19 of gestation and to decrease thereafter. Day 18 was also the only time from days 17 to 23 after conception when the testosterone concentration in individual pools from normal males was consistently higher than in matched female littermates. On day 17, mean testosterone concentrations in plasma of control males were not yet higher than in control females. The most striking change in males from stressed mothers is that they do not show the surge in testosterone on day 18 of gestation that characterizes unstressed males. Rather, stressed males have their highest testosterone titers on day 17.

On the basis of the above observations, we propose that day 18 of gestation represents a distinct and critical point in the process of sexual differentiation of the fetal rat brain. Specifically, we suggest that adequate masculinization of behavioral potentials requires, and may be initiated by, exposure of the developing CNS to an acute surge of testosterone secreted by the testes at a critical stage of fetal ontogeny, which is day 18 after conception in the rat. Behavioral masculinization is completed by sustained exposure through day 5 postpartum of the now androgen-sensitized CNS to concentrations of testosterone not markedly higher than those of normal females. The abnormal pattern of sexual behavior in male offspring of stressed mothers could result from the lack of testosterone surge on fetal day 18. After day 19, males from stressed mothers have testosterone titers comparable to those of unstresssed animals; however, their CNS may not be sufficiently sensitive to respond to the lower concentrations of testosterone secreted at these later stages. The finding that testosterone titers are significantly higher in male fetuses of stressed mothers than in control males on day 17 of gestation suggests that the testosterone surge is not eliminated in stressed fetuses, but occurs prematurely. Thus, the prenatal stress syndrome, characterized by impaired adult male copulatory behavior and an enhanced female lordotic potential, could result from a desynchronization between CNS maturation and patterns of testosterone secretion by the testes during fetal life.

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7. Details of the radioimmunoassay procedure can be found in (*6*). Briefly, after addition of tritiated testosterone (2600 to 3600 dis/min), 200-μl samples of plasma were extracted with 1.5 ml benzene saturated with deionized water and then with 1.5 ml methylene dichloride or twice with cyclohexane. The pooled extracts, containing two drops of olive oil, were dried under nitrogen and dissolved in 1 ml of isooctane. The extracts were chromatographed on Celite columns. The dried testosterone fractions were reconstituted in 1 to 2 ml of methanol and incubated overnight with [1,2,6,7-^{3}H]testosterone (approximately 4000 count/min) and an antiserum to testosterone (conjugated at the C-3 position). Free and bound steroids were separated from each other by activated charcoal. The samples were centrifuged at 7000 to 8000*g* for 10 minutes. The radioactivity in the supernatant was counted in a solution of toluene, liquiflor, and 2 percent methanol in a Packard scintillation counter. Duplicate measures on 25-, 50-, and 100-μl portions of the plasma samples were made. Representative samples from the different age and treatment groups were introduced into each assay.
8. This work was supported by grants HD-04688 and HD-09542 from the National Institute of Child Health and Human Development; by Research Scientist Development Award, Type II 1-K2-MH00049, from the National Institute of Mental Health (I.L.W.); and by the Rockefeller Foundation. We thank the following individuals who contributed to this study: R. Waniewski, R. Meisel, G. Dohanich, and D. Teti bred and collected blood from the animals. R. Waniewski, C. Hornidge, and, in particular, B. Brown carried out the radioimmunoassay. B. Ward advised us in the statistical analyses and critically read the manuscript. L. Loriaux of the National Institutes of Health provided the antiserum to testosterone.

13 August 1979; revised 10 October 1979

Interchangeability of Stress and Amphetamine in Sensitization

Abstract. *In view of similarities between the behavioral, biochemical, and electrophysiological effects of amphetamine and stress, we tested the hypothesis that presentation of a stressor, mild tail pressure, can sensitize an animal to the later effects of amphetamine, and vice versa. Our findings supported this hypothesis and suggest that amphetamine and at least some stressors may be interchangeable in their ability to induce sensitization. The data raise the possibility that stress might be a common variable contributing to both amphetamine psychosis and some forms of schizophrenia.*

In humans, repeated consumption of large doses of amphetamine or other stimulants often results in the progressive development of a psychotic syndrome notable for its resemblance to paranoid schizophrenia (*1*). Animals similarly treated with constant doses of stimulants also show a progressive enhancement (that is, sensitization) of certain behaviors [for example, stereotypy and locomotion (*2*)]. This apparent similarity between the sensitizing effects in animals and the gradual evolution of paranoid symptoms in humans has led to the suggestion that repeated stimulant administration may provide insight into some of the factors underlying amphetamine-induced psychosis and perhaps schizophrenia itself (*2, 3*). One such factor could be "stress." Acute psychotic episodes can be precipitated by stress in some schizophrenic individuals (*4*), and stress has been shown to reinstate amphetamine psychosis in abstinent individuals during remission (*5*). Moreover, the similarity in the neurochemical effects of stress and stimulants has prompted the suggestion that stimulants may produce their psychotogenic actions by imitating the effects of stress on the organism (*6*). These considerations, coupled with our own observations of a marked similarity between the acute behavioral, pharmacological, biochemical, and electrophysiological responses to a particular stressor, mild tail pressure (TP), and amphetamine administration (*7, 8*), led us to ask whether stress and amphetamine are interchangeable with regard to sensitization. We now report that repeated TP stress is sufficient to produce a virtually identical sensitization of amphetamine-induced sniffing as that seen during long-term amphetamine administration. Conversely, a single injection of amphetamine can result in a persistent sensitization of TP-induced behavior.

Male Sprague-Dawley rats (Zivic-Miller, Pittsburgh) weighing 200 to 300 g were used in these experiments. Mild TP (approximately 80 to 110 pounds per square inch) was applied 2.5 cm from the tip of the tail to 18 animals by means of a

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Table 1. Behavioral responses to TP in rats injected with haloperidol (0.4 mg/kg) at various times after they were injected with amphetamine. Data are expressed as percentages of trials in which TP behavior was observed for ten or more seconds. For each value the number of trials was $N \times 5$.

Time between amphetamine or vehicle injection and TP test with haloperidol (days)	Vehicle		Amphetamine			
			4 mg/kg		8 mg/kg	
	Percent	N	Percent	N	Percent	N
3	15	12	68*	12	70*	12
7	40	12	65†	12	72†	12
15	43	12	76†	12	82†	13
30	9	11	53*	12	43*	12

*$P < .001$. †$P < .02$ (t-test between amphetamine- and vehicle-treated animals after arcsine square root transformations of individual scores).

hand-held 25-cm sponge forceps insulated at the tips with foam rubber. Testing was done in shallow solution bowls 63.75 cm in diameter. Animals received four 1-minute TP trials during a 15-day period. Trials were separated by 8 to 10 minutes. Food pellets were present during TP, and eating, gnawing, or licking (predominantly eating) was observed on all but two of a total of 1008 trials ($N = 18$; 56 trials per animal). For details of the TP procedure see (*7*).

Twenty-four hours after the completion of the TP regimen the animals were injected intraperitoneally with either 3 or 5 mg of *d*-amphetamine sulfate per kilogram ($N = 9$ for each dose) and rated for stereotypy in their home cages according to the following scale: 0, inactive; 1, normal (alert); 2, increased locomotor behavior; 3, occasional stereotyped sniffing; 4, continual sniffing in a wide area; and 5, continual sniffing in a restricted area. Ratings took place every 15 minutes for the first hour after drug administration and every 30 minutes thereafter until 4 hours had elapsed. The rating of each animal at a given time point represents the mean score of two "blind" observers.

In addition to the animals subjected to TP, a second group (of equal weight) was injected for 16 days with *d*-amphetamine sulfate (either 3 or 5 mg/kg; $N = 8$ for each dose). Ratings, as described above, were made on days 1 and 16.

As shown in Fig. 1, A and B, repeated amphetamine treatment at each dose resulted in the previously reported enhancement of stereotyped sniffing (*2*) [3 mg/kg: day 1 versus day 16, $F(1, 14) = 18.93$, $P < .002$; 5 mg/kg: day 1 versus day 16, $F(1, 14) = 39.35$, $P < .002$]. A similar sensitization of sniffing behavior occurred in those animals that had been previously stressed with TP and then given only a single drug injection [unpinched versus repeated TP at 3 mg/kg: $F(1, 15) = 10.80$, $P < .01$; unpinched versus repeated TP at 5 mg/kg: $F(1, 15) = 23.59$, $P < .002$]. Comparison (on day 16) between animals given repeated injections of amphetamine (either dose) or TP plus a single injection of amphetamine indicated no significant differences in stereotyped sniffing behavior. There were no differences in body weight among any of the groups.

Since TP stress appears to sensitize an animal to a later injection of amphetamine we next asked whether amphetamine could conversely produce a sensitization of TP behavior. Because the behavior during TP occurs very rapidly and in virtually 100 percent of animals tested, it is difficult to observe a facilitation in this paradigm without first depressing TP behavior. Therefore, we tested the ability of amphetamine to diminish the effects of haloperidol, a drug known to antagonize TP behavior (*7*).

Twenty-four hours after screening for TP behavior (five 2-minute trials), animals received a single injection of amphetamine (either 4 or 8 mg/kg) or the saline vehicle. At various times after amphetamine treatment (3, 7, 15, and 30 days), animals were again tested for TP behavior (number of 2-minute trials out of five in which TP behavior was maintained for ten or more seconds) 1 hour after an intraperitoneal injection of haloperidol (0.4 mg/kg). For the TP testing the tester had no knowledge of the drug history of any of the animals or even the purpose of the study.

The results indicate that the effects of haloperidol were significantly reduced in all amphetamine-treated animals, regardless of whether this stimulant had been given 3, 7, 15, or 30 days prior to testing (see Table 1). The intensity of the amphetamine effect is also well illustrated by contrasting the frequency with which animals treated with amphetamine and vehicle (V) showed TP behavior on two or more trials [day 3: V = 1/12, amphetamine = 19/24 (doses 4 and 8 mg/kg combined), $\chi^2 = 16.46$, $P < .001$; day 7: V = 4/12, amphetamine = 19/24, $\chi^2 = 7.42$, $P < .01$; day 15: V = 4/12, amphetamine = 24/24, $\chi^2 = 20.22$, $P < .001$; and day 30: V = 1/11, amphetamine = 11/24, $\chi^2 = 4.61$, $P < .05$]. The fact that the behavioral effect obtained did not diminish over at least 15 days makes it unlikely that our results can be explained by unmetabolized or unexcreted amphetamine.

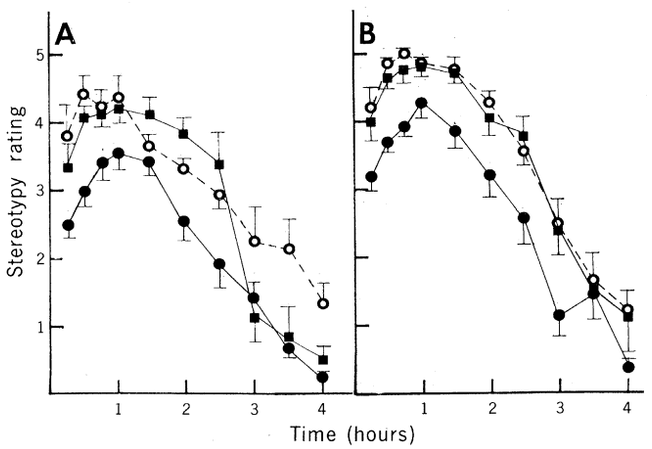

Fig. 1. The effects of repeated TP on amphetamine-induced sniffing on day 16 of the experiment. The rats were injected with 3 mg (A) and 5 mg (B) of amphetamine per kilogram of body weight. Symbols: ●, rats given amphetamine on day 1 ($N = 8$); ○, rats given amphetamine on days 1 through 16 ($N = 8$); and ■, rats subjected to repeated TP on days 1 through 15 and injected with amphetamine on day 16 ($N = 9$).

These data clearly demonstrate that (i) repeated TP stress can induce a sensitization to a later injection of amphetamine and (ii) a single injection of amphetamine can, conversely, induce a long-lasting sensitization of TP behavior, as reflected by a decreased response to haloperiodol. Collectively, these findings support the hypothesis that TP and amphetamine may be interchangeable in their capacity to induce sensitization. The potential importance of these findings depends on the extent to which they can be generalized to other stressors. In this regard, it has been demonstrated that other, more traditional stressors, such as foot shock and food deprivation, can also induce sensitization to amphetamine (*9*, *10*). Since amphetamine and stress can each induce a sensitization to the other, the question arises of whether the progressive influence of repeated stimulant administration may be due, in part, to the ability of these agents to simulate the effects of stress on the organism. Such a possibility would be greatly enhanced if it could be shown that repeated stress can sensitize an organism to a subsequent stress. Data from our laboratory are in accord with this possibility. For instance, we have shown that food deprivation for 72 hours can diminish the ability of haloperidol to antagonize TP behavior 15 days later (*10*) and that self-stimulation [another known stressor (*11*)] can induce ingestive behaviors that show progressive enhancement upon repetition of this stimulus (*12*).

Our findings might be clinically relevant in several respects. (i) They suggest that even a single administration of a relatively small dose of amphetamine can produce enduring behavioral consequences. Thus, even if individuals previously exposed to amphetamine never develop a psychotic syndrome, they may, nonetheless, display abnormal behavioral responses during stressful situations long after that exposure. (ii) If, as our data suggest, both amphetamine and stress can induce sensitization and may be interchangeable in this regard, it might be predicted that individuals with a vulnerability to stress (such as may occur in certain types of schizophrenia) (*4*) would show an enhanced response to amphetamine. Indeed, a number of studies have shown that schizophrenics appear to be especially sensitive to the psychotogenic effects of amphetamine as well as other stimulants (*13*). (iii) The present findings may also help in understanding the enigma of the extreme variability in amphetamine dosage required to induce psychotic reactions in individuals with no record of schizophrenia (*14*). Thus, those with a history of stress may be much more sensitive to the influence of amphetamine. The last two points are not intended to imply that other environmental, physiological, or psychological factors are not involved, but that stress may be one of many variables which predispose an individual to develop drug-related or naturally occurring psychoses (*15*).

Finally, it should be noted that since amphetamine-induced stereotypy is believed to be mediated by dopamine (*16*), TP sensitization of this response could suggest that repeated stress may sensitize brain dopamine mechanisms. Our recent report that TP and other activating stimuli alter the firing rates of midbrain dopamine-containing neurons is consistent with this possibility (*8*).

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15. The stressor used here (that is, TP) is a biological one, and produces both biochemical and neuroendocrine changes characteristic of classical noxious stressors [(*7*); S. M. Antelman *et al.*, unpublished observations]. The importance of physical or biological insults in precipitating schizophrenic episodes is indicated by both recent data implicating viral infection in a considerable proportion of schizophrenic patients [D. A. J. Tyrrell, T. J. Crow, R. P. Parry, E. Johnstone, I. N. Farrier, *Lancet* **1979-I**, 839 (1979); T. J. Crow, E. C. Johnstone, D. G. C. Owens, I. N. Ferrier, J. F. MacMillan, R. P. Parry, D. A. J. Tyrrell, *ibid.*, p. 842] and adoption data on monozygotic twins discordant for schizophrenia indicating that only 5.1 percent of the variance in outcome can be accounted for by psychosocial factors [D. Rosenthal, P. H. Wender, S. S. Kety, F. Schulsinger, J. Wilner, R. O. Rieder, *Arch. Gen. Psychiatry* **32**, 466 (1975)].
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29 May 1979; revised 22 October 1979

Schizophrenia: Elevated Cerebrospinal Fluid Norepinephrine

Abstract. *Concentrations of norepinephrine in cerebrospinal fluid are higher in schizophrenic patients, particularly in those with paranoid features, than in normal volunteer subjects of the same age. This observation supports recent reports of elevated concentrations of norepinephrine in specific brain areas adjacent to the cerebral ventricles of paranoid schizophrenic patients. Overflow of the amine from periventricular regions into the cerebrospinal fluid may reflect abnormally high release or diminished enzymatic destruction of norepinephrine in patients with schizophrenia.*

Indirect pharmacological evidence provides the basis for speculations about the role of catecholamines in schizophrenia (*1*). Neuroleptic agents effective in the management of schizophrenia block dopamine receptors and the limbic noradrenergic system that generates adenosine 3′,5′-monophosphate (cyclic AMP) (*2*). High doses of amphetamines, which enhance release of brain catecholamines, can produce symptoms similar to paranoid schizophrenia (*3*). Furthermore, reserpine, which depletes brain catecholamines, alleviates some of the symptoms

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