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Stress-Induced Facilitation of Acoustic Startle After d-Amphetamine Administration

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KOKKINIDIS, L. AND E. P. MACNEILL. *Stress-induced facilitation of acoustic startle after d-amphetamine administration.* PHARMAC. BIOCHEM. BEHAV. 17(3) 413–417, 1982.—Administration of d-amphetamine enhanced the startle response to an auditory stimulus. In contrast to saline treated mice, startle activity after amphetamine administration did not wane with repeated exposure to the auditory stimulus. Rather, the effects of amphetamine on startle activity increased as a function of stimulus presentation. Whereas exposure to isolation stress or inescapable shock had no effect on startle activity, both types of stress potentiated the effects of amphetamine on startle arousal. The observation that stress sensitized animals to later amphetamine administration is consistent with the effects of stress on other amphetamine behaviors, e.g., stereotypy. Results were related to the development of dopamine post-synaptic receptor supersensitivity after exposure to stress and were discussed in terms of the role played by stress in the expression of behavioral arousal. in the etiology of schizophrenia.

Acoustic startle Isolation stress Inescapable shock d-Amphetamine Norepinephrine Dopamine Dopamine post-synaptic receptors Behavioral arousal

IT is becoming increasingly apparent that amphetamine administration and exposure to stressful stimuli produce similar behavioral and neurochemical profiles. From the behavioral vantage, both stress and amphetamine induce patterns of stereotyped behavior [9,26] and among other things, facilitate rates of self-stimulation responding for electrical brain stimulation [10,13]. There is some evidence to suggest that stress may be a precipitating factor in the development of schizophrenia [12], and it is well documented that long-term exposure to amphetamine may result in a psychotic state that closely resembles paranoid schizophrenia [17, 23, 24]. From the neurochemical vantage, exposure to stress (e.g., inescapable shock, isolation stress) and long-term amphetamine administration deplete levels of norepinephrine, and dopamine in various regions of the brain [1, 4, 15, 22, 27]. Consistent with the observation that under some conditions the effects of stress parallel those of amphetamine, is the finding that amphetamine and stress may act synergistically on behavior. In particular, it appears that exposure to stress will sensitize the organism to later amphetamine administration [8]. For example, isolation stress was found to potentiate the stereotypic and locomotor response to amphetamine [11,21]. Likewise, apomorphine-induced stereotypy was facilitated by isolation stress [11].

The present investigation was designed to assess the effects of two forms of stress on acoustic startle after amphetamine administration. It is well documented that the startle reflex to an auditory stimulus is facilitated by amphetamine administration (for review see [5]). In order to determine whether exposure to stress would modify the behavioral response to amphetamine, as is the case with other amphetamine behaviors, e.g., stereotypy, the effects of isolation stress (Experiment 1) and inescapable shock (Experiment 2) on startle activity after amphetamine administration were evaluated.

METHOD

Subjects

One hundred and twenty Swiss mice procurred from the Animal Resources Centre, University of Saskatchewan, served as subjects. Mice were housed in standard polypropylene cages (3–5 per cage) and allowed free access to food and water. Subjects were 60–70 days of age and weighed 30–35 g at the time of testing. Animals were housed in a 12 hr light/dark cycle and behavioral testing was carried out during the light portion of the cycle.

Apparatus

Startle behavior was recorded in two acoustically insulated (styrofoam, 2.0 cm thick) circular chambers 28.0 cm in diameter and 21.0 cm high. The styrofoam floor of each chamber was positioned on an 8-W speaker (28.0 cm in diameter). Voltages produced by movements on the floor were fed to a Commodore PET Series 2001 Computer. The analogue signal from the speaker was amplified and digitized by an 8 bit A/D converter. The digitized output from the PET was printed out on a Data Terminal Mart printer. Only responses made during the tone presentation were measured and startle scores could vary from 1–5,000 units. The 2700 Hz tone (700 msec in duration, 5-msec rise-fall time) was generated by a Piezo Crystal Audio Transistor (Projects Unlimited, Dayton, Ohio) situated in the centre of the styrofoam roof of each chamber. The intensity of the tone in the chambers was 97 dB and background noise in the chambers was 44 dB.

Inescapable shock was administered in two identical white Plexiglas chambers that measured 28×14×10 cm. The grid floors of the chambers consisted of 0.32 cm stainless steel rods spaced 1.0 cm apart. Footshock (500 μA, scrambled) was administered by a Grason-Stadler Shock Generator, (E6070B, West Concord, MA).

Experiment 1—Isolation Stress

One-half of the sixty mice in Experiment 1 were removed from their home cages and were housed individually in standard mouse polypropolene cages for 10 days. The remaining half were removed from their home environments and were housed five to a cage. On test day (Day 11) both solitary and group housed mice were subdivided such that independent groups of mice (N=10 per group) received an intraperitoneal (IP) injection of saline, 1.0 or 3.0 mg/kg of d-amphetamine sulfate (salt weight). Immediately following the injection mice were placed in the startle chambers and allowed to explore freely for 10 min. Following the adaptation period animals were exposed to 160 presentations of the auditory stimulus with a 10 sec interval between tone presentations.

Experiment 2—Inescapable Shock

Sixty naive mice served as subjects in Experiment 2. One-half of the mice were placed individually in one of the two shock boxes and were exposed to 60 inescapable shocks. Each shock was 10 sec in duration and the interval between shock presentations was 60 sec. The remaining half of the mice (N=30) were placed in the shock boxes for a 70-min shock free period. Twenty-four hrs later mice in the shock and no-shock groups received an IP injection of saline, 1.0 or 3.0 mg/kg of d-amphetamine sulfate. Immediately after the injection mice were placed in the startle chambers and allowed a 10 min adaptation period. Mice were then exposed to 160 tone presentations with an intertrial interval of 10 sec.

RESULTS

Isolation Stress

Startle scores averaged over blocks of 20 trials were transformed ($\sqrt{x}$) in order to reduce the heterogeneity of variance. Analysis of variance of the transformed startle data yielded significant main effects for Housing Condition, $F(1,54)=9.06$, $p<0.01$ and Drug Treatment, $F(2,54)=121.98$, $p<0.01$, as well as significant Housing Condition × Drug Treatment, $F(2,54)=6.68$, $p<0.01$ and Drug Treatment × Trial Block $F(14,378)=9.46$, $p<0.01$, interactions.

Mean startle activity as a function of housing condition, drug treatment and trial block is depicted in Fig. 1. Newman Keuls multiple comparisons ($\alpha=0.05$) revealed that in group housed animals, d-amphetamine enhanced startle activity. Whereas mice tested with 1.0 mg/kg of d-amphetamine showed higher startle scores during trial blocks 7 and 8 as compared to saline treated mice, acoustic startle after 3.0 mg/kg of the drug was facilitated during the entire test session relative to the remaining groups. Moreover, as is evident in Fig. 1 saline treated mice showed a significant response decrement with repeated presentation of the startle stimulus. A similar response decrement was not observed when mice were tested with amphetamine. Instead of habituation to the startle stimulus, mice tested with 3.0 mg/kg of d-amphetamine exhibited increased startle scores after repeated exposure to the stimulus.

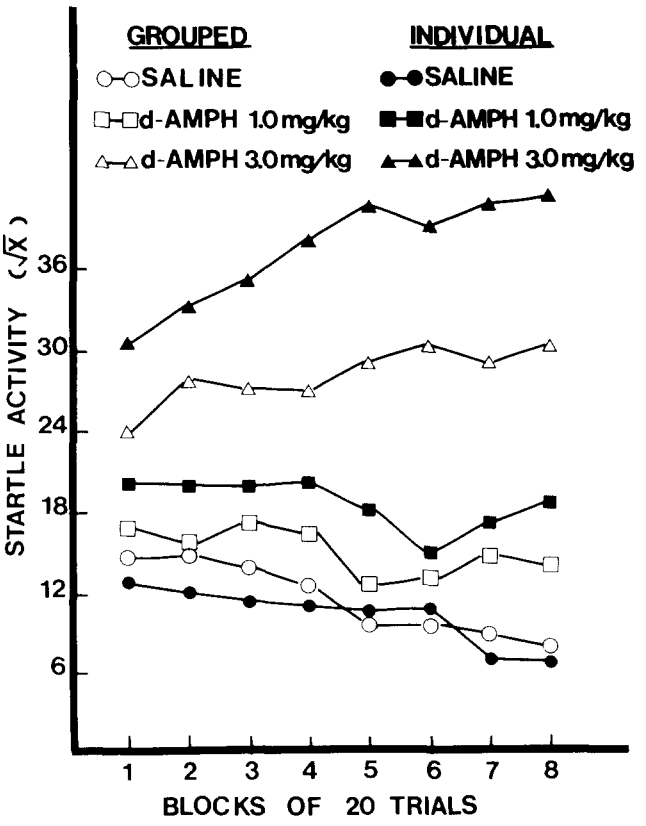

FIG. 1. Mean startle activity over blocks of 20 trials as a function of housing condition (grouped vs individual) and drug treatment (saline, 1.0 or 3.0 mg/kg of d-amphetamine).

As was the case for group housed animals, mice housed individually showed potentiated acoustic startle after amphetamine treatment. Solitary housed mice tested with either 1.0 or 3.0 mg/kg of the drug had higher startle scores relative to control animals during the entire test session. Moreover, whereas startle activity of individually housed mice tested with saline decreased with repeated stimulus presentation, acoustic startle after 3.0 mg/kg of d-amphetamine increased as a function of stimulus presentation (see Fig. 1).

Although amphetamine modified startle activity in both housing conditions, the drug was more potent in its behavioral consequences in mice that were housed individually. Acoustic startle after 1.0 mg/kg was significantly higher in individually housed animals as compared to group housed mice on trial blocks 2, 5 and 8. This was the case despite the fact that housing condition had no effect in mice treated with saline. The sensitizing effect of isolation stress was also apparent when startle activity after 3.0 mg/kg of the drug was considered. Startle activity after this dosage of the drug was significantly higher in individually housed mice as compared to animals housed in groups, during the entire test session.

In order to demonstrate that the effects of amphetamine on acoustic startle were not influenced by other drug-induced behaviors (e.g., locomotor activity), we conducted an experiment in which cage activity was sampled over 700 msec every 10 sec for 160 trials in the absence of an auditory stimulus (see [6]). Two groups of naive mice (N=10 per group) received an IP injection of either saline or 3.0 mg/kg

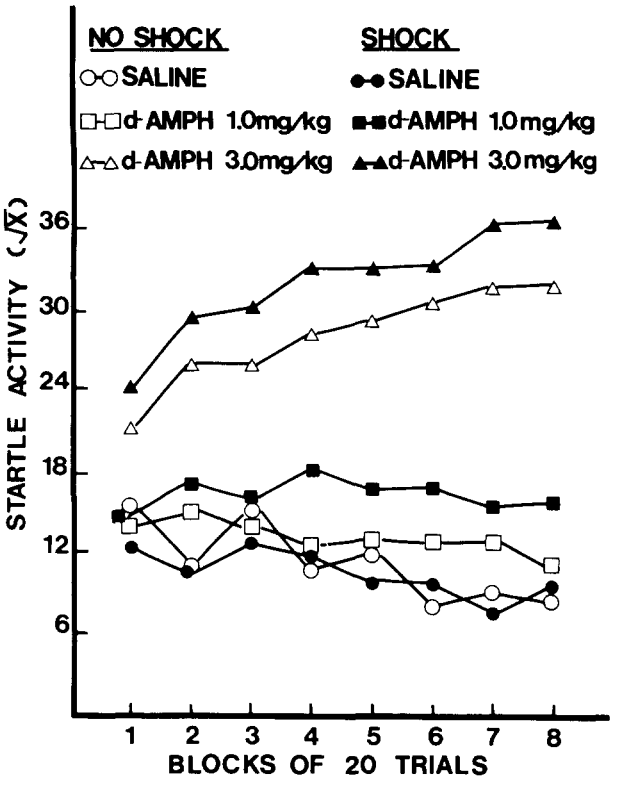

FIG. 2. Mean startle activity over blocks of 20 trials as a function of shock condition (inescapable shock vs no-shock) 24 hrs prior to administration of either saline, 1.0, or 3.0 mg/kg of d-amphetamine.

of d-amphetamine. All other specifications concerning subjects, apparatus and procedure were identical to those described in the methods section.

The results of this experiment demonstrated that the effects of amphetamine on acoustic startle were not biased by changes in drug-induced locomotor activity. The mean transformed cage activity collapsed over 160 trials was 6.1 ± 0.53 for saline treated mice, and 7.9 ± 0.93 for amphetamine treated animals. Although cage activity was slightly higher after amphetamine administration this effect was not statistically significant ($t = 1.5, p > 0.05$). Moreover, it is evident that cage activity after 3.0 mg/kg of amphetamine was considerably lower relative to the effects of the drug on startle activity which ranged from 24–36 units depending on housing condition (see Fig. 1).

Inescapable Shock

Mean startle activity as a function of shock treatment, drug treatment and stimulus presentation is shown in Fig. 2. Analysis of variance of the transformed startle scores ($\sqrt{x}$) yielded significant main effects for Drug Treatment, $F(2,54) = 42.57$, $p < 0.01$ and Trial Blocks $F(7,378) = 2.48$, $p < 0.05$, as well as a significant Shock × Trial Block interaction, $F(7,378) = 2.15$, $p < 0.05$, and a Drug Treatment × Trial Block interaction, $F(14,378) = 12.55$, $p < 0.01$.

Although the three way interaction involving shock treatment, drug treatment and trial block did not reach statistical significance, $F(14,378) = 1.16$, $p > 0.1$, Newman Keuls multiple comparisons ($\alpha = 0.05$) were carried out on the simple main effects involved in the interaction since an a priori prediction concerning the interaction had been made [28]. Relative to saline treated mice, 1.0 mg/kg of d-amphetamine administered to animals in the no-shock condition facilitated startle activity on trial blocks 2, 6 and 7, whereas 3.0 mg/kg of d-amphetamine potentiated acoustic startle during the entire test session. Moreover, saline treated mice in the no-shock condition exhibited decreased startle activity with repeated presentation of the startle stimulus. Conversely, the startle response to amphetamine after administration of 3.0 mg/kg of the drug increased with repeated stimulus presentations (see Fig. 2).

Amphetamine administration also modified acoustic startle when animals were exposed to inescapable shock. In particular, relative to control mice, 1.0 mg/kg of d-amphetamine enhanced startle activity on trial blocks 2–8. Mice tested with 3.0 mg/kg of the drug exhibited higher startle scores during the entire test session relative to the remaining groups.

Like isolation stress, inescapable shock had no observable effects on startle activity when mice were tested with saline. However, pre-exposure to shock sensitized animals to the effects of amphetamine. As depicted in Fig. 2, significantly greater startle activity was observed on trial blocks 4–6, and 8 when mice that had received prior exposure to shock were tested with 1.0 mg/kg of amphetamine relative to that of mice in the no-shock condition after the same dosage of the drug. Furthermore, mice exposed to inescapable shock and tested with 3.0 mg/kg amphetamine showed significantly higher startle scores on trial blocks 3–5, 7 and 8, as compared to no-shock animals tested with 3.0 mg/kg of the drug.

DISCUSSION

Consistent with previous reports, presentation of an auditory stimulus resulted in a startle reflex that was observed to wane with repeated stimulus presentation [5]. Moreover, administration of d-amphetamine potentiated the acoustic startle response [7,16]. Not only was startle activity enhanced by amphetamine administration, but in contrast to saline treated mice acoustic startle after amphetamine administration did not decrease as a function of stimulus presentation. Rather, mice tested with d-amphetamine exhibited increased startle activity with repeated exposure to the tone (see also [7]).

It is apparent from the results of the present study that the behavioral effects of amphetamine are sensitive to stress. That is, the effects of amphetamine on acoustic startle were facilitated when animals had been exposed to stress, despite the fact that exposure to stressful stimuli had little or no discernible effect on startle activity in the absence of the drug. This was the case for both isolation stress and inescapable shock, although from the behavioral vantage isolation stress was the more potent stressor. It appears, then, that there exists a synergistic behavioral interaction between stress and amphetamine that is not unlike that observed between chronic and acute exposure to the drug. In particular, the behavioral and neurochemical effects of stress are similar, in many respects, to those of long-term amphetamine treatment. Like chronic amphetamine treatment, exposure to various stressors (e.g., inescapable shock, isolation stress) reduced brain catecholamine levels [1, 4, 15, 22, 27]. Moreover, it is well documented that long-term amphetamine administration induces behavioral depression in both humans and animals [18]. The post-amphetamine de-

pression, however, is not observed when the organism is under the influence of the drug. Rather, long-term amphetamine treatment sensitizes animals to the locomotor, and stereotypy inducing effects of the drug, as well as to the facilitative effects of amphetamine on rates of self-stimulation responding supported from several brain regions (for review see [18]). Consistent with the behavioral consequences of chronic amphetamine treatment, inescapable shock and isolation stress produce behavioral depression in animals [1,27]. Furthermore, as is observed after long-term amphetamine treatment the locomotor and stereotypic responses to amphetamine were potentiated after exposure to stress [11,21]. We have demonstrated, previously, that the enhancing effects of amphetamine on startle arousal were facilitated by long-term amphetamine treatment [19], and the results of this investigation clearly show that inescapable shock and isolation stress potentiated the effects of amphetamine on acoustic startle.

The stressors employed in the present study have been shown to produce behavioral depression in other tasks [1], however, it is evident in the present study that both inescapable shock and isolation stress had no effect on startle arousal in the absence of drug treatment. It is likely that the inability of stress to depress startle arousal is related to the reflexive nature of the response elicited by the acoustic stimulus. In particular, the effects of inescapable shock on behavior are dependent upon the demands placed on the organism during the test situation. For example, Maier *et al.* [20] found that performance in a shuttle task was not modified by prior exposure to inescapable shock, but there was a marked increase in escape failures produced by inescapable shock when rats were required to make an FR-2 response in the shuttle task. It was argued that the effects of stress on behavior become evident only when the response associations necessary for successful performance are not easily attained. Thus, under conditions in which the required response is reflexive in nature, the detrimental effects of stress on subsequent performance will not become apparent [20].

Although the neurochemical consequences of stress and the neurochemical substrates of the startle response have been elucidated somewhat (for reviews see [1,5]), the underlying mechanisms involved in the synergism between stress and amphetamine are not well understood. One possibility that may account for the interaction between stress and amphetamine involves the effects of stress on dopamine and norepinephrine activity [2]. More, specifically, stress-induced interference with norepinephrine activity may, under certain conditions, facilitate dopamine dependent behaviors. According to this hypothesis, the severe and long-lasting depletion of norepinephrine levels observed after isolation stress and inescapable shock [1,27], should potentiate behaviors that are mediated primarily by dopamine [2]. In support of this position, manipulations that interfered with norepinephrine activity facilitated dopamine dependent behaviors. For example, inhibition of norepinephrine synthesis by either disulfiram or FLA-63 augmented the stereotypic response to amphetamine [2]. This model is an interesting one particularly since startle activity is modulated, in part, by dopamine [6]. In addition to dopamine, however, norepinephrine activity plays a major role in modulating acoustic startle [14], and is involved in the amphetamine-induced facilitation of startle activity, as well [16]. Thus, rather than potentiating acoustic startle, inhibition of norepinephrine synthesis by FLA-63 antagonized the startle response to amphetamine [16].

It seems that norepinephrine serves in an excitatory and not in an inhibitory capacity with respect to acoustic startle. Nevertheless, the fact remains that norepinephrine depletion ordinarily observed after isolation stress and inescapable shock [1,27], facilitated rather than antagonized amphetamine-induced startle activity. A likely explanation of these ostensibly enigmatic findings involves the effects of stress on dopamine post-synaptic receptor activity. In particular, after long-term isolation stress an increase in neuroleptic binding was observed in the striatum of rats, suggesting that isolation stress resulted in a proliferation of dopamine post-synaptic receptors [11]. In competing for the available dopamine, these receptors may become supersensitive resulting in an exaggerated behavioral response to subsequent dopamine release induced by amphetamine, or after direct receptor stimulation by apomorphine [11]. Since dopamine is an important substrate in modulating the effects of amphetamine on startle activity [5], it might well be the case that the sensitizing effect of stress to later amphetamine administration involves an increased neuronal efficacy of dopamine transmission.

The finding that exposure to stress sensitized animals to the facilitative effects of amphetamine on startle arousal may have important clinical implications. In particular, both amphetamine and idiopathic psychosis are characterized by excessive levels of behavioral arousal [12,18]. The fact that acoustic startle is a sensitive measure of arousal mechanisms, coupled with the finding that stress, like long-term amphetamine treatment, facilitated the effects of the drug on startle arousal [19], suggests that these manipulations may act on common substrates. Consistent with this point of view is the observation that stress plays a paramount role in the expression of certain symptoms characteristic of idiopathic psychosis [3]. Indeed, there is evidence to suggest that stressful situations may precipitate psychotic episodes in individuals suffering from schizophrenia [12], and in the same vein, exposure to stress may reinstate psychotic behavior among amphetamine abusers during periods of drug abstinence [25].

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