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Effects of Clonidine on Habituation and Sensitization of Acoustic Startle in Normal, Decerebrate and Locus Coeruleus Lesioned Rats

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Abstract. Rats were presented with startle-eliciting tones after injection of clonidine (0.01, 0.02, 0.04, 0.08, 0.5, 1.0 or 2.0 mg/kg) or saline. Clonidine potently depressed startle amplitude and the effect was monotonically related to dose. Pretreatment with piperoxane (10 mg/kg) antagonized this effect but pretreatment with phentolamine (10 mg/kg) did not. Clonidine still depressed startle in acutely decerebrate rats and in rats with bilateral ablation of the locus coeruleus. Clonidine did not interfere with sensitization to background noise and did not interfere with the ability to startle but instead improved within-session habituation. The results represent one of the few instances in the literature where a drug appears to improve habituation without directly interfering with the ability to respond. The possibility that clonidine might affect startle by stimulating central epinephrine rather than norepinephrine receptors is discussed.

Key words: Clonidine – Habituation – Startle – Sensitization – Locus coeruleus – Decerebrate – α-Adrenergic antagonists – Piperoxane – Phentolamine.

It has been reported that the α-adrenergic agonist, clonidine, depresses the amplitude of the acoustic startle response when doses of either 0.125 or 0.25 mg/kg are given to reserpinized rats (Fechter, 1974). Partly based on this observation, it was concluded that norepinephrine (NE) is inhibitory to startle.

The mechanism of action of clonidine is still not completely understood. Shortly after administration, clonidine acts like a peripheral α-adrenergic agonist, causing marked sympathomimetic signs which can be blocked by α- but not β-receptor antagonists. At later times, clonidine produces a centrally mediated hypotensive effect (cf. Kobinger, 1973), which accounts for its use clinically. In rats, high doses (above 100 μg/kg) potentiate spinal flexion reflexes, an effect attributed to a direct stimulation of spinal norepinephrine (NE) receptors (Andén et al., 1970). Behaviorally, high doses suppress exploration, conditioned avoidance, rotored performance and pain-elicited aggression (Laverty and Taylor, 1969).

Very low doses of clonidine given systematically (less than 10 μg/kg) or directly by iontophoresis, inhibit the firing rate of the NE containing neurons in the locus coeruleus (Svensson et al., 1975). This effect might explain the sedative actions of clonidine (Kleinlogel et al., 1975; Laverty and Taylor, 1969) as well as its effect of reducing NE turnover (Andén et al., 1970; Braestrup, 1974).

Recently it has been proposed that some of the central actions of clonidine might result from stimulation of central epinephrine (E) rather than NE receptors (Bolme et al., 1974). This hypothesis is based largely on the finding that the drug piperoxane, which in the peripheral nervous system may be an especially selective antagonist of E at α-adrenergic receptor sites (Bacq and Frederiq, 1934; DeVleeschhouver, 1935) has consistently been shown to block the central effects of clonidine (Bolme et al., 1974; Delbarre and Schmitt, 1971; Finch, 1974; Schmitt and Schmitt, 1970; Schmitt et al., 1971, 1973), but is much less potent in blocking the effect of clonidine on spinal flexion reflexes, where NE but not E receptors have been found (Bolme et al., 1974; Hökfelt et al., 1974). The hypothesis is supported by the fact that E terminals innervate the locus coeruleus cell bodies and, like clonidine, direct application of E onto locus cells inhibits their rate of firing (Cedarbaum and Aghajanian, 1976). This effect is antagonized by piperoxane.

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In addition, piperoxane given alone causes a marked increase in locus cell firing rates (Cedarbaum and Aghajanian, 1976), which might account for its ability to cause EEG activation (Fuxe et al., 1974).

Since very little is known about the possible roles of NE and E in modulating acoustic startle, the present study was designed to evaluate the effects of clonidine on startle more fully. In particular, we examined 1. both the time course and dose-response effects of clonidine on startle; 2. how the α-adrenergic antagonists phentolamine and piperoxane might alter the effects of clonidine on startle; 3. whether clonidine would still affect startle in acutely decerebrate rats; 4. whether clonidine would still affect startle in rats with bilateral lesions of the locus coeruleus; and 5. whether clonidine depresses startle by interfering with its motor aspects, or by reducing sensitization to background noise, or by accelerating rate of startle habituation.

EXPERIMENT I— EFFECTS OF VARIOUS DOSES OF CLONIDINE

Methods

Animals. In this and all subsequent experiments the animals were experimentally naive male albino rats of the Sprague-Dawley strain that weighed between 300 and 350 g. Upon receipt from the supplier (Charles River Co.) the rats were housed in group cages of 4 to 5 rats each in a large colony room that was maintained on a 12:12 light-dark schedule. Food and water were continuously available.

Apparatus. Five separate stabilimeter devices were used to record the amplitude of the startle response. Each stabilimeter consisted of an 8 × 15 × 15 cm Plexiglas and wire mesh cage suspended within a 25 × 20 × 20 cm steel frame. Within this frame the cage was sandwiched between four compression springs above, and a 5 × 5 cm rubber cylinder, below, with an accelerometer (M.B. 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 (M.B. Electronics model N 504), the output of which was proportionate to the velocity of accelerometer displacement.

The amplified signal was then fed to a specially designed sample and hold circuit. Basically this circuit consisted of 5 channels, one for each stabilimeter, and was used to sample the peak accelerometer voltage that occurred during a 200-ms 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 exposures was erased. In this way the amplitude of the startle response of 5 rats was recorded simultaneously and stored in one of each of the 5 channels. Immediately after the sample period the output of each of the 5 channels was digitized through a specially designed analog to digital convertor and fed into a 14-channel Newport Prnter. With 2 printing channels per cage startle amplitude could vary from 0 to 99 allowing appreciable resolution among various startle amplitudes.

The 5 stabilimeters were located in a 2.5 × 2.5 × 2 m, dark, ventilated sound attenuated chamber (Industrial Acoustic Co.-IAC). They were placed 1.1 m from an Altec high-frequency loud speaker, which was used to provide a 4000-Hz, 90-ms tone generated by a Hewlett Packard audio 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 ms. Background white noise was provided by a Grason-Stadler white noise generator. The intensity of the tone (120 db) and the white noise (46 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.

Procedure. A total of 70 rats were used. Prior to drug testing each rat was placed in a startle cage and 5 min later presented with 10 tones at a 20-s interstimulus interval (ISI). Based on the mean startle amplitude across the 10 tones the animals were divided into 7 groups of 10 rats each, with each group having similar means and variances.

On the first test day (2—4 days after the pretest), half the rats in each group were injected intraperitoneally (i.p.) with isotonic saline (1 ml) and half with clonidine hydrochloride, using doses of 0.01, 0.02, 0.04, 0.08, 0.5, 1.0 or 2.0 mg/kg, based on the weight of the salt. A different matched group was used for each dose. Immediately after the injections the rats were presented with 120 tones at a 20 s ISI.

Two days later the same procedure was repeated. In this case, however, rats previously injected with saline were now injected with clonidine and those previously injected with clonidine were now injected with saline. Each animal thus served as its own control with respect to saline vs. clonidine, while dosage was varied between animals. The order in which the various conditions were run within a day was varied so that the average time of day in which testing occurred was similar for all conditions.

Results and Discussion

Figure 1 shows an example of the change in startle at various times after an injection of the intermediate 0.08 mg/kg dose. In the saline condition startle showed a gradual increase in amplitude across the session, which is typical when tone presentation is initiated immediately after placing the rats in the chamber. In contrast, clonidine rapidly depressed startle with no indication of an initial excitatory effect. Within about 15 min startle was virtually abolished since the recorded output of the cages was at the lower limit of the measurement system. An overall analysis of variance at this dose showed a significant drug effect ($F = 45.03$, $df = 1/9$, $P < 0.001$) and a significant Drug × Trials interaction ($F = 15.70$, $df = 39/351$, $P < 0.001$).

Figure 2 shows the mean amplitude startle, combined over all 120 tones for each of the 7 doses of clonidine and the corresponding saline conditions. For all effective doses the time course of clonidine's action was qualitatively similar to the time course shown in Figure 1. At the very high doses, startle was depressed significantly within about 1—2 min after injection. At the lowest effective dose (0.02 mg/kg), significant depression did not occur until about 20 min after injection but then remained for the rest of the session.

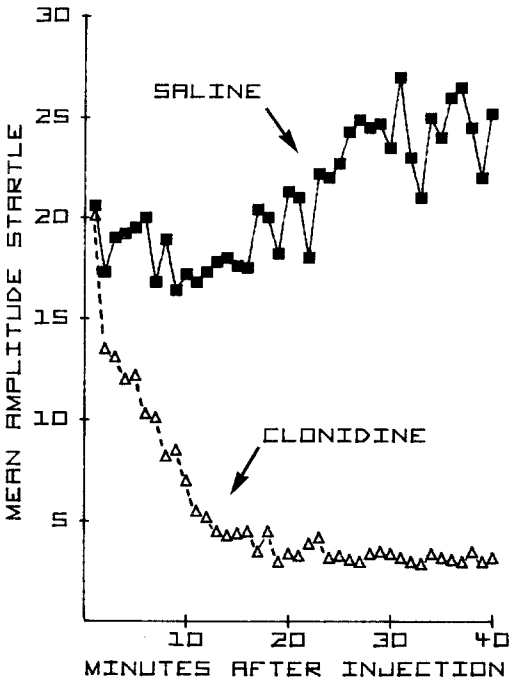

Fig. 1. Mean amplitude startle over successive 1-min intervals after injection of saline or clonidine (0.08 mg/kg)

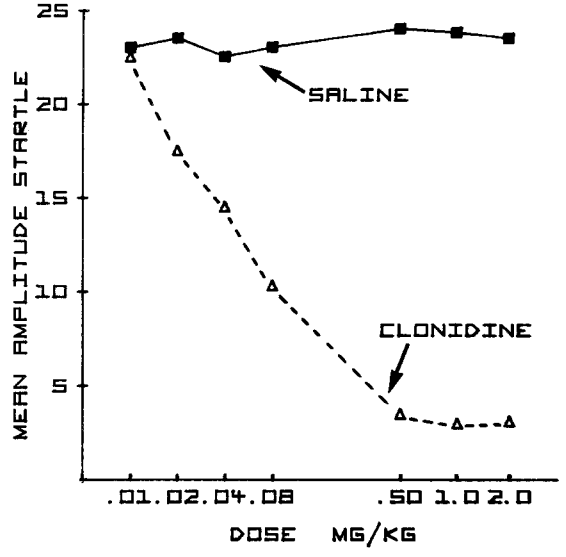

Fig. 2. Mean amplitude startle combined over all 120 tones (i.e. combined over the 40-min test session) after injection of saline or the various doses of clonidine

An overall analysis of variance revealed a significant Drug effect ($F = 198.14$, $df = 1/63$, $P < 0.001$) as well as a significant Drug × Dose interaction ($F = 8.79$, $df = 6/63$, $P < 0.001$) which was linerally related to dose ($F_{Lin} = 23.45$, $df = 1/63$, $P < 0.001$). In addition there was a significant Trials × Drug × Dose interaction ($F = 2.10$, $df = 234/2456$, $P < 0.001$), reflecting a faster onset of startle suppression, the higher the dose of clonidine.

EXPERIMENT II – EFFECTS OF PIPEROXANE AND PHENTOLAMINE ON CLONIDINE INHIBITION OF STARTLE

This study was designed to test whether the effects of a low dose of clonidine on startle are principally mediated centrally or peripherally. The central inhibitory effect of clonidine on the firing rate of neurons in the locus coeruleus (Svensson et al., 1975) is reversed acutely by the α-adrenergic receptor blocker, piperoxane (Cedarbaum and Aghajanian, 1976), but not by another α-adrenergic blocker phentolamine (unpublished observations), possibly because phentolamine fails to cross the blood barrier rapidly except in very high doses. Phentolamine does, however, block the peripheral effects of high doses of clonidine (Andén et al., 1973). Based on these observations, piperoxane but not phentolamine should attenuate the depressive effect of low doses of clonidine on startle if this effect is mediated centrally. Prior to this it was necessary to determine whether piperoxane or phentolamine themselves would have any effects on startle.

Methods

A total of 60 rats were matched into 6 groups of 10 rats, as previously described. One to 3 days later half the rats were injected with saline and half with either 0.31, 0.62, 2.5 or 10 mg/kg piperoxane or 10 or 20 mg/kg phentolamine. Immediately after the injections, rats were presented with 120 tones at a 20 s ISI. Two days later, the same procedures were repeated, except those rats previously injected with saline were now injected with the drug and vice versa.

To test how piperoxane and phentolamine might alter the depressive action of clonidine on startle, 40 rats were matched into 4 groups of 10 rats each, as previously described. Two or 3 days later half the rats were injected with saline and half with 10 mg/kg piperoxane. Fifteen minutes later half of the saline-pretreated rats and half of the piperoxane pretreated rats were injected with saline and half with 0.04 mg/kg clonidine and then immediately presented with 90 tones. Two days later the same procedures were repeated except rats previously given clonidine were now given saline and vice versa. Thus each rat served as its own control with respect to saline vs. clonidine, while pretreatment with piperoxane or saline varied between animals.

All of the above procedures were repeated with 40 more rats except in this case 10 mg/kg phentolamine rather than piperoxane was used. The particular doses of piperoxane and phentolamine were chosen since these doses (a) did not appreciably alter startle by themselves; (b) differentiate the two drugs in terms of their ability to block the central effects of clonidine on the firing of cells in the locus coeruleus; but (c) are known to block the peripheral effects of clonidine over comparable pre-injection test intervals.

Results and Discussion

Lower doses of piperoxane had a slight facilitory effect on startle amplitude. Relative to the saline con-

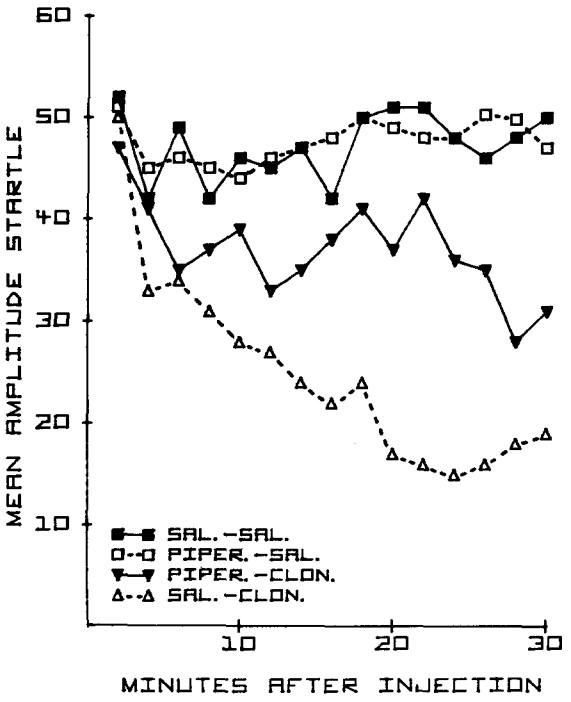

Fig. 3. Mean amplitude startle over successive 2-min intervals after injection of saline (*squares*) or 0.04 mg/kg clonidine (*triangles*) following injections 15 min earlier with either saline or 10 mg/kg piperoxane (see legend)

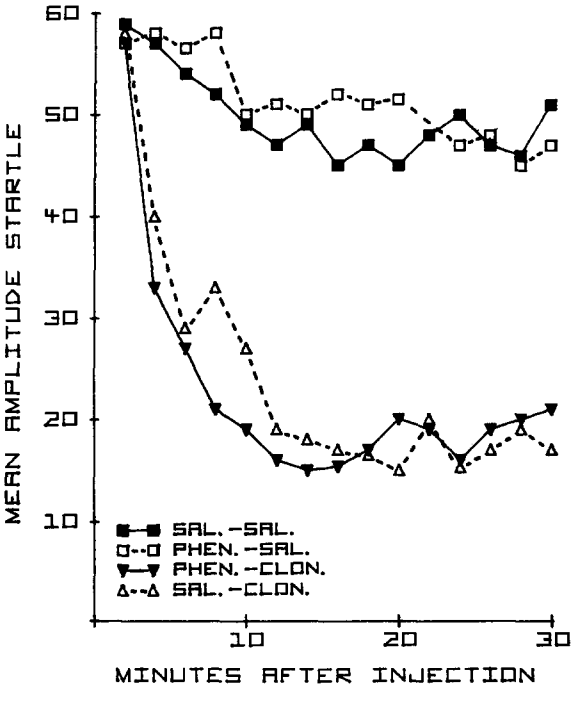

Fig. 4. Mean amplitude startle over successive 2-min intervals after injection of saline (*squares*) or 0.04 mg/kg clonidine (*triangles*) following injections 15 min earlier with either saline or 10 mg/kg phentolamine (see legend)

ditions piperoxane augmented startle 22.3, 29.9, 7.7 and 1.2% at doses of 0.31, 0.62, 2.5 and 10.0 mg/kg, respectively. Statistically the effects were only significant ($P < 0.05$) at the 0.31 and 0.62 mg/kg doses. The facilitory effects occurred almost immediately after injection and lasted for about 30 min.

Phentolamine had a weak biphasic effect in which startle was slightly augmented for the first 25 min and then slightly depressed thereafter. Both the early facilitory and later depressive effects were larger with the 20 compared to the 10 mg/kg dose and statistically significant only at the 20 mg/kg dose ($P < 0.05$).

Figure 3 shows that piperoxane attenuated the depressive effect of clonidine on startle by about 50% which was highly significant ($F = 21.45$, $df = 1/18$, $P < 0.001$). In contrast, Figure 4 shows the same dose of phentolamine did not detectably attenuate the depressive effect of clonidine on startle. Neither drug by itself altered startle significantly. In confirmation of other reports (Andén et al., 1973), preliminary work showed this dose of phentolamine essentially eliminated the hypothermia, piloerection and exophthalmus caused by a large 1 mg/kg dose of clonidine.

EXPERIMENT III – EFFECTS OF CLONIDINE ON HABITUATION AND SENSITIZATION

Clonidine could depress startle directly, by inhibiting the startle circuit itself. Alternatively, clonidine could depress startle indirectly by either inhibiting sensitization to background noise, an effect where startle is normally enhanced by exposing rats to background noise (Davis, 1974), or by facilitating habituation, an effect where startle is normally diminished by repetitive presentation of the startle stimulus (Prosser and Hunter, 1936). Thus far it has not been possible to distinguish among these alternatives since exposure to background noise, exposure to the startle-eliciting tones and the period of drug absorption have been, by design, confounded. To eliminate this confounding the following experiment was conducted.

Methods

Three groups of 20 rats each were used. One group was injected with clonidine (0.08 mg/kg) and then immediately presented with 120 tones similar to the first study. The other two groups were injected with either clonidine (0.08 mg/kg) or saline and placed in the test cages in the presence of the steady background noise. Twenty minutes later these groups were presented with 120 tones. This interval was chosen since the first study showed this dose of clonidine had essentially eliminated startle 20 min after injection (Fig. 1). In addition, this period of exposure to background noise markedly enhances startle to subsequent tones in normal rats and thus provides a sensitive index of noise sensitization (Davis, 1974).

Depending on the pattern of results over the first few tones after the 20-min injection-test interval it should be possible to determine more exactly how clonidine depresses startle. If clonidine has a direct effect, startle should be depressed on the very first tone 20 min after injection. That is, once the drug is absorbed sufficiently, its depressive effect on startle should be independent of prior exposure to repetitive tones. If clonidine inhibits sensitization to background noise, 20 min exposure to background noise should be less effective in facilitating startle after clonidine than after saline. Finally, if clonidine accelerates habituation, startle to the first tone 20 min after clonidine should be similar to that after saline (e.g. normal sensitization to noise), but the clonidine rats would then show a rapid decrease in startle over subsequent tones.

Results and Discussion

Figure 5 shows the startle amplitude of the various groups over successive blocks of 3 tones (i.e. 1-min periods). In the Clonidine-0 Group the results were essentially identical to those shown in Figure 1 in which the same injection-test schedule was used. As before, startle was virtually eliminated within 20 min of the injection and initiation of tones. In the Saline-20 Group initial startle amplitudes were high but then startle habituated appreciably over the rest of the session. Most important, however, the Clonidine-20 Group also had a relatively high level of startle over their first blocks of tones, which then decreased rapidly over the rest of the session. To determine how clonidine affected sensitization to background noise a more detailed analysis of the data over the first several tones in the various groups was carried out. These results are shown in Figure 6 where startle over each of the first 6 tones has been plotted. Also shown are the startle amplitudes over Tones 61–66 in the Clonidine-0 Group, which will be discussed below.

Figure 6 indicates that clonidine did not interfere with sensitization to background noise. Thus startle was facilitated after a 20-min exposure to noise as much or more after clonidine as after saline (Tone I in the Saline-20 and Clonidine-20 Groups). Thereafter, the clonidine rats showed a rapid decrease in startle compared to the saline rats and the difference in rate of response decrement was highly significant ($F = 10.89$, $df = 5/190$, $P < 0.001$).

Figure 6 also shows that the combination of clonidine plus 66 tones produced a greater suppression of startle than the combination of clonidine plus only 6 tones when testing occurred at the same time after injection. Thus startle amplitude over tones 61–66 in the Clonidine-0 Group, which were presented 20 min after injection, was significantly lower than startle over Tones 1–6 in the Clonidine-0 Group, which also were presented 20 min after injection ($F = 19.89$, $df = 1/38$, $P < 0.001$). This was also true when Tones 2–6 were compared to tones 62–66, where

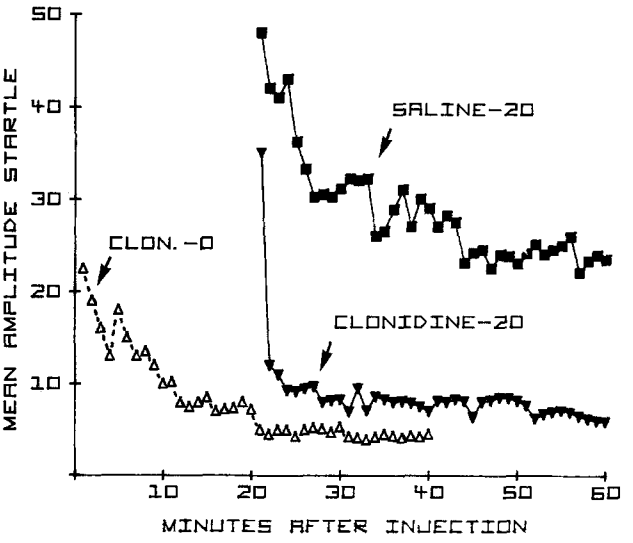

Fig. 5. Mean amplitude startle over successive 1-min intervals after injection of 0.08 mg/kg clonidine (*triangles*) or saline (*squares*) in animals tested either immediately (Clon.-0) or 20 min later (Saline-20, Clonidine-20)

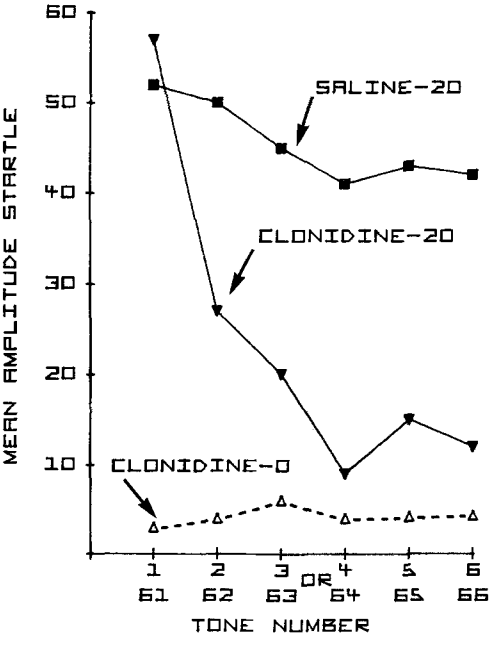

Fig. 6. Mean amplitude startle over the first 6 tones in the Saline-20 and Clonidine-20 Groups or over Tones 61–66 in the Clonidine-0 Group (See test for details)

the ISIs between all tones were equivalent in both groups ($F = 13.10$, $df = 1/38$, $P < 0.001$). The magnitude of the inhibitory effect of clonidine on startle was therefore dependent on previous exposure to the startle-eliciting tones, rather than simply the time since injection.

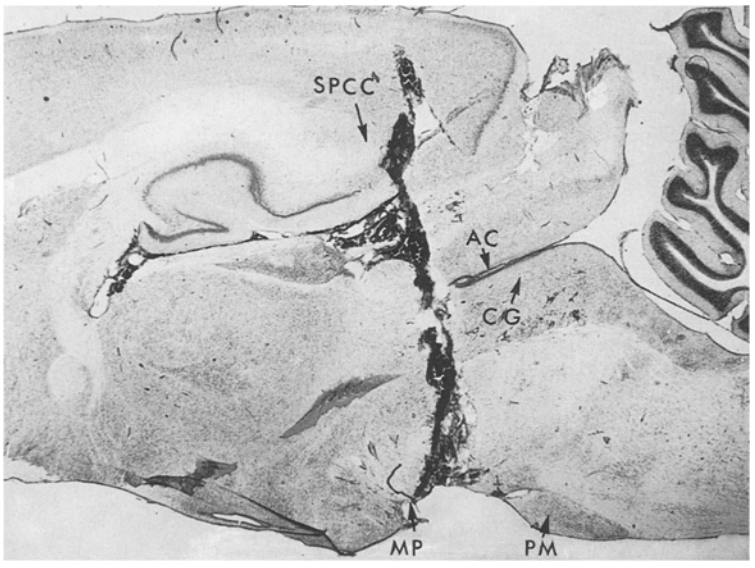

Fig. 7
Photomicrograph of a midsaggital section of the brain of a rat judged to have a complete transection. *SPCC* Spleniun of the corpus callosum; *AC* Aqueduct; *CG* Central grey; *MP* posterior nucleus of the mamillary bodies; *PM* medial nucleus of the pontine nuclei

EXPERIMENT IV – EFFECTS OF CLONIDINE IN THE ACUTELY DECEREBRATE RAT

A significant advantage of using the startle response for the analysis of drugs on behavior is that the acoustic startle can still be elicited in a totally decerebrate rat. By studying the effects of drugs on startle in this preparation, it is therefore possible to determine whether structures in the forebrain are required to mediate the effect of a given drug. For example, if clonidine failed to reduce startle amplitude in the decerebrate rat, forebrain structures would be implicated in mediating its action. On the other hand, if clonidine still suppressed startle in the decerebrate animal it could be concluded that receptors in the midbrain and below are sufficient to mediate its effect.

Methods

The method of transection has been described in detail elsewhere (Davis and Gendelman, 1976). Briefly, rats were anesthetized with halothane and placed in a stereotaxic instrument. A specially designed wire "knife" was inserted through a slot in the top side of the skull, and then moved laterally under the midsagittal sinus so that its tip touched the inside surface of the other side of the skull. It was then lowered through the brain until it touched the base of the skull, and then removed by reversing these steps. The plane of the transection was aimed to pass from the splenium of the corpus callosum down to the mamillary bodies. Previous work has shown that following complete transections in this plane, startle amplitude is relatively stable for about 60 – 100 min after surgery and not significantly different from non-transected rats (Davis and Gendelman, 1976).

Thirty-six rats were transected and 60 min later presented with 30 tones at a 20-s ISI. Testing was terminated if the rat a) died, b) became hyperreflexic and grossly changed its position in the cage, or c) did not startle above a mean amplitude of 20. Thirty rats met the criteria for testing and the experiment was continued by injecting with either saline or 0.04, 0.12, or 1.0 mg/kg clonidine, and then 90 more tones were presented.

Each day, 4 – 8 rats were tested this way. After testing, animals were given a lethal dose of chloral hydrate and perfused with saline followed by 10% formalin and their brains removed. Throughout the experiment, a running total was kept of the mean startle amplitudes over the 30 preinjection tones, and a gross estimate of the completeness of the transections was made by examining the base of the brains upon removal. Based on these two measures, it was decided on each subsequent day which of the four drug conditions to give, so that by the end of the experiment rats that had roughly comparable levels of startle during the pre-injection tones and that were provisionally judged to have complete transections would be distributed uniformly across the 4 drug conditions.

Results and Discussion

Of the 30 rats that met the criteria for testing, 16 were judged to have complete transections, based on examination of serial sagittal sections embedded in celloidin. Figure 7 shows a photomicrograph of a rat judged to have a complete transection.

Figure 8 shows the startle results of the groups before and after injection of saline or the various doses of clonidine. As designed, all 4 groups ended up with 4 rats, each of which had comparable levels of startle over the pre-injection tones and an overall analysis of variance found no significant group effect, no trials effect, and no interaction. Following the injections, however, clonidine significantly depressed startle in a dose-related fashion relative to the saline-injected rats ($F = 10.81$, $df = 3/12$, $P < 0.001$). Moreover, even under these conditions, both the magnitude and the time course of the dose-response effect of clonidine on startle were very similar in the decerebrate rat to that previously found in the intact animal.

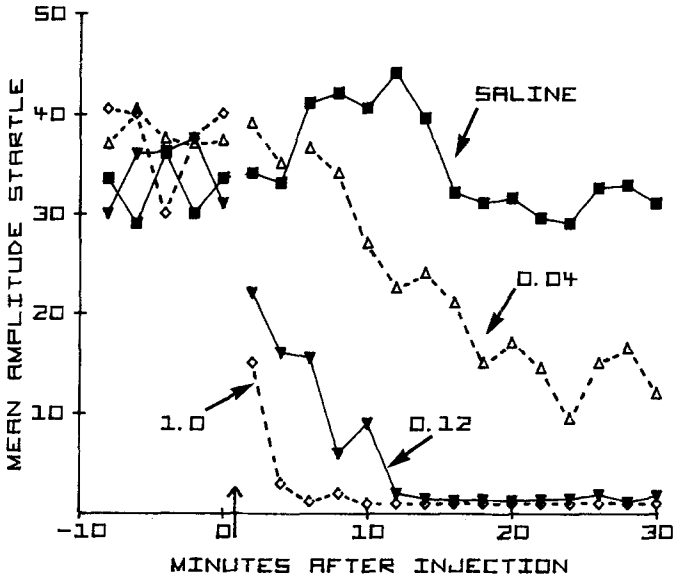

Fig. 8. Mean amplitude startle over successive 2-min intervals prior to and immediately after injection (*arrow*) of saline or either 0.04, 0.12 or 1.0 mg/kg clonidine in rats which had a complete brain transection 60 min earlier

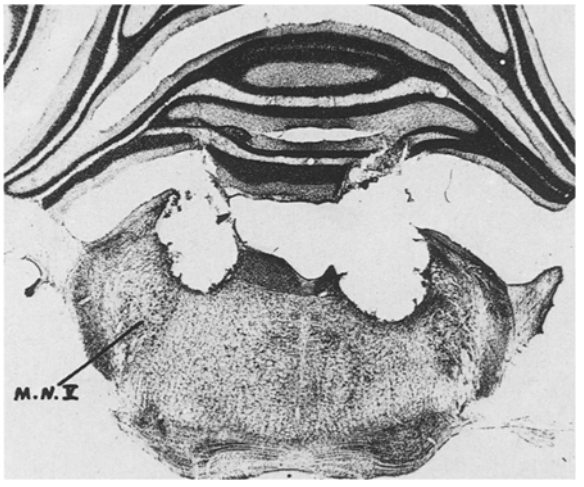

Fig. 9. Section through the mid-pons (Fig. A 8, Zeman and Innes, 1963) illustrating bilateral lesions of the locus coeruleus. Stain: Cresyl violet. *M.N.V.* Motor nucleus of the fifth nerve

EXPERIMENT V – EFFECTS OF CLONIDINE IN RATS WITH LOCUS COERULEUS LESIONS

Since the locus coeruleus is located below the level of the transection made previously and has both ascending and descending projections (Ungerstedt, 1971; Lindvall and Bjorklund, 1973; Swanson and Hartman, 1975), it was of interest to determine whether its ablation would alter the inhibitory effects of clonidine on startle.

Methods

Twenty rats were matched into 2 groups of 10 rats each as described for the first experiment. Two or 3 days later one group of animals was anesthetized with chloral hydrate and placed in a stereotaxic instrument An insulated 0.25 mm diameter stainless steel electrode with a 0.75 mm uninsulated tip was lowered 7.0 mm through a burr hole in the skull placed 1.1 mm lateral and 1.0 mm posterior to lambda, and then a 6 mA 100 kHz current was passed bilaterally for 60 s using a Grass Model LM 4 Radio Frequency Lesion Maker.

Two days later, half the animals in each group were injected with saline and half with clonidine (0.04 mg/kg) and then immediately presented with 120 tones. Twenty-four hours later the same test procedure was repeated except that rats previously injected with saline were now injected with clonidine and vice versa.

Results and Discussion

Six of the lesioned rats were judged to have complete bilateral lesions of the locus coeruleus and only their data were used for analysis. Figure 9 shows an example of the histology of one of these animals. The average pre-test scores of the 6 rats with complete lesions was somewhat lower than the average score of the original 10 animals. To allow a proper comparison, therefore, their data were compared with data from 6 unoperated animals that were selected so that their average pretest scores matched the scores of the 6 completely ablated locus rats. The startle results of the two groups are shown in Figure 10.

Complete bilateral ablation of the locus coeruleus did not appear to alter the depressive effect of clonidine on startle. Thus both the time course and the magnitude of clonidine's effect on startle were similar in the unoperated and locus lesioned rats. This was supported by an analysis of variance which found a significant Drug effect ($F = 30.10$, $df = 1/10$, $P < 0.001$), a significant Trials × Drug interaction ($F = 3.98$, $df = 19/190$, $P < 0.001$) without either a significant Lesion × Drug interaction ($F = 0.71$) nor a significant Lesion × Drug × Trials interaction ($F = 0.48$).

GENERAL DISCUSSION

The present results confirm the original observation of Fechter (1974) that clonidine depresses the acoustic startle reflex. In fact startle was extremely sensitive to clonidine since an i.p. dose as low as 20 μg/kg had a significant effect. The relationship between startle depression and clonidine dosage was a simple monotonic one over the range of doses used (10 μg/kg to 2 mg/kg). None of the doses ever produced an initial

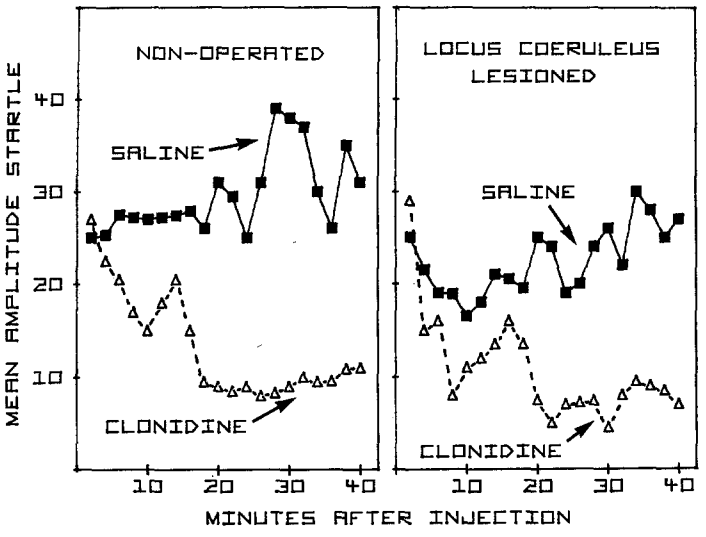

Fig. 10
Mean amplitude startle over successive 2-min intervals after injection of saline or clonidine in non-operated rats (*left panel*) and rats with complete bilateral lesions of the locus coeruleus (*right panel*)

increase in startle during the period of time when clonidine produces an initial increase in blood pressure. This indicates that changes in blood pressure are not correlated with clonidine's effect on startle.

The α-adrenergic antagonist, phentolamine, did not antagonize the depressive effect of clonidine on startle, at a dose which does antagonize the peripheral effects of clonidine in the rat (Andén et al., 1973). In contrast, another α-adrenergic antagonist, piperoxane, did antagonize the depressive effect of clonidine on startle, at a dose which does antagonize the central effect of clonidine (Cedarbaum and Aghajanian, 1976). This indicates that the depressive effects of clonidine on startle are probably mediated centrally.

Clonidine still depressed startle in acutely decerebrate rats. This indicates that structures at or below the level of the midbrain are sufficient to mediate clonidine's effect on startle.

Clonidine also depressed startle in rats with bilateral lesions of the locus coeruleus. This indicates that the NE-containing neurons of the locus coeruleus within the brainstem are not required to mediate the effect of clonidine on startle, so that further studies will be required to determine what these structures might be.

Complete bilateral ablation of the locus coeruleus itself produced about a 25% decrease in overall startle amplitude. Although this difference was not statistically significant, a recent report has also found that lesions of locus coeruleus depress air-puff elicited startle in rats (Geyer et al., 1976). Taken together the results suggest the locus coeruleus-NE system may have an excitatory effect on startle. The magnitude of this effect, however, is difficult to specify. Thus lesions of the locus coeruleus may indirectly alter the function of dopamine systems (Worth et al., 1976) which are now believed to modulate startle (Davis et al., 1975; Davis and Aghajanian, 1976), thereby obscuring the relationship between startle and NE. In addition, the present study was not designed to directly test the effects of locus coeruleus lesions on startle, so that more sensitive measures of habituation or sensitization are needed before definitive conclusions can be made.

A most important result was that clonidine appeared to depress startle by accelerating within-session habituation. This was inferred from the findings that clonidine (a) accelerated the rate of response decrement during repetitive tone presentation; (b) did not attenuate sensitization to background noise and (c) therefore did not impair the ability of rats to startle. Accelerated response decrement could not be attributed to progressive absorption of clonidine since presentation of tones rather than simply previous drug injection was required to produce a response decrement and prior exposure to several tones plus clonidine produced greater response attenuation than prior exposure to only a few tones plus clonidine.

Throughout the entire habitation literature, only a handful of reports suggest that certain drugs accelerate habituation. A particularly interesting finding was that implants of crystalline hydrocortisone acetate directly into the hypothalamus several days before testing accelerated within session startle habituation (Johnson, 1970, reported in Levine, 1971). The data were convincing since initial startle levels on each test day were not affected, but only the rate of startle decrement over subsequent stimuli. Based partly on this observation it was suggested that high circulating levels of adrenocorticotropic hormone (ACTH) retard habituation (Levine, 1971). Unfortunately, subsequent reports have failed to conform this hypothesis since

habituation has not been affected by adrenalectomy (Davis and Zolovick, 1972; Pearson and Vickers, 1974) nor by systemic administration of corticosteroids or ACTH (Pearson and Vickers, 1974).

Chlorpromazine has been reported to facilitate habituation of the EEG arousal response in cats (Key, 1961). However, the observed increase in response decrement probably resulted from a reduction in overall responsivity itself, rather than by accelerated habituation. Thus chlorpromazine has been shown to reduce the orienting reaction to sensory stimuli the first time the stimulus is presented (i.e. before any habituation could have occurred) and not to influence subsequent rates of habituation (File, 1973) or retention of habituation (File, 1974).

Lesions of the dorsal raphe nucleus or very low doses of *p*-chlorophenylalanine appear to accelerate habituation of electrically-elicited leg flexion in the rat (Pearson et al., 1975). The results were particularly interesting since the habituation curves of the lesioned and drug-treated rats actually crossed the control curves. With startle, however, depletion of serotonin via raphe lesions or *p*-chlorophenylalanine or *p*-chloroamphetamine seems to enhance sensitization without altering habituation (Connors et al., 1970; Davis and Sheard, 1974a, b; Geyer et al., 1976).

The only drug that has consistently been implicated in improving habituation is the anti-anxiety agent, chlordiazepoxide. For example chlordiazepoxide has been reported to facilitate habituation of the galvanic skin response in anxious patients (Lader and Wing, 1965), exploratory habituation in the rat (Iwahana and Sakama, 1972) and habituation of threat displays in Siamese fighting fish (Figler, 1973). In the latter experiment a subsequent test of dishabituation showed the drug did not simply interfere with the ability to respond. The possibility that the benzodiazepines, of which chlordiazepoxide is an example, alter transmission in GABAinergic inhibitory systems (Fuxe et al., 1975) makes them interesting candidates to be involved with habituation. Moreover, the observation that some of the central effects of clonidine can be antagonized by GABA antagonists (Gallager, personal communication) suggests that clonidine might accelerate startle habituation by indirectly altering GABA transmission and that the benzodiazepines would be an important class of drugs to test on startle habituation.

As mentioned earlier the mechanism of action of clonidine is still not completely understood. Because of this it cannot simply be concluded that NE is inhibitory to startle given the finding that clonidine depresses startle (Fechter, 1974). Thus low doses of clonidine may actually reduce the release of NE by inhibiting impulse flow in central NE containing neurons (Svensson et al., 1975). The fact that high doses of clonidine potentiate the spinal flexion reflex, an effect which is probably mediated by a direct stimulation of spinal NE receptors, suggests that stimulation of some NE receptors might actually enhance startle, since the flexion reflex is importantly involved in acoustically elicited startle. Similarly, the finding that lesions of the locus coeruleus depress air-puff elicited startle (Geyer et al., 1970) and perhaps acoustic startle also suggests that NE may be excitatory to startle. In fact, preliminary data in our laboratories indicates that increasing the availability of NE at central synapses by administration of NE uptake blockers (e.g. desipramine) or very low doses of *l*-amphetamine, which mainly alter the function of NE neurons (Bunney et al., 1975), does increase acoustic startle. Finally, the fact that clonidine still depresses startle in acutely decerebrate rats and in rats with bilateral lesions of the locus coeruleus indicates that neither forebrain NE receptors nor NE receptors on the NE containing neurons themselves (Svensson et al., 1975) are required to mediate clonidine's effect on startle.

It is possible, therefore, that clonidine might depress startle by stimulating central E receptors (Bolme et al., 1974). Epinephrine terminals have been found in the midbrain (Hökfelt et al., 1974) so that E receptors in the midbrain could be involved in mediating clonidine's effect in the acutely decerebrate rat. The fact that piperoxane, which may be a selective antagonist of E at α-adrenergic sites (Bacq and Frederiq, 1934; DeVleeschhoover, 1935) antagonized the depressive effect of clonidine on startle would be consistent with this hypothesis.

Taken together, therefore, these and other results indirectly suggest that stimulation of NE receptors (e.g. within the spinal cord) may actually be excitatory to startle, whereas stimulation of central E receptors (e.g. within the midbrain) may be inhibitory to startle. In addition, they suggest that E might be importantly involved in habituation of acoustic startle since clonidine appears to alter startle by accelerating within session habituation.

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