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Spinal modulation of the acoustic startle response: : behavioral, pharmacological and biochemical studies in the normal, supersensitive and desensitized animal

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SPINAL MODULATION OF THE ACOUSTIC STARTLE RESPONSE: BEHAVIORAL, PHARMACOLOGICAL AND BIOCHEMICAL STUDIES IN THE NORMAL, SUPERSENSITIVE AND DESENSITIZED ANIMAL

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by

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The experiments presented were all conducted in the laboratories of Dr. Michael Davis and Dr. Dorothy W. Gallager of Yale University and The Connecticut Mental Health Center. They represent a series of interrelated studies designed to evaluate the relationship between biochemistry and behavior. The first study in this series - Experiment 1 in this thesis - was carried out during my senior year at Yale College. It was included in this thesis as it is the most economical way to provide background information necessary for the understanding of the later experiments. The second and third experiments are comprised of work done during the summer prior to my second year of medical school as well as in May and June prior to beginning the third year. Parts of this work were presenteed at National Meetings of the Society for Neuroscience - in Cincinatti in 1980 and in Minneapolis in 1982.

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ABSTRACT

Spinal modulation of the acoustic startle response:
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A major goal of psychopharmacology is to understand at a cellular level how drugs interact with various chemical systems to affect behavior. To accomplish this a behavior is needed which is quantifiable, amenable to pharmacological manipulations and generated from a known neural circuit. The acoustic startle reflex of the rat is a quantifiable behavior sensitive to a wide variety of drugs which has its final central nervous system synapse in the lumbar spinal cord. Therefore, analysis of the biochemical effects of drugs infused into the spinal cord should provide a powerful tool for assessing the relationship between pharmacology, biochemistry and behavior.

Direct administration of serotonin, norepinephrine, and their agonists into the subarachnoid space of the lumbar spinal cord (intrathecal infusion) produced a dose dependent increase in acoustic startle magnitude. The effects were specific since noradrenergic antagonists prevented the increase in startle caused by noradrenergic agonists but not by serotonin agonists while serotonin antagonists blocked the facilitation caused by serotonin agonists but not by noradrenergic agonists. In contrast neither dopamine nor its agonist had any effect on startle magnitude at any of the doses tested. The magnitude of the behavioral facilitation caused by alpha-1-agonists correlated highly with the degree of alpha-1-adrenoceptor occupation

measured by ^{3}H-prazosin binding in lumbar spinal tissue. The more receptors that were occupied, the greater the behavioral change observed. Intrathecal administration of the noradrenergic neurotoxin 6-hydroxydopamine (6-OHDA) produced a 95% decrease in spinal norepinephrine and markedly enhanced the behavioral response to intrathecal noradrenergic agonists as well as the number of alpha-1-adrenoceptors (denervation supersensitivity). The correlation between the increased behavioral sensitivity to norepinephrine agonists and the increase in the number of alpha-1-adrenoceptors caused by 6-OHDA over time was 0.99.

Prior intrathecal infusion of the alpha-1-noradrenergic agonist phenylephrine prevented the excitatory effects of subsequent alpha-1 agonists infused 6 hours later on acoustic startle (behavioral desensitization). This desensitization was associated with a decrease in the number of alpha-1-adrenoceptor sites in the lumbar spinal cord.

Taken together these data indicate that by analyzing biochemical, pharmacological and behavioral processes in a specific region of the central nervous system, extremely high correlations can be found between biochemical (binding parameters) and behavioral (changes in acoustic startle response) measures. Moreover, they indicate that this model system may be particularly suitable for addressing more subtle questions about receptor mechanisms and behavior in the vertebrate. Eventually this system can be used to analyze the relationship between intracellular events and behavior.

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Introduction

Psychopharmacology is a science that attempts to bridge the gap between the model systems approach of the basic scientist and the more global observations and theories of behavioral scientists. By the use of simple behaviors amenable to observational, biochemical, physiological and pharmacological analyses conclusions that are made can be tested directly with a minimum of confounding variables. A major goal of psychopharmacology is to understand at a cellular level how drugs interact with various chemical systems to affect behavior. The central nervous system relies on these chemicals -- neurotransmitters -- for interneuronal communication. Drugs, which may mimic or antagonize these endogenous transmitters, are used as tools for probing transmitter systems. The resultant behavioral changes that accompany drug administration offer clues to how neurotransmitters modulate behavior. The more one is able to specifically examine the mechanisms of drug action on neuronal systems with the tools offered by biochemistry, physiology and pharmacology the clearer the information possible regarding how drugs affect neuronal processes which ultimately affect behavior.

Behaviors range in complexity with the simplest behaviors involving monosynaptic reflex arcs. The more simple the behavior the easier it should be to study. It is no wonder then that the most definitive work on the neuronal bases of drug action, neurotransmission and behavioral plasticity have been done in invertebrates, most specifically on the siphon withdrawal reflex of the Aplysia or sea slug (33). The first step in the endeavor was to

delineate the neural circuit mediating the behavior being measured. Following this it was possible to characterize the neurotransmitters modulating transmission along this circuit. Finally, it has been possible to begin to determine at a cellular level how changes produced by neurotransmitters at specific synaptic regions produce behavioral changes. Comparable levels of analysis have not yet been possible in intact vertebrate systems, primarily because few vertebrate behaviors are simple enough to have a neural circuit amenable to a similar kind of experimental analysis.

The short latency acoustic startle reflex in the rat is one vertebrate behavior which seems to provide an ideal system for studying behavioral and pharmacological plasticity. The startle reflex has proven to be a useful measure for studying how drugs effect sensory-motor reactivity (13). Acoustic startle amplitude is highly dependent on the characteristics of the eliciting stimulus and this tight degree of stimulus control allows nonzero baselines to be established against which to test drugs as well as lesions or electrical brain stimulation. The entire primary neural circuit has been recently worked out and shown to be quite simple involving only four synapses within the central nervous system (20). These are 1) posteroventral cochlear nucleus to 2) the dorsal and ventral nuclei of the lateral lemniscus to 3) the nucleus reticularis pontis caudalis and finally to 4) lower motor neurons of the spinal cord. With the circuit now delineated it is possible to determine exactly where and even how drugs act within this pathway to alter neuro transmission and ultimately affect behavior.

Generally, it has been assumed that drug effects are mediated by the brain, once peripheral effects have been ruled out. However, like most behaviors studied in psychopharmacology the startle response ultimately involves spinal motor neurons for its expression. These spinal motor neurons are known to receive dense modulating input from neurons located in the brainstem (10,12,47) and the importance of the spinal cord in mediating behavioral drug effects has been demonstrated (49,69,71). Yaksh and co-workers have described a method that allows direct infusion (intrathecal administration) of compounds onto the spinal cord in waking rats (70) and have shown these compounds to remain localized about the tip of the catheter with negligible diffusion into other areas of the CNS.

As the final synapse of the primary acoustic startle circuit occurs in the spinal cord it is now possible with the use of intrathecal catheters to infuse drugs directly onto a specific area of the central nervous systems involved in a specific and quantifiable behavior. It should also be possible to examine the area involved in the behavior by removing the tissue and biochemically determining at the cellular level how drugs interact with various neurotransmitter systems to affect behavior. The present series of experiments were designed towards this purpose.

Experiment 1: Spinal modulation of the acoustic startle response: the role of norepinephrine, serotonin and dopamine.

Systemic administration of serotonin (5-HT), norepinephrine (NE) or dopamine (DA) agonsits have been shown to increase both acoustic

and tactile startle (13). 5-Methoxydimethyltryptamine, a 5-HT agonist, given systemically produces large increases in acoustic startle magnitude (16). Combinations of drug which increase central nervous system levels of 5-HT such as L-tryptophan and pargyline (a monoamine oxidase inhibitor) also increase acoustic startle and this effect can be blocked by pretreatment with the 5-HT synthesis inhibitor p-chlorphenylalanine or by administration of varoius 5-HT antagonists (13).

Most available data suggest that norepinephrine is excitatory to acoustic startle. Drugs that increase availability of central NE by blocking its reuptake, such as moderate doses of desipramine or chlordesipramine, increase acoustic startle (19). Conversely, depletion of NE by lesions of the NE-containing cell bodies in the locus coeruleus depresses both acoustic and tactile (1) startle. In addition, the alpha-1 adrenergic antagonist phenoxybenzamine depresses startle at doses known to block adrenergic receptors (35). NE may also help mediate the excitatory effects of high doses of amphetamine since pretreatment with phenoxybenzamine blocks the excitatory effects of l-amphetamine (35) and pretreatment with the NE synthesis inhibitor FLA-63 blocks the excitatory effects of d-amphetamine (36).

High doses of d- and l-amphetamine in addition to releasing endogenous norepinephrine from nerve terminals also causes a marked release of dopamine. The excitatory effects of high dose amphetamine on acoustic startle is also blocked by pretreatment with the DA antagonists haloperidol or pimozide (35). Furthermore, the directly acting DA agonist apomorphine also increase acoustic startle and this

effect can be blocked by haloperidol or pimozide (14,35). The results of these systemic drug studies suport the idea that enhancement of DA transmission increases startle amplitude.

Anatomical studies indicate that spinal motor neurons receive dense input from 5-HT, NE and DA containing neurons located in the brainstem (10,12,14). Single unit recording studies indicate that both 5-HT and NE facilitate excitation of motor neurons produced by afferent stimulation (39,68). This increase in excitability occurred without a direct increase in the firing of these motor neurons consistent with a role for NE and 5-HT as neuromodulators within the spinal cord. Behavioral studies indicate that 5-HT or NE facilitate spinal reflexes in spinalized or decerebrate animals (2,3,40,52). Thus, it is possible that the excitatory effects on startle produced by systemic administration of monoamine agonists result from a direct modulatory action in the spinal cord.

Using a modification of the intrathecal technique developed by Yaksh and his coworkers the present experiments were designed to test the effects of intrathecal administration of 5-HT, NE, DA, and their agonists on the acoustic startle reflex.

Subjects

Male Sprague-Dawley, Charles River rats (350-450 g) were used in this and all subsequent experiments. Rats were housed in group cages (4-5 per cage) until intrathecal implantation at which time they were then housed singly. They were kept in a colony room with a reverse light-dark cycle (12h-12h). Food and water were continously

available.

Preparation of the Animals: Catheter Implantation

Animals were anesthetized with halothane and positioned with blunt ear bars in a Kopf stereotaxic instrument. The cisterna magna was exposed and the overlying membrane punctured. An 8.5-cm length of polyethylene tubing (PE 10) fused to a 3.5-cm length of PE-20 tubing was inserted into the cisterna magna and gently "snaked" down over the spinal cord to terminate above the lumbar enlargement as described by Yaksh and Rudy (70). The PE 20 portion of the catheter was secured between wound clips used to close the incision. The catheter was then filled with sterile saline and the rats allowed to recover for 1 week. For animals housed longer than four weeks with chronic catheters a slight modification of the implantation procedure was used (37). This involved drilling a small hole through the occipital and parietal bones of the skull and placing the PE 20 part of the catheter through these holes. This firmly anchored the catheter and prevented its accidental removal that frequently occurred in animals housed for more than four weeks catheterized in the usual way.

Measurement of acoustic startle amplitude.

Five separate stabilimeter devices were used to record the amplitude of the startle response. Each stabilimeter consisted of an 8 x 15 x 15 Plexiglass and wire mesh cage suspended within a 25 x 20 x 20 cm steel frame. Within this frame, the cage was sandwiched between four compression springs above and a 5 x 5 cm rubber cylinder below with an accelerometer (MB Electronics type 302) located between the

bottom of the cage and the top of the rubber cylinder. Cage movement resulted in displacement of the accelerometer and the resultant voltage was fed through a matched accelerometer amplifier (MB Electronics model N 504), the output of which was proportional to the velocity of accelerometer displacement.

The amplified signal then was fed to a specially designed sample-and-hold circuit. Basically, this current consisted of five channels, one for each stabilimeter, and was used to sample the peak accelerometer voltage that occurred during a 20-msec time band immediately after the onset of the startle-eliciting stimulus. Immediately prior to this sample period, each channel was discharged so that any spontaneous activity occurring between stimulus and stored in one of each of the five channels. Immediately after the sample period, the output of each of the five channels was digitized through a specially designed analog-to-digital convertor and fed into a PDP-11 computer. Startle amplitude could vary from 0 to 160, allowing appreciable resolution among various startle amplitudes.

The five stabilimeters were located in a 2.5 x 2.5 x 2 m, dark, ventilated, sound-attenuated chamber (Industrial Acoustic Co., IAC). They were placed 1.1 meters from an Altec high frequency loudspeaker, which was used to provide a 90-msec noise burst generated by a Grason-Stadler noise generator, amplified through an Altec 100-W power amplifier, and shaped through a Grason-Stadler electronic switch to have a rise-decay time of 5 msec. Background white noise provided by a Grason-Stadler white noise generator. The intensity of the noise burst (110 dB) and the background white noise (55 dB) was measured

with a General Radio model 1551-C sound level meter (A scale) by placing the microphone in each cage and positioning the cages to have comparable readings.

Testing Procedure

One week after implantation rats were placed in the stabilimeters and 5 min later a program began which gave startle-eliciting tones every 20 sec for 14 min. This constituted the pre-drug baseline. They were then removed from the test cages and infused with 18 ul of drug dissolved in sterile salilne (pH 7.4) at a rate of 5 ul/min. Ten ul of drug was actually delivered onto the spinal cord while the remaining 8 ul occupied the internal volume of the catheter. Right after infusion the rats were returned to test cages and immediately given tones every 20 sec for 1 hour. The post drug infusion scores for each rat were compared with their respective pre-drug baseline to determine the changes in startle amplitude following drug treatment. Rats were tested only once and then discarded. Following testing the catheters were removed and examined for possible leakage or clogging. Only results from animals with intact and unclogged catheters were included as data.

Results

Intrathecal administration of 100-200 ug of 5-HT (free base) increased startle by about 50% immediately after administration, but the effect only lasted for a few minutes. Pretreatment with the monoamine oxidase inhibitor pargyline (25 mg/kg) 1 hour earlier markedly enhanced the excitatory effect of 5-HT. Following pargyline,

5-HT (6.25, 25 or 50 ug) caused a dose-dependent increase in startle that now lasted for 30-40 minutes at the highest dose. An overall analysis of variance using each animal's postinfusion minus preinfusion change scores revealed a dose-dependent increase in startle amplitude over these conditions, $F(3/16) = 11.86$ $p < 0.001$, that was linearly related to the log dose given, $Flin(1/16) = 22.51$, $p < 0.001$.

Intrathecal administration of the direct 5-HT agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) produced a marked and immediate increase in acoustic startle amplitude. The left panel of Fig. 1 shows the time course for the most effective dose (50 ug) relative to the saline condition. The right panel of Fig. 1 shows the dose-response curve expressed as the mean change from the preinfusion baseline to the first 10 min postinfusion + S.E.M. An overall analysis of variance using these change scores revealed a significant dose-response effect, $F(3/8) = 11.44$, $p < 0.01$, that was linearly related to the log dose used, $Flin(1/18) = 30.17$, $p < 0.001$. Intrathecal NE immediately increased acoustic startle, but the effects decayed very rapidly, within about 4 min. All doses of NE that were used (12.5, 25, 50, and 100 ug free base) showed similar effects: a rapid initial increase in startle followed by an immediate decay. Analyses of the postinfusion-preinfusion change scores for each animal revealed a significant effect of NE, $F(1/15) = 11.56$, $p < .005$. Over this dose range, however, a significant dose-response relationship was not obtained.

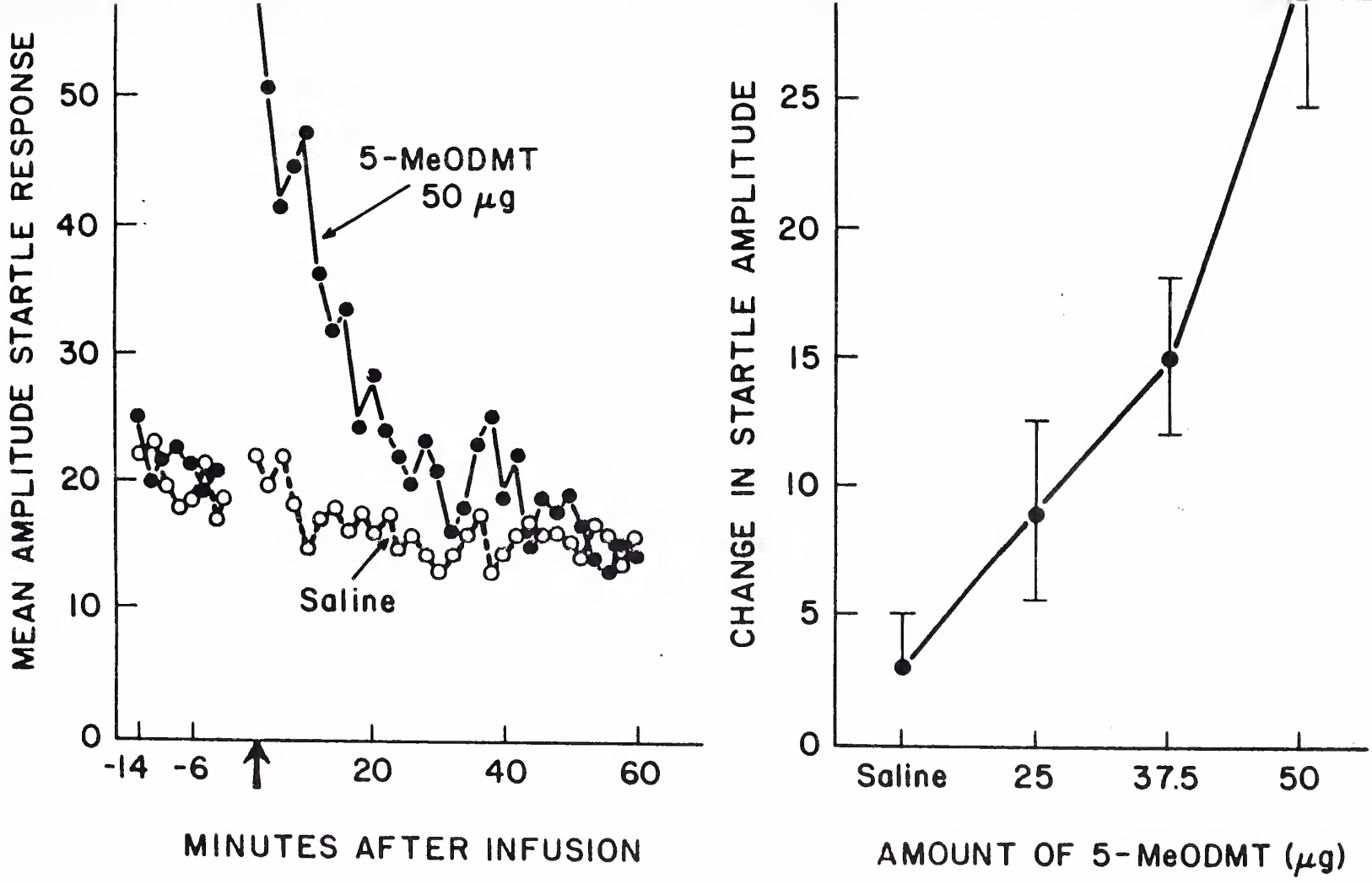

FIGURE 1. Mean amplitude startle response before and after intrathecal infusion of 50ug 5-MeODMT, n=4, relative to saline control n=4 (left panel). Mean change in startle amplitude (±S.E.M.) (the first 10 minutes postinfusion minus the predrug baseline) for 3 doses of 5-MeODMT and saline, n=4 at each point (right panel).

The left panel of Figure 2 shows the results of intrathecal infusion of 50 ug of the alpha-1 adrenergic agonist phenylephrine. Intrathecal phenylephrine increased startle amplitude for roughly 40 min following infusion. This effect was directly related to the dose that was used, $F(3/13) = 8.08$, $p < 0.001$, as shown in the right panel of Figure 2. In contrast to the alpha-1 adrenergic agonist phenylephrine, intrathecal administration of the beta-adrenergic agonist isoproterenol did not alter acoustic startle at any of the doses tested (25, 100, or 400 ug).

Table 1 shows the results of pretreating rats intraperitoneally with 5-HT or NE antagonists 15 minutes prior to the intrathecal infusion of either 5-MeODMT (50 ug) or phenylephrine (50 ug). A dose of 1.0 mg/kg of the alpha-1 adrenergic antagonist WB-4101 completely blocked phenylephrine's effect on startle but not that of 5-MeODMT. In contrast, a dose of 1.0 mg/kg of the 5-HT antagonist cyproheptadine completely blocked the effect of intrathecal 5-MeODMT but not that of phenylephrine. On the other hand, the beta-antagonist propranolol did not block the increase in startle amplitude caused by either 5-MeODMT or phenylephrine.

The preceeding experiments demonstrate that either 5-HT or NE can increase acoustic startle when applied directly onto the spinal cord. Excitation following intrathecal 5-HT or 5-HT agonists appears to be mediated through a 5-HT receptor. In the present study the direct acting 5-HT agonist, 5-MeODMT, increased acoustic startle when applied intrathecally and this effect was blocked by systemic administration of the 5-HT antagonist, cyproheptadine, but not by the

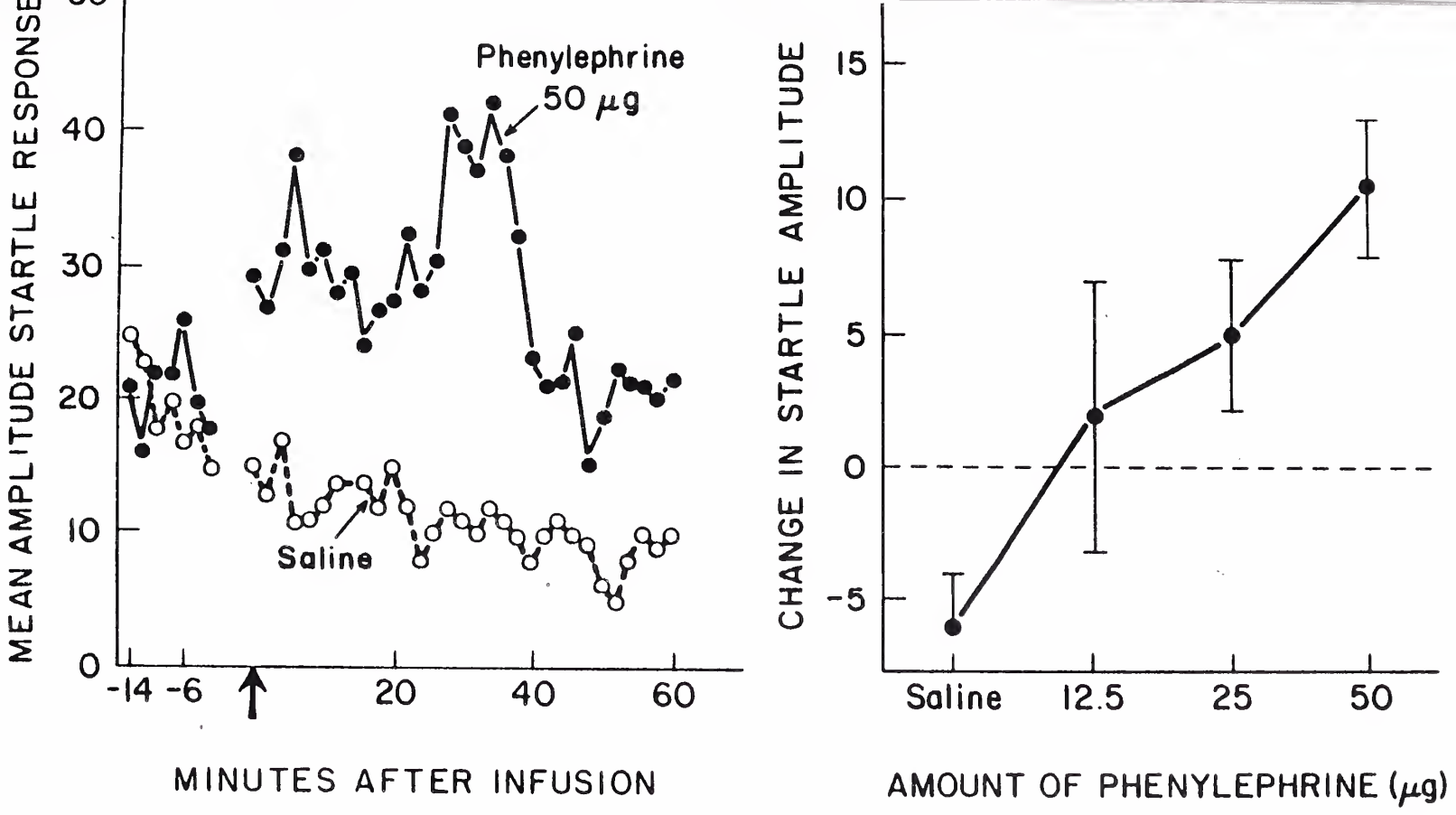

FIGURE 2. Mean amplitude startle response before and after intrathecal infusion of 50ug phenylephrine, n=6, relative to saline control, n=6 (left panel). Mean change in startle amplitude (±S.E.M) (the first 20 minutes postinfusion minus the predrug baseline) for 3 doses of phenylephrine and saline, n=6 at each point (right panel).

TABLE 1

Mean change in startle amplitude (± S.E.M.) from 0 to 20 minutes after intrathecal 5-MeODMT or phenylephrine following intraperitoneal administration of either saline, the alpha-1-noradrenergic antagonist WB-4101, the 5-HT antagonist cyproheptadine or the beta-noradrenergic antagonist propranolol.

All pretest drugs were given 15 minutes before intrathecal infusion of test drugs. Per cent change relative to preinfusion baseline is shown in parentheses.

Pretest drug	Test drug	
	5-MeODMT (50ug)	Phenylephrine (50ug)
Saline	26.1 ± 8.0 (+130%)	15.1 ± 5.2 (+116%)
WB-4101, 0.2mg/kg	—	12.2 ± 4.8 (+93%)
WB-4101, 1.0mg/kg	18.0 ± 4.3 (+98%)	-0.7 ± 3.0 (-10%)**
Cyproheptadine, 0.2mg/kg	9.0 ± 4.6 (+37%)	--
Cyproheptadine, 1.0mg/kg	-2.8 ± 1.6 (-8%)**	15.1 ± 3.2 (+111%)
Cyproheptadine, 5.0mg/kg	-5.2 ± 2.4 (-12%)**	--
Propranolol, 20mg/kg	20.5 ± 6.0 (+96%)	14.6 ± 4.0 (+101%)

**$P < 0.01$ relative to saline

alpha-1-adrenergic antagonist WB-4101. Other work has shown that the increase in startle produced by intrathecal 5-HT is blocked by systemic administration of the 5-HT antagonist, cinanserin (17).

Intrathecal NE appears to increase startle via stimulation of an alpha-1 receptor. Thus, the alpha-1 agonist phenylephrine increased startle when applied intrathecally and this effect was blocked by the alpha-1 antagonist WB-4101 but not by the beta-antagonist propranolol nor cyproheptadine. In contrast, intrathecal administration of the beta-agonist isoproterenol did not affect acoustic startle. In contrast to 5-HT and NE, neither DA nor the DA agonist apomorphine increased acoustic startle when given intrathecally.

This experiment clearly demonstrates the importance of the spinal cord as a target for mediating drug effects on behavior.

Experiment 2: Correlations between alpha-1 adrenergic stimulation of acoustic startle and alpha-1 adrenoceptor occupancy and number in rat lumbar spinal cord: supersensitivity vs normal state. For a drug to function it must be able to induce a change in a cellular system. One mechanism by which a drug alters neuronal function is by its joining with a specific recpetor on that cell. This drug-receptor complex may then lead to a change in cellular activity which may ultimately be expressed as a behavioral change.

The functional consequences of alterations in neurotransmitter receptors is important for the study of the neurochemical basis of behavior. Recently, in vitro high affinity binding of neurotransmitter substances to neuronal membranes has been used to

identify and characterize certain kinetic parameters of receptors such as affinity and number of recognition sites available for interaction with neurotransmitters or drugs (72). A relationship between alterations in neurotransmitter binding sites and behavior can be used to determine how binding parameters may relate to the functional state of the receptor (11). In order to make such a correlation a behavior is needed which is: 1) altered by specific pharmacological manipulations; 2) quantifiable; and 3) generated from a specific area of the CNS.

As has been shown, the acoustic startle response satisfies these three criteria. It is quantifiable and has been shown to be altered by both noradrenergic and serotonergic agonists applied locally to a specific area of the CNS. Alpha-1-noradrenergic receptors have been localized in the spinal cord and a specific alpha-1 induced increase in cAMP has been found in this tissue. In addition intrathecal d-amphetamine, which acts indirectly to increase noradrenergic transmission, has also been shown to increase the amplitude of aocustic startle (15). The excitatory effects of d-amphetamine -- like those of phenylephrine -- can be blocked by systemic administration of the alpha-1 antagonist, WB-4101, but not by the 5-HT antagonist cyproheptadine, or the dopamine antagonist haloperidol (15).

The acoustic startle reflex provides, therefore, a test system in which quantifiable changes in behavior can be measured following pharmacological manipulations in a limited part of the central nervous system. Examination of the lumbar spinal tissue in rats given intrathecal infusions of alpha-1-adrenergic agonists should thus

provide a method to determine the parameters of alpha-1-adrenergic binding that may be important in mediating changes in behavioral output.

Prazosin has been shown to have a preferential affinity for alpha-1-adrenoceptor sites both in the peripheral (9,41) and central nervous system (26,42,63). Hence, tritiated prazosin was used in these studies to evaluate the effects of various physiological and pharmacological manipulations on alpha-1-binding sites.

Correlations between behavior and binding were made using two different approaches. The first series of studies evaluated if there would be a correlation within animals, between the increase in the acoustic startle response produced by intrathecal administration of an alpha-1-agonist with the number of 3H-prazosin binding sites occupied by that agonist. If the magnitude of the behavior is related to receptor occupation, then increases in binding should be correlated to increases in behavior. The second series of experiments involved altering the receptors themselves. 6-OHDA lesions have been shown to cause significant increases in the number of alpha-1-adrenoceptors (54,56,61,62). If these receptors are involved in mediating the behavioral response, then an increase in the number of binding sites following denervation should be associated with an enhancement of the behaviorial response produced by an alpha-1-agonist. Moreover, depending on the correlation between these two measures, it should be possible to determine from these data the relationship between receptor occupation and pharmacological efficacy in the normal and denervated state.

d-Amphetamine and Phenylephrine: Receptor Occupation and Startle Change Correlation

One week following implantation rats were tested for acoustic startle following intrathecal administration of d-amphetamine or saline. Testing involved placing the rats in the stabilimeters and 5 min later giving startle eliciting noise bursts every 20 sec for 14 min to establish a pre-infusion baseline. Rats were then removed from the test cages and infused with 100 ug d-amphetamine dissolved in 10 ul of sterile saline followed by 8 ul of sterile saline to flush the d-amphetamine solution from the catheter and to occupy the catheter's internal volume. Immediately following infusion rats were returned to test cages and given noise bursts every 20 sec for 30 min. Previous work has shown that d-amphetamine's maximal effect on startle occurs about 30 min after the drug is infused. At the end of the 30 min test session each rat was decapitated and a 2.5 cm section of the lumbar cord was dissected out, weighed and frozen. The decapitation and dissection procedure took approximately 2-3 min. Lumbar cord segments weighed 214 + 22 mg per animal. Tissues were stored at -70 ^{o}C until assayed. Preliminary experiments showed no significant difference in binding between fresh tissue and tissue frozen under those conditions for at least 3 weeks. Assays were completed within this time period.

In a second group of rats the same procedures were repeated using phenylephrine as a direct alpha-1-adrenergic agonist instead of d-amphetamine. In addition, half the rats were treated with 6-OHDA to determine in a preliminary way if this would increase the effectiveness of phenylephrine and, if so, to determine the

correlation, within animals, between the response to phenylephrine and receptor binding in normal and denervated animals. To assure that the treatment groups would have similar initial startle amplitude prior to any treatment, all rats were placed in the stabilimeters and 5 min later presented with startle-eliciting noise bursts every 20 sec for 10 min. Based on the mean startle amplitude for each animal, the rats were divided into two groups with each group having similar mean startle amplitudes and variances. All of the rats within one group then received 20 ug of 6-OHDA dissolved in 10 ul of 0.1% ascorbic acid; the other matched group received an equal volume of the vehicle (0.1% ascorbic acid). The pH of both solutions was adjusted to 5.4 and the infusion rate was 10 ul/min. Following infusion the catheters were flushed with 8 ul of sterile saline. Thus, 10 ul of 6-OHDA or vehicle was actually delivered onto the spinal cord, while the remaining 8 ul of saline occupied the catheter's internal volume. Following infusion animals were returned to their home cages.

Animals were tested one week after receiving either 6-OHDA or vehicle, using either 25 or 50 ug of phenylephrine which was infused after a 14 min pre-infusion baseline. After the infusion the rats were returned to test cages and given noise bursts every 20 sec for 30 min. Experiment 1 showing phenylephrine's maximal effect on startle to occur from 15 to 30 min after the drug's infusion. At the end of the test session lumbar spinal cords were removed as described previously.

3H-Prazosin Binding: In Vitro Estimation of Receptor Occupation

3H-prazosin binding assays were carried out as a slight modification of the assay described by Greengrass and Bremner (26). In these studies lumbar cord sections were homogenized (Polytron PT-10/setting 8 for 20 sec) in 40 volumes (w/v) of ice cold 50 mM Tris-HCl pH 8.0 (at 25 C). One half of the resulting homogenate was removed and stored on ice until assay. This sample was labeled the "unwashed fraction". The remaining homogenate was further diluted with 200 volumes (w/v) of cold Tris buffer (pH 7.4), resuspended and centrifuged at 42,000 x g for 10 min; the resulting membrane pellet was resuspended and centrifuged two additional times. Preliminary experiments indicated that washing the membrane pellet three times was sufficient to remove residual phenylephrine from the assay which may have interfered with the binding of 3H-prazosin. Membranes were finally resuspended in 40 volumes (w/v) of 50 mM Tris-HCl buffer, (pH 8.0), labelled the "washed fraction". Aliquots (0.8 ml) of both the "washed" and "unwashed" fractions were incubated in the presence of ascorbic acid (0.1%), and single concentration of (0.7 to 1.0 nM) 3H-prazosin (Amersham-Searle S.A. 20.2 Ci/mM) and 0.05 M Tris HCl buffer (pH 8.0) to 1 ml total volume. Assays were analyzed in triplicate, two additional assay tubes contained 1 uM WB-4101 (WB Pharmaceuticals) to determine non-specific binding. Incubations were carried out at room temperature for 30 min; after incubation the samples were rapidly filtered through GF/B filters (Whatham Co., U.K.) under low vacuum. The filters were washed with three 8 ml aliquots of ice-cold 50 mM Tris buffer (pH 7.4) and counted in a liquid scintillation counter in 8 ml Econofluor (New England Nuclear). Results are reported as specific prazosin binding per mg protein, by

the method of Lowry et al. (14) representing total binding minus binding in the presence of 1 uM WB-4101. Each animal had a specific "unwashed" SB(UW) binding score and a specific "washed" SB(W) binding per mg protein score. An index of receptor occupation for each animal was calculated as the difference between the "washed" and "unwashed" specific binding of 3H-prazosin. Percent of total receptors occupied was determined by the formula % occupied:

$$\frac{SB(W) - SB(UW)}{Bmax} \times 100$$

Phenylephrine Dose Response Curve Following 6-OHDA

One week following implantation, a total of 48 animals were matched into four groups using the methods described above. Half the rats in each group were lesioned with 20 ug of intrathecal 6-OHDA, while the other half received an equal volume of vehicle. One week later animals were placed in the test cages and a preinfusion baseline was established. The animals were then removed from the test cages and received either saline, 12.5, 25, or 50 ug of phenylephrine, intrathecally. Immediately following infusion rats were returned to test cages and given noise bursts every 20 sec for 30 min. They were then discarded.

6-OHDA Time Course: Receptor Change, Startle Change Correlation

Animals were implanted and matched into two groups as described before. One group received 20 ug of 6-OHDA in 10 ul of 0.1% ascorbic

acid while the other matched group received an equal volume of vehicle. At various time intervals following the infusions (3 days, 1, 2, or 4 weeks) both vehicle and 6-OHDA groups were tested for acoustic startle following intrathecal administration of 12.5 ug of the alpha-1-agonist phenylephrine. This involved the same procedure of establishing a preinfusion baseline, removing the animals from the test cages, infusing all of them with 12.5 ug of phenylephrine and then returning them to the test cages for 30 min of startle eliciting noise bursts. Following testing lumbar cords were removed and prepared for the 3H-prazosin binding assay.

Scatchard Analysis

At specific times after 6-OHDA or vehicle administration and subsequent phenylephrine testing (3 days, 1, 2, or 4 weeks) animals were killed and lumbar spinal cord tissues for each treatment group were pooled . Pooled tissues were homogenized in cold 1: 200 (w/v) of Tris HCl buffer (pH 7.4), centrifuged at 42,000 x g for 10 min, resuspended and centrifuged two additional times. The resulting washed membrane pellets were resuspended in cold 1:40 (w/v) 50 mM Tris HCl buffer (pH 8.0). Aliquots (0.8 ml) of this homogenate were assayed for 3H-prazosin binding as described above in the presence of varying concentrations of 3H-prazosin (0.05 to 2.0 nM). Non-specific binding was assessed by the addition of 1 uM WB-4101. Quadruplicate samples and duplicate non-specific binding values were obtained for each concentration of 3H-prazosin. The maximum number of binding sites (Bmax) and apparent dissociation constant (KD) were estimated by Scatchard analysis. Protein concentrations were determined by the

method of Lowry et al. (38).

Determination of the Ki: Phenylephrine Competition

Pooled tissue for 6-OHDA lesioned and vehicle treated animals were analyzed for 3H-prazosin binding and its ability to be displaced by the in vitro addition of various concentrations of phenylephrine. Washed lumbar cord membranes were prepared as described above and incubated in the presence of 1 nM 3H-prazosin and various concentrations of phenylephrine (1 x 10-3 M to 1 x 10-6 M) in the presence or absence of 1 uM WB-4101.

Determination of Norepinephrine Content

Biochemical assay for catecholamines were performed using ligand chromatography with electron detection as described by Reinhard and Roth (50). Briefly, tissue samples (ca. 20 mg, wet weight) were sonically disrupted in 400 ul of 0.1 M perchloric acid containing 0.23 mM ascorbic acid (as an anti-oxidant), 75 nM dihydroxybenzyl amine; (Aldrich Chemical Co., Milwaukee, WI). The homogenates were centrifuged at 25,000 x g x 15 minutes and 300 ul aliquots of the clear supernatants were retained for subsequent analysis.

The supernates were adjusted to pH 8.6 and applied to miniature alumina columns (containing approximately 20 mg of acid-washed aluminum oxide). Passage of fluid through the columns was facilitated by centrifuging the columns (suspended in the mouths of 10 x 75 mm test tubes) at 50 x g x 2 min. The columns were washed with water (which was discarded) and eluted with 120 ul of 0.1 M oxalic acid; 50

ul of the eluate were applied to the LC for catechol analysis.

The chromatographic conditions for the model 420 Altex LC (equiped with a Bioanalytical System Inc., LC-3 amperometric detector) was as follows: the mobile phase was a 0.1 M NaPhosphate buffer (pH 3.2) which contained 0.2 mM octane sulfonic acid) (Regis Chemical Co., Morton Grove, IL), 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 4% (v/v) methanol; a flow rate of 1.5 ml/min was used through an Altex Ultrasphere - i.p. column. Compunds were identified on the basis of retention time and were quantitatively compared to authentic standards, run the same day.

RESULTS

The correlation between the increase in startle caused by 100 ug of intrathecal d-amphetamine and the 3H-prazosin binding sites occupied is shown in Figure 3. Occupation of binding sites was defined as the "washed" minus "unwashed" specific 3H-prazosin binding values corrected for protein concentrations. Within the d-amphetamine treated group the increase in startle could be directly correlated with the number of receptors occupied for each animal tested r (5) = 0.963, $p < .001$. The data also show that minimal occupation of alpha-1-adrenergic sites by the agonist is necessary to counteract a net decrease in startle normally observed in these test conditions (4,15). A significant correlation was also found between the increase in startle caused by either 25 or 50 ug of phenylephrine in both lesioned and non-lesioned animals and the 3H-prazosin binding sites occupied, r (16) = 0.50, $p < 0.02$. However, following the intrathecal

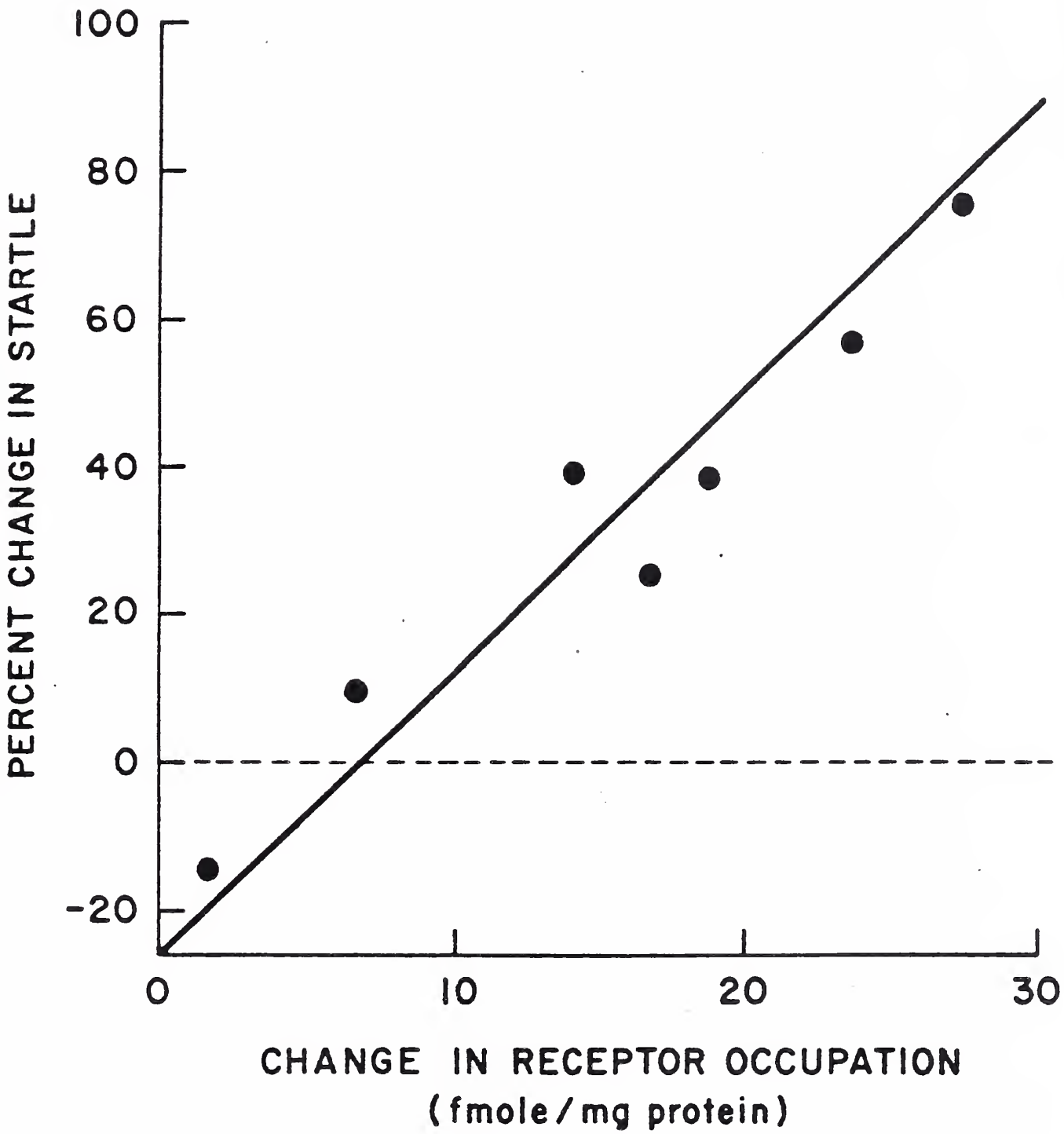

FIGURE 3. Correlation between estimated receptor occupation of individual animals (washed minus unwashed) and percent startle increase (mean amplitude startle measured from 15 to 30 minutes post-infusion following 100ug *d*-amphetamine minus mean startle amplitude over the 14 minute pre-drug baseline)..

administration of saline no significant difference in binding was observed between the 'unwashed' (98.9 fmoles of 3H-prazosin bound) and 'washed' (96.6 fmoles of 3H-prazosin bound) fractions when assayed at equivalent dilutions in the presence of 0.75 nM 3H-prazosin.

Various doses of phenylephrine were tested for their effects on acoustic startle response one week following either 6-OHDA lesion or sham-lesion. In both lesioned and unlesioned animals there was a significant effect of varying the dose of phenylephrine on the acoustic startle response, $\underline{F}$ (3/36) = 6.18, $p < .005$ (Figure 4). Moreover, there was a significant difference between the increase in acoustic startle caused by the 12.5 ug dose of phenylephrine in lesioned vs sham animals, $\underline{t}$ (10) = 3.31, $p < .01$, indicating that phenylephrine in a dose of 12.5 ug was able to induce a large increase in acoustic startle in lesioned rats but not in sham animals.

The magnitude of the excitatory effect of phenylephrine in acoustic startle increased as a function of the time after 6-OHDA administration, $\underline{F}$ (3,21) = 3.12, $p < 0.05$, this effect was linear over a time period from 3 days to 4 weeks, $\underline{F}$ lin (1,21) = 8.98, $p < 0.01$. An increase in the number of specific 3H-prazosin binding sites in animals lesioned with 6-OHDA at various time intervals (3 days, 1, 2, or 4 weeks) also occured (Table 2). A scatchard plot of data from 4-week 6-OHDA lesioned and sham-lesioned animals is shown in Figure 5. This analysis indicated that the increase in specific 3H-prazosin binding observed 4 weeks after pretreatment with 6-OHDA could be attributed to an increase in the total number of binding sites (Bmax) without a significant change in the dissociation constant (KD). Table

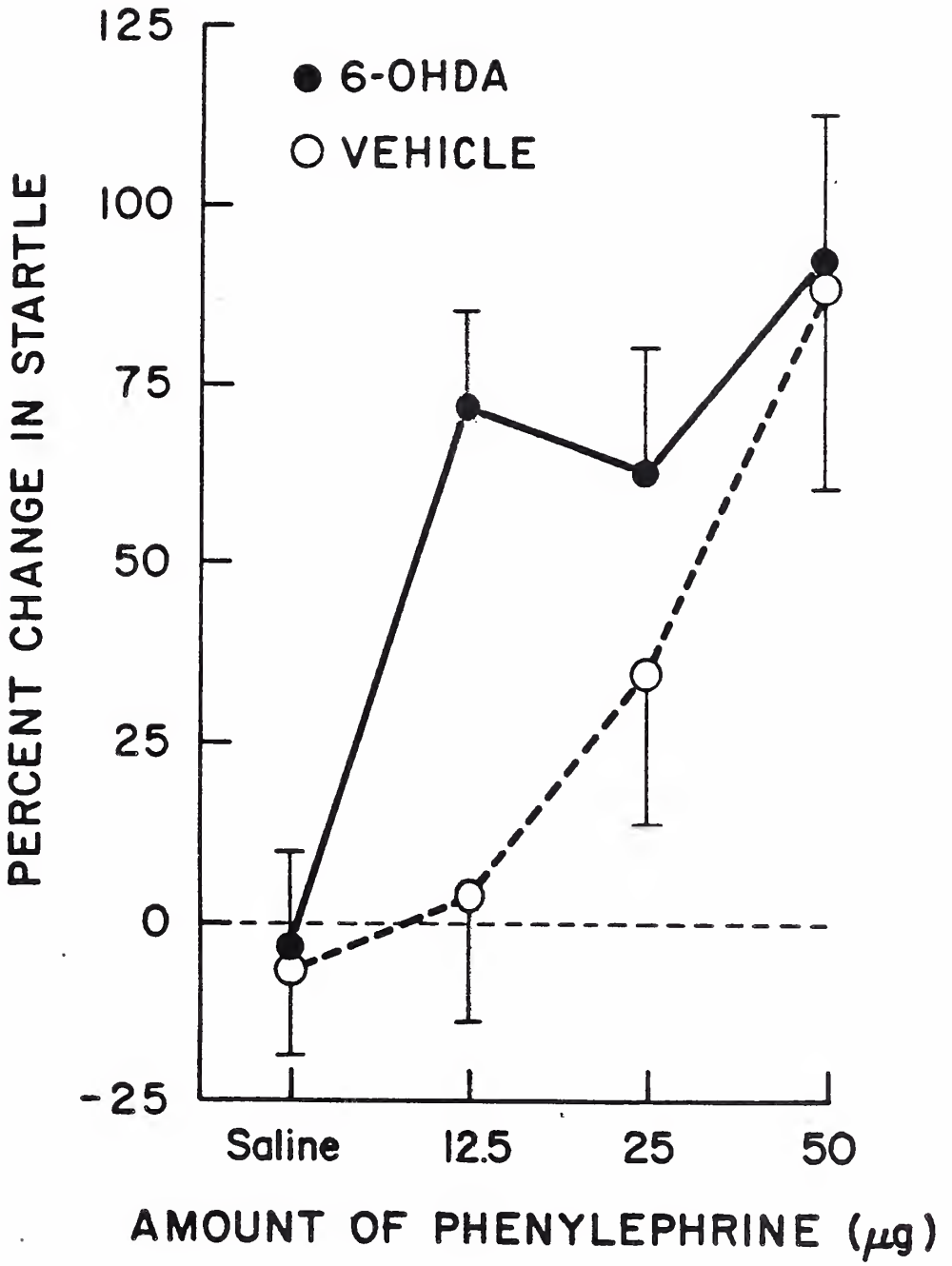

FIGURE 4. Mean change in startle amplitude (±S.E.M.) averaged from 15 to 30 minutes post-drug infusion minus the startle averaged over the 14 minute pre-drug period for three doses of phenylephrine and saline in both 1 week 6-OHDA lesioned and sham-operated animals, n=6 at each point.

TABLE 2

Dissociation constants (K_D), receptor densities (B_{max}) and phenylephrine's ability to inhibit [^{3}H]prazosin (1 nM) binding (K_i) of alpha-1-noradrenergic receptors in control and lesioned tissue at various time points.

Scatchard plots of [^{3}H]prazosin binding (0.05-2.0 nM) were determined from pooled tissue for each group at each time point. K_i values were calculated from the equation:

$$K_i = IC_{50} / \left(1 + \frac{\text{free radioligand}}{K_D}\right)$$

IC_{50} values were obtained from inhibition curves. 5-8 rats at each particular treatment.

	Vehicle			6-OHDA		
	K_D	B_{max}	K_i(nM)	K_D	B_{max}	K_i(nM)
3 days	0.30	47.9		0.30	51.6	
1 week	0.37	44.2	2905	0.30	53.8	3271
2 weeks	0.46	39.9		0.42	53.7	
4 weeks	0.38	43.5	4688	0.40	60.3	4511

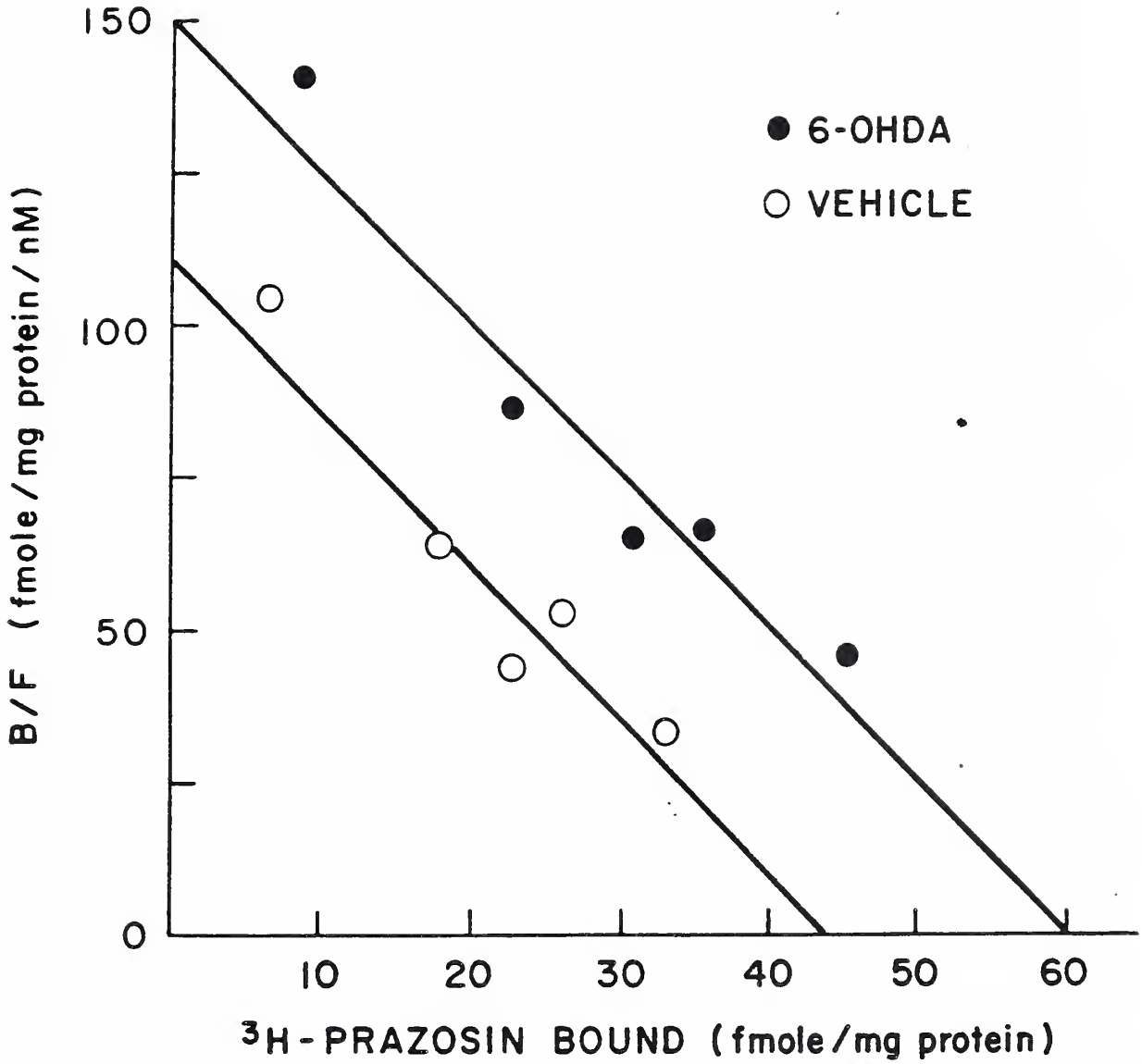

FIGURE 5. Scatchard analysis of [^{3}H]prazosin binding to rat lumbar spinal cord membranes from animals treated 4 weeks previously with either 6-OHDA or vehicle. B/F is the ratio of specific bound [^{3}H]-prazosin to free [^{3}H]prazosin. The slope of each individual plot gives an estimate of the equilibrium dissociation constant (K_D at 25°C) and the intersection with the abcissa gives a measure of the total number of binding sites (B_{max}). (Vehicle K_D=0.38 nM, B_{max}=43.5 fmol/mg protein; 6-OHDA K_D=0.40 nM, B_{max}=60.3 fmol/mg protein).

2 presents the observed KD and Bmax values in each of the 4 time point groups. The Ki values, coupled with constant KDs in sham vs. lesioned rats, indicates that the 6-OHDA lesion does not have a significant effect on receptor affinity.

A correlation between the change observed in the maximal number of binding sites (lesioned vs. sham) at each of the four time points, and the mean percent change in startle following an intrathecal dose of 12.5 ug phenylephrine is shown in Figure 6. A high correlation was found between the increase in the number of 3H-prazosin binding sites and the magnitude of the startle increase, $\underline{r}$ (2) = 0.99, $p < 0.01$.

An estimate for the number of receptors occupied was calculated by "washed" minus "unwashed" 3H-prazosin binding values (corrected for protein concentration) in animals exhibiting maximal behavioral changes in response to drug infusion. Comparing these numbers to the Bmax values obtained by Scatchard analysis of tissue from respective treatment groups gives the percentage of receptor occupied (Table 3). Occupation of approximately 30% of the total alpha-1-adrenoceptors is sufficient to produce a maximal increase in the startle response in both control and lesioned animals.

Howe and Yaksh (29) have demonstrated that intrathecal 6-OHDA depletes lumbar cord levels of NE within 1 day following treatment, peaking at 2 weeks (98% depletion) and continuing for at least 4 weeks after lesion. In the present experiments, we examined the localization of intrathecal 6-OHDA induced depletions of norepinephrine at various levels of the neuraxis. Intrathecal 6-OHDA

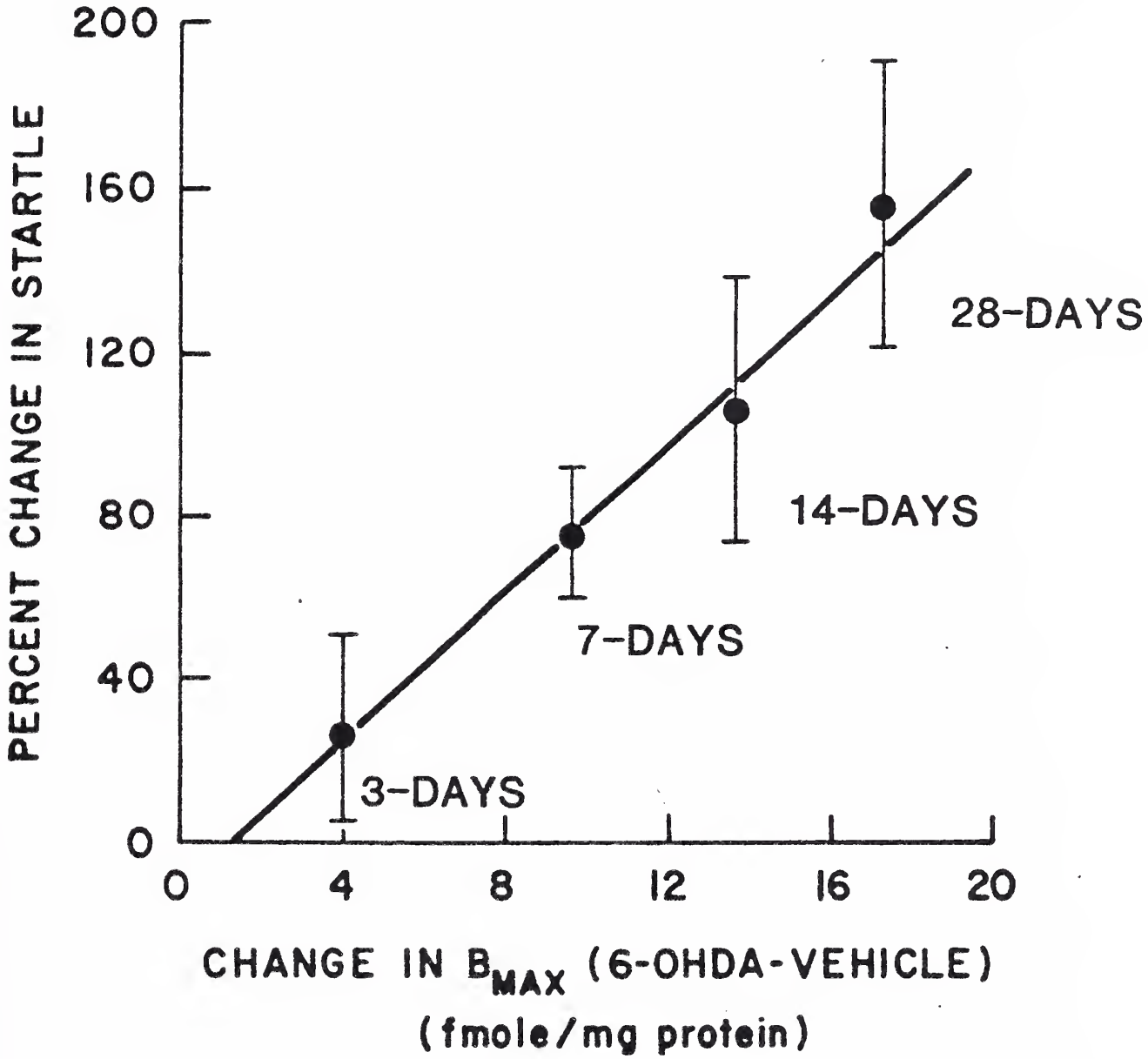

FIGURE 6. Correlation between increases in receptor number (B_{max}) as described by Scatchard analysis and mean percent startle increase in response to 12.5ug of intrathecal phenylephrine, ((startle averaged from 15 to 30 minutes post-drug infusion minus startle averaged over a 14 minute pre-drug period) x 100).

TABLE 3

Percentage of binding sites occupied in order to produce a significant increase in startle response over controls.

Individual binding scores were computed for each animal and the mean score for each treatment point was then compared to the B_{max} to get an estimate for the percentage of receptors occupied:

$$\frac{\text{(specific binding washed - specific binding unwashed)}}{B_{max}} \times 100$$

n = 5 at each treatment point.

	Mean receptors occupied (fmol/ mg protein)	B_{max} (fmol/mg protein)	Percentage receptors occupied
6-OHDA pretreatment (12.5ug PE)	13.7 ± 2.7	53.7	26%
Vehicle pretreatment(50 ug PE)	8.5 ± 0.8	36.9	23%
No pretreatment (100ug d-AMP)	13.2 ± 1.1	40.0	33%

lesions of the lumbar cord caused significant reductions in NE levels in the lumbar cord, t (16) = 9.85, $p < 0.001$, but not in the brain stem or cerebral cortex (Table 4). Within the spinal cord a gradient of NE depletion was found with greatest depletions in the lumbar and thoracic areas (96% depletion), and less in the more rostral cervical regions (33%). As previously reported (11), no significant alterations of 5-HT levels in the lumbar cord were observed following localized 6-OHDA injections (control = 218 + 25 ng/mg; lesion = 196 $\pm$ 19 ng/mg).

The purpose of the present study was to evaluate how changes in alpha-1- adrenoceptor binding parameters could be correlated with changes in functional alpha-1-adrenoceptor transmission, by measuring the effects of alpha-1-agonists on a behavioral reflex. Consistent with the results of experiment 1, intrathecal administration of the alpha-1-adrenergic agonist phenylephrine or d- amphetamine increased the amplitude of the acoustic startle reflex (15). The excitatory effect of intrathecal amphetamine appears to be due to its actions within the spinal cord since supraspinal (intraventricular) administration of amphetamine in equivalent doses does not increase startle (Commissaris and Davis, unpublished). In addition, Yaksh and Rudy (70) have shown that radioactive substances of varing lipid solubility infused into the lumbar enlargement of the rate spinal cord remain localized in the region around the tip of the cathether with little caudal to rostral diffusion when measured from 4-60 min after infusion. In the present study the magnitude of alpha-1-agonist induced increases in acoustic startle correlated positively, within

TABLE 4

Alterations in rat CNS norepinephrine levels after intrathecal administration of 6-OHDA.

Rats were infused intrathecally with 20ug of 6-OHDA in 10ul 0.1% ascorbic acid, sacrificed 2 weeks later, and the brain and spinal cord regions dissected and stored at -70°C. Control rats were administered the same volumes of vehicle solution. NE levels were determined as described in the text. Values are mean ± S.E.M. of 9 animals.

Region	Norepinephrine levels (ng/g wet weight)		
	Control	Lesion	Δ %
Cortex	240 ± 19	228 ± 24	-5
Brainstem	293 ± 11	286 ± 11	-2
Lumbar spinal cord	103 ± 10	4 ± 1**	-96

**Significantly different from control, $P < 0.001$.

animals, to the number of 3H-prazosin binding sites occupied by that agonist. Intrathecal administration of 6-OHDA caused a marked and selective decrease in spinal NE and an increase in the number of 3H-prazosin binding sites with no change in their affinity. From 3 days to 4 weeks after 6-OHDA the number of 3H-prazosin binding sites and the behavioral effects of phenylephrine increased linearly in a highly correlated fashion. Hence, under these conditions, changes in alpha-1-adrenoceptor number was closely associated with changes in functional alpha-1-adrenergic transmission.

The increase in acoustic startle following denervation correlated with a change in the number of receptors without any change in the affinity for the ligand. Behavioral supersensitivity has also been found following denervation or prolonged receptor blockade, which can be correlated with changes in receptor number rather than receptor affinity (11,44).

In summary, the present results document that a behavioral supersensitivity to an alpha-1 agonist produced by 6-OHDA is directly correlated with an increase in the number of alpha-1-adrenoceptors. Given the ability of this system to analyze supersensitivity leading to increased alpha-1 receptors and increased behavioral responsiveness it was then decided to test whether the opposite response could be observed and critically examined: the phenomenon of behavioral desensitization.

Experiment 3: Alpha-1 adrenergic stimulation of acoustic startle in desensitized and normal animals: behavior and binding studies.

Previous biochemical and physiological studies have shown that prolonged exposure of receptor sites to certain neurotransmitters or their agonists results in decreased responsiveness or desensitization to these substances (7,8,31,43,58). In a number of neurotransmitter systems, desensitization has been shown to be associated with decreases in the number of receptor sites as measured by radioligand binding techniques (7,31,43,53,58). At a behavioral level, desensitization or tachyphylaxis has been frequently observed. However, a number of mechanisms could account for such decreases in behavioral responsivity. To determine whether behavioral densensitization is associated with a decrease in receptor density it is necessary to examine a system in which behavioral changes in drug responsivity are highly correlated with changes in receptor number. As has just been shown denervation supersensitivity to the excitatory effects of the alpha-1- agonist phenylephrine on the acoustic startle reflex is highly correlated with the number of 3H-prazosin binding sites in the lumbar spinal cord. Thus the effects of intrathecal phenylephrine on the acoustic startle reflex represents a specific method to probe alpha-1- adrenoceptor sensitivity which is correlated with receptor density.

By exposing the animal to phenylephrine and then reintroducing it several hours later it was hoped that behavioral desensitization could be induced. Once induced, given the ease of analysis the acoustic startle system affords, it should be possible to observe whether or not a change in receptor density occurs.

Testing Procedure.

One week following implantation the rats were divided into two groups which received either intrathecal saline or 50 ug of phenylephrine dissolved in 10 ul of saline (pH 7.4). Six hours later the animals were placed in the startle test cages and 5 min later a program began which gave startle-eliciting noise bursts every 20 sec for 14 min. This constituted the pre-drug baseline. Based on the average startle performance across this baseline period the two pretreatment groups were divided into two groups that had roughly comparable baselines creating a total of four groups. The rats were then removed from their cages and infused a second time with either 50 ug phenylephrine (i.e., Groups SAL-PE and PE-PE) or saline (Groups SAL-SAL and PE-SAL). Two additional groups of rats were infused with phenylephrine or saline without any prior saline infusion to assess the importance of this treatment. All animals were tested for acoustic startle at approximately the same time of day in order to eliminate any possible influence of diurnal variation in the behavioral response. Immediately following the final infusion animals were returned to their test cages and given noise bursts every 20 sec for 30 min. Previous work has shown PE's maximal stimulatory effect occurs approximately 20 min following drug infusion. Therefore, the mean startle amplitude from 15 to 30 min after the drug infusion for each rat was compared with that rat's mean pre-drug infusion score to determine the change in startle amplitude following treatment.

As shown in Figure 7, intrathecal administration of 50 ug PE increased startle amplitude about 80% over the predrug baseline while saline treated animals showed the typical decline in startle amplitude

by about 40% over the same test period, indicative of habituation under these test conditions (4). Animals infused with saline 6 hrs previously (Group SAL-PE) were not significantly different in their responses to intrathecal phenylephrine compared to their respective non-preinfused counterparts (Group "--PE"; $p < .10$). Animals preinfused with PE, however, were barely responsive to another PE infusion 6 hrs later, and significantly different in their response to PE compared to rats pre-infused with saline, (Group SAL-PE vs. PE-PE: $t(14) = 5.59$, $p < .001$). The lack of effect of PE in these animals did not result from an elevation in their baselines produced by the earlier PE infusion. The effect of PE had dissipated by 6 hrs and in fact the baselines of Groups PE-PE and SAL-PE prior to their second infusion were essentially identical (data not shown). These results indicate marked desensitization following prior exposure to PE.

To assess the specificity of desensitization, a similar experiment was done using the indirect alpha-1-agonist d-amphetamine (dAMP) as the test drug 6 hrs following phenylephrine. Intrathecal d-amphetamine has been shown to increase startle and this effect can be blocked by WB-4101 but not by haloperidol, cyproheptadine or propranolol, indicating that an alpha-1- adrenergic receptor is involved (4,15). Fig. 8 confirms this in showing that both phenylephrine or d-amphetamine increase startle when given intrathecally in unpretreated animals. Most importantly, however, d-amphetamine had little effect in animals pretreated with phenylephrine as indicated by a significant difference in the response to dAMP in rats pretreated with saline vs PE (SAL-dAMP vs. PE-dAMP; t

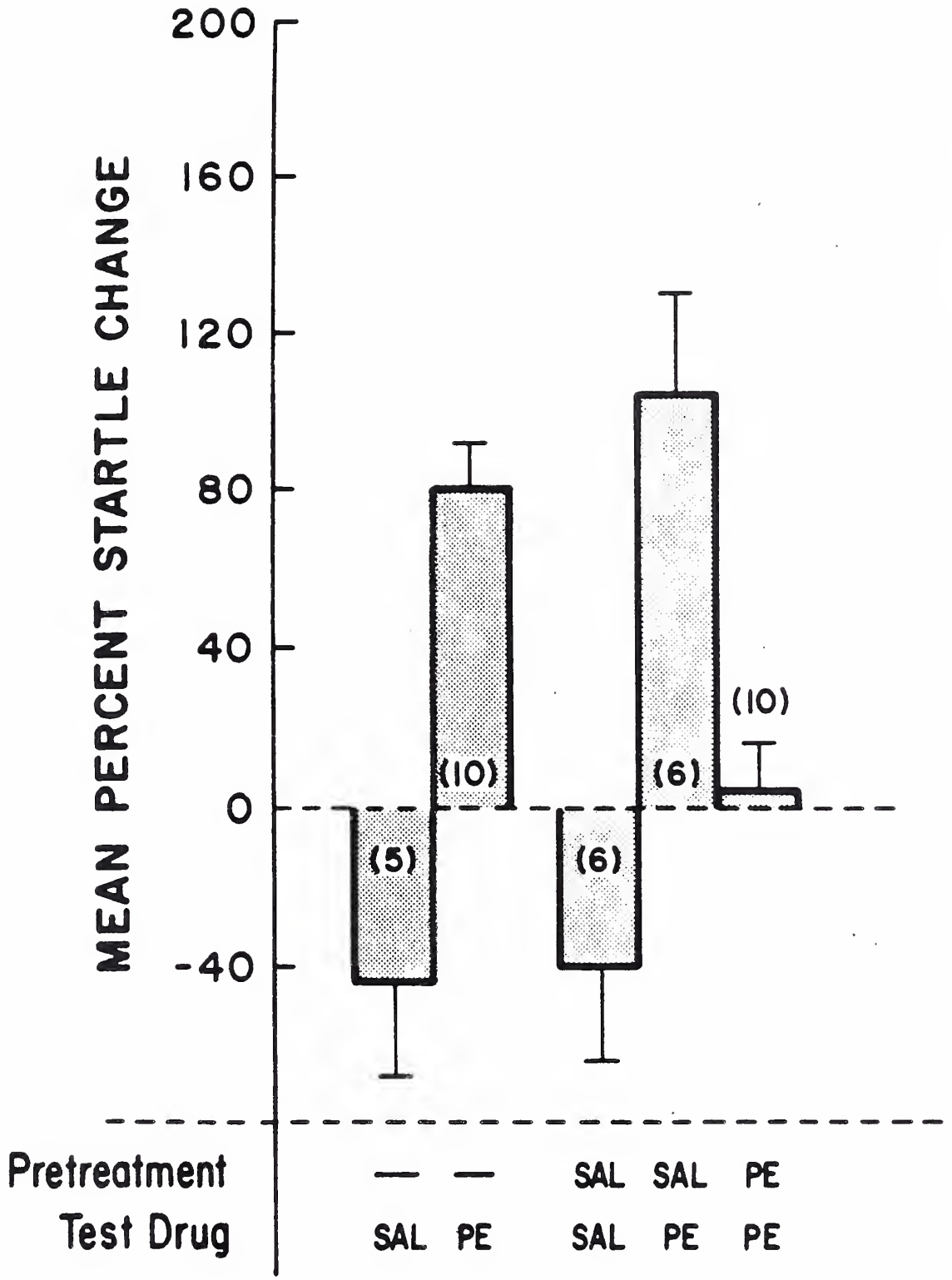

FIGURE 7. Mean percent change in startle amplitude following intrathecal infusion of the test drugs: saline (SAL) or phenylephrine (PE). The test drugs were given with no pretreatment or 6 hours after intrathecal infusion of PE. Percent change in startle amplitude from 15 to 30 minutes after the test drug with the mean startle amplitude over the 14 minute period prior to the test drug.

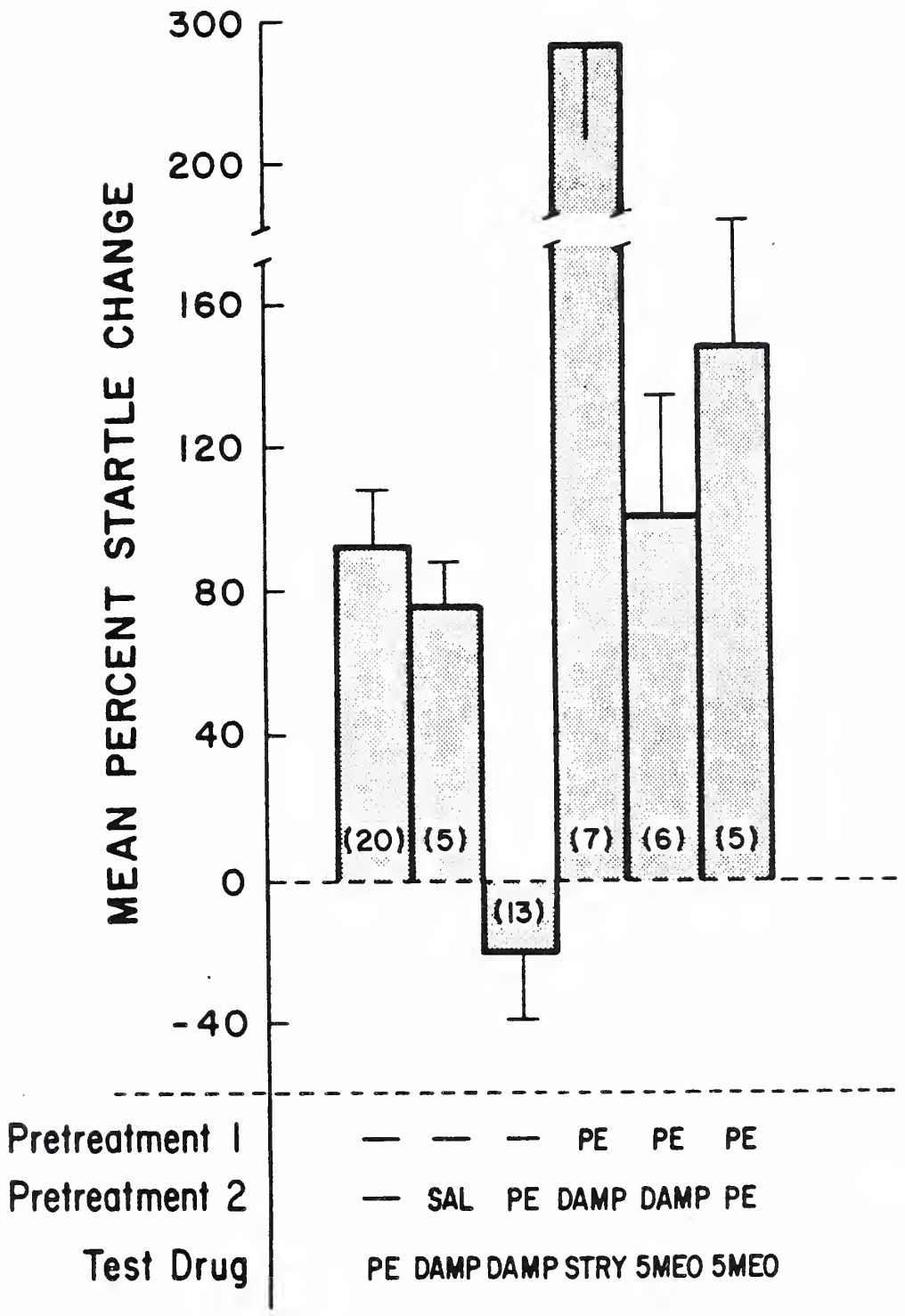

FIGURE 8. Mean percent change in startle to various test drugs (phenylephrine -- PE; d-amphetamine -- d-AMP; strychnine -- STRY; or 5-methoxydimethyltryptamine -- 5-MEO) after different pretreatments. Pretreatment 1 consisted of intrathecal infusion of PE 6 hours prior to Pretreatment 2. Pretreatment 2 consisted of intrathecal infusion of SAL, PE, or d-AMP 30 minutes prior to intrathecal infusion of the test drugs. Percent change in startle (±S.E.M.) was computed by comparing the mean startle amplitude from 15 to 30 minutes after the test drug with the mean startle amplitude over the 14 minute period prior to the test drug.

(16) = 4.41, $p < .001$). This indicates that cross tolerance occurred between phenylephrine and the indirectly acting alpha-1-adrenergic agonist d-amphetamine.

To more fully test specificity, the PE-dAMP and the PE-PE rats were given a final intrathecal infusion of either strychnine 6.25 ug (STRY - a glycine antagonist) or 5-methoxy-N,N-dimethyltryptamine 50 ug (5-MeoDMT - a 5-HT agonist). Both drugs have previously been found to increase acoustic startle when administered intrathecally to unpretreated rats (16,34). Fig. 8 shows that both drugs still increased startle in rats that were tolerant to d-amphetamine or PE ($t(6) = 2.67$, $p < .05$ for strychnine, $t(10) = 2.60$, $p < .02$ for 5-MeoDMT). This indicates that the desensitizing effect of prior exposure to alpha-1-agonists did not generalize to drugs that act through glycine or 5-HT receptors.

One possible explanation for the desensitization reported above is that the alpha-1-adrenergic receptors involved in the modulation of startle have decreased in number or undergone a conformational change following prior exposure to PE, rendering them less responsive in subsequent challenge tests. To evaluate this possibility Scatchard analysis of alpha-1-adrenoceptor sites and phenylephrine competition curves were performed on lumbar spinal cord tissue taken from other groups of animals 6 hrs following either intrathecal saline or 50 ug of intrathecal phenylephrine.

At 6 hrs following 50 ug of intrathecal phenylephrine or saline infusion animals were killed by decapitation and a 2.5 cm section of

lumbar cord was dissected out, weighed and frozen for subsequent ^{3}H-prazosin assays. Animals were sacrificed at the same time of day that the behavioral experiments were run in order to minimize reported circadian variations of B_{max} values (32). Lumbar spinal cord tissues for each treatment group (5-6 rats) were pooled and homogenized in cold 1:200 (w/v) of Tris HCl buffer (pH 7.4), centrifuged at 42,000 x g for 10 minutes, resuspended and centrifuged two additional times. Control experiments indicated that any phenylephrine remaining in the tissue was completely removed from the crude membrane preparations by these washing procedures. The resulting washed membrane pellets were resuspended in cold 1:40 (w/v) 50 mM Tris HCl buffer (pH 8.0). Aliquots (0.8 ml) of this homogenate were incubated in the presence of ascorbic acid (0.1%) and varying concentrations of ^{3}H- prazosin (0.05 to 1.0 mM) (Amersham-Searle, S.A., 20.2 Ci/mM) and 0.05 M Tris HCl buffer (pH 8.0) to 1.0 ml total volume. Non-specific binding was assessed by the addition of 1 uM WB-4101. Quadruplicate sample and triplicate non-specific binding values were obtained for each concentration of ^{3}H- prazosin. Incubations were carried out at room temperature for 30 min. After incubation the samples were rapidly filtered through GF/B filters (Waltham Co., U.K.) under low vacuum. The filters were washed with three 6 ml aliquots of ice cold 50 mM Tris buffer (pH 7.4) and counted in a liquid scintillation counter in 8 ml Econofluor (New England Nuclear). The maximum number of binding sites (B_{max}) and apparent dissociation constant (K_D) were estimated by Scatchard analysis. Values were obtained from four separate experiments, each with 5-6 animals per treatment group. Protein concentrations were determined by the method of Lowry et al. (38).

Pooled tissue from PE and saline pretreated animals were analyzed for ^{3}H- prazosin binding and its abililty to be displaced by the in vitro addition of various concentrations of phenylephrine. Washed lumbar cord membranes were prepared as described above and incubated in the presence of 1 nM ^{3}H-prazosin and various concentrations of phenylephrine (1 x 10(-3) M to 1 x 10(-6) M) in the presence or absence of 1 uM WB-4101.

The Scatchard plot in Fig. 9 shows a decrease in the number of prazosin binding sites in animals pretreated with phenylephrine 6 hrs prior to sacrifice without a significant change in the affinity of the receptor for the antagonist (Saline: B_{max} = 56.5 fmols/mg protein, K_D = 0.57 nM vs Phenylephrine B_{max} = 43.9 fmoles/mg protein, K_D = 0.48 nM). In addition, the Ki for phenylephrine was similar in PE pretreated (3.5 nM) and in saline pretreated (3.8 nM) animals ($p < 0.1$), indicating no change in the affinity of binding sites for phenylephrine following agonist pretreatment.

The purpose of the present study was to determine whether specific desensitization of alpha-1-receptors could be induced and observed in a behavioral paradigm: the acoustic startle response. Consistent with previous data, intrathecal administration of the alpha-1-adrenergic agonist phenylephrine or the indirect alpha-agonist d-amphetamine increased the amplitude of the acoustic startle response in animals receiving no pretreatment or an intrathecal saline pretreatment 6 hrs prior to startle testing. Such excitatory effects of intrathecal phenylephrine on startle can be blocked by systemic administration of the alpha-1-adrenergic antagonist WB-4101, but not

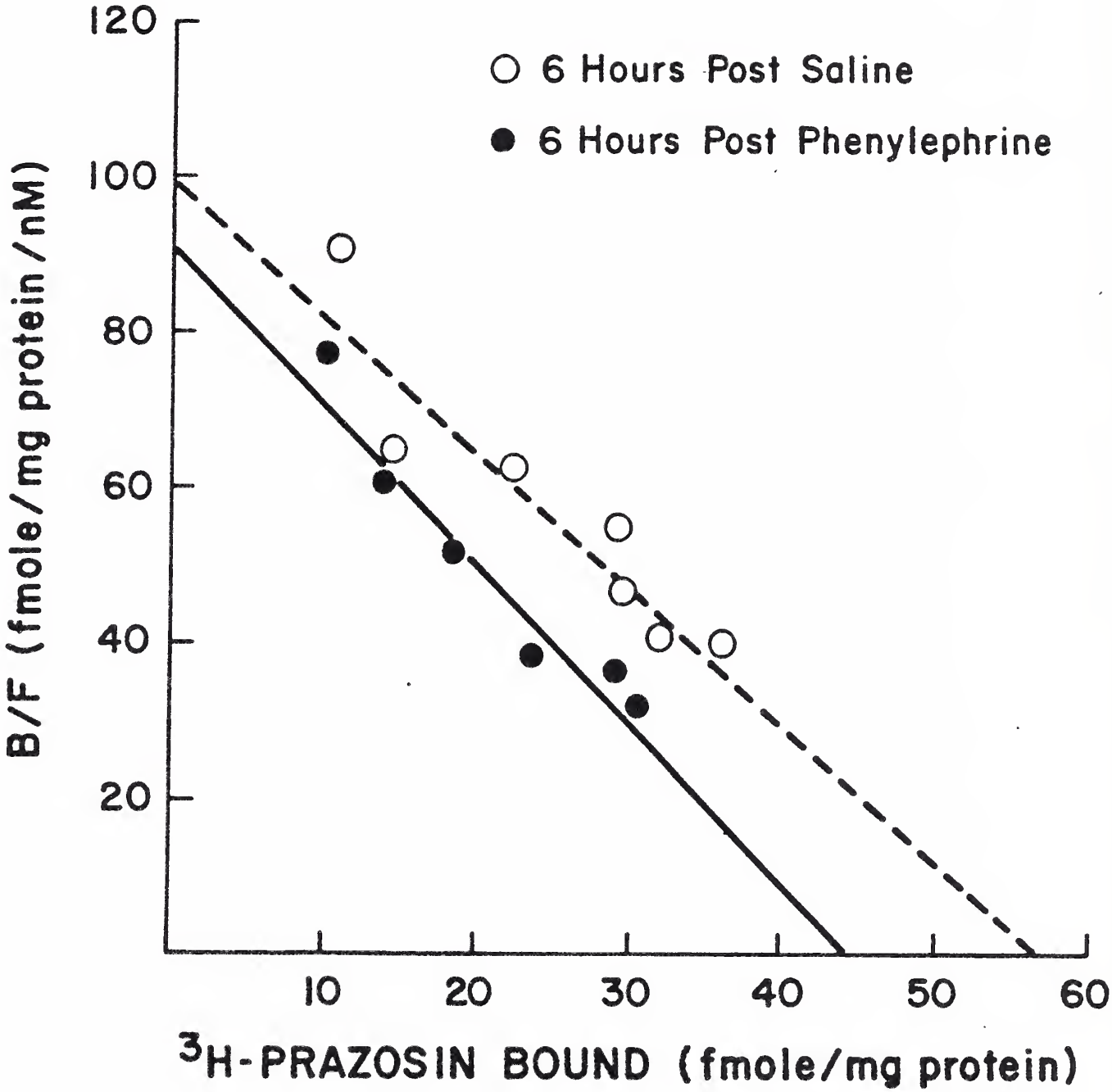

FIGURE 9. Scatchard analysis of [^{3}H]prazosin binding to rat lumbar spinal cord membranes from animals treated 6 hours previously with either intrathecal saline or 50ug of intrathecal phenylephrine dissolved in saline. B/F is the ratio of specifically bound [^{3}H]-prazosin to free [^{3}H]prazosin. The slope of each individual plot gives an estimate of the equilibrium dissociation constant (K_D at 25°C). The intersection aith the abcissa gives a measure of the total number of binding sites (B_{max}). The post saline K_D= 0.57 nM, B_{max}= 56.5 fmoles/mg protein. The post PE K_D= 0.48 nM, B_{max}=43.9 fmoles/mg protein.

by haloperidol, propranolol, yohimbine or cyproheptadine (4,15). This behavioral effect of phenylephrine represents, therefore, a specific method to probe alpha-1-adrenergic receptor sensitivity. In the present study however, pretreatment of animals with intrathecal phenylephrine dramatically decreased the normal stimulatory effect of an alpha-1-agonist infused 6 hrs later. These same animals, unresponsive to either phenylephrine or d-amphetamine, were still able to respond to intrathecal infusion of the glycine antagonist strychnine or the serotonin agonist 5-MeoDMT with significant increases in startle magnitude. These results satisfy, therefore, Waud's criteria for distinguishing specific from non-specific desensitization (66). Three representative drugs (an alpha-1-agonist, a serotonin agonist and a glycine antagonist) produce the same response (increase in startle magnitude) by interacting with three different receptors while desensitization of the response to one (alpha-1-agonist) is not accompanied by desensitization to the other two.

Using ^{3}H- prazosin as a marker for alpha-1-adrenergic binding sites it was shown, through Scatchard analysis, that the number of alpha-1-adrenergic binding sites was decreased 6 hrs following an intrathecal phenylephrine infusion without a change in the binding sites' affinity for the antagonist. In addition, agonist affinity was also unchanged as observed through the use of phenylephrine competition curves.

GENERAL DISCUSSION

The results of these experiments may be summarized as follows:

Experiment 1: showed that both serotonin and its agonist 5-Methoxy DMT and norepinephrine and its alpha-1 agonist phenylephrine produced dose dependent increases in acoustic startle amplitude when administered directly onto the lumbar spinal cord. These effects were specific as they were blocked by their respective antagonists but not by antagonists of other transmitter systems. Dopamine and its agonist apomorphine were without effect on acoustic startle when administered intrathecally.

Experiment 2: demonstrated that intrathecal administration of 6-OHDA caused a marked and selective depletion of spinal NE with a concomitant increase in the number of 3H-prazosin binding sites with no change in their affinity. The behavioral effects of phenylephrine increased linearly in a highly correlated fashion with the number of 3H-prazosin binding sites. Changes in alpha-1-adrenoceptor number was closely associated with changes in functional alpha-1 adrenergic transmission. In addition, a high correlation was found between receptor occupation and behavioral response to intrathecal d-amphetamine.

Experiment 3: showed that specific desensitization to alpha-1 receptors could be induced and observed in the acoustic startle response paradigm. The desensitization induced by pretreatment with phenylephrine was specific in that only the direct alpha-1 agonist phenylephrine and the indirect alpha-1 agonist d-amphetamine no longer proved efficacious in increasing startle amplitude while 5-MeODMT and

strychnine were still able to markedly augment acoustic startle amplitude. This desensitization was accompanied by a decrease in alpha-1 adrenergic binding sites without an observed change in receptor affinity.

The way in which serotonin, norepinephrine and their agonists enhance the startle response is most probably similar to that proposed for the modulation of cell firing in the facial motor nucleus (12). On these motor cells, iontophoretic administration of 5-HT or NE does not have any direct excitatory effects. However, such treatment does markedly increase excitation that is produced by electrical stimulation of afferents to these cells. Essentially identical modulatory effects of NE and 5-HT have also been reported for lower motor neurons in the spinal cord (18). In the case of acoustic startle, therefore, intrathecal administration of 5-HT or NE or their agonists should amplify the effects of the afferent volley to these neurons initiated by presentation of the acoustic startle stimulus. This interpretation is also consistent with the finding that 5-HT and NE increase the amplitude of spinal reflexes in spinal or decerebrate animals (2,3,40,52).

These effects also involve specific agonist receptor interactions as 5-HT antagonists block the effects of 5-MeODMT but not PE and alpha-1 antagonists block PE's effect but not 5-MeODMT. It is interesting to note that in the present study propranolol did not block the excitatory effect of intrathecal 5-MeODMT while previous work has shown that systemic pretreatment with propranolol antagonizes the increased startle caused by systemically administered 5-MeODMT

(16). Propranolol has also been shown to block the syndrome caused by systemically administered 5-MeODMT or 5-HT precursors and a monoamine oxidase inhbiitor (25). Microiontophoretic work has shown, however, that propranolol is ineffective in blocking the facilitation of the facial motor nucleus caused by direct application of 5-HT or 5-HT agonists (G.K. Aghajanian, personal communicati on). It would seem, therefore, that the antagonism caused by propranolol found in some studies may be a secondary effect related to systemic administration of both propranolol and 5-MeODMT.

In contrast to 5-HT and NE, neither DA nor the DA agonist apomorphine increased acoustic startle when given intrathecally. This is particularly interesting, since apomorphine is known to increase both acoustic and tactile startle when given systemically (14,24), and this effect can be blocked by either the DA antagonist, haloperidol (14) or the alpha-1 antagonist phenoxybenzamine (35). Perhaps apomorphine increases startle by acting through a DA receptor in the brain which is then expressed by release of NE in the spinal cord. This hypothesis could be tested by seeing whether intrathecal NE antagonists would block the effects of systemic apomorphine. In fact, the present results open up the possibility that a variety of drug or environment manipulations that increase startle (e.g., sensitization to background noise, potentiation due to prior conditioning) might be expressed by release of 5-HT and/or NE into the spinal cord. Again, the technique of administering intrathecal antagonists could be used to test this directly.

The second group of experiments allowed the information gathered in the first series to be applied in an effort to more clearly understand the role of receptors and the drug-receptor complex in mediating specific behavior. It offered a number of opportunities to determine the relationship between the degree of receptor occupation and behavioral output. Since in these studies, in vitro membrane preparation was required, the true degree of in vivo receptor occupation could not be determined. During homogenization and incubation, drug which was not initially bound to receptors in vivo could compete for binding sites in the in vitro assay. However, our 'washed' minus 'unwashed' procedure does give an estimate for the maximimum possible drug occupation of binding sites in the spinal cord membrane preparation. In each case, the percentage of alpha-1-adrenergic binding sites estimated to be occupied in animals exhibiting full potentiation of startle was about 30%. This figure was calculated when either d- amphetamine or phenylephrine was used to increase startle. It also occurred in animals treated with 6-OHDA. Moreover, the Ki values for phenylephrine in lesioned and non-lesioned animals were similar, despite the large increase in receptor number after 6-OHDA treatment. Thus, the ability of phenylephrine to displace 3H-prazosin in in vitro preparations of tissue from lesioned and non-lesioned animals was constant. These data support the concept of 'spare receptors', in which only a fraction of the total number of receptors need to be occupied to give a full biological effect, consistent with data in other transmitter systems. Thus Paul et al., (48) has shown that a 30% occupation of benzodiazepine binding sites was sufficient to protect against drug induced seizures in mice.

Venter (64) found that only 10% of cardiac beta receptors were needed to provide the full inotropic and cyclic AMP responses elicited by isoproterenol. Furchgott (23) using irreversible alpha adrenergic receptor antagonists found that more alpha receptors existed than were required for complete tissue activation in certain peripheral systems.

In the present experiments, the magnitude of startle facilitation produced by a rather low dose of phenylephrine 4 weeks after 6-OHDA was actually higher than the maximal degree of facilitation seen after any dose in normal rats. Functionally, therefore, denervation resulted in an increase in the efficacy of phenylephrine along with an increase in potency. For example, in normal rats, the maximal startle increase caused by intrathecal phenylephrine was about 100% above baseline even at the highest doses tested. In contrast, a dose of only 12.5 ug increased startle by about 160% in rats treated with 6-OHDA 4 weeks earlier.

There may be several reasons why higher doses of phenylephrine are not able to increase startle above about 100% in normal rats. One possibility is that high doses were observed to elicit behaviors such as twitching and panting which may compete with the expression of the startle response. Thus, at high doses, these behaviors may subtract from the increase in startle expected. However, if these competing behaviors are not due to specific occupation of alpha-1-receptor sites, then one should not expect supersensitivity of these behaviors to develop following alpha-1-receptor site proliferation. Equivalent receptor occupancy in normal and 6-OHDA treated rats would result in a

greater absolute number of binding sites occupied by phenylephrine in denervated rats. Since the magnitude of startle change is directly related to the absolute number of alpha-1-binding sites, then a low dose of phenylephrine could result in a large (supranormal) increase in startle without eliciting incompatable behaviors.

Another possibility to explain the lack of large doses of agonists in bringing out maximal behavioral changes is the phenomenon of receptor desensitization, involving changes in receptor recognition sites (28), and/or coupling steps beyond the receptor (21,22). Such desensitization processes are both time and dose dependent (22). Thus the inability to see large changes in startle following high doses of phenylephrine could result from desensitization. In 6-OHDA-treated rats, comparable numbers of receptors could be occupied by lower and hence less desensitizing doses of phenylephrine.

The results of the third series of experiments do indeed show that desensitization can occur in intrathecally implanted animals and that this desensitization does involve changes in the number of reeptor recognition sites. These results support the work of others who have shown decrease in receptor number to accompany desensitization. Kababian et al. (31) working with the rat pineal beta-adrenergic-receptor, observed rapid subsensitivity that developed within hours and which correlated with progressively fewer beta-adrenergic-binding sites. Mickey et al. (43) studying catecholamine tolerance in the purified frog erythrocyte system, also found a correlation between desensitization and declining beta-adrenergic-receptor binding. These workers hypothesized that a

reduction in receptor binding is a major mechanism in the induction of desensitization to catecholamines. As in our study, receptor affinity for either agonist or antagonist was reported to be unchanged. Thus it appears that decrease in receptor number can occur in desensitized systems. However, in our studies, only a small absolute decrease in receptor binding density (22%) accompanied the complete desensitization of the behavioral resonse to phenylephrine. Experiment two showed that occupation of a minimum number of alpha-1 adrenoceptor sites by the agonist is necessary to produce a net increase in the startle response. It is therefore possible that only a small decrease in receptor density could result in a sub-threshold activation of alpha-1 adrenoceptors for changing startle. Thus small decreases in receptor number could result in desensitization of the behavioral response, as observed in the present study. However, it is also possible that, in addition to changes in receptor density, coupling steps beyond the receptor may be involved in the desensitization processes (22). Such possibilities are currently under investigation. From the present study, however, it is clear that the ability to study behavior, pharmacology and biochemistry in the acoustic startle system makes it a powerful tool in the study of receptor mechanisms and other aspects of central nervous system plasticity.

REFERENCES

1. Adams, L.M. and Geyer, M.A., Effects of 6-hydroxydopamine lesions of locus coeruleus on startle in rats, Psychopharmacology, 73 (1981) 394-398.

2. Anden, N.E., Jukes, M.G. and Lundberg, A., Spinal reflexes and monoamine liberation, Nature (Lond.), 202 (1964) 1222-1223.

3. Anderson, E.G. and Shibuya, T., The effects of 5-hydroxytryptamine and l-tryptophan on spinal synaptic activity, J. Pharmacol. Exp. Ther., 153 (1966) 352-360.

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

5. Astrachan, D.I., Davis, M. and Gallager, D.W., Behavior and binding: correlations between alpha-1- adrenergic stimulation of acoustic startle and alpha-1- adrenoceptor occupancy and number in rat lumbar spinal cord, Brain Research, 260 (1983) 81-90.

6. Astrachan, D.I., Davis, M. and Gallager, D.W., Behavior and binding: desensitization to alpha-1 adrenergic stimulation of acoustic startle is associated with a decrease in alpha-1 adrenoceptor binding sites, Brain Research, 276 (1983) 183-187.

7. Axelrod, J. and Zatz, M., The Beta-adrenergic receptor and the regulation of circadian rhythms in the pineal gland. In Biochemical Actions of Hormones, Academic Press, New York, 1977, pp. 249-267.

8. Ball, N., Danks, J.L., Dorudi, S. and Nasmyth, P.A., Desensitization by noradrenaline of responses to stimulation of pre- and postsynaptic adrenoceptors, Brit. J. Pharmacol., 76 (1982) 201-210.

9. Cambridge, D., Davey, M.J., and Massingham, R., Prazosin, a selective anatagonist of postsynaptic alpha-adrenoceptors, Brit. J. Pharmacol., 59 (1977) 514 P.

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

11. Creese, I., Burt, D.R. and Snyder, S.H., Dopamine receptor binding enhancement accompanies lesion-induced behavioral supersensitivity, Science, 197 (1977) 596-598.

12. Dahlstrom, A. and Fuxe, K., 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, Suppl. 247 (1965) 1-36.

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

14. Davis, M. and Aghajanian, G.K., Effect of apomorphine and haloperidol on the acoustic startle response in rats, Psychopharmacology, 47 (1976) 217-223.

15. Davis, M. and Astrachan, D.I., Spinal modulation of acoustic startle: opposite effects of clonidine and d-amphetamine, Psychopharmacology, 75 (1981) 219-225.

16. Davis, M., Astrachan, D.I., Gendelman, P.M. and Gendelman, D.S., 5-Methoxy-N,N-dimethyltryptamine: spinal cord and brainstem mediation of excitatory effects on acoustic startle, Psychopharmacology, 70 (1980) 123-130.

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

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

19. Davis, M., Gallager, D.W. and Aghajanian, G.K., Tricyclic antidepressant drugs: attentuation of excitatory effects of d-lysergic acid diethylamide (LSD) on the acoustic startle reflex, Life Sci., 20 (1977) 1249-1258.

20. Davis, M., Gendelman, D.S., Tischler, M.D. and Gendelman, P.M., A primary acoustic startle circuit: lesion and stimulation studies, J. Neurosci., 2 (1982) 791-805.

21. Exton, J.H., Molecular mechanisms involved in alpha-adrenergic responses, Molec. Cellular Endocrin., 23 (1981) 233-264.

22. Fishman, P.H., Mallorga, P. and Tallman, J.F., Catecholamine-induced desensitization of adenylate cyclase in rat glioma C6 cells: evidence for a specific uncoupling of beta-adrenergic receptors from a functional regulatory component of adenylate cyclase, Molec. Pharmacol., 20 (1981) 310-318.

23. Furchgott, R.F., The pharmacology of vascular smooth muscle, Pharmacol. Rev., 7 (1955) 183-265.

24. Geyer, M.A., Peterson, L.R., Rose, G.J., Horwitt, D.D., Light, R.R., Adams, L.M., Zook, J.A., Hawkins, R.L. and Mandell, A.J., The effects of LSD and mescaline derived hallucinogens on sensory integrative function: tactile startle, J. Pharmacol. Exp. Ther., 207 (1978) 837-847.

25. Green, A.R. and Grahame-Smith, O.G., (-) Propranolol inhibits the behavioral responses of rats to increased 5-hydroxytryptamine in the central nervous system, Nature (Lond), 262 (1976) 594-596.

26. Greengras, S.P. and Bremner, R., Binding characteristics of 3H-prazosin to rat brain alpha-adrenergic receptors, Eur. J. Pharmacol., 55 (1979) 323-326.

27. Gudelsky, G.A., Thornburg, J.E. and Moore, K.E., Blockade of alpha-methyltryrosine induced supersensitivity to apomorphine by chronic administration of L-DOPA, Life Sci., 16 (1975) 1331-1338.

28. Heidmann, T. and Changeux, J.P., Structural and functional properties of the acetylcholine receptor protein in its purified

and membrane-bound states, Ann. Rev. Biochem., 47 (1978) 317-357.

29. Howe, J.R. and Yaksh, T.L., 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. Ther., 200 (1982) 311-321.

30. Jones, D.J. and McKenna, L.F., Alpha-adrenergic receptor mediated formation of cyclic AMP in rat spinal cord, J. Cyclic Nucl. Res., 6 (1980) 133-141.

31. Kababian, J.W., Zata, M., Romero, J.A. and Axelrod, J., Rapid changes in rat pineal beta-adrenergic receptor: alterations in l-(3H)-alprenolol binding and adenylate cyclase, Proc. Nat. Acad. Sci., 72 (1975) 3735-3739.

32. Kafka, M.S., Wirz-Justice, A. and Naber, D., Circadian and seasonal rhythms in alpha- and beta- adrenergic receptors in the rat brain, Brain Research, 207 (1981) 409-419.

33. Kandel, E.R., Grass Lecture Monograph I: A cell-biological approach to learning, Soc. Neuroscience, Maryland, 1978.

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

35. Kehne, J.H. and Sorenson, C.A., The effects of pimozide and phenoxybenzamine pretreatments on amphetamine and apomorphine

potentiation of the acoustic startle response in rats, Psychopharmacology, 58 (1978) 137-144.

36. Kokkinidis, L. and Anisman, H., Acute and chronic effects of d-amphetamine behavioral and neurochemical specificity, Psychopharmacology, 59 (1978) 285-292.

37. Lopachin, R.M., Rudy, T.A. and Yaksh, T.L., An improved method for chronic catheterization of the rat spinal subarachnoid space, Physiol. Behav., 27 (1981) 559-561.

38. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the Folin phenol reagent, J. Biol. Chem., 198 (1951) 265-275.

39. McCall, R.B. and Aghajanian, G.K., Serotonergic facilitation of facial motoneuron excitation, Brain Research., 169 (1979) 11-27.

40. Maj, J., Palider, W. and Baran, L., 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 Trans., 38 (1976) 131-147.

41. Massingham, R., Dubocovich, M.L., Shepperson, N.B. and Langer, S.Z., In vivo selectivity of prazosin but not of WB-4101 for postsynaptic alpha-1-adrenoceptors, J. Pharmacol. Exp. Ther., 217 (1981) 467-474.

42. Miach, P.J., Dausse, J.P., Cardot, A. and Meyer, P.M., 3H-prazosin binds specifically to 'alpha-1'-adrenoceptors in rat

brain, Naunyn-Schmied Arch. Pharmacol., 312 (1980) 23-26.

43. Mickey, J.V., Tate, R., Mullikin, D. and Lefkowitz, R.J., Regulation of adenylate cyclase coupled beta adrenergic receptor binding sites by beta adrenergic catecholamines in vitro, Mol. Pharmacol., 12 (1976) 409-419.

44. Mishra, R.K., Marshall, A.M. and Varmuza, S.L., Supersensitivity in rat caudate nucleus: effects of 6-hydroxydopamine on the time course of dopamine receptors and cyclic AMP changes, Brain Research, 200 (1980) 47-57.

45. Nahorski, S.R., Behavioral supersensitivity to apomorphine following cerebral dopaminergic denervation by 6-hydroxydopamine, Psychopharmacologia (Berl.), 42 (1975) 159-162.

46. Nickerson, M., Receptor occupancy and tissue response, Nature, (Lond.), 178 (1956) 697-698.

47. Nygren, L. and Olson, L., 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 Research., 132 (1977) 85-93.

48. Paul, S.M., Syapin, P.J., Paugh, B.A., Moncada, V. and Skolnick, P., Correlation between benzodiazepine receptor occupation and anticonvulsant effects of diazepam, Nature, 281 (1979) 688-689.

49. Reddy, S.V.R. and Yaksh, T.L., Spinal noradrenergic terminals system mediates antinociception, Brain Research., 189 (1980)

391-401.

50. Reinhard, J.R. and Roth, R.H., Alpha receptor mediated inhibition of serotonin synthesis and metabolism, in vivo, by clonidine, J. Pharmacol. Exp. Ther., 221 (1982) 541-546.

51. Samanin, R., Mennini, T., Ferraris, A., Bendotti, C. and Borsini, F., Hyper- and hyposensitivity of central serotonergic receptors: (3H)serotonin binding and functional studies in the rat, Brain Res., 189 (1980) 449-451.

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

53. Schwartz, J.C., Costentin, J., Martres, M.P., Protais, P. and Baudry, M., Modulation of receptor mechanisms in the CNS: hyper and hyposensitivity to catecholamines, Neuropharmacol., 17 (1978) 665-685.

54. Skolnick, P., Stalvey, L.P., Daly, J.W., Hoyler, E. and Davis, J.N., Binding of alpha-adrenergic ligands to cerebral cortical membranes: effect of 6-hydroxydopamine treatment and relationship to the responsiveness of cyclic AMP-generating systems in two rat strains, Eur. J. Pharmacol., 47 (1978) 201-210.

55. Sporn, J.R., Harden, T.K., Wolfe, B.B. and Molinoff, P.B., Beta-adrenergic receptor involvement in 6-hydroxydopamine induced supersensitivity in rat cerebral cortex, Science, 194 (1976) 624-626.

56. Sporn, J.R., Wolfe, B.B., Harden, T.K. and Molinoff, P.B., Supersensitivity in rat cerebral cortex: pre- and postsynaptic effects of 6-hydroxydopamine at noradrenergic synapses, Molec. Pharmacol., 13 (1977) 1170-1180.

57. Starke, K., Endo, T. and Taube, H.D., Relative pre- and postsynaptic potencies of alpha-adrenoceptor agonists in the rabbit pulmonary artery, Naunyn-Schmied. Arch. Pharmacol., 291 (1975) 55-78.

58. Strittmater, W.J., Davis, J.N. and Lefkowitz, R.J., alpha-Adrenergic receptors in rat parotid cells: desensitization of receptor binding sites and potassium release, J. Biol. Chem., 252 (1981) 5478-5482.

59. Thornburg, J.E. and Moore, K.E., Supersensitivity to dopamine agonists following unilateral 6-hydroxydopamine induced lesions in mice, J. Pharmacol. Exp. Ther., 192 (1975) 42-49.

60. Trendelenburg, U., Mechanisms of supersensitivity and subsensitivity to sympathomimetic amines, Pharmacol. Rev., 18 (1966) 629-640.

61. U'Prichard, D.C., Bechtel, W.D., Rouot, B.M. and Snyder, S.H., Multiple apparent alpha-noradrenergic receptor binding sites in rat brain: effects of 6-hydroxydopamine, Molec. Pharmacol., 16 (1979) 47-60.

62. U'Prichard, D.C., Reisine, T.D., Mason, S.T., Fibiger, H.C. and Yamamura, H.I., Modulation of rat brain alpha- and

beta-adrenergic receptor populations by lesions of the dorsal noradrenergic bundle, Brain Research, 187 (1980) 143-154.

63. U'Prichard, D.C. and Snyder, S.H., Distinct alpha-noradrenergic receptors differentiated by binding and physiological relationships, Life Sciences, 24 (1979) 79-88.

64. Venter, J.C., High efficiency coupling between beta-adrenergic receptors and cardiac contractility: direct evidence for "spare" beta adrenergic receptors, Molec. Pharmacol., 16 (1979) 429-440.

65. Waddington, J.L., Cross, A.J., Longden, A., Owen, F., and Poulter, M., Functional distinction between DA-stimulated adenylate cyclase and 3H-spiperone binding sites in rat striatum, Eur. J. Pharmacol., 58 (1979) 341-342.

66. Waud, D.R., Pharmacological receptors, Pharmacol. Rev., 20 (1968) 49-88.

67. Weiss, G.T. and Davis, M., Automated system for acquisition and reduction of startle response data, Pharmacol. Biochem. Behav., 4 (1976) 713-720.

68. White, S.R. and Neuman, R.S., Facilitation of spinal motoneuron excitability by 5-hydroxytryptamine and noradrenaline, Brain Research, 188 (1980) 119-128.

69. Yaksh, T.L., Direct evidence that spinal serotonin and noradrenaline terminals mediate the spinal antinocipceptive effects of morphine in the periaqueductal gray, Brain Res., 160

(1979) 180-185.

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

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

72. Yamamura, H.I., Enna, S.J. and Kuhar, M.J. (Eds.), Neurotransmitter receptor binding, Raven Press, N.Y., 1978, pp. 195.

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