were 7% - 15%.

Using another behavioral paradigm, the acoustic startle reflex, a correlation between the increse in the number of α_1-adrenergic receptor sites following specific lesions of spinal noradrenergic neurons with 6-hydroxydopamine and behavioral activation following administration of intrathecal phenylephrine, has been found (Astrachan, Davis, and Gallager, in press). The present findings indiate that there are consistent (though perhaps variable in magnitude) increases in α_1-adrenergic receptor density following transection.

In addition, in the one experiment carried out, there appeared to be a decrease in the number of ^{3}H-clonidine labelled α_2-adrenergic receptor sites in the lumbar spinal cord. If clonidine was exerting its depressant behavior effect by acting on α_2-adrenergic receptors in the spinal cord, then one would argue that its increased efficacy as a α_1-adrenergic agonist might be due to a decrease in its α_2-adrenergic receptor-mediated inhibitory properties. However, since intrathecal

administration of α_2-adrenergic agonist oxymetazoline was not effective in depressing the FR (Expt. 3), this explanation seems unlikely.

Experiment 7: Effects of intrathecal phenylephrine on the FR in intact and spinalized rats. If α_1-adrenergic supersensitivity occurs within two hours following transection, then a drug that has predominantly α_1-adrenergic agonist properties should show a greater facilitatory effect on the FR in spinalized rats compared to intact rats. The purpose of this experiment was to compare the effects of the α_1-adrenergic agonist phenylephrine on the FR in intact vs. spinalized rats.

Methods. Since phenylephrine is lipophobic, the drug was administered intrathecally to assure its delivery across the blood-brain barrier into the spinal subarachnoid space. Rats were implanted with intrathecal catheters as described before. On the day of testing the baselines of 12 rats ('intact' group) were measured and then they were infused with either vehicle, 25, 50, or 100 ug phenylephrine dissolved in 10 ul vehicle. Following 30 minutes of testing, the rats were sacrificed and their catheters examined for patency. A second group of 12 intrathecally-implanted rats ('spinalized' group) were spinally transected, with care being taken not to damage the intrathecal catheters in the process of severing the cord. (Subsequent examinations of the catheters following sacrifice revealed no damaged catheters.) Two hours following transection, rats were tested identically to the 'intact' group for their response to intrathecal

vehicle or phenylephrine. All rats were tested only once.

Results and discussion. Fig. 11 shows the mean change scores (30 minutes post-injection minus 5 minutes pre-injection) for the FR in the intact (open circles) and spinalized (closed circles) groups following injection of vehicle, 25, 50, or 100 ug phenylephrine. Inspection of the curves would seem to indicate that phenylephrine enhances the FR in both preparations. However, an overall ANOVA of the change scores revealed that the drug effect was significant only in the 'spinalized' group ($F = 4.13$; $df = 3,8$; $p<.05$) and not in the 'intact' group ($F = 1.28$; $df = 3,8$; n.s.). Subsequent contrasts in the spinalized group revealed a significant linear trend ($F_{lin} = 4.41$; $df = 1,8$; $p<.05$) and a significant quadratic trend ($F_{quad} = 8.07$; $df = 1,8$; $p<.025$). Furthermore, intrathecal treatment with the α_1-adrenergic antagonist WB-4101 (100 ug) following the termination of the 30 minute test session (at a time when the excitatory effect of phenylephrine was still apparent) produced a rapid blockade of the effect (data not shown). Moreover, systemic pretreatment with another α_1-adrenergic antagonist, prazosin, also prevented any sign of phenylephrine activation. Thus the excitatory effect of phenylephrine on the FR in spinalized rats is attributable to α_1-adrenergic activation.

It should be emphasized, however, that the excitatory effect of phenylephrine in the spinal rat was very weak in comparison to the clonidine effect on the FR (Fig. 12). At no time was a robust, "clonidine-like" effect seen. This finding is difficult to understand, given that the clonidine-induced facilitation of the FR was blocked by

prazosin (Expt. 2). It is possible that clonidine is facilitating the reflex by acting through stimulation of some non-adrenergic system, and that the blockade by prazosin is non-specific. Studies of prazosin antagonism of drug effects on the FR that are mediated through other neurochemical systems have not been reported. However, it has been reported that blockade of serotonin receptors with several putative antagonists (cyproheptadine, metergoline) failed to influence the effects of clonidine on the FR in rats (Maj et al., 1976). Furthermore, the clonidine activation is also blocked by another α_1-adrenergic antagonist (phenoxybenzamine; Austin et al., 1976), providing further support for the idea that blockade of α_1-adrenergic receptors is critical. Alternatively, clonidine might be facilitating the FR not by acting on spinal receptors, but rather by acting through a peripherally-mediated mechanism (e.g. an α_1-adrenergic mediated pressor effect). However, systemic injection of methoxamine, which mimics the pressor effect of clonidine (Goodman and Gilman, 1980), did not produce any activation of the FR in spinalized rats. Furthermore, Nozaki et al. (1977) reported that intravenous methoxamine produced a weak, variable (and statistically non-significant) facilitatory effect on the FR. Austin et al. (1976) reported that clonidine applied directly to a flexor muscle (the psoas) failed to mimic the excitatory effect of systemic injection, whereas direct application of clonidine to the surface of the exposed spinal cord produced a rapid activation of the reflex. Phenylephrine's lipophobic nature (and hence its difficulty in crossing lipid membranes) provides another explanation for its poor efficacy in facilitating the FR. Even with intrathecal

infusions, the drug may have difficulty in saturating the relevant receptor sites in the lumbar spinal cord that are necessary for the α_1-adrenergic mediated activation of the FR to occur.

The original purpose of this experiment was to assess for signs of α_1-adrenergic supersensitivity following spinal transection. Given the weak nature of the results, it is important to look more closely at Expt. 5, in which a progressive increase in the excitatory effect of clonidine was seen at various times after transection.

Experiment 8: Effects of re-exposure to halothane anesthetic on the clonidine induced facilitaton of the FR in spinalized rats. A potentially serious objection to the time course study is that the anesthesia under which the spinal transection was performed might have had residual effects which would interfere with the expression of the clonidine-induced facilitation of the FR at early points following transection. Although, behaviorally, the rats appeared to be normal very soon (within 5-10 minutes) after the termination of the anesthesia, subtle residual effects might be occurring. This experiment was designed to assess for residual effects of the halothane anesthetic.

Methods. Rats (n=3) were transected as described previously. One and one-half hours following termination of the operation, the rats were re-exposed to and maintained under halothane anesthesia for 15 minutes. Ten minutes following the termination of anesthesia, the rats were placed in the apparatus and tested in a manner identical to the

procedure used in Expt. 5. That is, the rats received a 5 minute baseline, after which they were injected with 0.5 mg/kg clonidine and tested for an additional 30 minutes.

Results and discussion. The halothane re-exposure completely prevented the expression of the clonidine-induced excitation of the FR. Normally, this facilitation is maximal 2 hours following transection. The mean change scores (5 minutes pre-injection minus 30 minutes post-injection) were: $0.2 \pm .1$ (re-exposed group) and 14.2 ± 1.7 (rats not re-exposed to halothane, data from Expt. 2). There was a statistically reliable difference between these two groups ($t = 6.03$; $p<.01$), supporting the conclusion that re-exposure to halothane interfered with the expression of the facilitatory effects of 0.5 mg/kg clonidine on the FR.

Recently, it has been reported that a brief (5 minute) exposure of mice to halothane anesthetic produced signs of decreased cerebral energy metabolism in the brain, some of which still persisted 10 minutes following termination of anesthesia (a time at which the mice showed behavioral recovery) (McCandless and Wiggins, 1981). Such changes might contribute to the interfering effects of halothane found in the present study.

The results of this experiment make the outcome of the time-course study (Expt. 5) difficult to interpret. It is clear that prior exposure to halothane can interfere with the expression of the excitatory effects of clonidine on the FR. Although the present experiment does not provide a precise quantification of the residual

effects of halothane that might have accounted for the time course found in Expt. 5, it nonetheless indicates that such residual effects cannot be ruled out.

These findings emphasize the need to evaluate the effects of clonidine on the FR following transection in a preparation in which anesthesia can be ruled out as a confounding factor. One procedure which could be used to produce a rapid, 'transection-like' blockade of spinal impulse flow is 'spinal block' with a spinally-administered local anesthetic. The purpose of the next experiment was to use intrathecally-infused procaine to achieve such a spinal block, and thereby determine if the excitatory effects of clonidine would be immediately "unmasked" following rapid cessation of spinal impulse flow.

Experiment 9: Intrathecal procaine-induced reversal of effects of clonidine. This experiment was designed to determine if, at a time of maximal or near-maximal depression of the FR in the intact rats, a sudden blockade of spinal impulse flow by intrathecally-administered procaine could produce a shift of the clonidine effect from inhibition to excitation.

Methods. Reversible spinal block was accomplished by intrathecal infusion of a 20% procaine solution. Intrathecal catheters were constructed as previously described, except that the length of the PE 10 tubing was shorter (5.5 cm), to allow infusion into the thoracic sub-arachnoid space (determined by dye injection). Three to four days

following implantation, rats were placed into the test apparatus and given a 5 minute baseline. They were then injected IP with either vehicle (n=3) or 2.0 mg/kg clonidine (n=3), and then tested for an additional 20 minutes. At this point (the time of near-maximal depression by clonidine), the contents of the intrathecal catheter (4 ul saline) were infused, followed by 1 - 2 ul of procaine. Testing was carried out for an additional 15 minutes.

Results and discussion. As seen in the bottom panel of Fig. 13, rats treated with 2.0 mg/kg clonidine showed a depression from baseline, in contrast to rats that had been injected with IP vehicle. However, when the clonidine-injected rats were infused with 1 - 2 ul procaine, there was a sudden shift of the FR from inhibition to excitation. This excitation occurred either immediately or within a minute or two following procaine infusion, and was accompanied by paralysis of the hindlimbs. The excitatory effect was transient, lasting 4-5 minutes before the response levels returned to their original low levels. The decay of the excitation was accompanied by a loss of complete paralysis (that is, return of muscle tone and/or some spontaneous leg movements).

In contrast, when the vehicle pretreated rats were infused with procaine, a different response was seen. Following procaine infusion, the FR showed a rapid, 'transection-like' decline in response amplitude to near zero levels (top panel of Fig. 13). During this period of diminished responsiveness, the rats showed clear hindlimb paralysis. Recovery from the anesthetic (as indicated by a return of muscle tone and some movement) was not immediately accompanied by a return to

baseline, though one rat that was tested for a longer time period did regain normal levels of responsiveness.

One might argue that the procaine-induced excitation in the clonidine pretreated rats was due not to an active, clonidine-induced excitatory process, but rather to a non-specific 'release' from tonic supraspinal inhibition. However, this argument is refuted by the results obtained from the vehicle pretreated rats, in which no signs of excitation following procaine infusion were seen. Rather, these animals showed an abrupt decline in response amplitude to near zero levels.

Despite the positive results obtained with acute infusions of procaine, there were several technical and theoretical problems with the technique that limited its use. First, although initial pilot work indicated that prolonged spinal block could be readily achieved, in practice the exact levels of procaine needed for effective, prolonged anesthesia proved to be difficult to titrate. Repeated infusions of procaine often produced respiratory failure, probably due to diffusion of the anesthetic to respiratory centers in the cervical cord. Some rats tolerated even larger doses of procaine quite well, though no clear factor underlying the mixed responses was evident. Furthermore, depressant effects seen following prolonged administration might be attributable to diffusion of the anesthetic to the lumbar cord, where direct interference with the FR circuitry could take place. Second, in the strictest sense, anesthetic-induced blockade of impulse flow is not equivalent to the transections produced in previous experiments, since it involves a chemical, not mechanical, disruption of impulse flow in

the spinal cord. What is needed is a technique by which a mechanical transection could be performed without the interference of a general anesthetic. In the next experiment, such a technique was used to determine whether spinal transection can "unmask" an excitatory effect of clonidine on the FR.

Experiment 10: Spinal ligation-induced reversal of effects of clonidine on the FR in intact rats.

Methods. Under halothane anesthesia, the spinal cord was exposed using procedures described previously. However, instead of severing the spinal cord, the following procedure was carried out. The spinal cord was constantly bathed in saline, to maintain its viability. A suture thread was inserted into the eye of a curved suture needle. Holding the sharp end of the needle with hemostats, the blunt end was carefully passed underneath the spinal cord, and the thread was retrieved with a pair of fine, pointed tweezers. Care was taken not to put too much upward pressure on the spinal cord, since this was found to induce some spinal paralysis. The thread was tied loosely around the cord and the ends brought up through the incision in the skin. The exposed spinal cord was then covered with a layer of vaseline and gauze. The skin was sutured shut, and the rat allowed to recover for at least 4-1/2 hours before testing.

Rats that underwent this operation showed a range of recoveries, varying from 'poor' (complete or near complete paralysis) to 'fair' (clear presence of voluntary motor control, but some residual

motor/muscle tone abnormalities), to 'excellent' (physically indistinguishable from unoperated rats). Animals in 'poor' condition were not included in the following study, since they appeared to be functionally transected.

A test procedure similar to that used in the procaine study (Expt. 9) was used. Rats were given a 5 minute baseline period, injected with either 2.0 mg/kg clonidine (n=3) or vehicle (n=3), and then were treated for an additional 20 minutes. At this point, the rats were mechanically transected by tightening the thread that was tied around the spinal cord (hereafter referred to as 'spinal ligation'). Following spinal ligation, the FR was measured for an additional 20 minutes. Spinal ligation occurred a minimum of 4-1/2 hours following exposure to halothane anesthesia.

Results and discussion. Fig. 14 shows the effects of ligation of the spinal cord in rats that had been treated with either vehicle (open circles) or 2.0 mg/kg clonidine (closed circles). Prior to ligation, clonidine produced a depression of the FR relative to the pre-injection baseline, whereas vehicle injection had little effect. Immediately following the spinal ligation, all rats showed a transient (approximately 30 seconds in duration) period of spontaneous flexions and extensions of the hindlimbs and tail slapping. This phenomenon corresponds to the kicking reflex that has been reported to occur following decapitation (referred to as the 'post-decapitation reflex' or PDR; Pappas et al., 1980). During, and for a few minutes after the

cessation of the PDR, the FR was greatly augmented in both groups.[2] However, whereas vehicle treated groups showed a rapid and precipitous decline to near zero levels of responding (like typical transected or procaine-treated rats), the clonidine treated rats' FR remained elevated throughout the entire post-transection test period. This elevation was relative to both the immediate pre-transection level and also relative to the original baseline level. Thus, spinal ligation produced an immediate shift in the effect of clonidine from inhibition to excitation. Preliminary data indicates that 1.0 mg/kg pretreatment with the α_1-adrenergic antagonist prazosin 1/2 hour before testing attenuated (but did not block) the excitation induced by 2.0 mg/kg clonidine. The lack of complete blockade by prazosin (unlike the findings of Expt. 2) might be attributable to the fact that a high dose (2.0 mg/kg) of clonidine was used in the present experiment (alternatively, a longer pretreatment time might be warranted, since

---

[2] This transient period of excitability could be attributed to either a release from a tonic, supraspinal inhibition (Kamut and Sheth, 1971) or it could be due to a transection-induced release of spinal neurotransmitters (i.e. the monoamines) that are facilitatory to spinal reflexes. An argument against the former is the finding in the previous experiment that intrathecal procaine failed to produce a similar, transient hyperexcitability in vehicle treated rats, but rather caused an abrupt decline in response amplitude. Currently, there is no direct proof for the latter hypothesis. However, it has been shown that immediately after peripheral axons have been cut or crushed, they fire off a high-frequency repetitive volley ('injury discharge'). This discharge lasts for seconds, or at most minutes, after which the fibers fall silent. The fibers are still electrically excitable, and may at later times, take on a spontaneous discharge of their own (Kirk, 1974; Wall et al., 1974). It would thus not be surprising to find signs of enhanced noradrenergic activity (i.e. increased release) in the lumbar cord shortly following transection.

prazosin does not readily pass the blood-brain barrier). Nonetheless, a partial attenuation is consistent with the notion that clonidine is, at least in part, exerting its effects through an activation of α_1-adrenergic receptors.

The results of Expt. 5 suggested that clonidine's excitatory effect on the FR was maximal at two hours following transection. However, the last three experiments indicate (1) that the lack of an excitatory clonidine effect at early times following transection was attributable to the interfering effects of the halothane anesthesia, and (2) that spinal block or ligation immediately unmasks an α_1-adrenergic mediated excitatory effect of clonidine. Nevertheless, if there is a progressive augmentation of the clonidine excitation following transection, then this should be manifest by a greater response to clonidine at two hours following 'spinal ligation' than at an early point after 'ligation'.

Experiment 11: Effects of clonidine on the FR 15 minutes or 2 hours following spinal ligation. The purpose of this experiment was to evaluate the effects of 2.0 mg/kg clonidine at either 15 minutes or 2 hours following spinal ligation.

Methods. Rats were prepared as described in the previous study. Four hours following initial surgery, rats were spinally ligated and either 15 minutes or 2 hours later, placed in the test apparatus. Following a 5 minute baseline, rats were injected with 2.0 mg/kg clonidine (IP) and tested for an additional 30 minutes.

Results and discussion. At the time of testing, all rats showed complete hindlimb paralysis typical of spinalized rats. Following clonidine administration, both groups showed a marked enhancement of the FR. The change scores (5 minutes pre-injection minus 30 minutes post-injection) ± standard error of the mean were: 17.3 ± 3.7 (group ligated 15 minutes before testing) and 22.6 ± 1.9 (group ligated 2 hours before testing). An independent t-test revealed no significant difference in these change scores between groups ($F = 1.28$, $df = 4$, n.s.).

Experiment 12: ^{3}H-prazosin and ^{3}H-clonidine binding at 15 minutes or 2 hours following spinal ligation. A supraspinal hypothesis could be invoked to explain the immediate shift in the effects of clonidine from inhibition to excitation following transection (spinal ligation). If supersensitivity does rapidly occur, then one might expect to see changes in α_1-adrenergic binding at either 15 minutes or 2 hours following spinal ligation. The purpose of the present experiment was to evaluate this possibility.

Methods. Twelve rats with threads tied around their spinal cords were prepared as before. Four hours following the operation, the cord was tied off (spinal ligation). Rats were sacrificed either 15 minutes (n=3) or 2 hours (n=3) later, and their lumbar cords were dissected out and frozen at -70°C. In addition, a 'sham-operated' group (n=3), in which threads were implanted but transection was not carried out, and an 'untreated group' (n=3; naive rats taken directly from the animal

room) were similarly sacrificed. One day later, the lumbar cord samples were prepared for combined ^{3}H-prazosin/^{3}H-clonidine assay (procedure described in general methods section). However, a Scatchard analysis was not carried out. Rather, specific binding was measured in tissue from each individual animal in the presence of a single concentration of either ^{3}H-prazosin (1.0 nM) or ^{3}H-clonidine (4.0 nM). Similar procedures to those previously described were used to perform the assays.

Results and discussion. Fig. 15 shows that the mean specific binding of ^{3}H-prazosin or ^{3}H-clonidine was not different across the four groups. If anything, the 2 hour transected ras showed a slight decrease in ^{3}H-prazosin binding and a slight elevation in ^{3}H-clonidine binding. However, an overall one-factor ANOVA revealed no change in ^{3}H-prazosin binding ($F = 1.02$; $df = 3,8$; n.s.) or in ^{3}H-clonidine binding ($F = .45$; $df = 3,8$; n.s.). Mean binding values for individual groups were: unoperated controls - 63.1; sham-operated - 57.4; 15 minute group - 57.1; 2 hour group - 49.2 fmole/mg protein (^{3}H-prazosin binding); and unoperated controls - 91.0; sham-operated - 89.1; 15 minute group - 98.7; 2 hour group - 105.9 fmole/mg protein (^{3}H-clonidine binding).

Thus, using the 'spinal ligation' procedure in which halothane anesthesia is less likely to be a confounding factor, changes in α_1-adrenergic and α_2-adrenergic binding were not seen. The fact that no change occurred at the 2 hour time point was surprising, since the previous binding experiments (Expt. 6) indicated that there were

increases in the number of ^{3}H-prazosin binding sites. There are no obvious explanations for the discrepancy between these two experiments. Perhaps the changes found in the previous experiment are attributable to an interaction between the halothane anesthetic and the transection procedure. Nonetheless, the findings of the present experiment do indicate that changes in α_1-adrenergic and α_2-adrenergic receptor binding are not necessary for the occurrence of the shift in the effect of clonidine on the FR from inhibition in the intact rat to excitation in the transected rat.

The data gathered thus far indicate that, in the normal rat, clonidine produces an inhibition of the FR that is, at least in part, mediated by an activation of α_2-adrenergic receptors. Furthermore, some supraspinal influence (either an inhibitory system activated by clonidine or another, independent system that is tonically active) prevents clonidine from expressing an α_1-adrenergic mediated activation of the FR at the spinal level. Transection produces a 'release' from this inhibitory influence, allowing the excitatory effects to occur. The task of determining the locus (loci) of the supraspinal modulatory systems would be greatly facilitated if one could evaluate the effects of clonidine in a 'reduced' preparation. Decerebration neurally isolates the caudal brainstem from the forebrain, allowing one to rule out forebrain contributions to observed effects of drugs on behavior.

Experiment 13: Effects of clonidine on the FR in acutely-decerebrated rats. The purpose of this experiment was to determine if the effects of clonidine on the FR were altered in the acutely-decerebrated rat.

Methods.

Decerebration. Rats were anesthetized with halothane and placed in a Kopf stereotaxic instrument. The nose bar was set at -3.5. A midline incision was made over the skull and the skin and fascia were retracted. A hole was made with a large burr drill at the farthest lateral point on the top of the skull, such that the center of the hole was 1.5 cm anterior to lambda. Decerebrations at this level extend from the splenium of the corpus callosum down to the mammillary bodies. To decerebrate, the tip of a retractable wire knife (see Davis and Gendelman, 1977, for detailed construction procedure) was lowered into the hole and the wire extended to its full 12 mm length. It was then lowered until it reached the base of the brain. The knife was then moved back up until it touched the top of the skull and then pulled straight up until it came out of the skull. This procedure caused the wire to deflect from its normal angle, as it slid along beneath the top surface of the skull, and allowed the most inaccessible areas directly below the top of the skull to be transected as well. By withdrawing the knife quite slowly at this step, it was usually possible to avoid injury to the sagittal sinus.

Testing. Two hours following decerebration, the rats were placed in the test apparatus, and a 5 minute baseline period was taken. Rats were then injected IP with either 2.0 mg/kg clonidine (n=3) or vehicle (n=3) and tested for an additional 30 minutes. At the end of testing, rats were sacrificed and their brains removed for histology.

Results and discussion. Following decerebration, the rats showed tonic extension of the hindlimbs, characteristic of 'decerebrate rigidity'. Fig. 16 shows the effects of either 2.0 mg/kg clonidine (closed circles) or vehicle (open circles) on the FR in decerebrate rats. Clonidine still depressed the reflex in the decerebrate animal. An independent t-test comparing the change scores (30 minutes post-injection minus 5 minutes pre-injection) revealed a significant difference between the vehicle and clonidine injected groups ($t = 2.7$; $df = 4$, $p<.05$).

These data indicate that, even in the decerebrate preparation, clonidine exerts a potent depressant effect on the FR, with no sign of excitation. Thus, the structure (or structures) that mediates the depressant effect of clonidine and that prevents a clonidine-induced excitatory effect from being expressed is present in the caudal brainstem.

CHAPTER 3

GENERAL DISCUSSION

The results of these experiments may be summarized as follows:

Expt. 1 showed that clonidine produced a dose-related depression of the FR in intact rats, whereas it produced a dose-related excitation of the FR in spinally-transected rats. Thus, clonidine was found to depress the FR just as it does a wide range of other behaviors, provided the FR was measured in the intact rat. Expt. 2 showed that the excitatory effects of clonidine on the FR in spinally-transected rats were mediated by an activation of α_1-adrenergic receptors, since the α_1-adrenergic antagonist prazosin was more effective in blocking the effect than was the α_2-adrenergic antagonist piperoxane. Conversely, the clonidine-induced depressant effect on the FR in intact rats was attenuated by piperoxane, but not affected by prazosin pretreatment, supporting the conclusion that this effect was mediated by an activation of α_2-adrenergic receptors. Expt. 3 showed that intraventricular, but not intrathecal administration of the α_2-adrenergic agonist oxymetazoline was effective in depressing the FR in intact rats, and this effect was attenuated by pretreatment with the α_2-adrenergic piperoxane. Expt. 4. These data support the notion that the clonidine stimulation of supraspinal α_2-adrenergic receptors was responsible for exerting its depressant effect on the FR in the intact rat. Expt. 5 showed that the facilitatory effect of clonidine on the FR in spinalized rats increased in magnitude as a function of time

following transection, with a maximal activation occurring at two hours following transection. These data were interpreted as being consistent wth the notion that a rapid development of supersensitivity, rather than a release from supraspinal inhibition, underlied the shift of the clonidine effect from inhibition to excitation in the transected rat. Expt. 6 showed that at two hours following transection (a time of maximal excitation of the FR in the spinalized rat), there was an increase in the number of α_1-adrenergic receptor sites in the lumbar spinal cord. This biochemical index was consistent with the development of α_1-adrenergic supersensitivity. Expt. 7 found that intrathecal administration of the α_1-adrenergic agonist phenylephrine produced only a weak excitatory effect in spinalized rats and little, if any effect in intact rats. These data did not support the hypothesis that α_1-adrenergic supersensitivity developed following transection. Expt. 8 showed that the halothane anesthetic used in the operations was capable of interfering with the expression of the clonidine excitatory effect on the FR, thus making the results of Expt. 5 ambiguous. Expt. 9 found that a blockade of spinal impulse flow with intrathecal procaine caused an immediate shift in the effect of clonidine from inhibition to excitation, consistent with the idea that spinal transection eliminates a supraspinal, inhibitory influence that normally prevents the α_1-adrenergic excitatory effect of clonidine from being expressed. Expt. 10 showed that 'spinal ligation' in intact, behaving rats similarly produced an immediate shift in the effects of clonidine from inhibition to excitation. Expt. 11 showed that the excitatory effects of clonidine in rats that had undergone prior spinal

ligation were near maximal whether the rats were tested 15 minutes or 2 hours later. These data were again consistent with a 'release' hypothesis. Expt. 12 showed that α_1-adrenergic and α_2-adrenergic receptor binding in the spinal cord did not change in rats that had been spinally-ligated two hours or fifteen minutes before. The lack of a significant binding change provided further evidence against the supersensitivity hypothesis, and indicated that the binding changes seen in Expt. 6 might be related to an anesthetic-transection interaction. Expt. 13 showed that clonidine still depressed the FR in the decerebrate rat, indicating that the mechanism that prevents clonidine induced excitation from being expressed does not require forebrain structures.

The overall aim of the proposed studies was to gain an understanding of why clonidine acts like an α_1-adrenergic agonist to excite the FR in spinally-transected rats when, in other behavioral paradigms (e.g. the acoustic startle reflex) it exerts α_2-mediated depressant effects. Phenomenologically, the resolution to this paradox is clear: the α_1-adrenergic excitatory effect of clonidine was seen only in rats that were spinally-transected (either physically or functionally, with local anesthetic-induced spinal block). When injected into intact rats, clonidine produced a potent depression of the FR, which was attributable to the activation of supraspinal α_2-adrenergic receptors. Thus, in the intact rat, clonidine produced an effect on the FR qualitatively similar to that seen on acoustic startle and other behaviors.

These results suggest that caution should be exercised when

interpreting pharmacological data gathered using the spinalized preparation, in that qualitatively different drug effects may be seen in the spinalized vs. intact organism. Certainly, the rationale for employing spinalization is reasonable: cutting the spinal cord effectively eliminates any contribution of supraspinal actions of systemically-administered drugs. However, alternative methods for localizing drug action to the spinal cord (i.e. the intrathecal technique) may provide information that is more relevant to understanding spinal neurochemical modulation in the intact organism.

In this regard, almost all of the evidence that has been cited in support of clonidine's α_1-adrenergic agonist property has utilized the spinalized preparation. Drug antagonism studies (reported previously and in the present study) clearly show that clonidine's facilitatory effect is due to its spinal α_1-adrenergic agonist property. Yet this property is evident only when the influence of supraspinal structures is eliminated. Why doesn't stimulation of spinal α_1-adrenergic receptors by clonidine produce excitation of the FR in the intact rat?

The initial hypothesis entertained was that spinalization-induced disruption of descending impulse flow in noradrenergic neurons produced a rapid "denervation supersensitivity" in spinal α_1-adrenergic receptors that accentuated the α_1-agonist properties of clonidine. Indeed, receptor binding parameters and the time course for the onset of clonidine's facilitatory effect supported this hypothesis. However, several subsequent findings (primarily the lack of functional evidence for supersensitivity following intrathecal phenylephrine administration) strongly argued against a supersensitivity explanation.

Nevertheless, other studies have provided evidence for the long-term development of supersensitivity following transection. Nozaki et al. (1978) found that the effects of the α_1-adrenergic agonist methoxamine on the FR were greatly enhanced in rats that had been transected for two months (vs. one day). The effect of methoxamine was blocked by pretreatment with the α_1-adrenergic antagonist phenoxybenzamine, supporting the conclusion that the effect was attributable to an activation of α_1-adrenergic receptors. Nygren and Olsen (1976) used a different approach to demonstrate the development of α_1-adrenergic supersensitivity in spinal receptors. These authors found that prior depletion of spinal NE with intracisternally-administered 6-hydroxydopamine enhanced the excitatory effect of clonidine on the FR in acutely-spinalized rats. Thus, either long-term spinalization or long-term depletion of spinal NE produced clear signs of spinal α_1-adrenergic supersensitivity. In another behavioral paradigm, the acoustic startle response, an enhanced excitatory response to phenylephrine has been found following chronic (a week or more) depletion of spinal NE by means of intrathecally-administered 6-hydroxydopamine. Furthermore, the enhanced response to phenylephrine correlated highly with the increase in the number of α_1-adrenergic binding sites in the lumbar spinal cord, as measured with [^{3}H]-prazosin. Thus, in this case, changes in receptor binding reflected functional signs of supersensitivity (Astrachan, Davis and Gallager, in press).

In lieu of a supersensitivity explanation, the bulk of the evidence supports the hypothesis that, in the intact rat, clonidine

activation of supraspinal α_2-adrenergic receptors engages an inhibitory system that depresses the FR and prevents the expression of clonidine's spinal α_1-adrenergic excitatory effects. Spinal ligation or spinal block removes the supraspinal inhibitory influence, allowing the immediate expression of the spinal α_1-adrenergic excitatory effects of clonidine. What evidence is there for the existence of a supraspinal inhibitory system with which clonidine might interact?

In a series of now-classic studies, Magoun and Rhines investigated the effects of electrical stimulation of different areas in the brainstem reticular formation on spinal reflex activity. In addition to finding areas that, when stimulated, produced an excitation of spinal reflexes, they noted that stimulation of the ventro-medial bulbar reticular formation produced a profound inhibitory influence on spinal reflexes (Magoun and Rhines, 1946). This stimulation inhibited extensor and flexor reflexes, as well as inhibiting reflex activity initiated by stimulation of supraspinal structures, such as the motor cortex. The inhibition produced by stimulation was present following complete transection of the rostral medulla, indicating that a direct descending pathway was involved. Recently, fast-conducting axons from the ventro-medial reticular formation have been shown to descend into the spinal cord via the dorsolateral funiculus (Field and Basbaum, 1978). In addition to the classic "depressor area" of Magoun and Rhines, another area, designated the "magnocellular tegmental field" (located adjacent to the raphe nuclei) has been found to exert a depressant effect on somatic reflexes (Engberg, Lundberg and Ryall, 1968). This area gives rise to axons that descend into the spinal cord

via the ventrolateral funiculi. Whereas the ventromedial reticular formation exerts its influence almost exclusively on cranial motor nuclei and ventral horn motor neurons, the tegmental field appears to exert more widespread influences, especially in the dorsal horn (Field and Basbaum, 1978).

Postural atonia (i.e. suppression of spinal postural reflexes) can be produced (1) spontaneously in chronic decerebrate animals; (2) by injection of an anticholinesterase in acute decerebrates; and (3) during REM sleep in intact animals (cf. Pompeiano and Hoshino, 1976). This suppression of reflex activity has been attributed to the activation of the bulbospinal inhibitory system originating in the gigantocellular tegmental field ("FTG"), one of the "depressor areas" just described. Furthermore, Pompeiano and Hoshino (1976) suggested that a dynamic interaction exists between the nucleus locus coruleus/subcoeruleus and the FTG, whereby increased noradrenergic cell firing <u>inhibits</u> the FTG, and decreased noradrenergic activity releases the FTG from inhibition (thus causing it to become active and produce reflex depression). Since cholinergic neurons are found in the FTG, it was proposed that activation of these neurons (either by administration of an anticholinesterase or by reduced noradrenergic activity) produced postural atonia. The authors' hypothesis was supported by good correlations between single cell activity (in the locus coeruleus/subcoeruleus and FTG) and periods of postural atonia induced by the anticholinesterase eserine. That is, high FTG activity (and low locus coeruleus activity) corresponded to atonia, whereas the reverse pattern accompanied recovery. Interestingly, somatosensory stimulation

(which is known to activate the locus coeruleus) reversed eserine-induced atonia.

This work was carried out in cats, though it is possible that such a relationship exists in the rat as well. A "NE-FTG" interaction might shed light upon the mechanism underlying the depressant effect of clonidine in the intact rat. Specifically, if the locus coeruleus tonically inhibited the FTG, then reduction of noradrenergic neuronal firing following clonidine administration would be expected to "release" the FTG, allowing it to exert a depressant effect on spinal reflex activity. Such a relationship would lead to the predictions that:

1. Administration of eserine into the FTG would mimic the depressant effect of systemic clonidine on the FR in the intact rat.
2. Lesions of the locus coeruleus/subcoeruleus would depress the FR (by disinhibiting the FTG).
3. Clonidine administration to a locus coeruleus/subcoeruleus lesioned rat would <u>not</u> "unmask" an excitatory effect on the FR (since the descending inhibitory system would be tonically engaged).
4. Lesions of the FTG (or its output) would produce a spinalization-like "unmasking" of the α_1-adrenergic excitatory effect of clonidine on the FR.

In support of this overall schema, (1) it was found that depression of the FR in intact rats became evident at lower doses than did excitation in the spinalized rats; and (2) preliminary data

indicates that bilateral lesions of the locus coeruleus/subcoeruleus produce the predicted results (a depression of the FR, and no excitatory effect following clonidine administration). However, the most compelling evidence would be the "unmasking" of clonidine's excitatory effect following lesions to the FTG.

The problem with this proposed mechanism is that, at high doses, clonidine would be predicted to act on post-synaptic α_1-adrenergic receptors to inhibit the FTG (and thus reduce or reverse the depression of the FR). At the doses tested in the current study, there was no evidence for such a reversal. (Perhaps a very high dose of clonidine would produce an excitatory effect in the intact rat.) Another explanation is that clonidine, at high doses, might be having depressant effects mediated through other neurotransmitter receptors, such as histamine (Sastry and Phillis, 1977).

Alternatively, clonidine might be stimulating supraspinal α_2-adrenergic receptors that are located other than presynaptically. Recent work indicates that a large percentage of the total number of α_2-adrenergic receptors are located postsynaptically to noradrenergic neurons (Dausse et al., 1981; U'Pritchard et al., 1978). Thus, clonidine could activate (through a post-synaptic α_2-adrenergic receptor) a depressor system that would account for the observed effects on the FR. Importantly, high doses of clonidine would <u>not</u> be expected to produce a 'shift' of the effects of clonidine. Furthermore, direct infusion of oxymetazoline into the depressor area in intact rats would be predicted to mimic the depressant effects of systemic clonidine.

If lesions of a particular inhibitory area produced a "release" of the excitatory effects of clonidine on the FR, then it would be interesting to see if other behaviors which are normally inhibited by clonidine (e.g. locomotor activity, startle) would now show an α_1-mediated excitation.

With regard to locomotor activity, Grillner and his colleagues (Grillner, 1975; Forssberg and Grillner, 1973) have shown that clonidine administered to spinally-transected cats suspended above a moving treadmill produces patterned locomotor movements. Since this activation was blocked by phenoxybenzamine, the authors concluded that stimulation of noradrenergic receptors in the spinal cord activates the pattern generator for locomotion. Furthermore, the clonidine-induced stepping is similar to the locomotor activation seen when a region of the brainstem (designated the "mesencephalic locomotor region", or MLR) is electrically-stimulated in low-decerebrate cats (which normally do not spontaneously locomote). Since the locomotion induced by MLR stimultion is blocked by phenoxybenzamine, and since the MLR is closely-situated anatomically to the nucleus locus coeruleus (Steeves et al., 1980), the authors concluded that MLR stimulation produced locomotion by releasing NE in the spinal cord. Curiously, however, it has not been reported whether clonidine administration in the low-decerebrate preparation produces locomotor stimulation. Given other reports that the drug depresses locomotion in intact rats (as well as exerting general depressant effects on behavior), it would not be surprising to find that clonidine was ineffective. If this were the case, one might postulate that MLR stimulation was, in addition to

releasing spinal NE, also disengaging an independent descending inhibitory system that would normally prevent α_1-adrenergic stimulation in the spinal cord from having excitatory effects on locomotor behavior.

A recent report has shown that a developmental change to the locomotor effects of clonidine takes place (Reinstein and Issacson, 1977). Prior to postnatal day 7, rats show a marked stimulation following clonidine administration, followed thereafter by a shift in the response to inhibition. This finding is consistent with the notion that an inhibitory system which prevents the spinal α_1-adrenergic effects of clonidine from being expressed is not functional at early ages. If this were the case, then it would be predicted that intact baby rats would show an excitatory FR response following clonidine administration. That is, functionally, baby rats should respond to clonidine like spinally-transected adults.

Beyond lesion studies, it would be of interest to investigate pharmacological and, possibly, environmental conditions which would similarly produce a "release" of clonidine's α_1-adrenergic effect. Pharmacological conditions have been reported in which clonidine produces α_1-adrenergic mediated excitatory effects. For example, clonidine was found to depress tactile startle when administered alone (Geyer, 1978), but when given in combination with d-amphetamine, it produced a potentiation of the excitatory effect of amphetamine (Handley and Thomas, 1977). This potentiation was blocked by the α_1-adrenergic antagonist phenoxybenzamine, supporting the conclusion that an activation of α_1-adrenergic receptors was involved. In other

studies, clonidine has been found to potentiate the excitatory effects of apomorphine on locomotor activity (Strombom, 1975) and, again, this effect was blocked by phenoxybenzamine. Apomorphine and d-amphetamine share the common property of enhancing dopaminergic transmission (apomorphine, by a direct agonist action at dopamine receptors, and d-amphetamine, by increasing release and decreasing uptake into the presynaptic terminal (see Groves and Rebec, 1976)). Thus, in the case of locomotion and tactile startle, an enhancement of dopaminergic transmission would appear to produce a shift in the effects of clonidine from inhibition to an α_1-mediated excitation. It would be of interest to see if a similar shift on the FR would be seen if apomorphine was administered together with clonidine. Such a shift would be consistent with the idea that increasing dopaminergic transmission in the brain "turns off" a tonically-active brainstem inhibitory system, which in turn enables clonidine to exert an excitatory behavioral stimulation through an activation of spinal α_1-adrenergic receptors.

Both dopaminergic (Antelman and Caggiula, 1976) and noradrenergic (Weiss et al., 1981) systems have been implicated in mediating an organism's behavioral response to stress-inducing stimuli. The ultimate behavioral outcome when an animal is exposed to stress in all likelihood represents the dynamic interaction of both systems. Given the schema described in the preceding paragraphs, one could postulate that in the presence of an aversive stimulus, an activation of dopaminergic neurons could disengage a brainstem inhibitory system, thus "unmasking" the effects of a concomitant release of spinal NE.

The resulting behavioral response (facilitated reflexes/locomotion) would represent an important example of an interaction between supraspinal and spinal systems in mediating an integrated, adaptive response.

SUMMARY

1. Clonidine depresses the flexor reflex in intact rats through a stimulation of supraspinal α_2-adrenergic receptors that are located in the caudal regions of the brainstem.
2. Clonidine facilitates the flexor reflex in spinalized rats through a stimulation of spinal α_1-adrenergic receptors.
3. In the intact rat, the depressant effect of supraspinal α_2-adrenergic stimulation "overrides" the excitatory effect of spinal α_1-adrenergic receptor stimulation.
4. Spinalization removes the influence of the supraspinal α_2-adrenergic system, allowing the excitatory effect of spinal α_1-adrenergic receptor stimulation to be expressed.

BIBLIOGRAPHY

Aghajanian, G.K. (1980) Mescaline and LSD facilitate the activation of locus coeruleus neurons by peripheral stimuli. Brain Res., 186, 492-498.

Ahlman, H., Grillner, S., & Udo, M. (1971) The effect of 5-HTP on the static fusimotor activity and the tonic stretch reflex of an extensor muscle. Brain Res., 27, 393-396.

Albuquerque, E.X., & Smith, C.M. (1964) Specificity of excitation of muscle spindle afferents by cholinergic substances. J. Pharmacol. Exp. Therap., 146, 344-353.

Anden, N.E., Corrodi, H., & Fuxe, K. (1971) Hallucinogenic drugs of the indolealkylamine type and central monoamine neurons. J. Pharmacol. Exp. Therap., 179, 236-249.

Anden, N.E., Corrodi, H., Fuxe, K., & Hokfelt, T. (1968) Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide. Brit. J. Pharmacol., 34, 1-7.

Anden, N.E., Corrodi, H., Fuxe, K., Hokfelt, T., Rydin, C., & Svensson, T. (1970) Evidence for a central noradrenaline receptor stimulation by clonidine. Life. Sci., 9, 513-523.

Anden, N.E., Gomes, C., Persson, B., & Trolin, G. (1978) R-28935 and prazosin: effects on central and peripheral alpha-adrenoceptor activity and on blood pressure. Nauyn-Schmied. Arch. Pharmacol., 302, 399-306.

Anden, N.E., Grabowska, M., & Strombom, U. (1975) Different alpha-adrenoceptors in the central nervous system mediating biochemical and functional effects of clonidine and receptor blocking agents. Acta. Physiol. Scand. Suppl., 431, 1-24.

Anden, N.E., Haggendahl, J., Magnusson, T., & Rosengren, E. (1964) The time course of the disappearance of noradrenaline and 5-hydroxytryptamine in the spinal cord after transection. Acta. Physiol. Scand., 62, 115-118.

Anden, N.E., Jukes, M.G.M., & Lundberg, A. (1966) A. Spinal reflexes and monoamine liberation. Nature (Lond.), 202, 1222-1223.

Anden, N.E., Jukes, M.G.M., Lundberg, A., & Vylickly, L. (1966) A new spinal flexor reflex. Nature (Lond.), 202, 1344-1345.

Anden, N.E., Jukes, M.G.M., & Lundberg, A. (1966) The effect of DOPA on the spinal cord. 2. A pharmacological analysis. Acta. Physiol. Scand., 67, 387.

Anderson, R.J., & Raines, A. (1976) Suppression of decerebrate rigidity by phenytoin and chlorpromazine. Neurology, 26, 858-862.

Astrachan, D.I., & Davis, M. (1981) Spinal modulation of the acoustic startle response: the role of norepinephrine, serotonin, and dopamine. Brain Res., 206, 223-228.

Astrachan, D.I., Davis, M., & Gallager, D.W. (1982) Behavior and binding: correlations between α_1-adrenergic stimulation of acoustic startle and α_1-adrenoceptor occupancy and number in rat lumbar spinal cord. Brain Res. (in press).

Ault, B., & Evans, R.H. (1978) The action of catecholamines on the isolated hemisected spinal cord of the immature rat. J. Physiol. (Lond.), 278, 41-42.

Austin, J.H., Nygren, L.G., & Fuxe, K. (1976) A system for measuring the noradrenaline receptor contribution to the flexor reflex. Medical Biol., 54, 352-363.

Baez, L.A., & Petroff, F. (1975) Amphetamine effects on motor behavior of chronic decerebrated rats. Neurosci. Abstr., 1, 244.

Baker, R.G., & Anderson, E.G. (1970a) The effects of 1-3,4,-dihydroxyphenylalanine on spinal reflex activity. J. Pharmacol. Exp. Therap., 173, 212-223.

Baker, R.G., & Anderson, E.G. (1970b) The antagonism of the effects of L-3,4-dihydroxyphenylalanine on spinal reflexes by adrenergic blocking agents. J. Pharmacol. Exp. Therap., 173, 224-231.

Banna, N.R., & Anderson, E.G. (1968) The effects of 5-hydroxytryptamine antagonists on spinal neuronal activity. J. Pharmacol. Exp. Therap., 162, 319-325.

Barasi, S., & Roberts, M.H.T. (1973) The action of 5-hydroxytryptamine antagonists and precursors on bulbospinal facilitation of spinal reflexes. Brain Res., 52, 385-388.

Barasi, S., & Roberts, M.H.T. (1974) The modification of lumbar motoneurone excitability by stimulation of a putative 5-hydroxytryptamine pathway. Brit. J. Pharmacol., 52, 339-348.

Barbeau, H., & Bedard, P. (1981) Similar motor effects of 5-HT and TRH in rats following chronic spinal transection and 5,7-dihydroxytryptamine injection. Neuropharmacol., 20, 477-481.

Barber, R.P., & McLaughlin, B.J. (1980) GABAergic synapses in the spinal cord. In, The Spinal Cord and Its Reaction to Traumatic Injury (W.F. Windle, ed.) Marcel Dekker: New York, pp. 189-198.

Barber, R.P., Vaughn, J.E., Saito, K., McLaughlin, B.J., & Roberts, E. (1978) GABAergic terminals are presynaptic to primary afferent terminals in the substantia gelatinosa of the rat spinal cord. Brain Res., 141, 35-55.

Barnes, C.D., Fung, S.J., & Adams, W.L. (1979) Inhibitory effects of substantia nigra on impulse transmission from nociceptors. Pain, 6, 207-215.

Barnes, C.D., Fung, S.J., & Gintautas, J. (1980) Brainstem noradrenergic system depression by cyclobenzaprine. Neuropharmacology, 19, 221-224.

Bartholini, G., Halfely, W., Jalfre, M., Keller, H.H., & Pletscher, A. (1972) A.: Effects of clozapine on cerebral catecholaminergic neurone systems. Brit. J. Pharmacol., 46, 736-740.

Beall, J.E., Martin, R.F., Applebaum, A.E., & Willis, W.D. (1976) Inhibition of primate spinothalamic tract neurons by stimulation in the region of the nucleus raphe magnus. Brain Res., 114, 328-333.

Bedard, P., Barbeau, H., Barbeau, G., & Filion, M. (1979) Progressive increase of motor activity induced by 5-HTP in the rat below a complete transection of the spinal cord. Brain Res., 169, 393-397.

Bednarczyk, B., & Vetulani, J. (1977) Stimulatory and inhibitory action of clonidine on the locomotor activity in the rat. Pol. J. Pharmacol. Pharm., 29, 219-229.

Belcher, G., Davies, J., & Ryall, R.W. (1976) Glycine mediated inhibitory transmission of group I-A excited inhibitory interneurons by Renshaw cells. J. Physiol., 256, 651-662.

Bell, J.A., & Martin, W.R. (1974) Studies of tryptamine and lysergic acid diethylamide (LSD) on cutaneous C-fiber and polysynaptic reflexes in the cat. J. Pharmacol. Exp. Therap., 190, 492-500.

Bell, J.A., & Martin, W.R. (1977) The absence of an effect of l-tryptophan on the C-fiber reflex in the chronic spinal cat. Life Sci., 20, 1087-1090.

Bell, J.A., Martin, W.R., Sloan, J.W., & Buchwald, W.F. (1976) The effect of l-tryptophan on spinal cord C-fiber reflexes. J. Pharmacol. Exp. Therap., 196, 373-379.

Bjorklund, A., & Skagerberg, G. (1979). Evidence for a major spinal cord projection from the diencephalic (A11) dopamine cell group in the rat using transmitter-specific fluorescent retrograde tracing. Brain Res., 177, 170-175.

Bowker, R.M., Westlund, K.N., & Coulter, J.D. (1981) Origins of serotonergic projections to the spinal cord in rat: an immunocytochemical-retrograde transport study. Brain Res., 226, 187-199.

Brautigam, M., Flosbach, C.W., & Herken, H. (1980) Depression of reserpine-induced muscular rigidity in rats after administration of lisuride into the spinal subarachnoid space. Nauyn-Schmiedeberg's Arch. Pharmacol., 315, 177-179.

Brodshaw, C.M., Pun, R.Y.K., Slater, N.T., & Szabadi, E. (1981) Comparison of the effects of methhoxamine with those of noradrenaline and phenylephrine on single cerebral cortical neurones. Brit. J. Pharmacol., 73, 47-54.

Brown, J., & Handley, S.L. (1980) Effects on the pinna reflex of drugs acting at alpha-adrenoceptors. J. Pharmacol., 32, 436-437.

Buterbaugh, G.G. (1977) A role for central serotonergic systems in the pattern and intensity of the convulsive response of rats to electroshock. Neuropharmacology, 16, 707-709.

Carlsson, A., Falck, B., Fuxe, K., & Hillarp, N.A. (1964) Cellular localization of monoamines in the spinal cord. Acta. Physiol. Scand., 60, 112-119.

Carlsson, A., Magnuson, T., & Rosengren, E. (1963) 5-hydroxytryptamine of the spinal cord normally and after transection. Acta. Physiol. Scand., 15, 359.

Carstens, E., Fraunhoffer, M., & Zimmerman, M. (1981) Serotonergic mediation of descending inhibition from midbrain periaqueductal gray, but not reticular formation, of spinal nociceptive transmission in the cat. Pain, 10, 149-167.

Carstens, E., Klumpp, D., Randic, M., & Zimmerman, M. (1981) Effect of iontophoretically applied 5-hydroxytryptamine on the excitability of single primary afferent C- and A-fibers in the cat spinal cord. Brain Res., 220, 151-158.

Cavero, I., & Roach, A.G. (1980) The pharmacology of prazosin, a novel antihypertensive agent. Life Sci., 27, 1525-1540.

Cedarbaum, J., & Aghajanian, G.K. (1976) Noradrenergic neurons of the locus coeruleus: inhibition by epinephrine and activation by the α_1-antagonist piperoxane. Brain Res., 112, 413-420.

Cedarbaum, J., & Aghajanian, G.K. (1977) Catecholamine receptors on locus coeruleus neurons: pharmacological characterization. Europ. J. Pharmacol., 44, 375-385.

Charlton, G., Brennan, M.J.W., & Ford, D.M. (1981) Alterations in neurotransmitter receptor binding in the spinal cord after transection. Brain Res., 218, 372-375.

Clineschmidt, B.V., Pierce, J.E., & Sjoerdsma, A. (1971) Interactions of tricyclic antidepressants and 5-dihydroxyindolealkylamine precursors on spinal monosynaptic reflex transmission. J. Pharm. Exp. Therap., 179, 312-323.

Clough, D.P., & Hatton, R. (1981) Hypotensive and sedative effects of α-adrenoceptor agonists: relationship to α_1- and α_2-adrenoceptor potency. Brit. J. Pharmacol., 73, 595-604.

Commissiong, J.W. (1981) Spinal monoaminergic systems: an aspect of somatic motor function. Fed. Proc., 40, 277-2777.

Commissiong, J.W., Galli, C.L., & Neff, N.H. (1978) Differentiation of dopaminergic and noradrenergic neurons in the rat spinal cord. J. Neurochem., 30, 1095-1099.

Commissiong, J.W., Gentleman, S., & Neff, N.H. (1979) Spinal cord dopaminergic neurons: evidence for an uncrossed nigrospinal pathway. Neurpharmacol., 18, 565-568.

Commissiong, J.W., Hellstrom, S.O, & Neff, N.H. (1978) A new projection from locus coeruleus to the spinal ventral columns: histochemical and biochemical evidence. Brain Res., 148, 207-213.

Commissiong, J.W., & Neff, N.H. (1979) Commentary - current status of dopamine in the mammalian spinal cord. Biochem. Pharmacol., 28, 1569-1573.

Commissiong, J.W., & Sedgwick, E.M. (1974) A pharmacological study of the adrenergic mechanisms involved in the stretch reflex of the decerebrated rat. Brit. J. Pharmacol., 50, 365-374.

Commissiong, J.W., & Sedgwick, E.M. (1979) Modulation of the tonic stretch reflex by monoamines. Europ. J. Pharmacol., 57, 83-92.

Connor, H.E., Drew, G.M., Finch, L., & Hicks, P.E. (1981) Pharmacological characteristics of spinal alpha-adrenoceptors in rats. J. Auton. Pharmac., 1, 149-156.

Connor, H.E., & Finch, L. (1981) Postsynaptic alpha-adrenoceptors mediate effects of intrathecal clonidine. Europ. J. Pharmacol., 76, 97-100.

Corr, P.B., Shayman, J.A., Kramer, J.B., & Kipniss, R.J. (1981) Increased alpha-adrenergic receptors in ischemic cat myocardium--a potential mediator of electrophysiological derangements. J. Clin. Invest., 67, 1232-1236.

Curtis, D.R., Duggan, A.W., Felix, D., & Johnston, G.A.R. (1971) Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord of the cat. Brain Res., 32, 69-96.

Curtis, D.R., Duggan, A.W., & Johnston, G.A.R. (1969) Glycine, strychnine, picrotoxin and spinal inhibition. Brain Res., 14, 759-762.

Dahlstrom, A., & Fuxe, K. (1965) Evidence for the existence of monoamine neurons in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal neuron systems. Acta. Physiol. Scand., 64, 1-36.

Dausse, J.P., Quan-Bui, K.H., & Meyer, P. (1981) α_1 and α_2-adrenoceptors in rat cerebral cortex: effects of neonatal treatment with 6-hydroxydopamine. Europ. J. Pharmacol., 70, 297-302.

Davey, M.J. (1980) Relevant features of the pharmacology of prazosin. J. Cardiovascular Pharmacol., 2, S287-S301.

Davidoff, R.A., Graham, L.T., Shank, R.P., Werman, R., & Aprison, M.H. (1967) Changes in amino acid concentrations associated with loss of spinal interneurons. J. Neurochem., 14, 1025-1031.

Davis, M. (1980) Neurochemical modulation of sensory-motor reactivity: acoustic and tactile startle reflexes. Neurosci. Biobehav. Rev., 4, 241-263.

Davis, M., & Aghajanian, G.K. (1976) Effects of apomorphine and haloperiodol in the acoustic startle response in rats. Psychopharmacology, 47, 217-233.

Davis, M., Astrachan, D.I., Gendelman, P.M., & Gendelman, D.S. (1980) 5-Methoxy-N,N-dimethyltryptamine: spinal cord and brainstem mediation of excitatory effects on acoustic startle. Psychopharmacology.

Davis, M., & Astrachan, D.I. (1981) Opposite effects of clonidine and d-amphetamine on the acoustic startle response in rats. Psychopharmacology.

Davis, M., Astrachan, D.I., & Kass, E. (1980) Excitatory and inhibitory effects of serotonin on sensorimotor reactivity measured with acoustic startle. Science, 209, 521-523.

Davis, M., Cedarbaum, J.M., Aghajanian, G.K., & Gendelman, D.S. (1977) Effects of clonidine on habituation and sensitization of acoustic startle in normal, decerebrate and locus coeruleus lesioned rats. Psychopharmacology, 51, 243-253.

Davis, M., Svensson, T.H., & Aghajanian, G.K. (1975) Effects of d- and l-amphetamine on habituation and sensitization of the acoustic startle response in rats. Psychopharmacology (Berl.), 43, 1-11.

Davis, M., Gendelman, D.S., Tischler, M.D., & Gendelman, P.M. A primary acoustic startle circuit. J. Neuroscience (in press).

Delbarre, B., Schmitt, H. (1973) A further attempt to characterize sedative receptors activated by clonidine in chickens and mice. Europ. J. Pharmacol. 22, 355-359.

Delina-Stula, A., Baumann, P., & Buch, O. (1979) Depression of exploratory activity by clonidine in rats as a model for the detection of relative pre- and potsynaptic central noradrenergic receptor selectivity of α-adrenolytic drugs. Nauyn-Schmiedeberg's Arch. Pharmacol., 307, 115-122.

Drew, G.M. (1976) Effects of alpha-adrenoceptor agonists and antagonists at pre- and post-synaptically located alpha-adrenoceptors. Brit. J. Pharmacol., 62, 468-469.

Eichbaum, F.W., Slemer, O., & Yasaka, W.J. (1975) Postdecapitation convulsions and their inhibition by drugs. Expt. Neurology, 49, 802-812.

Engberg, I., Lundberg, A., & Ryall, R.W. (1968) The effect of reserpine on transmission in the spinal cord. Acta Physiol. Scand., 72, 115-122.

Enna, S.J., & Maggi, A. (1979) Biochemical pharmacology of the GABAergic agonists. Life Sci., 1727-1738.

Fields, H.L., & Bausbaum, A.I. (1978) Brainstem control of spinal pain-transmission neurons. Ann. Rev. Physiol., 40, 217-248.

Forssberg, H., & Grillner, S. (1973) The locomotion of the acute spinal cat injected with clonidine i.v. Brain Res., 50, 184-186.

Franklin, K.B.J., & Herberg, L.J. (1977) Presynaptic α-adrenoceptors: the depression of self-stimulation by clonidine and its restoration by piperoxane but not by phentolamin or phenoxybenzamine. Europ. J. Pharmacol., 43, 33-38.

Fung, S.J., & Barnes, C.D. (1981) Evidence of facilitatory coeruleospinal action in lumbar motoneurons of cats. Brain Res., 216, 299-311.

Fuxe, K., Holmstedt, B., & Jonsson, G. (1972) Effects of 5-methoxy-N,N,-dimethyltryptamine on central monoamine neurons. Europ. J. Pharmacol., 19, 25-34.

Gallaher, D.W., Kehne, J.H., Wakeman, E.A., & Davis, M. (1983) Developmental changes in pharmacological responsivity of the acoustic startle reflex: effects of picrotoxin. Physhopharmacology (in press).

Gentleman, S., Parenti, M., Commissiong, J.W., & Neff, N.H. (1981) Dopamine-activated adenylate cyclase of the spinal cord: supersensitivity following transection of the cord. Brain Res., 210, 217-275.

Georgiev, V.P., & Rawlow, A. (1978) Does picrotoxin interact with the 5-HT system in the flexor reflex? Agressologie, 19, 321-324.

Gilbert, R.F.T., Emson, P.C., Hunt, S.P., Bennett, G.W., Marsden, C.A., Sandberg, B.E.B., Steinbusch, H.W.M., & Verhofstad, A.A.J. (1982) The effects of monoamine neurotoxins on peptides in the rat spinal cord. Neuroscience, 7, 69-87.

Goldstein, B.D., & Anderson, E.G. (1981) Effects of the serotonin agonist, quipazine, on spinal reflexes in the rat. Neuroscience Abstracts, 186.13, 583.

Greengrass, P., & Bremner, R. (1979) Binding characteristics of ^{3}H-prazosin to rat brain α-adrenergic receptors. Europ. J. Pharmacol., 55, 323-326.

Grillner, S. (1975) Locomotion in vertebrates: central mechanism and reflex interaction. Physiol. Rev., 55, 247-296.

Groves, P.M., & Rebec, G.V. (1976) Biochemistry and behavior: some central actions of amphetamine and antipsychotic drugs. Ann. Rev. Psychol., 27, 91-111.

Guyenet, P.G. (1980) The coeruleospinal noradrenergic neurons: anatomical and electrophysiological studies in the rat. Brain Res., 189, 121-133.

Hayes, A.G., & Tyers, M.B. (1979) Effects of intrathecal and intracerebroventricular injections of substance P on nociception in the rat and mouse. Brit. J. Pharmacol., 66, 488P.

Headley, P.M., Duggan, A.W., & Griersmith, B.T. (1978) Selective reduction by noradrenaline and 5-hydroxytryptamine of nociceptive responses of cat dorsal horn neurones. Brain Res., 145, 185-189.

Hodge, C.J., Aprkarian, A.V., Stevens, R., Vogelsang, G., & Wisnicki, H.J. (1981) Locus coeruleus modulation of dorsal horn unit responses to cutaneous stimulation. Brain Res., 204, 415-420.

Hornung, R., Presek, P., & Glossman, H. (1979) Alpha adrenoceptors in rat brain: direct identification with prazosin. Nauyn-Schmied. Arch. Pharmacol., 308, 223-230.

Howe, J.R., & Yaksh, T.L. (1982) Changes in sensitivity to intrathecal norepinephrine and serotonin after 6-hydroxydopamine (6-OHDA), 5,6-dihydroxytryptamine (5,6-DHT) or repeated monoamine administration. J. Pharmacol. Exp. Therap., 220, 311-321.

Jacobs, B.J., & Klemfuss, H. (1975) Brain stem and spinal cord mediation of a serotonergic behavioral syndrome. Brain Res., 100, 450-457.

Jankowska, E., Lund, S., Lundberg, A., & Pompeiano, O. (1968) Inhibitory effects evoked through ventral reticulospinal pathways. Arch. Ital. Biol., 6, 124-140.

Johnston, G.A.R. (1978) Neuropharmacology of amino acid inhibitory transmitters. Ann. Rev. Pharmacol. Toxicol., 18, 269.

Jones, D.J., & McKenna, L.F. (1980) Norepinephrine-stimulated cyclic AMP formation in rat spinal cord. J. Neurochem. 34, 467-469.

Jordan, L.M., Kenshalo, D.R., Martin, R.F., Haber, L.H., & Willis, W.D. (1979) Two populations of spinothalamic tract neurons with opposite responses to 5-hydroxytryptamine. Brain Res., 164, 342-346.

Jordan, L.M., McCreas, D.A., Steeves, J.D., & Menzies, J.E. (1977) Noradrenergic synapses and effects of noradrenaline on interneurons in the ventral horn of the cat spinal cord. Canadian J. Pharmacol., 55, 399-412.

Jurna, I. (1981) Spinal reflexes: what can they tell the pharmacologist? Trends in Pharmacol. Sci., 2, 241-243.

Kamut, U.G., & Sheth, U.K. (1971) The role of central monoamines in decapitation convulsion in mice. Neuropharmacology, 10, 229.

Karoum, F., Commissiong, J.W., Neff, N.H., & Wyatt, R.J. (1980) Biochemical evidence for uncrossed and crossed locus coeruleus projections to the spinal cord. Brain Res., 196, 237-241.

Kawasaki, K., & Matsushita, A. (1981) Sensitive depressant effect of benzodiazepines on the crossed extensor reflex in chloralose-anesthetized rats. Life Sci., 28, 1391-1398.

Kayaalp, S.O., & Neff, N.H. (1980) Regional distribution of cholinergic muscarinic receptors in spinal cord. Brain Res., 196, 429-436.

Kehne, J.H., Gallager, D.W., & Davis, M. (1981) Strychnine: brainstem and spinal mediations of excitatory effects on acoustic startle. Europ. J. Pharmacol., 76, 177-186.

Kirk, E.J. (1974) Impulses in dorsal spinal nerve rootlets in cats and rabbits arising from dorsal root ganglia isolated from the periphery. J. Comp. Neurol., 2, 165-176.

Kobinger, W. (1978) Central α-adrenergic systems as targets for hypotensive drugs. Rev. Physiol. Biochem. Pharmacol., 81, 39-100.

Kobinger, W., & Pichler, L. (1981) α_1- and α_2-adrenoceptor subtypes: selectivity of various agonists and relative distribution of receptors as determined in rats. Europ. J. Pharmacol., 73, 313-321.

Kostyuk, P.G., & Vasilenko, D.A. (1979) Spinal interneurons. Ann. Rev. Physiol., 41, 115-126.

Krivoy, W.A., Couch, J.R., Stewart, J.M., & Zimmerman, E. (1980) Modulation of cat monosynaptic reflexes by substance P. Brain Res., 202, 365-372.

Langer, S.Z. (1977) Presynaptic receptors and their role in the regulation of transmitter release. Brit. J. Pharmacol., 60, 481-497.

Langer, J., Seeber, U., Kuschinsky, K., & Sontag, K.H. (1979) Effect of haloperidol on reflex activation of rat α-motoneurons. Nauyn-Schmied. Arch. Pharmacol., 308, 149-154.

Little, K.D., DiStefano, V., & Leary, D.E. (1957) LSD and serotonin effects on spinal reflexes in the cat. J. Pharmacol. Exp. Therap., 119, 161.

LaMotte, C.C., deLanerolle, N.C., & Johns, D. (1981) Serotonin-positive neurons in monkey spinal cord. Neurosci. Abstr., 186.8.

Llinas, R., & Terzuolo, C.A. (1965) Mechanism of supraspinal action upon spinal cord activities. Reticular inhibitory mechanisms upon flexor motoneurons. J. Neurophysiol., 28, 413-421.

Lundberg, A. (1965) Monoamines and spinal reflexes. In D.R. Curtis and A.K. McIntyre (eds.). Studies in Physiology, Springer, Berline, pp. 186-190.

MacDonald, J.F., & Pearson, J.A. (1979) Some observations on habituation of the flexor reflex in the rat: the influence of strychnine, bicuculline, spinal transection, and decerebration. J. Neurobiol., 10, 67-78.

Magoun, H.W., & Rhines, R. (1946) An inhibitory mechanism in the bulbar reticular formation. J. Neurophysiol., 9, 165-171.

Maj, J., Palider, W., & Rawlow, A. (1977) The influence of mescaline on the flexor reflex of the hindlimb of the spinal rat. J. Pharm. Pharmacol., 29, 177-178.

Maj, J., Palider, W., & Rawlow, A. (1977) The effect of serotonergic compounds on the hind limb flexor reflex in the spinal rat. Pol. J. Pharmacol., 29, 253-257.

Maj, J., Palider, W., & Rawlow, A. (1978) The effect of haloperidol, spiperone, and pimozide on the flexor reflex of the hind limb of the spinal rat. J. Neural Transmission, 43, 113-120.

Maj, J., Palider, W., & Baran, L. (1976) The effects of serotonergic and antiserotonergic drugs on the flexor reflex of the spinal rat: a proposed model to evaluate the action on the central serotonin receptor. J. Neural Transmission, 38, 131-147.

Marley, E., & Vane, J.R. (1967) Tryptamines and spinal cord reflexes in cats. Brit. J. Pharmacol., 31, 447-465.

Martin, W.R., & Eades, C.G. (1967) Pharmacological studies of spinal cord adrenergic and cholinergic mechanisms and their relation to physical dependence on morphine. Psychopharmacol. (Berl.), 11, 195-223.

Martin, W.R., & Sloan, J.W. (1970) Effects of infused tryptamine in man. Psychopharmacologia (Berl.), 18, 231-237.

Marwaha, J., Commissaris, R., Kehne, J., & Davis, M. (1982) The effects of intrathecal administration of clonidine on neuronal activity in nucleus locus coeruleus. Neurosci. Abstr. (in press).

Mason, S.T., & Fibiger, H.C. (1979) Physiological function of descending noradrenaline projections to the spinal cord: role in post-decapitation convulsions. Europ. J. Pharmacol., 57, 29-34.

Mason, S.T., & Fibiger, H.C. (1979) Regional topography within the noradrenergic locus cooeruleus as revealed by retrograde transport of horseradish peroxidase. J. Comp. Neurol., 187, 703-724.

Maxwell, D.R. (1975) Some aspects of the pharmacology of decerebrate rigidity. Gen. Pharmac., 6, 1-7.

McCandless, D.W., & Wiggins, R.C. (1981) Cerebral energy metabolism during the onset and recovery from halothane anesthesia. Neurochemical Research, 6, 1319-1326.

Menkes, D.B., Baraban, J.M., & Aghajanian, G.K. (1981) Prazosin selectively antagonizes neuronal responses mediated by α_1-adrenoceptors in brain. Nauyn-Schmied. Arch. Pharmacol., 317, 273-275.

Menon, M.K., & Vivonia, C.A. (1981) Muscimol-induced myoclonic jerks in mice. Neuropharmacoloby, 20, 441-444.

Menon, M.K., & Vivonia, C.A. (1981) Serotonergic drugs, benzodiazepines and baclofen block muscimol-induced myoclonic jerks in a strain of mice. Europ. J. Pharmacol., 73, 155-161.

Mountcastle, V.B. (1974) Effects of spinal transection. In: Medical Physiology. V.B. Mountcastle (ed.), St. Louis, C.V. Mosley, Co., 662-667.

Myslinski, N.R., & Anderson, E.G. (1978) The effect of serotonin precursors on alpha- and gamma-motoneuron activity. J. Pharmacol. Exp. Therap., 204, 19-26.

Neuman, R.S., & White, S.R. (1982) Serotonin-like actions of quipazine and CPP on spinal motoneurons. Europ. J. Pharmacol., 81, 46-49.

Nicoll, R.A. (1977) Excitatory action of TRH on spinal motoneurons. Nature, 265.

Nozaki, M., Bell, J.A., & Martin, W.R. (1977) Facilitation of the flexor reflex by a central α-adrenergic action of amphetamine in the acute and chronic spinal rat. Fed. Proc., 36, 352.

Nozaki, M., Bell, J.A., & Martin, W.R. (1980) Noradrenergic action of amphetamine following degeneration of descending monoaminergic fibers in the spinal cord. Psychopharmacology, 67, 25-29.

Nozaki, M., Bell, J.A., Vaupel, D.B., & Martin, W.R. (1977) Responses of the flexor reflex to LSD, Tryptamine, 5-hydroxytryptophan, methoxamine, and d-amphetamine in acute and chronic spinal rats. Psychopharmacology, 55, 13-16.

Nozaki, M., Vaupel, D.B., & Martin, W.R. (1977) A pharmacologic comparison of 3,4-methylene dioxyamphetamine and LSD in the chronic spinal dog. Europ. J. Pharmacol., 46, 339-349.

Nutt, J.G., Marquez, M.A., & Woodward, W.R. (1980) Effects of baclofen, GABA mimetics and adrenergic agents on the flexor reflex of the chronic spinal rat. Neurosci. Abstr., 6, 394.

Nygren, L.G., & Olson, L. (1976) On spinal noradrenergic receptor supersensitivity: correlation between nerve terminal densities and flexor reflexes various times after intracisternal 6-hydroxydopamine. Brain Res., 116, 455-470.

Nygren, L.G., & Olson, L. (1977) A new major projection from locus coeruleus: the main source of noradrenergic nerve terminals in the ventral and dorsal columns of the spinal cord. Brain Res., 132, 85-93.

Nygren, L.G., Fuxe, K., Jonsson, G., & Olson, L. (1974) Functional regeneration of 5-hydroxytryptamine nerve terminals in the rat spinal cord following 5,6-dihydroxytryptamine induced degeneration. Brain Res., 78, 377-394.

Piercey, M.F., & Schroeder, L.A. (1981) Sensory and motor functions of spinal cord substance P. Science, 214, 1361-1362.

Piercey, M.F., Dobry, P.J.K., Schroeder, L.A., & Einspahr, F.J. (1981) Behavioral evidence that substance P may be a spinal cord sensory neurotransmitter. Brain Res., 210, 407-412.

Proudfit, H.K., & Anderson, E.G. (1974) New long latency bulbospinal evoked potentials blocked by serotonin antagonists. Brain Res., 65, 542-546.

Proudfit, H.K., & Hammond, D.L. (1981) Alterations in nociceptive threshold and morphine-induced analgesia by intrathecally administered amine antagonists. Brain Res., 218, 393-399.

Ramana Reddy, S.V., & Yaksh, T.L. (1980) Spinal noradrenergic terminal system mediates antinociception. Brain Res., 189, 391-401.

Ramana Reddy, S.V., Maderdrut, J.L., & Yaksh, T.L. (1980) Spinal cord pharmacology of adrenergic agonist-mediated antinociception. J. Pharmacol. Exp. Therap., 213, 525-533.

Reinstein, D.K., & Isaacson, R.L. (1977) Clonidine sensitivity in the developing rat. Brain Res., 135, 378-382.

Roberts, D.C.S., Mason, S.T., & Fibiger, H.C. (1978) Selective depletion of spinal noradrenaline abolishes post-decapitation convulsions. Life Sci., 23, 2411-2412.

Roberts, D.C.S., McCaughran, J.A., & Fibiger, H.C. (1979) Behavioral effects of 6-hydroxydopamine-induced depletion of spinal noradrenaline. Can. J. Physiol. Pharmacol., 57, 1223-1228.

Sastry, B.S.R., & Phillis, J.W. (1977) Evidence that clonidine can activate histamine H_2-receptors in rat cerebral cortex. Neuropharmacol., 16, 223-225.

Sastry, B.S.R., & Sinclair, J.G. (1976) Serotonin involvement in the blockade of bulbospinal inhibition of the spinal monosynaptic reflex. Brain Res., 115, 427-436.

Sastry, B.S.R., & Sinclair, J.G. (1976) The blockade of presynaptic and postsynaptic bulbospinal inhibition of the cat spinal monosynaptic reflex by imipramine. Brain Res., 110, 399-402.

Sastry, B.S.R., & Sinclair, J.G. (1976) Tonic inhibitory influence of a supraspinal monoaminergic system on recurrent inhibition of an extensor monosynaptic reflex. Brain Res., 117, 69-76.

Sastry, B.S.R., & Sinclair, J.G. (1977) Tonic inhibitory influence of a supraspinal monoaminergic system on presynaptic inhibition of an extensor monosynaptic reflex. Brain Res., 124, 109-120.

Sautter, R., Kow, L.M., Pfaff, D.W., & Zemlan, F.P. (1981) Denervation supersensitivity of the bulbospinal serotonin system: 5-HT_1 receptor binding and functional data. Neurosci. Abstr., #6.8.

Schwartz, J.C., Costentin, J., Martres, M. Profais, P., & Baudry, M. (1978) Review: modulation of receptor mechanisms in the CNS: hyper- and hypo-sensitivity to catecholamines. Neuropharmacol., 19, 665-685.

Sinclair, J.G., & Sastry, B.S.R. (1974) Supraspinal monoaminergic involvement in the blockade of recurrent inhibition of the monosynaptic reflex. Neuropharmacol., 13, 741-747.

Sinclair, J.G., & Sastry, B.S.R. (1974) The blockade of bulbospinal inhibition by imipramine, desipramine and pargyline. Neuropharmacol., 13, 643-650.

Skolnick, P., Daly, J.W., & Segal, D.S. (1978) Neurochemical and behavioral effects of clonidine and related imidazolines: interactions with α-adrenoceptors. Europ. J. Pharmacol., 47, 451-456.

Sloviter, R.S., Drust, E.G., & Connor, J.D. (1978) Specificity of a rat behavioral model for serotonin receptor activation. J. Pharmacol. Exp. Therap., 206, 339-347.

Sorenson, C.A., George, S.A., & Friedman, A.J. (1978) The effects of d-amphetamine on gamma efferent activity in the acute decerebrate rat. Brain Res., 143, 387-391.

Starke, K., & Docherty, J.R. (1980) Recent developments in α-adrenoceptor research. J. Cardiovasc. Pharmacol., 2, 269-286.

Steeves, J.D., Schmidt, B.J., Skovgaard, B.J., & Jordan, L.M. (1980) Effect of noradrenaline and 5-hydroxytryptamine depletion on locomotion in the cat. Brain Res., 185, 349-362.

Strahlendorf, J.C., Strahlendorf, H.K., Kingsley, R.E., Gintautas, J., & Barnes, C.D. (1980) Facilitation of the lumbar monosynaptic reflexes by locus coeruleus stimulation. Neuropharmacology, 19, 225-230.

Strombom, U. (1975) On the functional role of pre- and postsynaptic catecholamine receptors in brain. Acta. Physiol. Scand., 43, 289-298.

Strombom, U., & Svensson, T.H. (1980) Clonidine: attenuation of sedative action by facilitated central noradrenergic transmission. J. Neural Transmission, 47, 29-40.

Summers, R.J., Jarrot, B., & Louis, W.J. (1980) Selectivity of a series of clonidine-like drugs for α_1- and α_2-adrenoceptors in rat brain. Neuroscience Letters, 20, 347-350.

Sypniewska, M. (1979) The effect of baclofen on the hind limb flexor reflex of the spinal rat. Pol. J. Pharmacol. Pharm., 31, 493-501.

Taber, C.A., & Anderson, A.G. (1973) Paradoxical blockade by p-chlorophenylalanine of 5-hydroxytryptophan facilitatory actions on spinal reflexes. J. Pharmacol. Exp. Therap., 187, 229-238.

Takano, K., & Neumann, K. (1972) Effect of glycine upon stretch reflex tension. Brain Res., 36, 474-475.

Takano, K., & Student, J.C. (1978) Effect of diazepam on the gamma motor system indicated by the response of the muscle spindle of the triceps surae muscle of the decerebrate cat to the muscle stretch. Nauyn-Schmied. Arch. Pharmacol., 302, 91-101.

Tilson, H.A., Chamberlain, J.H., Gylys, J.A., & Buyniski, J.P. (1977) Behavioral suppressant effects of clonidine in strains of normotensive and hypertensive rats. Europ. J. Pharmacol., 43, 99-105.

Ungerstedt, U. (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. Acta. Physiol. Scand., 367, 1-48.

U'Prichard, D.C., Bechtel, W.D., Ruot, B.M., & Snyder, S.H. (1978) Multiple apparent alpha-noradrenergic receptor binding sites in rat brain: effect of 6-hydroxydopamine. Molecular Pharmacology, 16, 47-60.

Vaupel, D.B., & Martin, W.R. (1976) Actions of methoxamine and tryptamine and their interactions with cyproheptadine and phenoxybenzamine on cat spinal cord segmental reflexes. J. Pharmacol. Exp. Therap., 196, 87-96.

Wall, P.D., & Devor, M. (1978) Physiology of sensation after peripheral nerve injury, regeneration, and neuroma formation. In Physiology and Pathobiology of Axons (S.G. Waxman, ed.). New York: Raven Press, 377-388.

Weiss, J.M., Goodman, P.A., Losito, B.G., Corrigan, S., Charry, J.M., & Bailey, W.H. (1981) Behavioral depression produced by an uncontrollable stressor: relationship to norepinephrine, dopamine, and serotonin levels in various regions of rat brain. Brain Res. Reviews, 3, 167-205.

White, S.R., & Neuman, R.S. (1980) Facilitation of spinal motoneurone excitability by 5-hydroxtryptamine and noradrenaline. Brain Res., 188, 119-127.

Yaksh, T.L. (1979) Direct evidence that spinal serotonin and noradrenaline terminals mediate the spinal antinociceptive effects of morphine in the central periaqueductal gray. Brain Res., 160, 180-185.

Yaksh, T.L., & Wilson, P.R. (1979) Spinal serotonin terminal system mediates antinociception. J. Pharmacol. Exp. Therap., 208, 446-453.

Yaksh, T., Hammond, D.L., & Tyce, G.M. (1981) Functional aspects of bulbospinal monoaminergic projections in modulating processing of somatosensory information. Fed. Proc., 40, 2786-2794.

Yaksh, T.L., & Rudy, T.A. (1976) Chronic catheterization of the spinal subarachnoid space. Physiol. Behav., 17, 1031-1036.

York, D.H. (1972) Potentiation of lumbo-sacral monosynaptic reflexes by the substantia nigra. Exper. Neurol., 36, 437-448.

APPENDIX

Table 1. Comparative effects of various drugs on the flexor and startle reflexes.

TABLE 1

SPINAL ACTION	DRUG	EFFECT ON FLEXOR REFLEX (SYSTEMIC INJECTION)	EFFECT ON STARTLE REFLEX (INTRATHECAL INJECTION)
INCREASE SEROTONERGIC TRANSMISSION	d5-HT	INCREASE	INCREASE
	LSD	INCREASE	INCREASE
	MESCALINE	INCREASE	INCREASE
	PARA-CHLOR-AMPHETAMINE	INCREASE	INCREASE
INCREASE DOPAMINERGIC TRANSMISSION	APOMORPHINE	NO CHANGE	NO CHANGE
DECREASE GLYCINERGIC TRANSMISSION	STRYCHNINE	INCREASE	INCREASE
DECREASE GABAERGIC TRANSMISSION	PICROTOXIN	INCREASE	INCREASE
INCREASE NORADRENERGIC TRANSMISSION	d-AMPHETAMINE	INCREASE	INCREASE
	METHOXAMINE	INCREASE	INCREASE
	PHENYLEPHRINE	-	-
	CLONIDINE	INCREASE	INCREASE

Table 2. Effects of vehicle or 10 mg/kg piperoxane (I.P.) pretreatment on vehicle or oxymetazoline (intraventricular) treatment. Data are expressed as mean change scores (30 minutes post infusion minus 5 minutes pre-infusion) ± S.E.M.

TABLE 2

		Intraventricular Treatment	
		Vehicle	Oxymetazoline
Intraperitoneal Pretreatment	vehicle	2.3 ± 1.1	-4.3 ±1.1
	piperoxane	-1.3 ± .9	1.2 ± .7

Fig. 1. Mean amplitude flexor reflex following i.p. injection of 0.5 mg/kg clonidine (left panels) or vehicle (right panels) in spinalized (n=18, top panels) or intact (n=27, lower panels) rats. Three stimulus intensities (60v, 30v, 15v) were used.

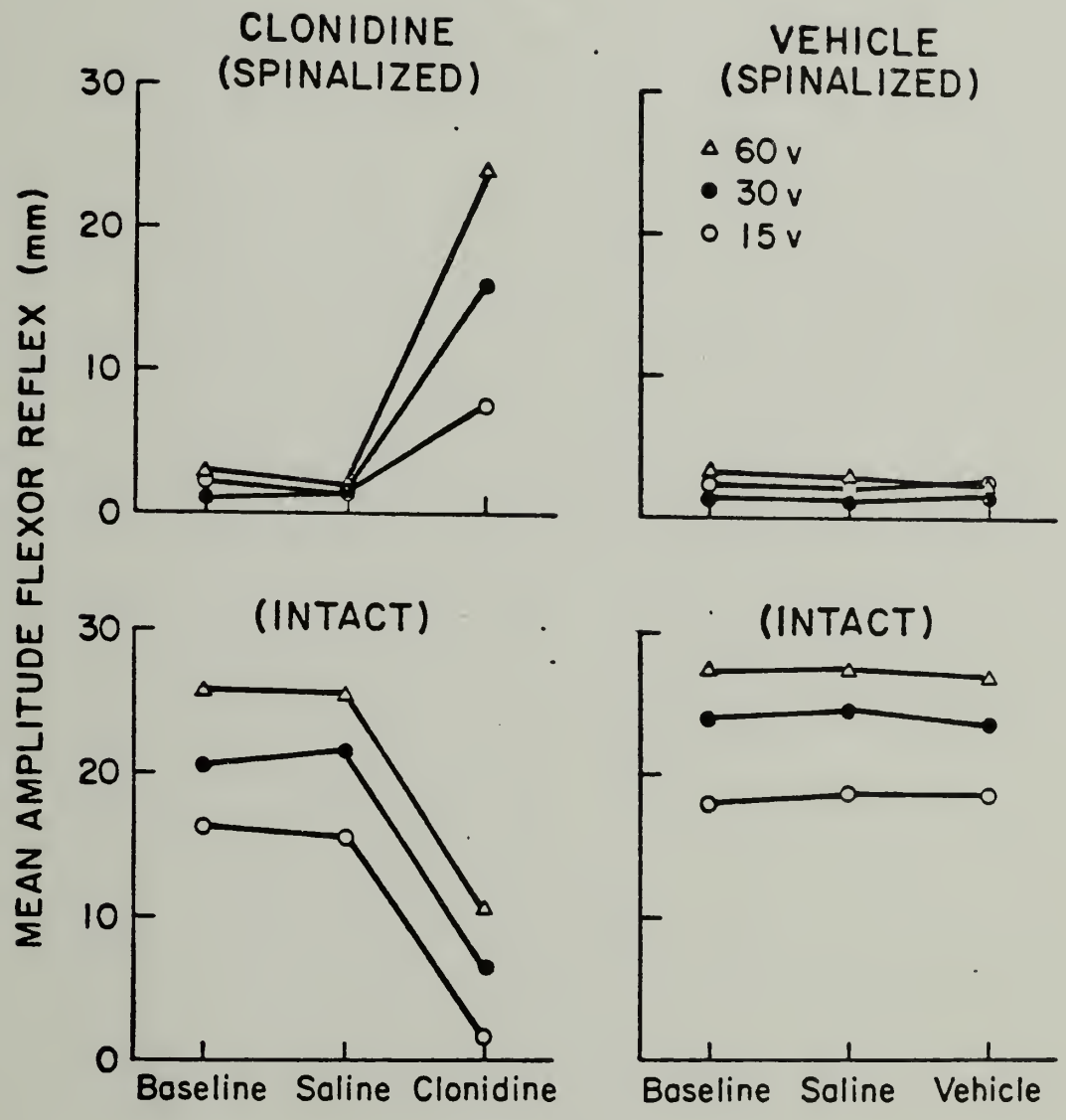

CLONIDINE
(SPINALIZED)
VEHICLE
(SPINALIZED)
△ 60 v
● 30 v
○ 15 v
(INTACT)
(INTACT)
MEAN AMPLITUDE FLEXOR REFLEX (mm)
30
20
10
0
Baseline
Saline
Clonidine
Vehicle

Fig. 2. Mean change scores (clonidine minus saline) for rats treated with clonidine (.007 - 2.0 mg/kg, i.p.) or vehicle in spinalized (upper panel) or intact (lower panel) rats.

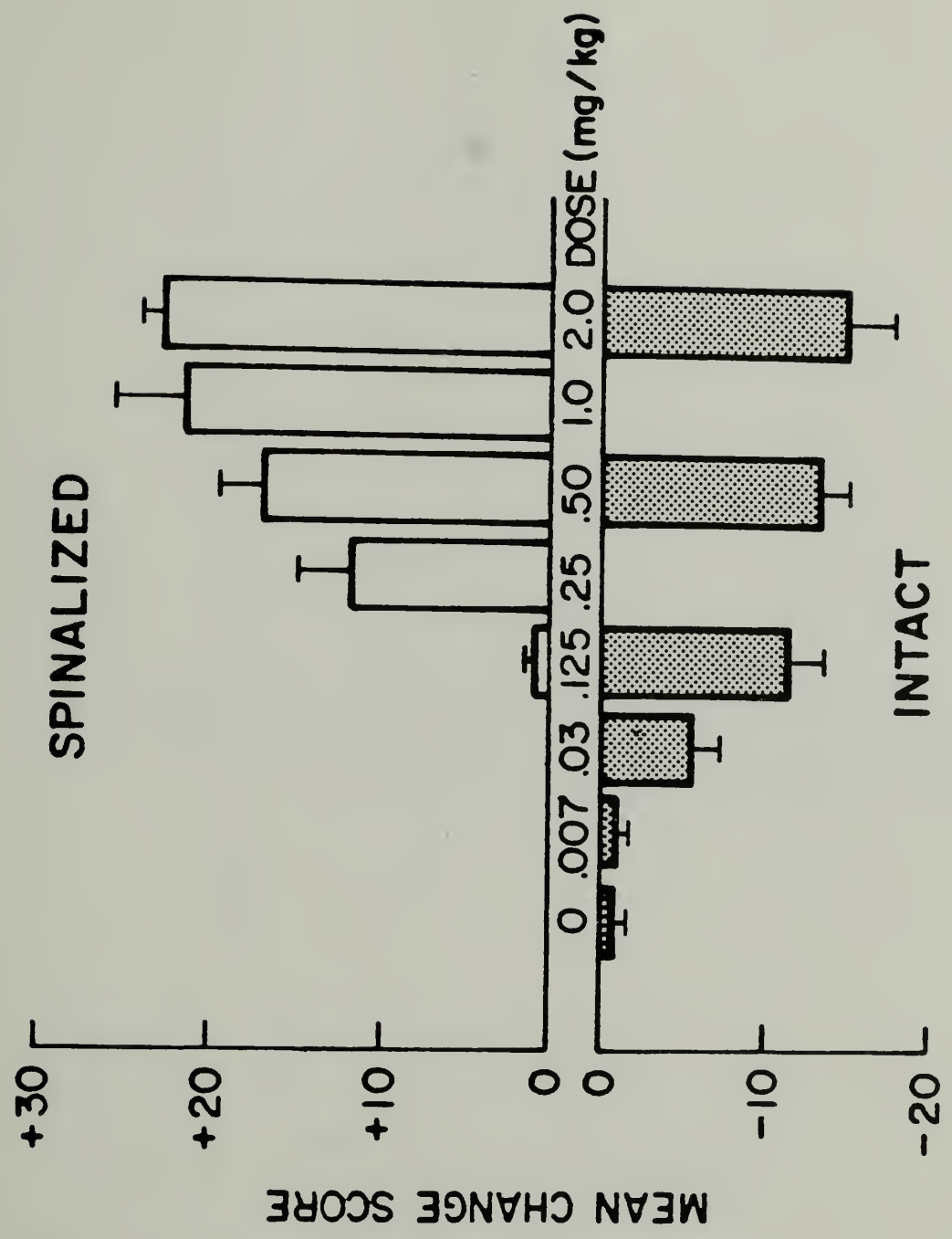

SPINALIZED
INTACT
MEAN CHANGE SCORE
+30
+20
+10
0
0
-10
-20
0
.007
.03
.125
.25
.50
1.0
2.0
DOSE (mg/kg)

Fig. 3. Mean amplitude flexor reflex following treatment with clonidine (.007 - 2.0 mg/kg, i.p.) or vehicle in spinalized (closed circles) or intact (open circles) rats. Each point represents the average across the three stimulus intensities.

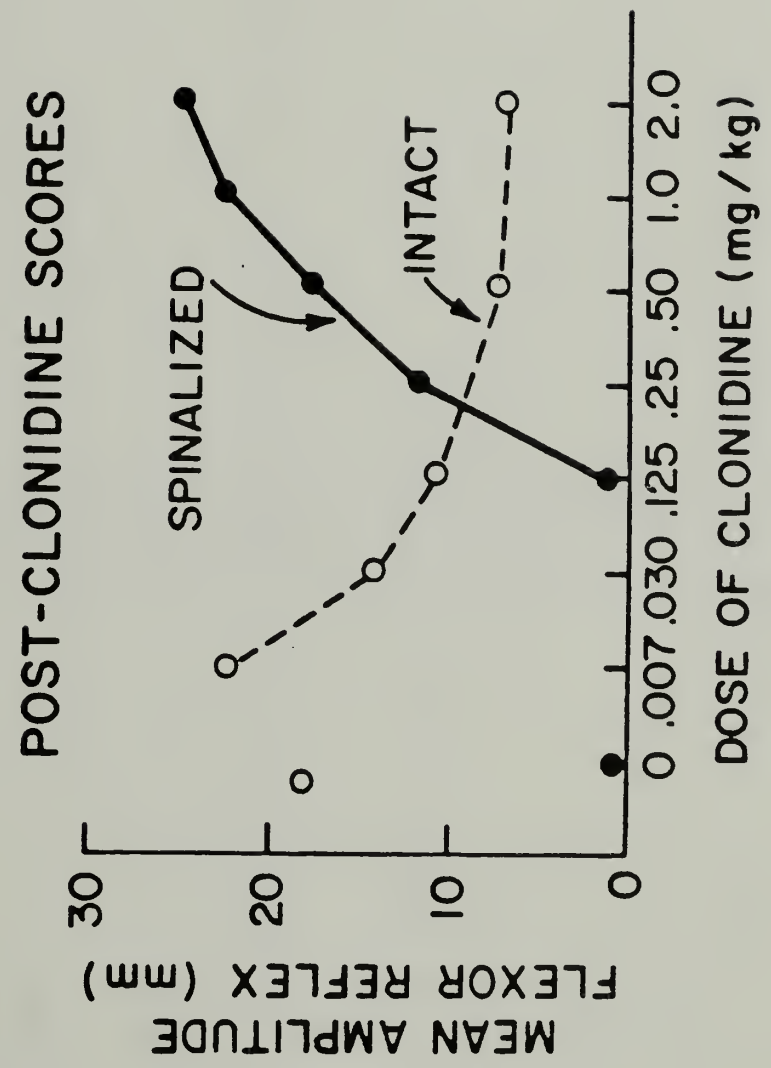

POST-CLONIDINE SCORES
MEAN AMPLITUDE
FLEXOR REFLEX (mm)
30
20
10
0
SPINALIZED
INTACT
0 .007 .030 .125 .25 .50 1.0 2.0
DOSE OF CLONIDINE (mg/kg)

Fig. 4. Mean amplitude flexor reflex following vehicle (left panels), piperoxane (middle panels) or prazosin (right panels) pretreatment and 0.5 mg/kg (i.p.) clonidine (closed circles) or vehicle (open circles) treatment in spinalized (upper panels) or intact (lower panels) rats.

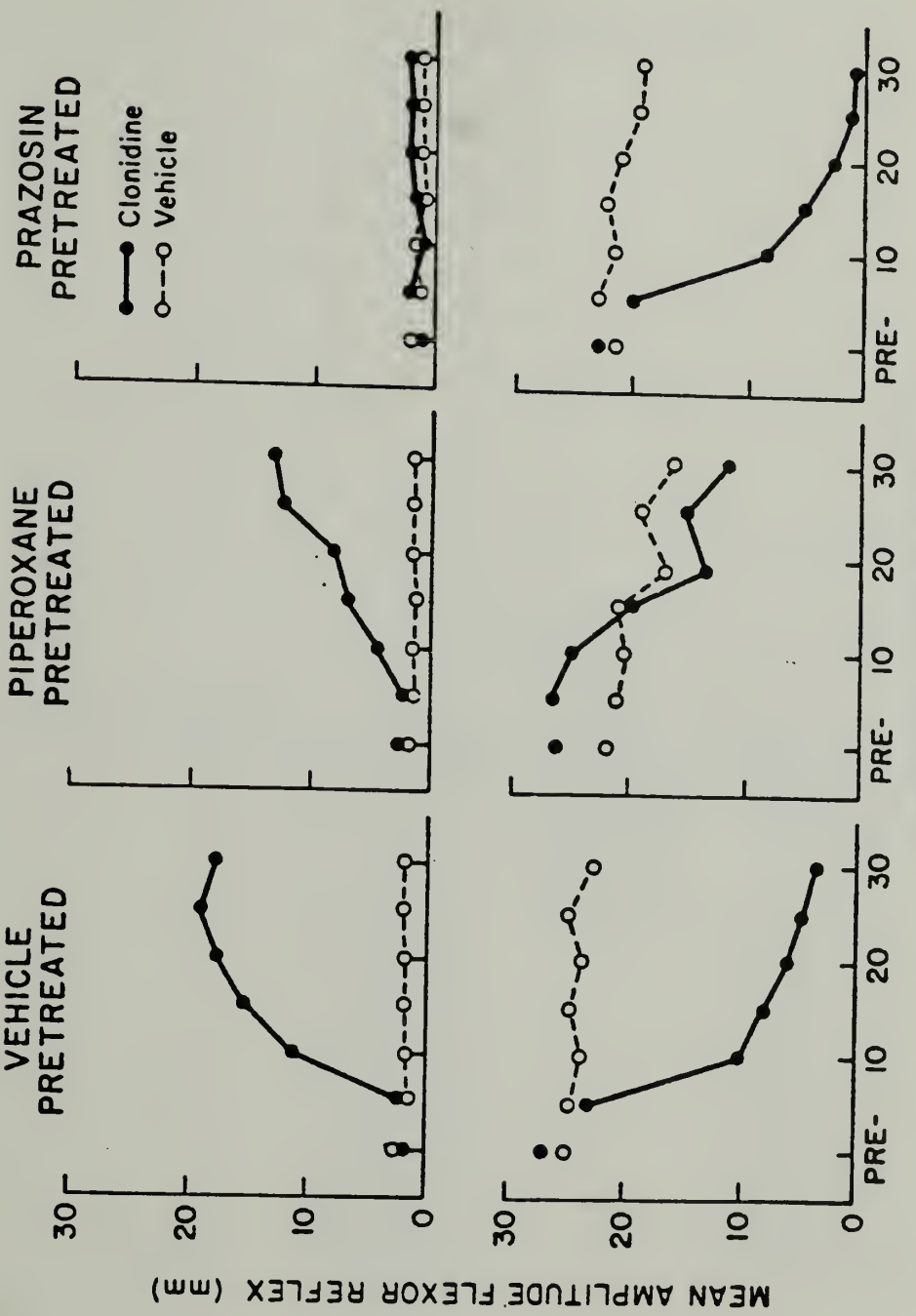

VEHICLE PRETREATED
PIPEROXANE PRETREATED
PRAZOSIN PRETREATED
Clonidine
Vehicle
MEAN AMPLITUDE FLEXOR REFLEX (mm)
TIME AFTER TREATMENT (min)
PRE-
0
10
20
30

Fig. 5. Mean change scores (30 minutes post treatment minus 5 minutes pretreatment) for vehicle, piperoxane, or prazosin pretreated spinalized (top row) or intact (bottom row) rats.

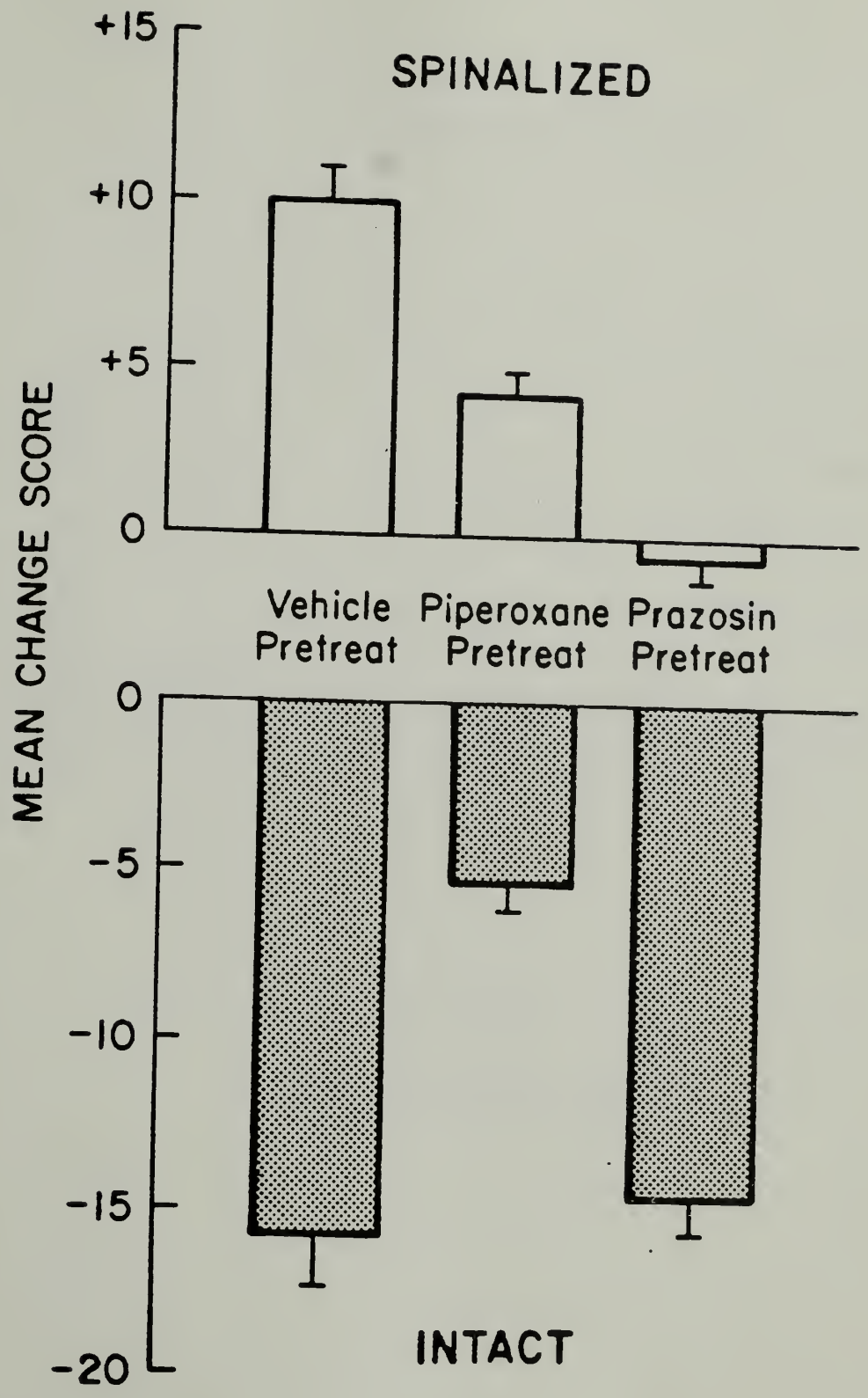

+15
SPINALIZED
+10
+5
0
MEAN CHANGE SCORE
Vehicle Pretreat
Piperoxane Pretreat
Prazosin Pretreat
0
-5
-10
-15
-20
INTACT

Fig. 6. Mean change scores (30 minutes post-injection minus 5 minutes pre-injection) following intraventricular (closed circles) or intrathecal (open circles) infusion of oxymetazoline (e.25 ug - 50 ug) or vehicle in intact rats.

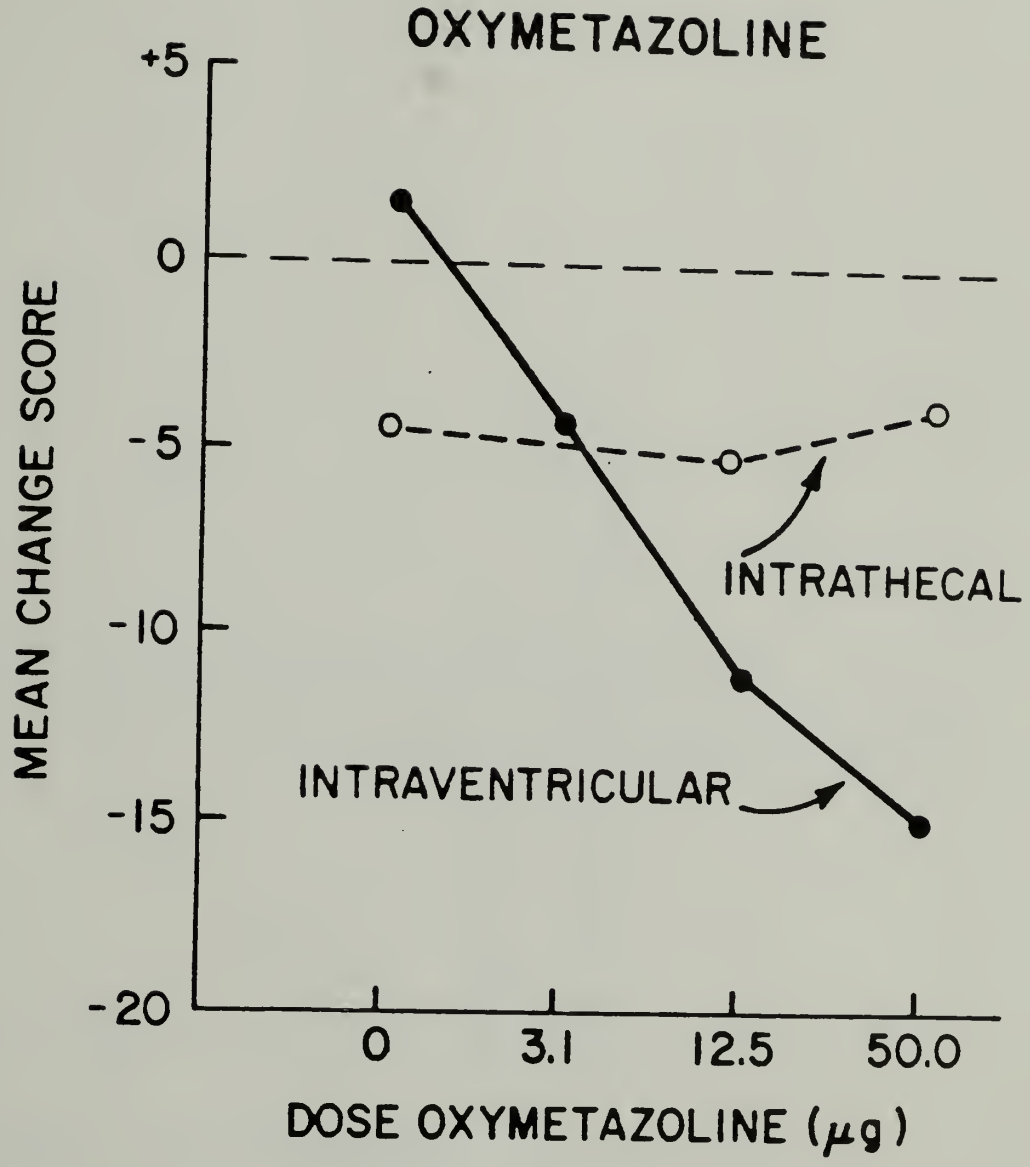

OXYMETAZOLINE
+5
0
-5
-10
-15
-20
MEAN CHANGE SCORE
INTRATHECAL
INTRAVENTRICULAR
0
3.1
12.5
50.0
DOSE OXYMETAZOLINE (μg)

Fig. 7. Mean change scores (30 minutes post-injection minus 5 minutes pre-injection) following treatment with 0.5 mg/kg (i.p.) clonidine in rats spinalized 10 minutes, 30 minutes, 2 hours, or 6 hours before testing (n=3 in each group).

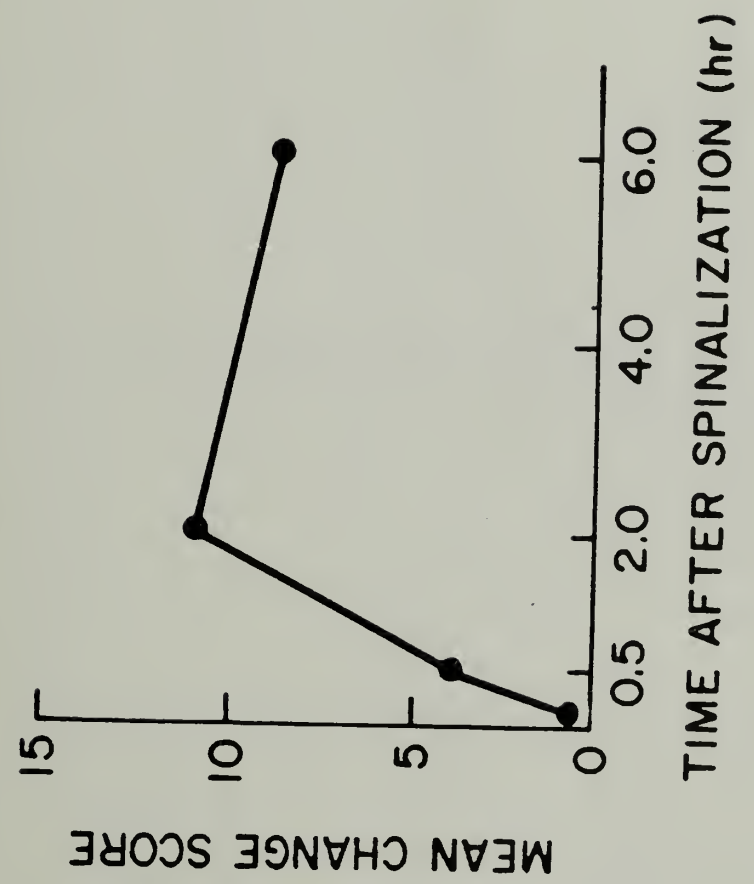

MEAN CHANGE SCORE
15
10
5
0
0.5
2.0
4.0
6.0
TIME AFTER SPINALIZATION (hr)

Fig. 8. Scatchard analysis of ^{3}H-prazosin binding in rats that were either spinalized (closed circles) or exposed to anesthetic (open circles) two hours before sacrifice.

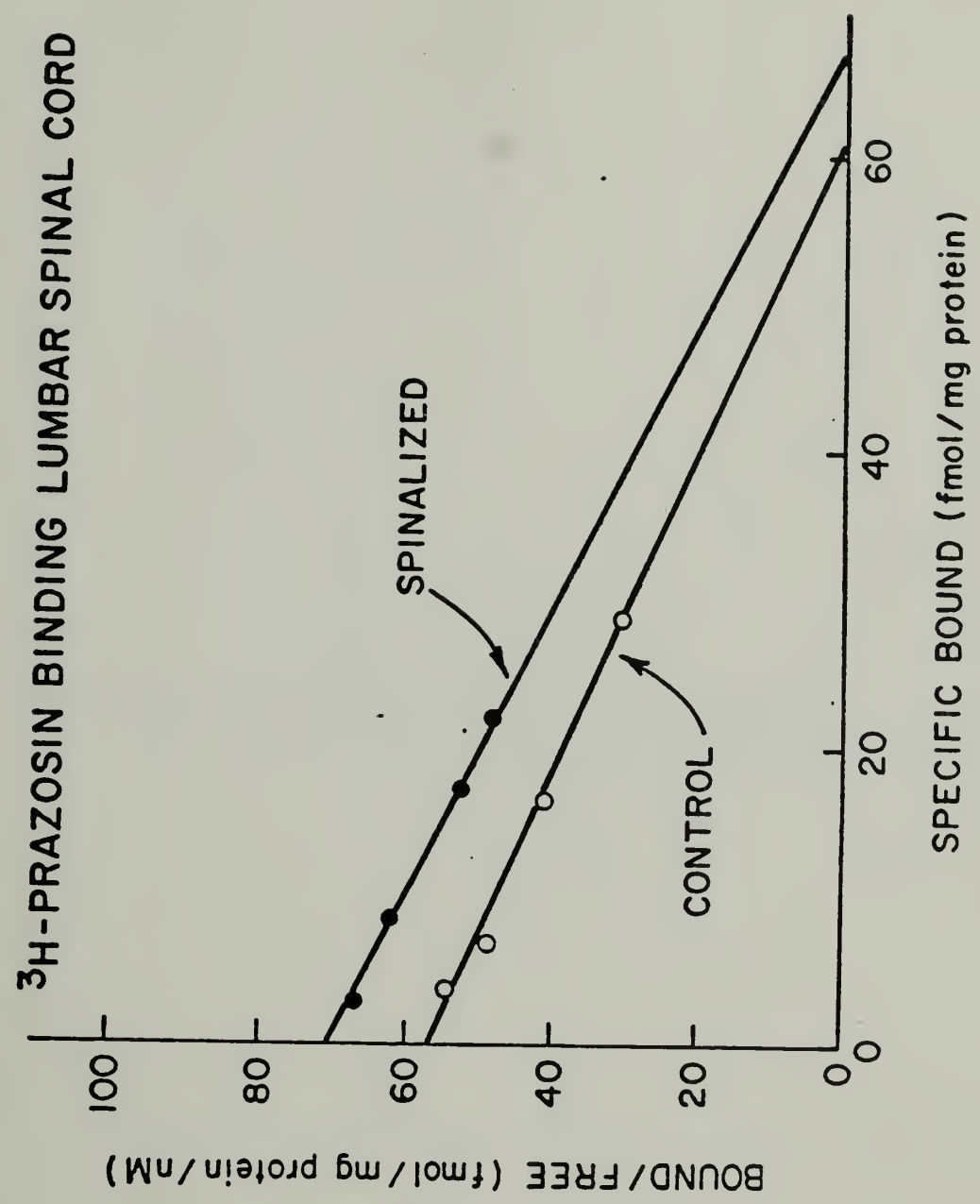

^{3}H-PRAZOSIN BINDING LUMBAR SPINAL CORD
SPINALIZED
CONTROL
BOUND/FREE (fmol/mg protein/nM)
SPECIFIC BOUND (fmol/mg protein)
0
20
40
60
80
100
0
20
40
60

Fig. 9. Scatchard analysis of ^{3}H-clonidine binding in rats that were either spinalized (closed circles) or exposed to anesthetic (open circles) two hours before sacrifice.

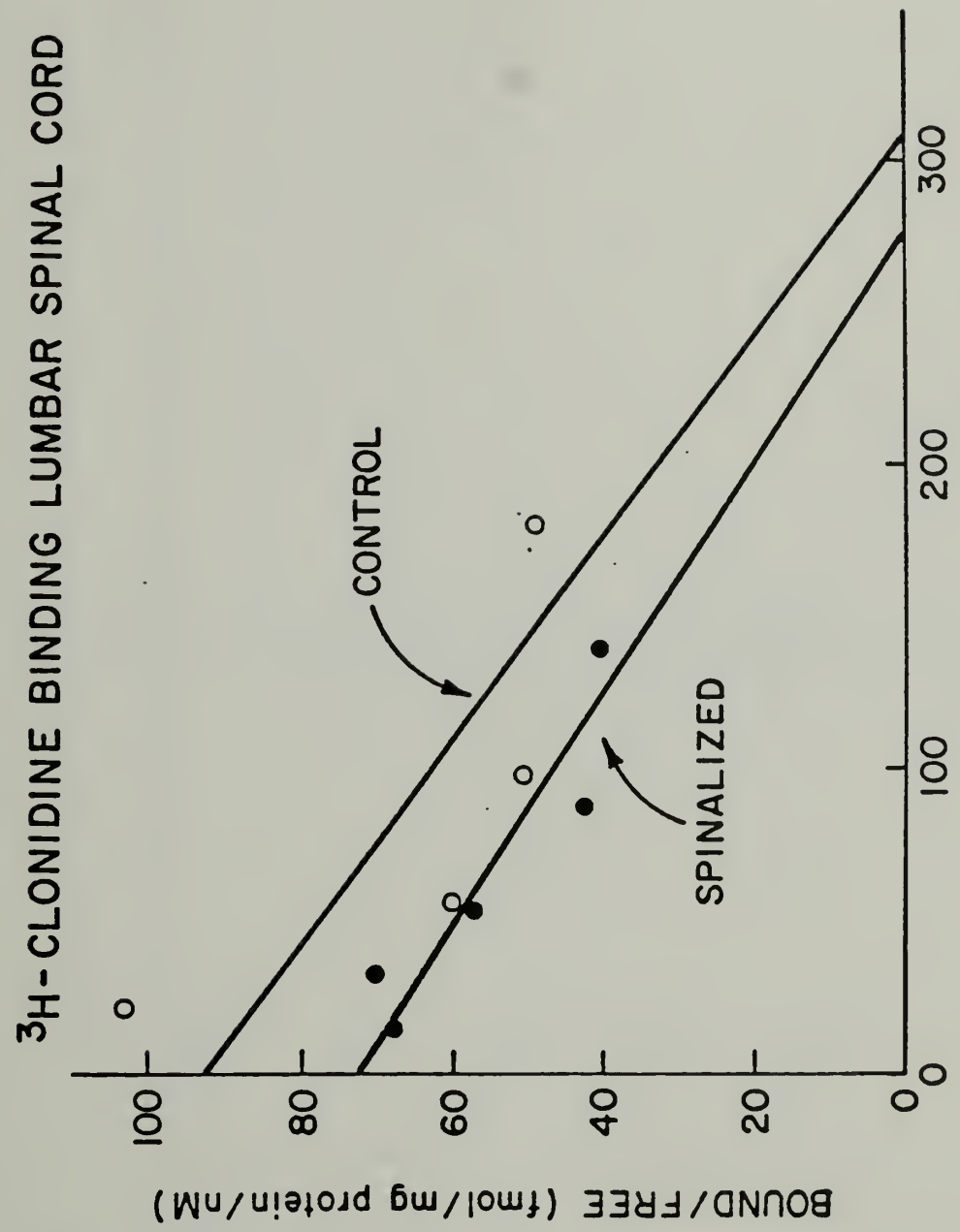

^{3}H-CLONIDINE BINDING LUMBAR SPINAL CORD
CONTROL
SPINALIZED
BOUND/FREE (fmol/mg protein/nM)
SPECIFIC BOUND (fmol/mg protein)
0
20
40
60
80
100
0
100
200
300

Fig. 10. Scatchard analysis of ^{3}H-prazosin binding in rats that were either spinalized (low-thoracic, closed circles) or exposed to anesthetic (open circles) two hours before sacrifice.

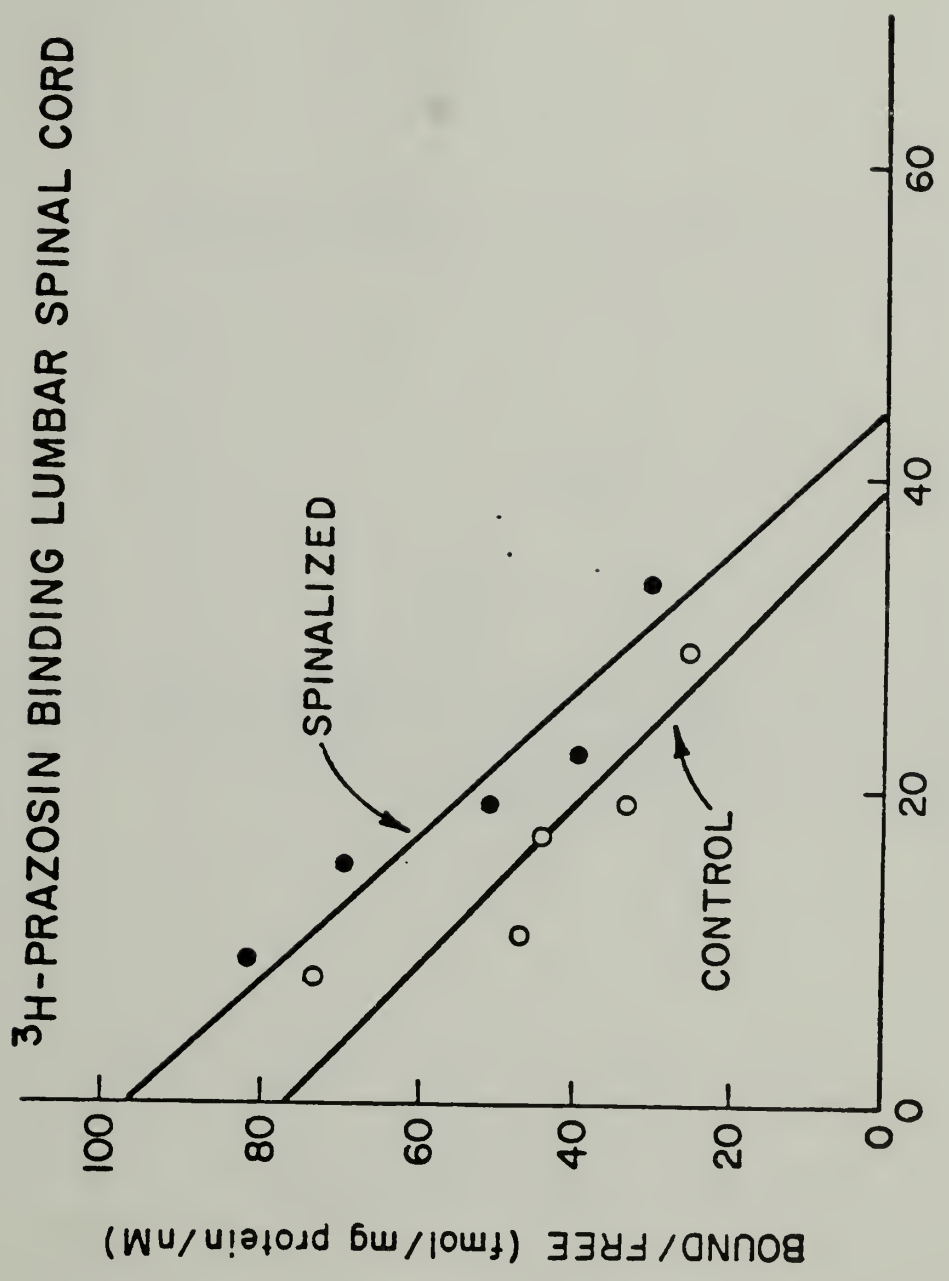

^{3}H-PRAZOSIN BINDING LUMBAR SPINAL CORD
SPINALIZED
CONTROL
BOUND/FREE (fmol/mg protein/nM)
SPECIFIC BOUND (fmol/mg protein)
0
20
40
60
80
100
0
20
40
60

Fig. 11. Mean change scores (30 minutes post-injection minus 5 minutes pre-injection) following intrathecal infusion of phenylephrine (25 - 100 ug) or vehicle in spinalized (closed circles) or intact (open circles) rats (n=3 in each group).

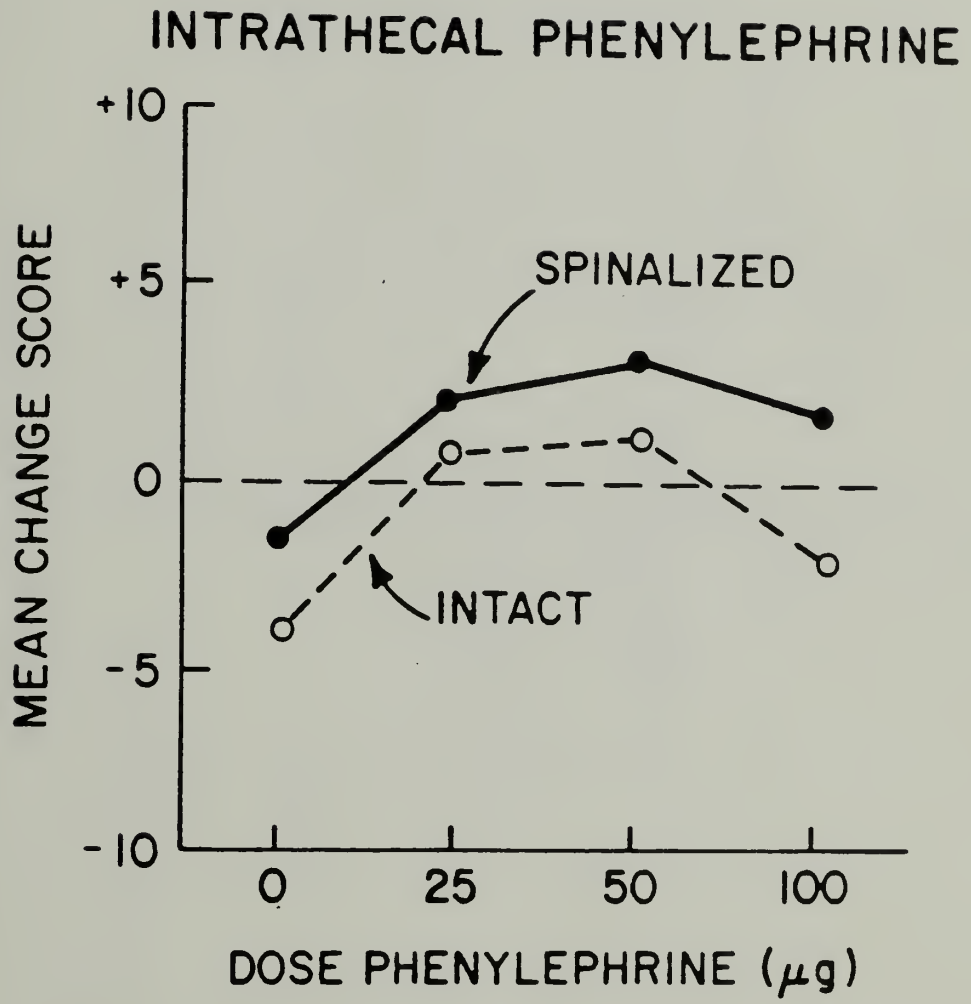

INTRATHECAL PHENYLEPHRINE
+10
+5
0
-5
-10
MEAN CHANGE SCORE
SPINALIZED
INTACT
0
25
50
100
DOSE PHENYLEPHRINE (μg)

Fig. 12. Comparison of effects of intrathecal phenylephrine with i.p. clonidine (0.5 mg/kg, data from Expt. 1).

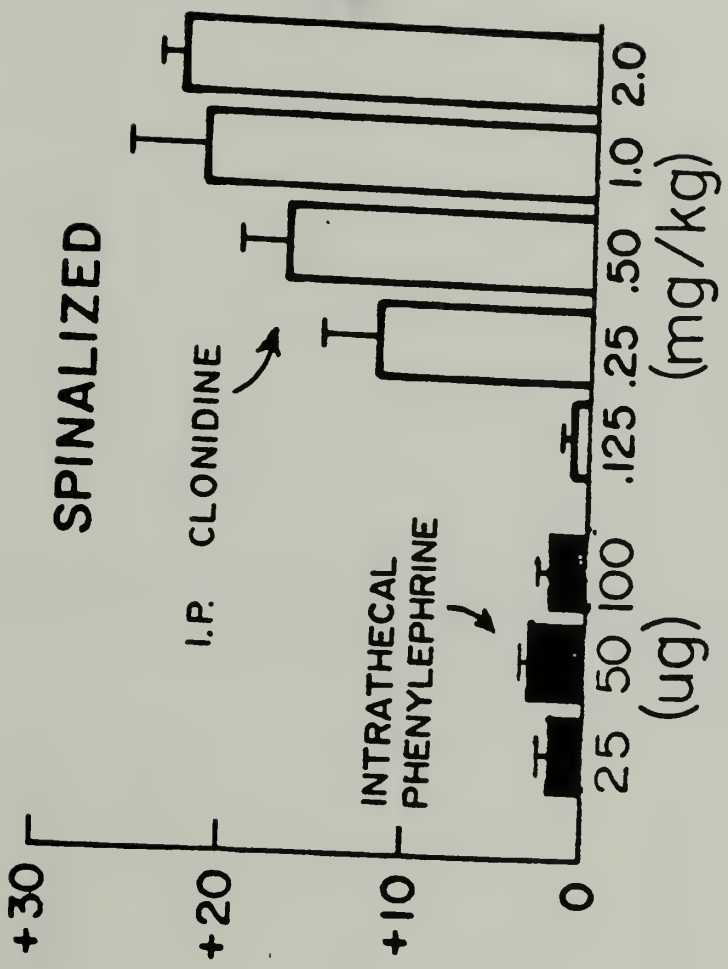

SPINALIZED
I.P. CLONIDINE
INTRATHECAL PHENYLEPHRINE
+30
+20
+10
0
25 50 100 (ug)
.125 .25 .50 1.0 2.0 (mg/kg)
DOSE

Fig. 13. Mean amplitude flexor reflex following intrathecal procaine in rats pretreated wih 2.0 mg/kg clonidine (i.p.,, lower panel) or vehicle (upper panel) (n=3 in each group).

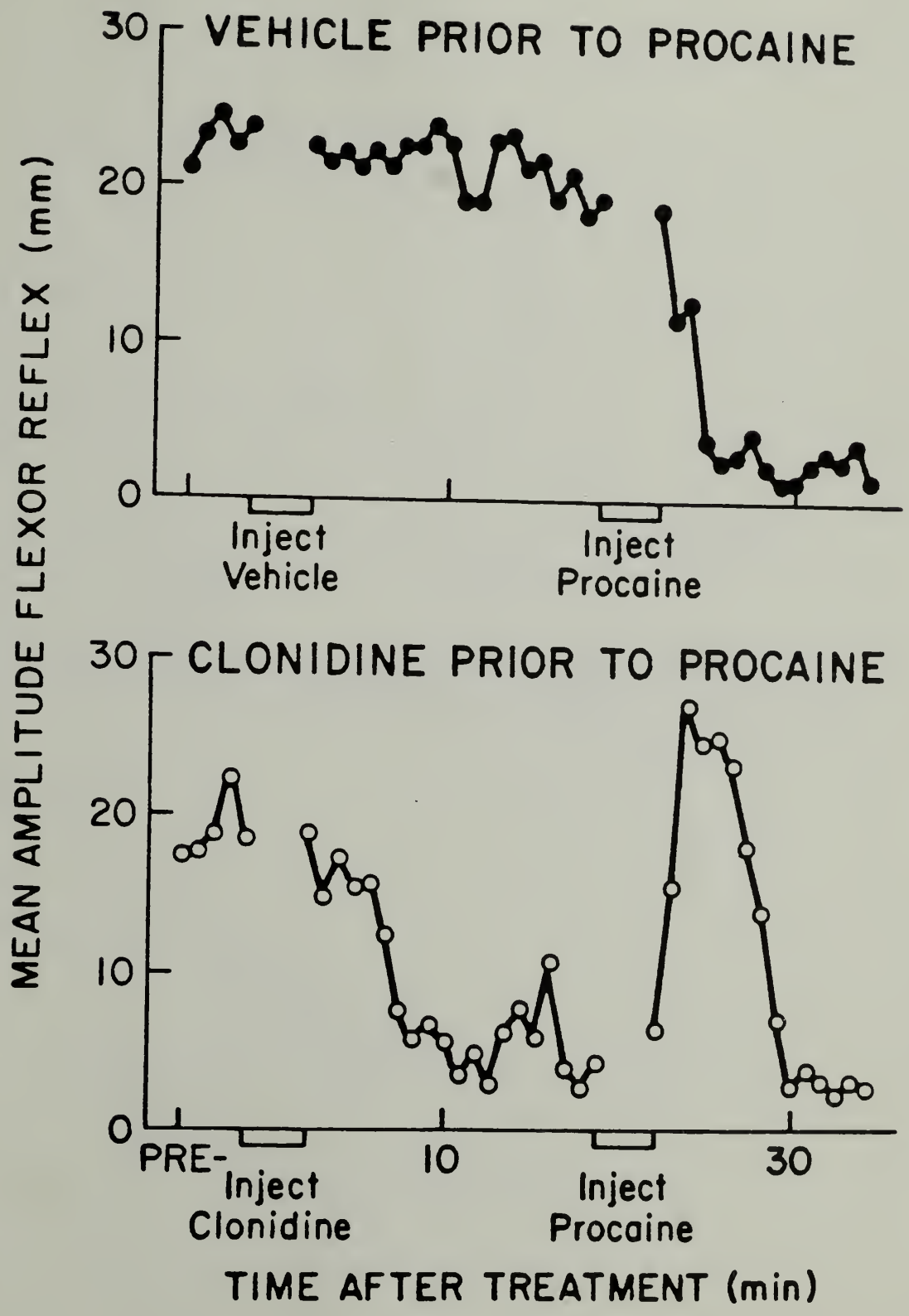

VEHICLE PRIOR TO PROCAINE
30
20
10
0
Inject Vehicle
Inject Procaine
CLONIDINE PRIOR TO PROCAINE
30
20
10
0
PRE-
Inject Clonidine
10
30
Inject Procaine
MEAN AMPLITUDE FLEXOR REFLEX (mm)
TIME AFTER TREATMENT (min)

Fig. 14. Mean amplitude flexor reflex following spinal ligation in rats pretreated with 2.0 mg/kg clonidine (i.p., lower panel) or vehicle (upper panel) (n=3 in each group).

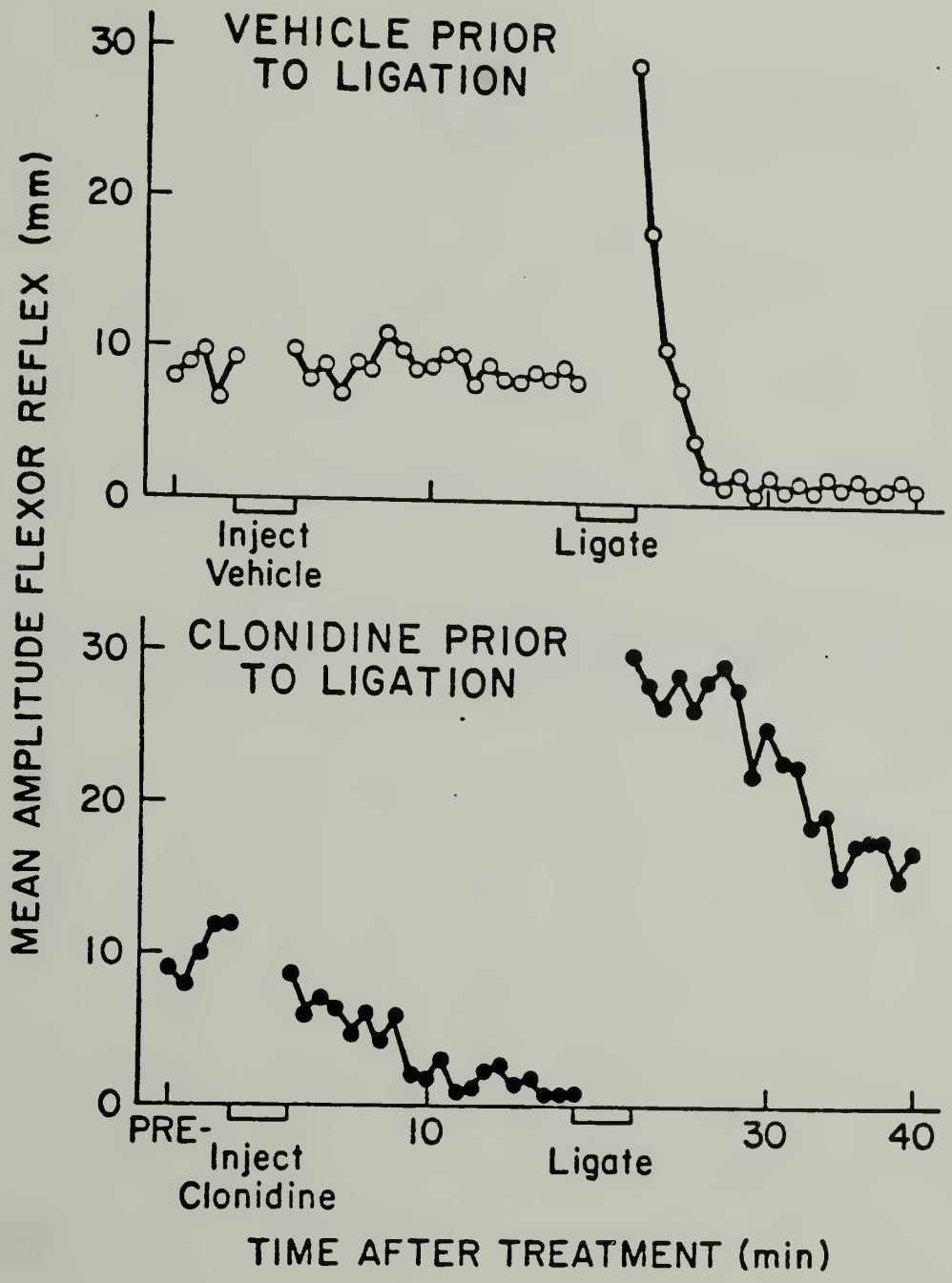

VEHICLE PRIOR
TO LIGATION
30
20
10
0
Inject
Vehicle
Ligate
CLONIDINE PRIOR
TO LIGATION
30
20
10
0
PRE-
Inject
Clonidine
10
30
40
Ligate
MEAN AMPLITUDE FLEXOR REFLEX (mm)
TIME AFTER TREATMENT (min)

Fig. 15. ^{3}H-prazosin (upper panel) and ^{3}H-clonidine (lower panel) binding in rats that were untreated, sham-operated but not ligated, ligated 15 minutes before sacrifice, or ligated 2 hours before sacrifice (n=3 in each group).

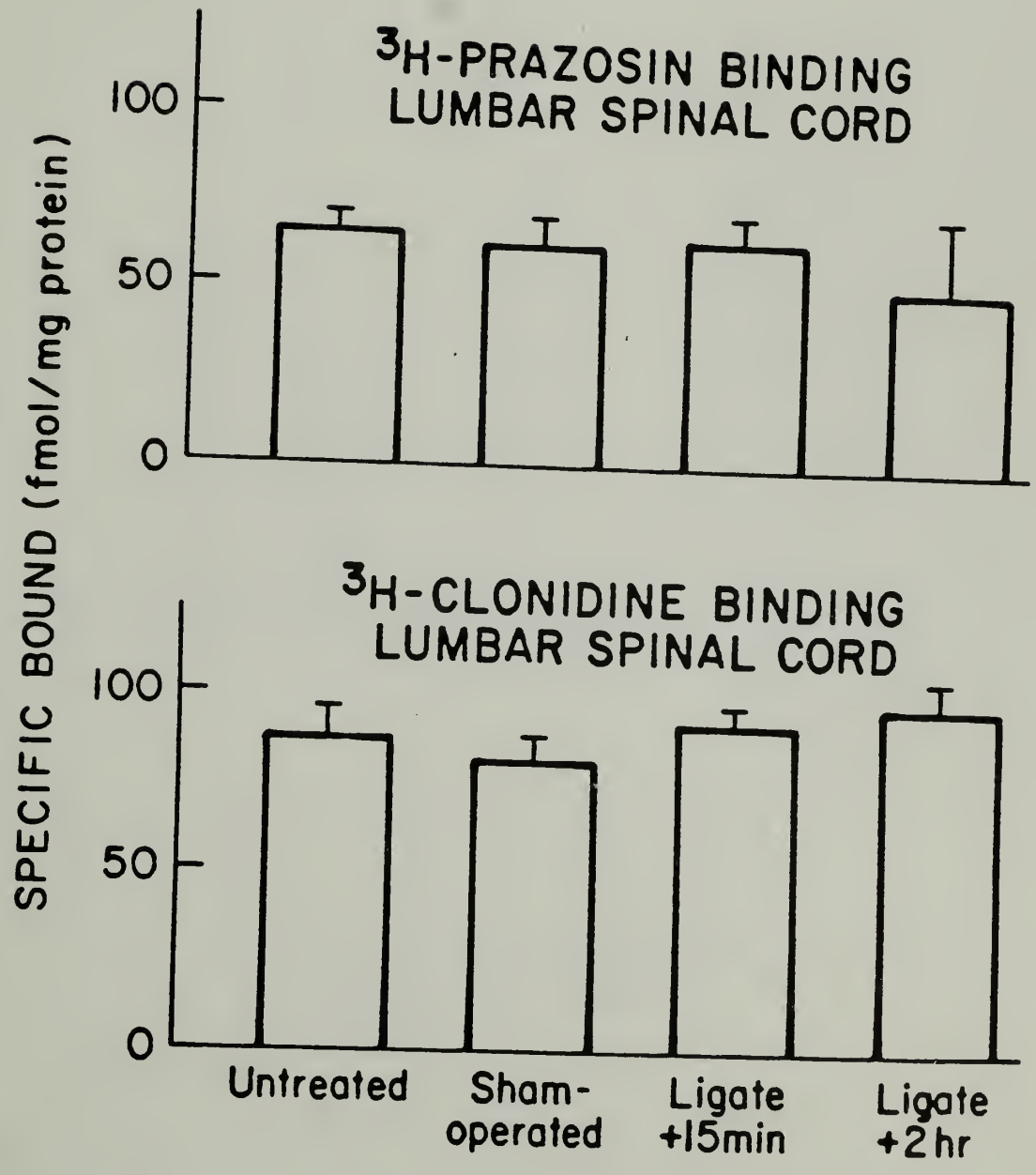
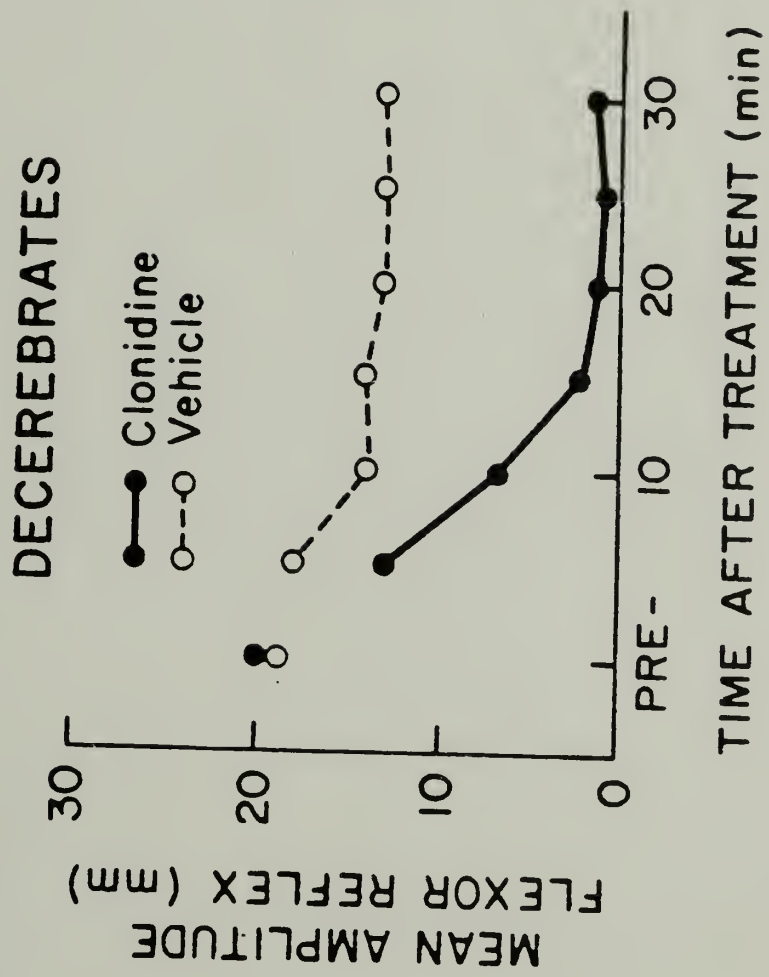

^{3}H-PRAZOSIN BINDING
LUMBAR SPINAL CORD
100
50
0
^{3}H-CLONIDINE BINDING
LUMBAR SPINAL CORD
100
50
0
SPECIFIC BOUND (fmol/mg protein)
Untreated
Sham-operated
Ligate +15min
Ligate +2hr

Fig. 16. Effects of 2.0 mg/kg clonidine (closed circles) or vehicle (open circles) in rats decerebrated two hours before testing (n=3 in each group).


DECEREBRATES
Clonidine
Vehicle
MEAN AMPLITUDE
FLEXOR REFLEX (mm)
30
20
10
0
PRE-
10
20
30
TIME AFTER TREATMENT (min)