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## Amphetamine's Effects on Terminal Excitability of Noradrenergic Locus Coeruleus Neurons are Impulse-Dependent at Low But Not High Doses

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The actions of amphetamine in the locus coeruleus and its terminal fields in the frontal cortex were studied using extracellular recording to measure terminal excitability, firing rate and the probability of antidromic action potential invasion of the somatodendritic region in urethane anesthetized rats. At low dose (0.25 mg/kg), amphetamine increased terminal excitability. In comparison, subsequent administration of the highest dose (5.0 mg/kg, i.v.) of amphetamine tested suppressed neuronal firing and blocked antidromic action potential invasion of the somatodendritic region. Despite the absence of impulse traffic, high dose amphetamine reversed the effect of low dose amphetamine in the terminal field and decreased terminal excitability. The  $\alpha_2$  antagonist, yohimbine (0.5 mg/kg, i.v.), reversed the effects of high dose amphetamine on terminal excitability and somatodendritic invasion without re-instating neuronal firing. Noradrenergic autoreceptor agonists are known to decrease terminal excitability, whereas antagonists are known to increase terminal excitability. Thus, since low dose amphetamine produces the same effect on terminal excitability that antagonists do, it appears that low dose amphetamine may reduce autoreceptor activation by reducing norepinephrine release in frontal cortex as a consequence of inhibiting locus coeruleus neuronal firing. In contrast, high dose amphetamine acts like autoreceptor agonists do and decreased terminal excitability. Hence high dose amphetamine may increase norepinephrine release, even in the absence of impulse traffic.

### INTRODUCTION

Amphetamine has long been considered to promote release and block re-uptake of catecholamine neurotransmitters<sup>2,6,7,15,20,28</sup>. However, recent studies using synaptosomal preparations have demonstrated that the potency of amphetamine for inducing release is greater for dopaminergic than noradrenergic neurons, whereas amphetamine appears to be a potent re-uptake blocker in both types of catecholamine neurons<sup>3,20,27</sup>. Since systemically administered amphetamine inhibits noradrenergic neuronal firing in the locus coeruleus even at quite low doses<sup>9</sup>, it is possible that amphetamine-induced norepinephrine release may not be great enough to counteract the decrease in action potential-induced release, thus producing a net decline in norepinephrine release. Two electrophysiological studies of amphetamine's effects suggest that in vivo amphetamine does, under certain conditions, reduce norepinephrine release

from terminals of locus coeruleus neurons. Huang and Maas<sup>16</sup> observed an increase in firing rate of hippocampal neurons following 0.1 mg/kg, i.v., or 0.5 mg/kg, i.p., amphetamine. This effect did not occur in animals in which the locus coeruleus had been destroyed. Since norepinephrine exerts a potent inhibition on these cells, they suggested amphetamine, at this dose, reduces norepinephrine release within the hippocampus by decreasing locus coeruleus neuronal firing thus leading to excitation. Higher doses had mixed excitatory and inhibitory effects.

Results from our laboratory also suggest low dose systemic amphetamine may reduce norepinephrine release in frontal cortex<sup>24,25</sup>. These studies examined activation of the presynaptic receptors sensitive to the neuron's own transmitter that are believed to exist on the synaptic terminals of many catecholaminergic neurons<sup>21,29</sup>. Biochemical studies reveal that activating these autoreceptors modulates synaptic efficiency by diminishing neurotransmitter release and

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slowing neurotransmitter synthesis<sup>10,18</sup>. In our experiments, the activation of these presynaptic receptors is monitored by measuring the electrical excitability of the presynaptic terminals as indicated by the amount of stimulating current required to initiate antidromic action potentials in locus coeruleus neurons. Activation of the noradrenergic presynaptic receptors on locus coeruleus axon terminals by agonist drugs infused into frontal cortex terminal fields decreases the electrical excitability of those terminals. Conversely, antagonists increase terminal excitability. When amphetamine was infused into the frontal cortex near the stimulating electrode, it acted in the same manner as agonists did and decreased electrical excitability. In contrast, when low doses of amphetamine (< 1 mg/kg, i.v.) were administered systemically, amphetamine had the same effect as antagonists did and increased terminal excitability. It was suggested that, since the ED<sub>50</sub> for inhibition of firing of locus coeruleus neurons is 0.24 mg/kg, i.v.<sup>9</sup>, systemic low dose amphetamine inhibits cell firing but may not sufficiently facilitate release to counteract the loss of impulse traffic.

The present study was designed to examine the actions of amphetamine at various systemically administered doses at synaptic sites within the nucleus locus coeruleus and its frontal cortex terminal fields to determine whether the ability of amphetamine to release norepinephrine at higher doses can overcome the apparent diminution of release caused by the profound inhibition of noradrenergic neuronal firing. Changes in the activation of presynaptic receptors in frontal cortex were estimated by measuring changes in terminal excitability. This measure may also index the amount of transmitter available within the synaptic cleft and so indirectly indicate the degree of postsynaptic receptor activation. The activation of receptors in the locus coeruleus was examined by measuring firing rate and the likelihood of somatodendritic invasion of the antidromic action potential from the initial segment. Somatodendritic invasion is thought to reflect the degree of hyperpolarization of the neuronal cell body<sup>14</sup>.

#### MATERIALS AND METHODS

Male Sprague–Dawley rats ( $n = 60$ ) weighing between 250 and 400 g were anesthetized with 1.3 g/kg

urethane, i.p. The general methods of preparation, stimulation and recording were as previously described<sup>24,25</sup>. The coordinates<sup>19</sup> of the dorsal noradrenergic pathway stimulating electrode were 2.0 mm anterior to lambda, 0.9 mm lateral to the midline and 5.7–6.0 mm ventral to the cortical surface. For stimulation of the frontal cortex, the electrode was implanted 3.0 mm anterior to bregma, 2.5 mm lateral to the midline and 1.7 mm deep. For recording from the locus coeruleus, a large hole was centered 2.0 mm posterior to lambda and 1.0 mm lateral to the midline, for an electrode approach of 15° off the coronal plane. Several previously described criteria<sup>22–25</sup> were used to identify locus coeruleus neurons. Animals were immobilized with gallamine triethiodide (50 mg/kg, i.p.) and respired on a Harvard Apparatus Rodent respirator at 80–90 strokes/min.

Responses elicited by electrical stimulation of the dorsal pathway and frontal cortex were considered to be antidromic provided they were extinguished following collision with spontaneous action potentials. The antidromic threshold was defined as the minimum current sufficient to elicit an antidromic response on 100% of the non-collision trials. In addition, the proportions of antidromic responses to several lower currents were determined. Thresholds were determined with both ascending and descending current series, with steps of less than 5% of the threshold value. After a stable threshold was determined, 0.25 mg/kg amphetamine was injected i.v. and the threshold was redetermined. If the recording remained stable either 1.0 mg/kg, 2.5 mg/kg, or 5.0 mg/kg, i.v., was administered 5–10 min after the first injection. The threshold was again determined. Many of those animals receiving 5.0 mg/kg amphetamine later (5–10 min) received 0.5 mg/kg yohimbine. D-Amphetamine sulfate (dextro-amphetamine sulfate, Smith, Klein & French) was administered i.v. via the femoral vein. The doses ranged from 0.25 to 5.0 mg/kg of the salt in a volume of 0.9% saline corresponding to 1.0 ml/kg for i.v. injection. Yohimbine was administered as 0.5 mg/kg in 0.9% saline. One cell was studied per animal.

The changes in each dependent variable produced by 0.25 mg/kg amphetamine and 0.5 mg/kg yohimbine were analyzed by a *t*-test of the difference between related means. The effects of 1.0, 2.5 and 5.0

mg/kg amphetamine were analyzed by 2-way analysis of variance with dose (1.0, 2.5 and 5.0) as one factor and condition (pre-drug, 0.25 mg/kg, test dose as levels for analyzing firing rate and somatodendritic invasion; difference between 0.25 mg/kg and pre-drug and between 0.25 mg/kg and test dose as the two levels for analyzing the threshold data) as a repeated factor. Multiple post-hoc comparisons were made using the appropriate error term from the analysis of variance to perform the Tukey honestly significant difference (hsd) test.

## RESULTS

### *Changes in terminal excitability*

Terminal excitability was estimated by measuring the current required to antidromically activate locus coeruleus neurons by electrical stimulation of the synaptic terminal field in frontal cortex.

The i.v. administration of 0.25 mg/kg amphetamine reliably decreased the threshold current relative to the pre-injection threshold, indicating an increase in terminal excitability ( $X = -9.7\%$ ; S.E.M. = 1.1;  $t = 10.1$ ;  $df = 59$ ;  $P < 0.01$ ). Fig. 1 shows the antidromic response of a typical locus coeruleus neuron. Prior to drug administration, a current of 1.34 mA was just sufficient to elicit an antidromic action potential to every stimulus (Fig. 1A); at a lower intensity of 1.09 mA, antidromic responses were evoked to only 7.7% of the stimuli (Fig. 1B). Within 30 s of the i.v. administration of 0.25 mg/kg amphetamine, this latter current (1.09 mA) became sufficient to elicit an antidromic response 100% of the time (Fig. 1C).

Subsequent injections of either 1.0 mg/kg or 2.5 mg/kg amphetamine failed to cause any further change in threshold current, as compared to the current required after 0.25 mg/kg (1.0 mg/kg:  $X =$

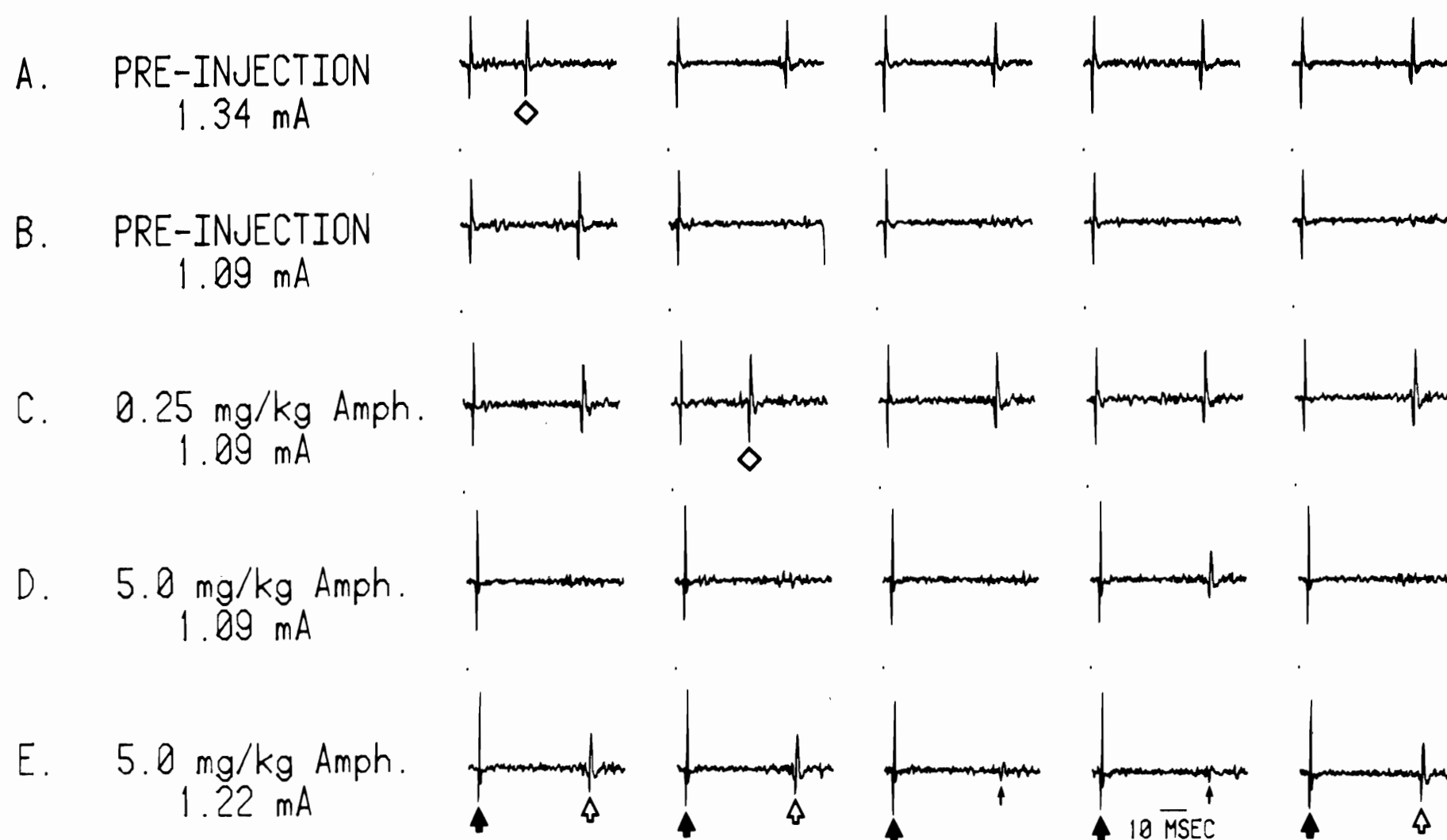


Fig. 1. Antidromic responses to electrical stimulation of the frontal cortex terminal field of a locus coeruleus neuron before and after i.v. administered amphetamine. Collisions of the antidromic response with spontaneously occurring action potentials are marked by diamonds in A and C. A: prior to drug injection, 1.34 mA evoked antidromic responses to every stimulus. B: 1.09 mA evoked an antidromic response only 7.7% of the time. C: after 0.25 mg/kg amphetamine, i.v., 1.09 mA was sufficient to evoke a response to every stimulus. D: 5.0 mg/kg, i.v., reversed this lowering of the threshold, causing only 32.5% responding to 1.09 mA. E: raising the threshold for 100% antidromic activation to 1.22 mA. 5.0 mg/kg amphetamine caused many of the antidromic responses to fail to invade the somatodendritic region, so that only the small initial segment spike occurred (marked by small arrows in E). The stimulus is marked with large black arrows and the full, initial segment plus somatodendritic, spike is marked with large white arrows.

-1.4%; S.E.M. = 2.5;  $n = 10$ ; 2.5 mg/kg:  $X = -3.0\%$ ; S.E.M. = 3.1;  $n = 6$ ; Tukey hsd, n.s.). In contrast, the highest dose of amphetamine tested did alter terminal excitability. The subsequent injection of 5.0 mg/kg amphetamine reversed the effect of 0.25 mg/kg amphetamine (analysis of variance: interaction - dose  $\times$  condition,  $F_{2,33} = 3.95$ ,  $P < 0.05$ , Tukey hsd test,  $P < 0.05$ ), reliably increasing the current threshold ( $X = 9.4\%$ , S.E.M. = 1.7,  $n = 20$ ) so that it often exceeded the pre-injection threshold. As shown for a typical locus coeruleus neuron in Fig. 1C, after 0.25 mg/kg amphetamine, a current of 1.09 mA sufficed to elicit an antidromic potential to each stimulation of the terminal field. Administration of 5.0 mg/kg amphetamine made this current insufficient; it produced antidromic responses to only 32.5% of the stimuli (Fig. 1D) and the threshold current rose to 1.22 mA (Fig. 1E). This dose of amphetamine elevated the threshold current in 16 of the 20 cells tested.

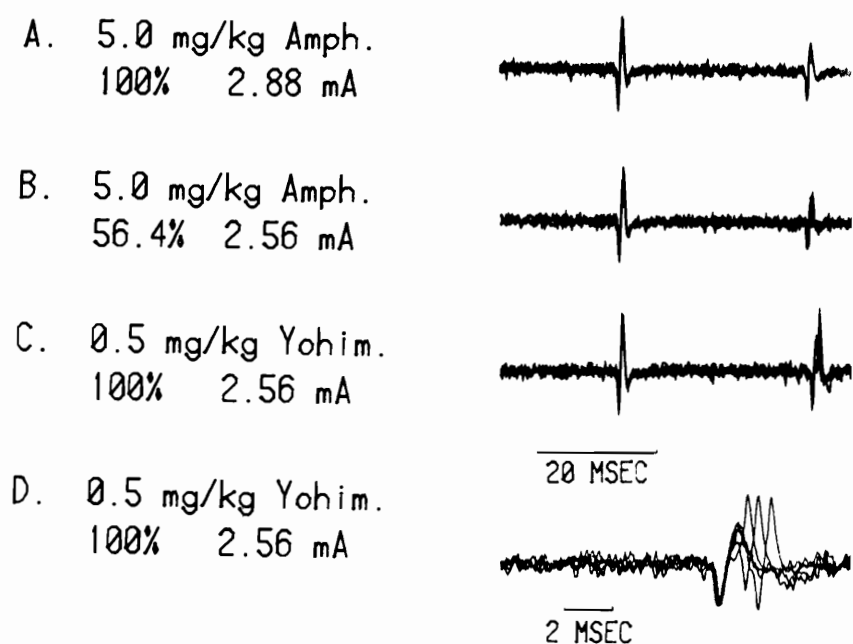


Fig. 2. Antidromic responses of a locus coeruleus neuron to electrical stimulation of the frontal cortex terminal field after 5.0 mg/kg amphetamine, i.v. Each tracing is 5 trials superposed. A: 100% antidromic responding required 2.88 mA after 5.0 mg/kg amphetamine (the animal had also received an earlier injection of 0.25 mg/kg amphetamine). Only the initial segment spike is seen. B: a lower current (2.56 mA) elicited antidromic responses to 56.4% of the stimuli (note misses in the overlaid traces). C: administration of 0.5 mg/kg yohimbine, i.v., made this current sufficient to activate an antidromic response with every stimulus. Furthermore, it increased the latency of the response, and allowed the somatodendritic antidromic spike to occur. D: the antidromic response after 0.5 mg/kg yohimbine is shown on an expanded time scale. All 5 stimuli evoked initial segment spikes. In two cases, a somatodendritic spike follows with a 1-2 ms delay. In one case a full initial segment plus somatodendritic spike was initiated immediately after the initial segment antidromic action potential.

The elevation in threshold produced by 5.0 mg/kg amphetamine was counteracted by the subsequent administration of 0.5 mg/kg yohimbine. In all 7 cases in which yohimbine was administered, the threshold current declined ( $X = -9.8\%$ ; S.E.M. = 1.4;  $t = 14.1$ ;  $df = 6$ ;  $P < 0.01$ ). An example of this effect is shown in Fig. 2 for a different cell than in Fig. 1. Here a current of 2.88 mA produced 100% antidromic responding after 5.0 mg/kg amphetamine (Fig. 2A) and 2.56 mA produced 56.4% responding (Fig. 2B). Administration of 0.5 mg/kg yohimbine lowered the threshold so that this latter current (2.56 mA) evoked an antidromic response to every stimulus (Fig. 2C).

The sequence of changes in terminal excitability are shown in Fig. 3 for one experiment. In this figure, excitability curves, showing the proportion of antidromic responses as a function of stimulating current, are compared for the different stages of the experiment. Prior to drug administration, the percentage of the stimuli that evoked antidromic responses was determined over a range of stimulating currents from approximately 1.9 to 2.5 mA; this excitability curve is

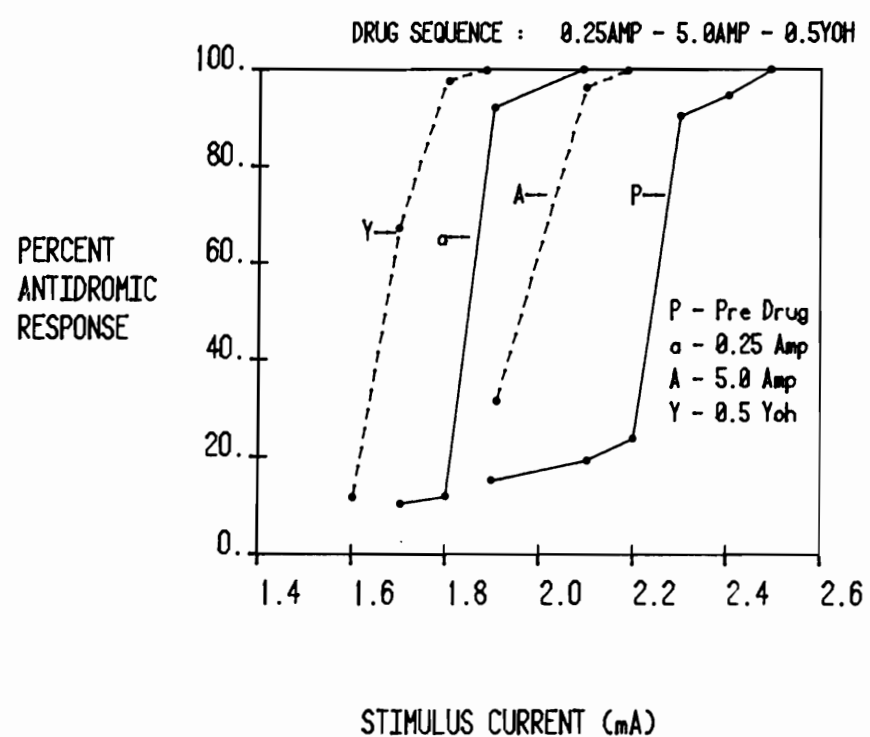


Fig. 3. Effects of i.v. administered amphetamine and amphetamine plus yohimbine on the terminal excitability of a locus coeruleus neuron. An initial injection of 0.25 mg/kg, i.v., amphetamine shifted the excitability curve to the left (compare line P to line a), indicating less current was required to activate antidromic responses. Subsequent administration of a high dose of amphetamine (5.0 mg/kg) partially reversed this effect (compare line a with line A). Subsequent administration of 0.5 mg/kg yohimbine countered the effect of 5.0 mg/kg amphetamine, lowering the current required for antidromic activation (compare line A with line Y).



labeled 'P' in Fig. 3. The administration of 0.25 mg/kg amphetamine shifted the excitability curve, labeled 'a', to the left indicating a reduction in the current required to activate the terminals. 5.0 mg/kg amphetamine partially reversed this effect, shifting the curve, labeled 'A', back to the right. The subsequent administration of 0.5 mg/kg yohimbine clearly reversed this effect, and shifted the curve, labeled 'Y', to the left.

Additional animals received 1.0 mg/kg ( $n = 2$ ), 2.5 mg/kg ( $n = 2$ ), and 5.0 mg/kg ( $n = 7$ ) but in a different sequence of drug injection. Results from these experiments were similar to those described above.

#### *Changes in the latency of the antidromic response*

We previously noted that the latency of the antidromic response declines following low doses of amphetamine<sup>24,25</sup>.

Prior to drug injection, the latency of the antidromic response ranged from 27.5 ms to 58.0 ms, with a mean of 41 ms (S.E.M. = 1). Following administration of 0.25 mg/kg amphetamine, the latency of the antidromic response declined by at least 0.5 ms for 45 cells and was unchanged for the rest. The variability in the latency of the antidromic response also declined.

Subsequent administration of 1.0 mg/kg amphetamine or 2.5 mg/kg amphetamine further decreased the antidromic response latency by at least an additional 0.5 ms in 11 of 16 cells.

Inconsistent changes in latency were observed following administration of 5.0 mg/kg amphetamine. The changes in latency following subsequent injection

of 0.5 mg/kg yohimbine were also unreliable. These effects are summarized in Table I.

#### *Changes in the somatodendritic invasion by the antidromic action potential*

The antidromic action potential waveform seen in locus coeruleus neurons consists of 3 distinguishable components: an initial segment (or A) potential, the somatodendritic (or B) potential, and a third inflection, the C potential<sup>22</sup>. In anesthetized but otherwise undrugged animals, the antidromic action potential usually invades the soma and dendrites of locus coeruleus neurons so that a full (A+B+C) action potential is seen<sup>22</sup>. Since blockade of invasion results from hyperpolarization of the soma<sup>14</sup>, the probability of somatic invasion may be used as an indirect measure of the polarization of the cell.

Prior to drug injection, 97.4% (S.E.M. = 1.2) of all antidromic responses invaded the soma. In those cases where invasion failed, only the small A-spike was seen (Figs. 1 and 2). As shown in Fig. 4, administration of 0.25 mg/kg amphetamine caused a very slight reduction in the probability of antidromic invasion ( $X = 96.1\%$ , S.E.M. = 1.4,  $t = 2.27$ ,  $df = 52$ ,  $P < 0.05$ ). The subsequent injection of 1.0 mg/kg, 2.5 mg/kg, or 5.0 mg/kg amphetamine reduced the probability of antidromic invasion in a dose-dependent manner, to only 32.9% of all antidromic responses after 5.0 mg/kg amphetamine (analysis of variance, interaction: dose  $\times$  condition -  $F_{4,60} = 7.63$ ,  $P < 0.01$ ; Tukey hsd,  $P < 0.05$ ).

Subsequent administration of 0.5 mg/kg yohimbine partially reversed this effect of 5.0 mg/kg am-

TABLE I

*The effects of various doses of i.v. administered amphetamine and amphetamine plus yohimbine on antidromic response latencies of locus coeruleus neurons*

Latency	Dosage				
	0.25 A	0.25 A + 1.0 A	0.25 A + 2.5 A	0.25 A + 5.0 A	0.25 A + 5.0 A + 0.5 Y
Decreased	45* (1.2 + 0.1)	7 (1.1 + 0.2)	4 (1.6 + 0.1)	8 (1.1 + 0.4)	3 (0.5 + 0.0)
Increased	0	0	0	3 (3.2 + 1.9)	2 (0.8 + 0.2)
No change	9	3	2	6	2
n	54	10	6	17	7

\* The number of cells showing increases or decreases in latency were tabulated if a change of at least 0.5 ms occurred relative to the latency in the preceding condition. Numbers in parentheses are the mean change, in ms, + S.E.M. The notation 0.25 A + 1.0 A, for example, indicates that 1.0 mg/kg amphetamine was administered after the animal received 0.25 mg/kg amphetamine, and the change caused by 1.0 mg/kg amphetamine is given relative to the latency of the antidromic response after 0.25 mg/kg.

phetamine ( $t = 2.4$ ,  $df = 6$ ,  $P < 0.05$ ). After yohimbine administration, full somatodendritic spikes comprised 63.9% of all antidromic responses. An example of this effect is shown in Fig. 2. In this cell, after 0.25 mg/kg amphetamine, but prior to administration of 5.0 mg/kg amphetamine, full somatodendritic spikes were seen for all responses (not shown). After 5.0 mg/kg amphetamine, only initial segment (A) spikes were observed (Fig. 2A). The subsequent administration of 0.5 mg/kg yohimbine partially reversed this effect, producing 15.8% full, somatodendritic, spikes (Fig. 2B). A second injection of 0.5 mg/kg yohimbine raised this to 77.7% full spikes. This figure also illustrates that although the somatodendritic spike is re-instated, the invasion from the initial segment is often delayed. Fig. 2D shows two somatodendritic spikes occurring at different delays following the initial segment action potential. A full spike, including both A and B spikes, that occurred immediately after the antidromic A spike is also shown.

#### Changes in firing rate

Prior to drug administration, the locus coeruleus

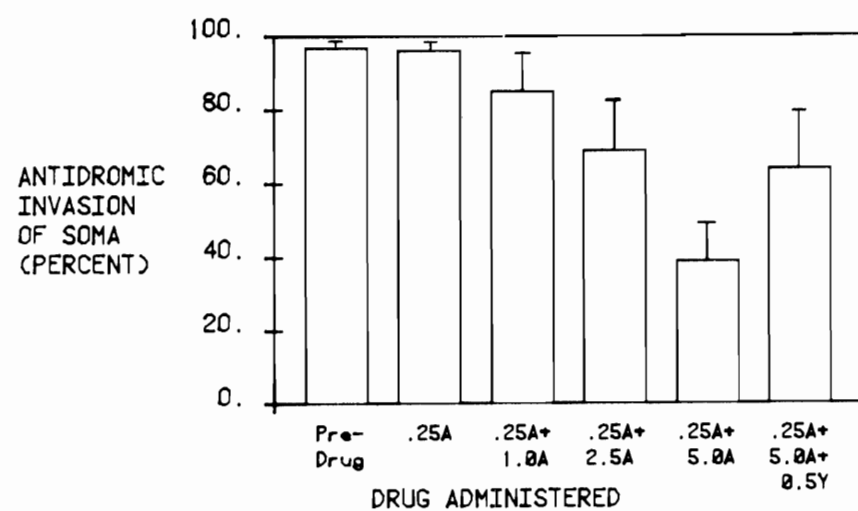


Fig. 4. Amphetamine reduced the likelihood that the antidromic action potential elicited from frontal cortex would invade the somatodendritic region of locus coeruleus neurons from the initial segment. The grand means for pre-drug ( $n = 54$ ) and after 0.25 mg/kg amphetamine ( $n = 54$ ) are shown since the means for these conditions did not differ among the individual groups. Groups of rats received 1.0 mg/kg amphetamine ( $n = 10$ ), 2.5 mg/kg amphetamine ( $n = 6$ ) or 5.0 mg/kg amphetamine ( $n = 17$ ) after receiving 0.25 mg/kg. 0.25 mg/kg amphetamine, i.v., slightly reduced the probability of full, somatodendritic, antidromic spikes occurring. The higher doses of amphetamine caused progressively greater resistance to antidromic somatodendritic invasion. Subsequent administration of 0.5 mg/kg yohimbine partially reversed this effect of 5.0 mg/kg amphetamine. The notation used is explained in the legend to Table I. S.E.M. bars are shown.

neurons fired in a range from 0.05 to 5.78 spikes per second, with a mean rate of 1.69 spikes/s (S.E.M. = 0.16,  $n = 50$ ). This value is slightly lower than those reported by others<sup>8,11,34</sup> probably because it was measured during the stimulation eliciting the antidromic response. Since antidromic activation inhibits locus coeruleus neurons for several hundred milliseconds<sup>1,33,34</sup>, and since we stimulated once per second, our measure underestimates the nominal firing rate.

Administration of 0.25 mg/kg amphetamine reduced the firing rate by 43% to a mean of 0.91 spikes per second ( $t = 7.67$ ,  $df = 47$ ,  $P < 0.01$ ). Subsequent administration of 1.0 mg/kg, 2.5 mg/kg, or 5.0 mg/kg amphetamine significantly depressed firing rate and almost completely silenced the cells (analysis of variance: main effect, condition -  $F_{2,52} = 35.5$ ,  $P < 0.01$ ; Tukey hsd: higher doses differ from 0.25 mg/kg,  $P < 0.05$ ). There was no significant difference in firing rate after 1.0, 2.5 and 5.0 mg/kg amphetamine (analysis of variance: main effect, dose -  $F_{2,26} = 0.42$ , ns). The firing rate after each of these doses is plotted in Fig. 5.

Although 0.5 mg/kg yohimbine reversed the effect of 5.0 mg/kg amphetamine on antidromic threshold current and on the probability of somatodendritic invasion of the antidromic potential, it did not reverse

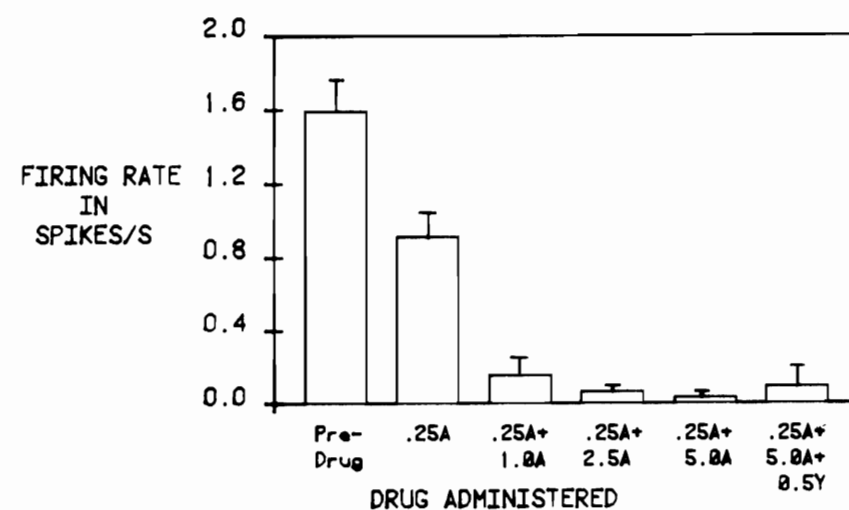


Fig. 5. Amphetamine caused an inhibition of firing of locus coeruleus neurons. The grand means for pre-drug ( $n = 54$ ) and for 0.25 mg/kg amphetamine ( $n = 54$ ) are shown since the means for the individual groups did not differ among themselves. Subgroups received injections of either 1.0 mg/kg amphetamine ( $n = 10$ ), 2.5 mg/kg amphetamine ( $n = 6$ ), or 5.0 mg/kg amphetamine ( $n = 17$ ) after receiving 0.25 mg/kg amphetamine. 0.25 mg/kg amphetamine caused approximately a 40% reduction in firing rate. The 3 higher doses caused an almost complete suppression of cell firing. Of those animals receiving 5.0 mg/kg amphetamine, 7 received a further injection of 0.5 mg/kg yohimbine, i.v. This dose of yohimbine was insufficient to re-instate neuronal firing. S.E.M. bars are shown.

the inhibition of neuronal firing that 5.0 mg/kg amphetamine produced ( $t = 0.9$ ,  $df = 6$ , ns; Fig. 5).

## DISCUSSION

### *Action of amphetamine on locus coeruleus neurons*

Within the nucleus locus coeruleus, amphetamine acts as a noradrenergic agonist and powerfully suppresses locus coeruleus neuronal firing. Our lowest tested dose of amphetamine, 0.25 mg/kg, i.v., caused a 43% decline in firing, a value close to the  $ED_{50}$  for 50% inhibition of 0.24 mg/kg reported by Enberg and Svenson<sup>9</sup>. Subsequent administration of higher doses of amphetamine, 1.0 mg/kg, 2.5 mg/kg and 5.0 mg/kg, produced profound, and in most cells, complete suppression of locus coeruleus neuronal firing.

### *Action of amphetamine on presynaptic receptors*

In contrast to amphetamine's dose-dependent increase in noradrenergic receptor activation within the locus coeruleus, as shown by the inhibition of locus coeruleus neuronal firing and failure of somatodendritic action potential invasion, amphetamine's action on presynaptic receptors located in the frontal cortex terminal field of locus coeruleus neurons is, instead, biphasic. A low dose of amphetamine increases the electrical excitability of this terminal field, decreases latency of the antidromic response and reduces the latency variability of the response. These effects are similar to those produced by noradrenergic antagonists infused directly into the frontal cortex and confirm similar effects previously reported for amphetamine on locus coeruleus neurons<sup>25</sup>. We have interpreted increases in terminal excitability as reflecting a depolarization or change in conductance of the presynaptic membrane<sup>12,13,24,25,32</sup>. Depolarization is believed to increase axonal conduction velocity<sup>31</sup> and so should decrease the latency of the antidromic response. These results suggest that low dose amphetamine may reduce autoreceptor activation, indicating a reduction in the synaptic availability of norepinephrine at frontal cortex noradrenergic terminals.

Neither of the two intermediate doses tested changed terminal excitability when they were administered after 0.25 mg/kg amphetamine. However the highest dose tested, 5.0 mg/kg, i.v., reversed the effect of 0.25 mg/kg amphetamine and decreased ter-

terminal excitability, often beyond the terminal's pre-drug level of excitability. Therefore, at this highest dose, amphetamine is acting like a noradrenergic agonist, probably via  $\alpha_2$  receptors since this decrease in terminal excitability can be reversed by yohimbine.

The biphasic action of amphetamine in the frontal cortex terminal field may reflect the interaction of amphetamine's ability to provoke release and block re-uptake at frontal cortex terminals and the competing effect of amphetamine powerfully reducing impulse traffic. The loss of impulse traffic is important because amphetamine has been shown to facilitate stimulus-evoked release in noradrenergic neurons<sup>17,26</sup> whereas in the absence of impulse traffic, such as in *in vitro* preparations, amphetamine is relatively ineffective at provoking non-stimulus evoked norepinephrine release<sup>3,17,27</sup>. Hence, the powerful inhibition of locus coeruleus neuronal firing produced by low dose amphetamine may counteract its weak norepinephrine releasing action in the terminal field and so result in an overall reduction in the amount of norepinephrine available at pre- and postsynaptic noradrenergic receptors in frontal cortex.

The highest dose of amphetamine tested appears to increase the synaptic availability of norepinephrine in the frontal cortex. Since neuronal activity is completely suppressed at this dose, amphetamine may be provoking non-impulse dependent release. As shown in release studies using synaptosomal preparations, high doses of amphetamine are capable of promoting norepinephrine release<sup>3,17,27</sup>. Two other observations are consistent with this interpretation. First, yohimbine reverses the effect of high dose amphetamine on terminal excitability, indicating that the excitability change is mediated by activation of  $\alpha_2$  receptors. Second, in the locus coeruleus, 1.0 mg/kg amphetamine almost completely inhibits neuronal firing and impulse traffic is essentially absent after doses of 2.5 and 5.0 mg/kg. The higher doses, however, appear to produce greater hyperpolarization of locus coeruleus neurons as indicated by the greater likelihood of failure of somatodendritic invasion by the antidromic action potential with increasing dose. This additional hyperpolarization beyond that necessary to inhibit neuronal firing is mediated via  $\alpha_2$  receptors since the likelihood of somatodendritic invasion can be increased, after being depressed by 5.0

mg/kg amphetamine, by yohimbine. Hence increasing doses of amphetamine appear to promote greater norepinephrine release within the locus coeruleus, despite the absence of impulse traffic along the axon.

In this experiment, antidromic action potentials are being initiated once per second. It is probable that these action potentials are releasing norepinephrine and that amphetamine may be facilitating this release. The results presented here, however, cannot be attributed entirely to this source of release for several reasons. In others' studies in which locus coeruleus cells are not being stimulated, amphetamine powerfully inhibits neuronal firing<sup>9</sup> and this inhibition could be reversed by yohimbine. Therefore, the observed inhibition of neuronal firing caused by low dose amphetamine cannot be attributed to release provoked by our stimulation. Furthermore, in the frontal cortex we observed an apparent reduction in norepinephrine availability, even though our regular stimulation should, to some degree, compensate for the decline in impulse traffic. Nor can the effects observed at higher doses be attributed to this stimulation. Impulse traffic is essentially lost after 1.0 mg/kg amphetamine (the frequency of stimulation being 10 times or more greater than the reduced firing rate), therefore the differences in the effects of 1.0, 2.5 and 5.0 mg/kg cannot be attributed to differences in impulse traffic, whether orthodromic or antidromic.

#### *Comparison of the effects of amphetamine on noradrenergic and dopaminergic neurons*

Substantia nigra dopamine neurons respond differently to amphetamine than do locus coeruleus noradrenergic neurons. At doses ranging from 0.25 to 2.0 mg/kg, i.v., amphetamine decreased the excitability of nigral terminals within the neostriatum<sup>12</sup>, the

same effect that is produced by direct infusion of amphetamine or apomorphine into the terminal fields<sup>32</sup>. Hence, at every dose, amphetamine appeared to increase dopamine autoreceptor activation in contrast to amphetamine's biphasic action on noradrenergic autoreceptors. Several factors may account for this apparent difference between cell types. Dopaminergic substantia nigra neurons are more resistant to amphetamine-induced reduction in impulse traffic, having an ED<sub>50</sub> for 50% inhibition of 0.8–1.6 mg/kg, i.v.<sup>4,5,30</sup>. Although amphetamine appears to inhibit stimulus-evoked release of dopamine but not of norepinephrine<sup>17</sup>, amphetamine provokes substantial non-stimulus evoked dopamine release at much lower concentrations than it provokes norepinephrine release<sup>3,17,20,27</sup>. Hence, the dynamics of amphetamine's actions on dopaminergic and noradrenergic neurotransmission are different.

The results presented here suggest that systemically administered amphetamine has different actions at different sites on the same cell, and that the specific action depends upon dose. At low doses, amphetamine appears to reduce the postsynaptic influence of noradrenergic neurons on their distant targets as well as on their terminal autoreceptors by profoundly inhibiting neuronal firing. In contrast, amphetamine at higher doses can increase norepinephrine release and so increase both the presynaptic and postsynaptic influence of locus coeruleus neurons.

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