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Behavioral Activation of Rats during Intraventricular Infusion of Norepinephrine

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Abstract. Rats were infused with norepinephrine or saline intraventricularly in a free-field situation and while performing a continuous avoidance task (Sidman avoidance). The results indicate that central administration of norepinephrine may lead to behavioral activation in the rat.

Over the past decade, a number of studies have suggested that there is a relationship between central norepinephrine and behavioral states.[1] Most of these studies have involved the indirect manipulation of brain norepinephrine with drug or precursor administration. It has been suggested that metabolic manipulations which result in an increase in functional levels of brain norepinephrine produce behavioral excitation or stimulation while those drugs that decrease the amount of functional norepinephrine result in depression or sedation. However, studies in this area have resulted in conflicting findings. For example, evidence from studies of antidepressant drug effects on both the level of brain norepinephrine and its patterns of metabolism have indicated that these drugs *increase* functional norepinephrine in the brain of the rat,[2] whereas, studies in the chick suggest that antidepressant agents lead to patterns of norepinephrine metabolites consonant with a *decrease* in functional norepinephrine. This latter finding was interpreted as being consistent with the demonstrated behavioral depressing effect of norepinephrine in the chick.[3] Large, single dose, intraventricular administration of norepinephrine alone has been shown in a number of studies in various species, including man, to produce behavioral depression.[4] However, when disulfiram (a dopamine-B-hydroxylase inhibitor) preceded this treatment in the rat, the resulting response rate decrement could be reversed by central administration of norepinephrine.[5]

In the present study, chronic intraventricular infusion of relatively small doses of norepinephrine was accomplished in the rat during the measurement of activity in a free-field situation and performance on a continuous avoidance schedule (Sidman avoidance).[6] This method of administration was utilized in order to achieve a more gradual and relatively more stable elevation of norepinephrine than is possible by a single, large injection. The demonstration, in this study, of an increase in behavioral responsiveness during chronic infusion of norepinephrine (without the possible confounding effects of other drugs simultaneously influencing behavior) represents direct evidence in support of an activating role of norepinephrine in behavior.

Continuous avoidance: For the Sidman avoidance task the subjects were 12

male Sprague-Dawley rats obtained from Simonson Laboratories. The rats were approximately 150 days old at the time of testing.

The apparatus consisted of operant boxes and automated programming and recording equipment. In addition to the automated recording of avoidances and shocks, periodic observations were made of the performing rats. In order to infuse for relatively long periods of time, while at the same time allowing the animals relatively free movement, it was necessary to utilize a harness assembly. This consisted of a counter weight, a water-type Swivel M 120 (Sage Instrument Co.) and a modified Saddle and Spring assembly, model 1602 (Lehigh Valley Electronics, Inc.).[7] The drugs were dissolved in isotonic saline and administered by means of an infusion pump.[8]

Training began when the rats were approximately 60 days old. The schedule was the same as that used by Heise and Boff[9] with the exception that the shock-shock interval was 5 sec and the shock duration and intensity were 3 sec and 0.8 mA, respectively. The rats were placed in this task once every 3 days for 4 hr. At approximately 120 days, each rat was permanently implanted with a cannula unilaterally placed in the lateral ventricles (DeGroot coordinates: A 5.4, ML 2.0 H+3). After approximately 40 days of adaptation to the saddle, the infusion sessions were initiated.[10]

Following this adaptation period, the subjects received continuous infusion of isotonic saline, initiated after a 45-min warm-up period. No significant differences in avoidance responding were found between this saline session and previous noninjection sessions. During the following session, all rats were infused at a rate of 20 μl/hr with either isotonic saline (saline control animals; $n = 6$) or one of two doses of DL-norepinephrine hydrochloride: 0.5 μg/μl and 3.0 μg/μl ($n = 3$ in each group). Subjects were assigned to each of the groups on the basis of their previous performances (matched groups). The influence of norepinephrine on avoidance responding was evaluated in terms of a ratio between each animal's avoidance responses on the second infusion session (saline or norepinephrine) and avoidance responding on the first infusion session (saline).

The effect of both doses of norepinephrine and of saline, at each of the three time intervals is presented in Figure 1. For the dose of 0.5 μg/μl of norepinephrine it was found that the level of responding was significantly higher than saline control for the whole 3-hr session (Mann-Whitney U, $p < 0.05$ at 1 to 3 hr). For the 3.0 μg/μl dose of norepinephrine, only the avoidance responses in the first and second hour were found to be significantly greater than saline control (Mann-Whitney U, $p < 0.05$ at 1 and 2 hr). Although the response levels at the third hour for 3.0 μg/μl were not found to be significantly different from saline control, the mean level of avoidance responding was below that of the saline control. Thus, for the higher doses, there appears to be a decreasing tendency over time to make avoidance responses. Observations made of the animals while they were performing the task indicate that, at least initially, the decline appears to occur concomitantly with an increase in ambulation. An increase of this sort might be expected to interfere with any task in which the appropriate response requires the animal to remain relatively immobile. Thus, the initial decline in avoidance responding might be due to the emergence of competing

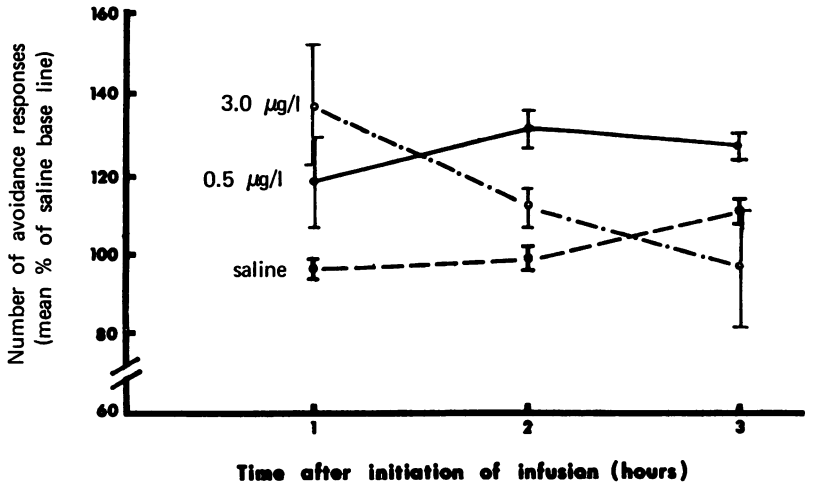

FIG. 1.—Effect of continuous infusion of two doses of DL-norepinephrine and saline on avoidance responding (Sidman). Results are expressed as the mean percentage of change in avoidance responding when compared with saline baseline (each animal was used as his own control). The dose of 0.5 μg/μl resulted in a consistent increase in avoidances throughout the 3-hr test session. At 3.0 μg/μl, there was an initial increase in avoidance responding followed by a decreasing tendency to respond. The brackets indicate the standard error of the mean for each point.

responses. However, at the end of the session, especially during the last half hour, most of the animals injected with the higher dose appeared somnolent and responded only in the presence of shock and immediately following its termination. In addition, when these animals were removed from the experimental chambers, they were found to be flaccid. Thus, after some period of norepinephrine infusion, the behavioral effects appear to be similar to those found after the acute administration of relatively high doses.[4] It may be postulated that an increasing amount of norepinephrine at the synapse eventually results in a depolarization block[11] rather than continued activation of the neural systems involved.

Activity: The subjects were 27 adult male albino rats. Adaptation to the saddle was initiated 2 days after the implantation of the intraventricular cannula.[12] The dose levels and rates of infusion were the same as those described for Sidman avoidance.

The apparatus consisted of a chamber (25 × 15 × 10 in.) with a floor suspended by springs. The floor was connected by a rigid bar to a record player cartridge. The index of general activity was obtained by counting and then estimating the area of the voltage amplitudes generated by the movement of the suspended platform. The measure of locomotor activity (crossovers from one side of the apparatus to the other) was obtained by microswitches placed on the sides of the suspended floor.[7]

Figure 2 shows the effect on general activity of infused norepinephrine (0.5 μg/μl, $N = 5$ and 3.0 μg/μl, $N = 11$) and saline ($N = 11$) in the activity cage for a 2-hr test period. The data are presented as mean differences in activity from

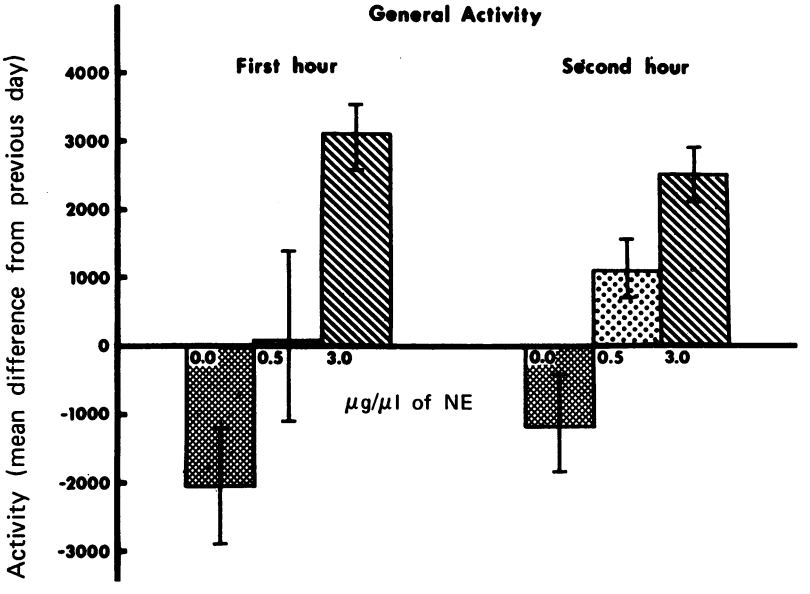

FIG. 2.—Effect of two concentrations of infused norepinephrine and saline on general activity level during a 2-hr test period in an activity cage. The data are presented as the mean difference from the previous session ± SEM. There is a dose-dependent increase in general activity for both the first- and second-hour intervals. Saline controls showed the typical decrease in activity on successive days in the experimental chamber.

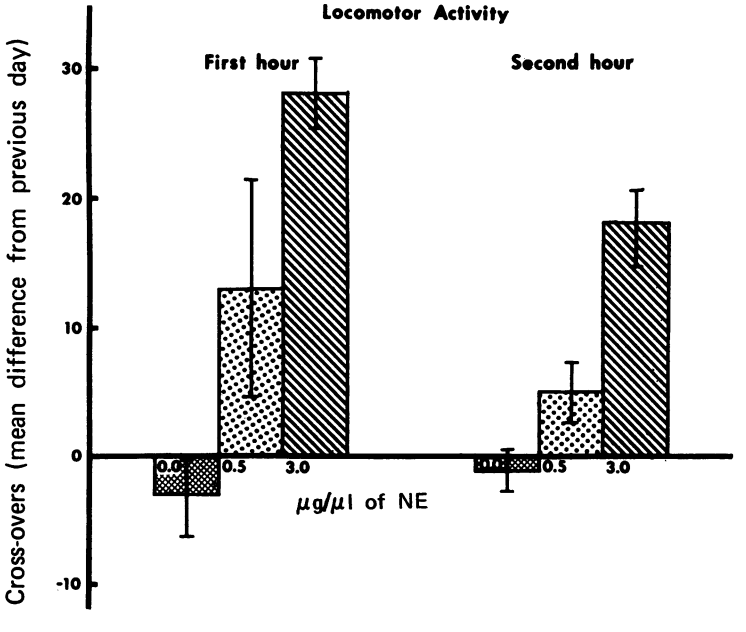

FIG. 3.—Effect of two concentrations of infused norepinephrine and saline on mean difference in crossovers from previous session ± SEM. There is a dose-dependent increase in crossovers for both first- and second-hour intervals.

the previous day in which all animals were infused with saline. For both the first and second hour intervals, there was a significant increase in general activity with increasing levels of infused norepinephrine ($f = 8.22$, $p < 0.01$ and $f = 10.33$, $p < 0.01$ for the first and second hours, respectively). The decrease in activity found for the saline group was consistent with the decreasing tendency shown by all animals on successive days in the experimental chamber.

The effect of infused norepinephrine on locomotor activity is presented in Figure 3. As with the index of general activity, locomotor activity was found to increase as a function of increasing levels of norepinephrine for both the first ($f = 19.12$, $p < 0.01$) and second ($f = 18.38$, $p < 0.01$) hour intervals. These findings (in addition to periodic observations) indicate that the primary effect of the infused norepinephrine was to increase the extent of ambulation. No bizarre or abnormal behavior patterns such as circling or ataxic movements were observed.

The findings from all three measures of behavioral arousal support the notion that centrally administered norepinephrine produces behavioral activation. This is the first direct demonstration of the behavioral activating effects of intraventricularly administered norepinephrine that did not involve the concomitant use of drugs.

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[2] Glowinski, J., and J. Axelrod, *Nature*, **204**, 1318 (1964).

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[5] Wise, D., and L. Stein, *Science*, **163**, 299 (1969).

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[7] A more complete description of this apparatus is in preparation (D. S. Segal, Ph.D. thesis).

[8] Warburton, D. M., and R. W. Russell, *Life Sci.* (1969) in press.

[9] Heise, G. A., and E. Boff, *Psychopharmacologia (Berl.)*, **3**, 264 (1962).

[10] Adaptation consisted of daily 30-min sessions during which the animals were placed in the saddle and allowed to move freely. After approximately 20 days, the rats were left in the saddle while performing the avoidance task. The daily adaptation sessions were discontinued prior to testing. Animals which successfully avoided shock in this task rarely left the vicinity of the lever.

[11] McLennan, H., *Synaptic Transmission* (New York: Saunders, 1963).

[12] Adaptation consisted of daily 30-min sessions during which animals were placed in the saddle and allowed to move freely. After approximately 10 days, the rats were placed in the activity chambers on four consecutive days for 2-hr periods. During the third and fourth days the animals were infused with saline and then saline or one of the two doses of norepinephrine, respectively.