Chinese Journal of Physiology 45(2): 75-87, 2002

Amphetamine Induced Sensitization in Acoustic Startle: Lack of Blockade by Adrenalectomy and Alpha-helical CRF_{9-41}

Der-Yow Chen and K. C. Liang

Department of Psychology
National Taiwan University
Taipei, Taiwan, 106 ROC

Abstract

The present study utilized the acoustic startle response to evaluate the sensitization effect of repeated administration of amphetamine (AMPH). Intraperitoneal injections of AMPH induced a dose-dependent enhancement of startle: 5.0 mg/kg caused a robust effect, 1.0 or 3.0 mg/kg caused a negligible effect. Sensitization was generated by repeated administration of 5.0 mg/kg AMPH for 7 consecutive days and tested on the 8th and 9th days with challenge of saline and 3 mg/kg AMPH. The results showed that rats receiving chronic injections of AMPH, but not saline, showed significant enhancement of startle to 3.0 mg/kg AMPH, and this effect lasted at least for a month. To explore the role of the hypothalamo-pituitary-adrenal axis in this sensitization effect, rats received adrenalectomy, adrenal demedullation, or sham adrenal operation, and then were subjected to acute or chronic injections of 5.0 mg/kg AMPH. Removal of the whole adrenal gland or only the medulla abolished neither the startle enhancing effect of AMPH injected acutely nor the sensitization effect of AMPH injected chronically. In addition, intracerebroventricular infusion of a CRF antagonist, α-helical CRF_{9-41}, prior to the challenge test failed to alter the sensitization effect of AMPH. These findings suggest that neither adrenal hormones nor CRF was indispensable for induction/expression of AMPH-induced sensitization in acoustic startle.

Key Words: adrenal cortex, adrenal medulla, corticosteroid, epinephrine, CRF, emotion abnormality, the hypothalamo-pituitary-adrenal axis

Introduction

Repeated treatments of certain psychostimulants intermittently would sensitize behavioral responses to subsequent exposure of these drugs. For example, an acute injection of amphetamine (AMPH) increased locomotion or stereotyped behavior in rats, and repetitive administration of the drug further potentiated these effects (22, 42, 65). Pre-exposure to cocaine or AMPH also potentiates their subsequent influences on rotational behavior in animals bearing unilateral 6-hydroxydopamine lesions (17, 57). Evidence has suggested that some sensitized effects involve the dopaminergic pathway that projects from the ventral tegmental area to the nucleus accumbens. This dopaminergic projection is also implicated in the reinforcing effects of addictive drugs, as attested by findings from self-administration or conditioned place preference studies (67). Repeated administration of addictive drugs further enhanced their potencies in these tasks (20, 34, 50). These findings have led to a view that drug-induced sensitization of psychomotor behavior could model for the development of compulsive drug-seeking behavior in addiction (58, 60).

Chronic use of psychostimulants also causes progressive emotional disturbance and contributes to psychiatric disorders including anxiety and affective or psychotic syndromes in drug addicts. Behavioral sensitization could play a role in evolution of pathological emotional changes (52, 53). For example, the post-traumatic stress disorder (PTSD) is conceived as sensitization of fear responses to stress (16, 54, 69)

Corresponding author: to K. C. Liang, Department of Psychology, National Taiwan University, 1 Roosevelt Rd., Sec. 4, Taipei, Taiwan, 106, R.O.C. Email: kcliang@ms.cc.ntu.edu.tw
Received: April 24, 2002; Revised: May 17, 2002; Accepted: May 18, 2002.

based on that a single catastrophic event induces PTSD just as a single injection of psychostimulant induces sensitization (59). Despite this apparent parallelism and the cross-sensitization between stress and AMPH in affecting locomotion (1, 35), the relevance of locomotor sensitization to evolving emotion abnormality is essentially obscured in terms of underlying mechanisms.

To tackle this issue, we develop a sensitization of acoustic startle paradigm in rats. The startle reflex involves rapid twitches of facial and body muscles evoked by sudden appearance of intense stimuli. It serves to protect the organism against imminent danger or harmful stimuli. This paradigm has advantages over locomotor sensitization. First, the pathway subserving this swift reflex has been traced in the nervous system (10), which allows better delineation of how it may be affected by AMPH and pinpointing the synaptic plasticity underlying sensitization. Further, and probably the most important, the acoustic startle response is very sensitive to emotional states of fear or anxiety (9, 30). Enhanced startle to acoustic or other stimuli has been used as a clinical criterion for diagnosing heightened anxiety in PTSD patients. An early study has shown that an acute injection of AMPH enhanced the acoustic startle response (11). Kokkinidis and his colleagues have demonstrated that in mice chronic administration of AMPH induced sensitization of acoustic startle (26-28). However, few data are available for rats. Thus, it is imperative to replicate and extend such findings in rats.

Stress induces emotional disturbance and contributes to relapse in human addicts (29). Animal studies show that brief exposure to intermittent footshock reinstated self-administration behavior (51). In this regard, it is worth noting that prenatal stress facilitated sensitization of motor responses to AMPH (19). Stress involves activation of the hypothalamo-pituitary-adrenal (HPA) axis. Consistently, chronic administration of corticosterone enhances sensitization induced by AMPH in locomotion (14, 48), and activation of glucocorticoid receptors appears to be needed for AMPH-induced behavioral sensitization (12, 55). Further, AMPH causes release of corticotropin-releasing factor (CRF) in the brain (66), and also sensitizes the HPA axis several weeks later (62). Intracerebroventricular (icv) infusion of CRF in rats produced a pronounced dose-dependent enhancement of the acoustic startle reflex, which was reversed by administration of a CRF antagonist, α-helical CRF_{9-41} (40). This effect could be further augmented by stress or chronic injections of corticosterone (33, 49). Thus, CRF, either by working directly in the brain or by releasing corticosteroid peripherally, might also be involved in AMPH-induced sensitization of the acoustic startle.

On the other hand, evidence discordant with a mediating role of corticosterone in behavioral sensitization is also available. A study reported that sensitization of AMPH-induced stereotyped behavior was accompanied with reduced rather than elevated plasma levels of corticosterone (46). Further, Schmidt and his colleagues reported that chronic exposure to AMPH or morphine enhanced locomotor responses to later AMPH challenge, yet in the former case increase of ACTH and corticosterone and in the latter decrease of both hormores were observed (64). These findings suggested that the sensitized response to AMPH challenge could be dissociated from the HPA activity. Conversely, evidence has shown that norepinephrine mediates the excitatory effect of high doses of AMPH on startle (8, 24). Systemic injections of AMPH enhanced retention in avoidance tasks by releasing adrenal epinephrine (43), which influences memory through engaging the central noradrenergic function (36, 38, 39). Thus, the adrenal medulla rather than the adrenal cortex may play a more pivotal role in the AMPH-induced sensitization.

The present study addressed the above issues. Experiment I was designed to replicate the acute enhancing effect of AMPH on startle. Experiment II was designed to study whether this effect could be sensitized after repeated administration of AMPH. Experiment III was designed to evaluate the effects of adrenalectomy and adrenal demedullation in sensitization. Experiment IV investigated the role of CRF in expression of startle sensitization by examining whether icv infusion of α-helical CRF_{9-41} blocked the sensitized responses to an acute AMPH challenge.

Materials and Methods

Subjects

Male Sprague-Dawley rats weighing 350 to 450 grams were used. After receiving from the National Experimental Animal Breeding Center (Nankang, Taiwan, ROC), they were individually housed in the air-conditioned vivarium with free access to food and water. Throughout the study, a 12:12 hr light-dark cycle was maintained with lights on at 12:00 noon. Behavioral tests were always carried out in the light phase. All procedures adhered to the Guidelines for Care and Use of Experimental Animals of the Chinese Psychological Association.

Surgery

Adrenalectomy and adrenal demedullation. Animals were first injected intra-peritoneally with 0.3 mg/kg atropine sulfate (Sigma, St. Louis, MO, U.S.A.) and 10 minutes later with 45 mg/kg sodium

pentobarbital (MTC Pharmaceuticals, Cambridge, Ontario, Canada). An incision was made caudal to the last rib on the lateral body wall of a fully anaesthetized rat. For adrenalectomy (ADX), the adrenal gland and adjacent adipose tissue were totally removed. For adrenal demedullation (ADMX), the adrenal gland was clamped and medulla tissue was removed with iris scissors (3). Afterwards, the remaining cortex was replaced into the abdominal cavity. For sham operation, both adrenals were clamped, but no tissue was removed. The muscle wall was sutured and the skin was clipped. After surgery, the ADX rats were maintained on 1% saline. Animals were allowed to recover for one week prior to behavioral assessments.

Cannula implantation. After fully anaesthetized, the rats was placed onto a stereotaxic instrument (David Kopf Instruments, DKI-900, Tujunga, CA, U.S.A.) and the skull was exposed. A 12-mm 26-guage stainless cannula was implanted into the lateral ventricle (AP 0.0 mm, ML +1.2 mm, DV -3.5 ~ -4.5 mm). Two jewelery screws were implanted to serve as anchors. The whole assembly was affixed onto the skull with dental cement. The stainless cannula was connected to a 10-cm polyethylene tube (Intramedic PE-20, Sparks, MD, USA), which was filled with artificial cerebrospinal fluid (ACSF) and sealed by heat to prevent contamination. A small plastic cylinder was inserted to protect the juncture between cannula and PE tube. After a recovery period of at least one week, rats were subjected to startle assessment.

Drugs and Drug Administration

D-Amphetamine hydrochloride (Sigma, St. Louis, MO, U.S.A.) was dissolved in saline to the appropriate concentration and was administered intraperitoneally. Alpha-helical CRF_{9-41} (Sigma, St. Louis, MO, U.S.A.) was dissolved in ACSF (10 μg/μl) and was infused into the lateral cerebral ventricle.

The icv infusion device was constructed as follows: A piece of 50-cm PE-20 tubing was connected to a 25-μl microsyringe (Hamilton 702, Reno, NV, USA) on one end and it was cemented to a 26-guage needle on the other. The syringe and tubing were first filled with distilled water. About 10 μl of ACSF was introduced into the cannula from the injection needle, followed by α-helical CRF_{9-41} or ACSF. Tiny air bubbles were placed between different solutions to prevent intermixing. The injection needle was inserted into the PE tube of icv cannula and infusion of drug was administered to a conscious rat held gently. Care was taken to minimize stress for the animal. The infusion was administered at a rate of 2.5 μl/min through a syringe pump (Carnegie Medicin, CMA/100, Stockholm, Sweden). Five microliter of ACSF or α-helical CRF_{9-41} was infused into the lateral ventricle and then followed by 8 μl ACSF to flush the dead volume in the PE tubing of icv cannula. The catheter was sealed again by heat immediately after the end of infusion.

Behavioral Apparatus

The startle response was measured in a startle apparatus (San Diego Instrument, San Diego, U.S.A.) as described in a previous study (5). Briefly, the animal was constrained in a Plexiglas cylindrical tube (length 20 cm, diameter 10 cm) with an accelerometer sensor attached on the base. The whole set-up was enclosed in a ventilated, sound-attenuating cabinet (length 38 cm, width 38 cm, and height 55 cm). The acoustic startle stimuli were high-intensity white noise bursts and were delivered by a speaker 30 cm above the animal. After the stimulus onset, the sensor sampled startle-induced vibration for 200 ms. The vibration was transduced into voltage signals and then digitized online by an IBM-PC compatible computer. The startle amplitude of each trial was defined as the maximum vibration within the 200 ms period. All data were reserved for off-line analyses.

Measurement of the Acoustic Startle Responses

Matching. At the beginning of each experiment, rats were first matched for their startle amplitude. Briefly, animals were placed into the startle apparatus with a continuous 55 dB background noise. Five minutes later, 30 noise bursts (40 ms in duration) with a 30-s inter-trial interval (ITI) were presented. The intensities were 95, 105, and 115 dB with 10 bursts at each intensity. Noise bursts were presented in a balanced and quasi-random order. The mean startle amplitude across the 30 noise-burst trials was calculated for each animal and was used to assign rats into various groups such that different groups would have comparable mean startle amplitude.

Startle sessions. Rats were placed into the apparatus with a background noise of 45 dB. Following a 5-min acclimation period, the startle test session began. It was separated into two segments: The first one contained 60 trials, and the second one contained 120 trials. The ITI was 30 s. Drug administration was interposed between the two segments of a session.

Induction and Expression of Sensitization by Amphetamine

To induce AMPH sensitization, rats were subjected to repeated AMPH injections for 7 consecutive days. In each daily startle session of this induction phase, different group of rats received vehicle or 5.0 mg/kg of AMPH immediately after the 60th startle

trial. The expression of AMPH sensitization was evaluated one or two days after the last chronic treatment. During this expression phase, all rats were injected with both vehicle and AMPH (3.0 mg/kg) in alternating days. Half of the group was challenged with vehicle first and then with AMPH, while the other half received the two injections in a reverse order.

Data Analysis

Sensitization was evaluated by the startle responses to 3.0 mg/kg AMPH in comparison with those to saline in the challenge phase. The last 20 startle responses before the injection were averaged to serve as the pre-injection baseline. The effect of AMPH for an individual subject was represented by a change score, which equals to the post-injection startle amplitude minus the pre-injection baseline as depicted in Figure 1. Mean change scores were calculated for blocks of 10 trials. These data was analyzed by mixed-design analyses of variance (ANOVA). Post-hoc tests for individual comparisons were conducted according to the adjusted procedures for accurate comparison in the mixed-design ANOVA (25) and a significant level of .05 was adopted.

Results

Experiment I: The Effect of Acute Amphetamine on Acoustic Startle Responses

To evaluate the acute effect of AMPH on acoustic startle, four groups of naive rats (n = 8 in each group) received injections of saline, 1.0 mg/kg, 3.0 mg/kg, or 5.0 mg/kg AMPH. Figure 2 shows that the control group had lower startle than the pre-injection baseline after saline injection. An injection of 5.0 mg/kg AMPH enhanced acoustic startle well above the baseline level, and the enhancement grew along with time. Lower doses (1.0 or 3.0 mg/kg) produced less prominent effects. The data were analyzed by a two-way mixed-design ANOVA with Drug as the between-subject variable and Block as the within-subject variable. The analysis revealed that the main effects for Drug and Block were statistically significant ($F(3,28) = 2.95$, $p < .05$; $F(11,308) = 4.08$, $p < .0001$, respectively), so was the Drug×Block interaction ($F(33,308) = 1.55$; $p < .05$). Post-hoc tests indicated that only 5.0 mg/kg AMPH significantly enhanced startle ($p < .05$), while the effect of 1.0 or 3.0 mg/kg failed to reach statistical significance.

Experiment II: Amphetamine-induced Sensitization of Acoustic Startle

To examine whether repeated administration of AMPH induced sensitization of acoustic startle, two groups of rats received chronic injections of saline (n = 8) or 5.0 mg/kg AMPH (n = 7) during the 7-day induction phase. They were then challenged in the expression phase. Figure 3 shows that 5.0 mg/kg AMPH enhanced acoustic startle in each daily session of the induction phase, and this enhancing effect did not diminish with repeated administration. The data were analyzed by a two-way mixed-design ANOVA with Drug as the between-subject variable and Day as the within-subject variable. The Drug main effect was statistically significant ($F(1,13) = 28.99$, $p < .001$), but the Day effect and Drug×Day interaction effect were not ($F(6,78) = 0.87$, $F(6,78) = 1.39$, respectively). Post-hoc tests indicated that the Drug effect was significant in each day ($p < .001$ for Day 1 to Day 4, $p < .05$ for Day 5 to Day 7). Trend analyses showed neither linear nor quadratic tendency in the Drug effect over the 7-day period (all ps > .20).

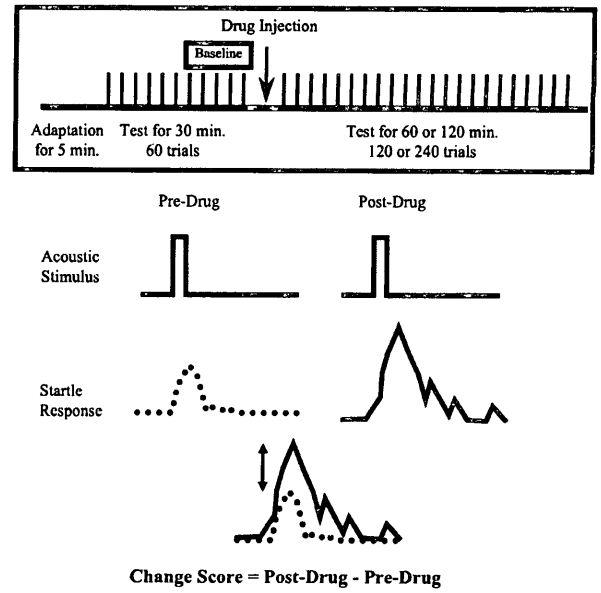

Fig. 1. A diagram illustrating the startle assessment procedure and data processing.

Expression of sensitization is shown in Figure 4: In comparison with saline challenge, 3.0 mg/kg of AMPH significantly enhanced startle in rats receiving repeated administration of AMPH, but not in rats repeatedly exposed to saline in previous days. The effects were analyzed by a 3-way mixed-design ANOVA with Chronic Injection as the between-subject variable and Challenge as well as Block as two within-subject variables. The main effects of Chronic Injection and Challenge were both significant ($F(1,13) = 6.82$, $p < .05$; $F(1,13) = 9.64$, $p < .01$), but their interaction was not ($F(1,13) = 1.56$, $p > .05$). Post-hoc tests showed that only rats received 5.0 mg/kg AMPH injections during the induction phase

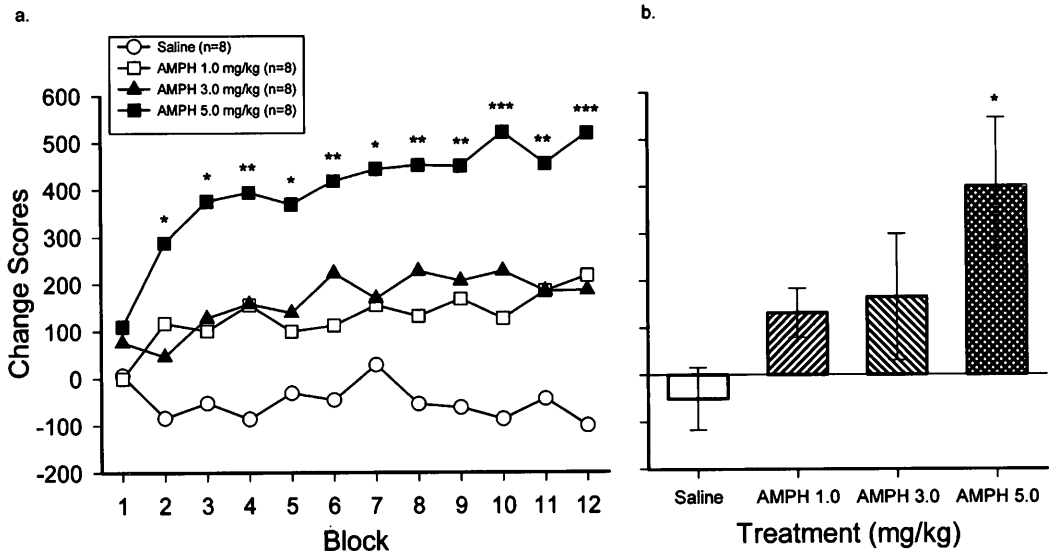

Fig. 2. The effect of acute amphetamine (AMPH) on acoustic startle responses. a. Time course of the startle-enhancing effect induced by an acute injection of AMPH. Mean change scores were calculated for blocks of 10 trials in 5 min. b. Averaged enhancement on acoustic startle over the whole period of one hour after injection. Mean change scores were collapsed for all trials. (*p < .05, **p < .01, ***p < .001, different from the scores of the saline group)

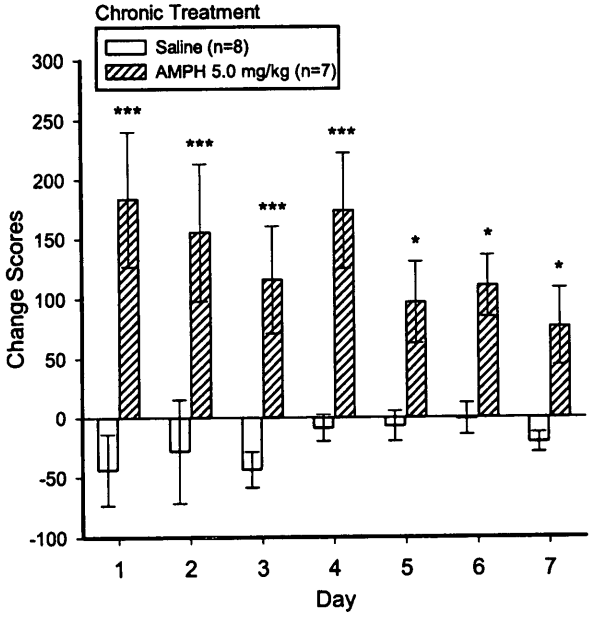

Fig. 3. AMPH (5.0 mg/kg) significantly enhanced startle in the induction phase. (*p <. 05, **p < .01, ***p < .001, different from the scores of the saline group)

displayed sensitized startle to a challenge of 3.0 mg/kg AMPH ($p < .05$), but rats received saline during the induction phase did not. The startle responses significantly varied across time as indicated by a significant Block main effect ($F(11,143) = 4.52$, $p < .001$). The Block×Challenge and three-way interactions were also significant ($F(11,143) = 5.24$, $p < .001$; $F(11,143) = 2.46$; $p < .01$, respectively). For rats with AMPH pre-exposure, post hoc tests showed that 3.0 mg/kg AMPH significantly increased startle responses in comparison to saline challenge at Blocks 3 and 5-12 (all ps < .01). In contrast, AMPH challenge did not significantly enhance startle re-sponses across all blocks in rats with saline pre-exposure.

To examine whether AMPH induced sensitization of acoustic startle could last for a long time, an additional group of rats received injections of 5.0 mg/kg AMPH (n = 8) during the 7-day induction phase and were challenged one month after the last chronic treatment. Results in Figure 5 shows that 3.0 mg/kg AMPH significantly enhanced acoustic startle one month after drug withdrawal, indicating a significant long-term sensitization. The data were analyzed by a two-way mixed design ANOVA with Drug as the between-subject variable and Block as the within-subject variable. The Drug effect was statistically significant ($F(1,7) = 12.32$, $p < .01$), but the Block main effect and Block×Day interaction effect were not ($F(11,77) = 0.81$, $F(11,77) = 1.13$, respectively). Post-hoc tests indicated that the drug effect was significant in all blocks except for the first one (all ps < .05).

Experiment III: Adrenalectomy and Adrenal Demedullation Failed to Attenuate the Acute and Sensitization Effects of Amphetamine on Startle.

To evaluate the effect of adrenalectomy and

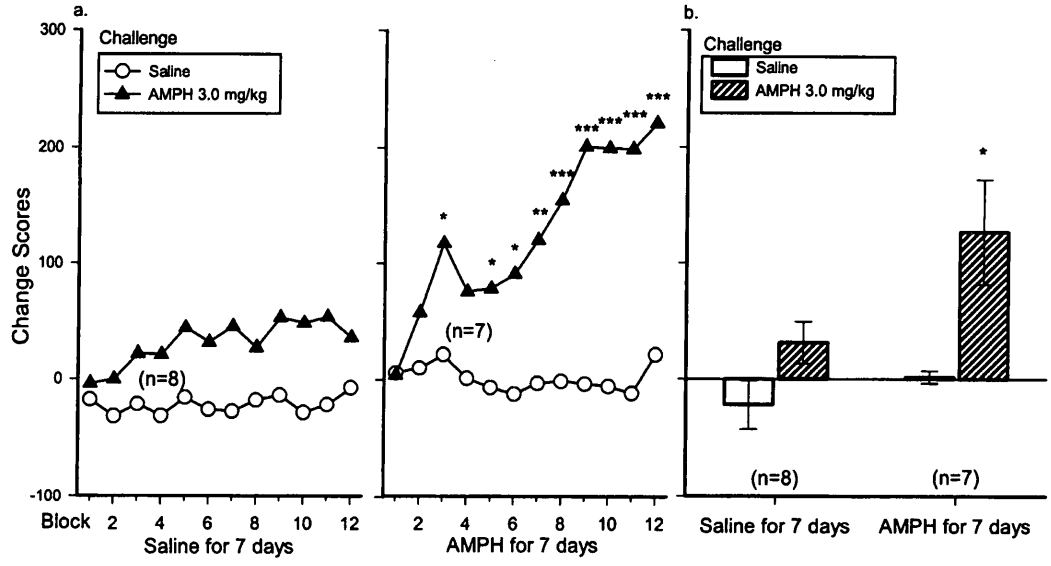

Fig. 4. Rats receiving AMPH during acoustic startle sessions for consecutive 7 days developed sensitization. a. Time course of the responses to a challenge dose of AMPH or saline. b. Averaged effect of the challenge treatment on acoustic startle over the 1-hr period after injection. (*p < .05, ** p < .01, ***p < .001, different from the scores under saline challenge in the correspondent group)

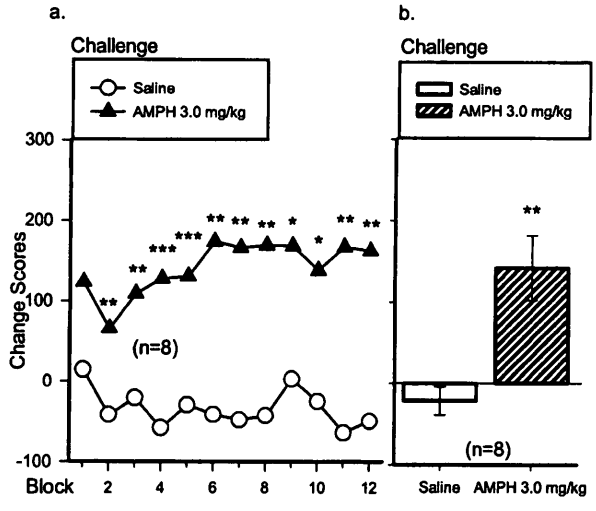

Fig. 5. One month after AMPH withdrawal, rats expressed significant sensitization in the challenge phase. a. Time course of the responses to a challenge dose of AMPH or saline. b. Averaged effect of the challenge treatment on acoustic startle over the 1-hr period after injection. (*p < .05, **p < .01, ***p < .001, different from the scores under saline challenge in the correspondent group)

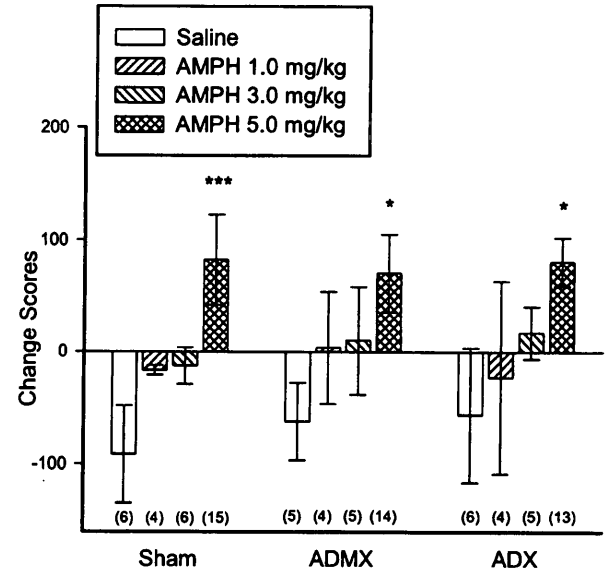

Fig. 6. Effects of adrenalectomy and adrenal demedullation on startle-enhancement induced by an acute injection of AMPH. (*p < .05, **p < .01, ***p < .001, different from the scores of the correspondent saline group)

adrenal demedullation on augmentation of startle induced by an acute injection of AMPH, three groups of rats were subjected to adrenalectomy (ADX, n = 28), adrenal demedullation (ADMX, n = 28), or sham operation (n = 31). After recovery, rats in each group were subdivided into four groups receiving saline or AMPH at a dose of 1.0, 3.0, 5.0 mg/kg during the startle test session (n = 6, 4, 6, 15 for sham operation; n = 5, 4, 5, 14 for ADMX; n = 6, 4, 5, 13 for ADX).

Results shown in Figure 6 indicate that acute administration of 5.0 mg/kg AMPH enhanced startle, and this enhancing effect was not attenuated by ADX or ADMX. The data was analyzed by a three-way mixed-design ANOVA with Drug and Surgery as two between-subject variables and Block as the within-subject variable. The analysis revealed a significant main effect of Drug ($F(3,75) = 7.24$, $p < .001$), yet the Surgery main effect and Drug×Surgery interaction

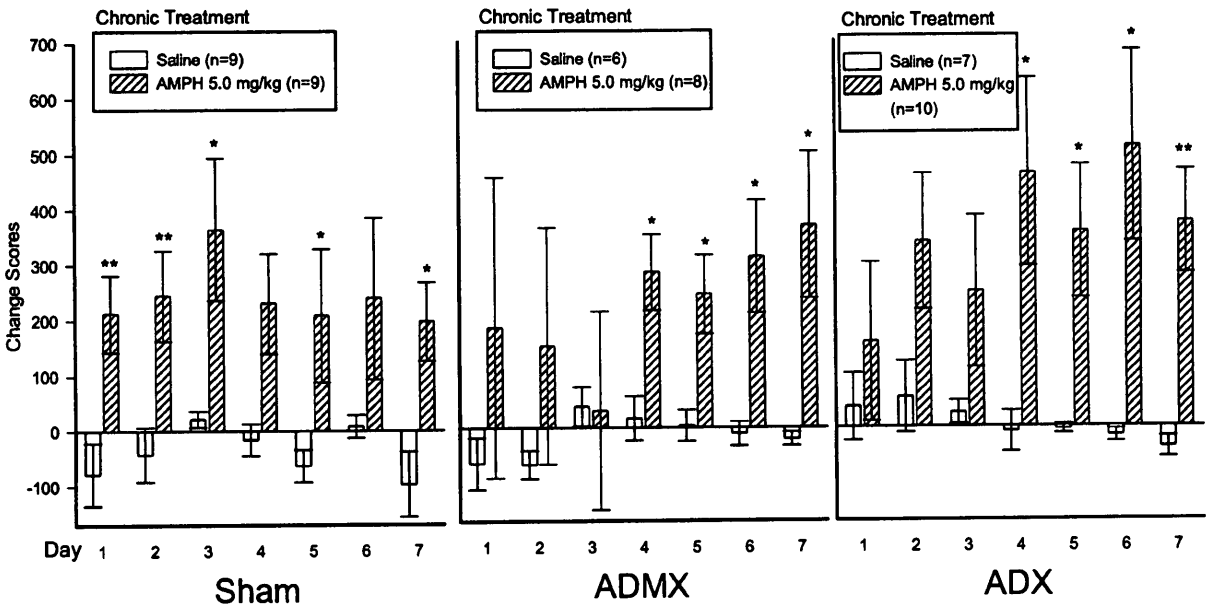

Fig. 7. For rats subjected to ADX, ADMX, or sham operation, AMPH (5.0 mg/kg) significantly enhanced startle in the induction phase. (*p < .05, **p < .01, different from the scores of the correspondent saline group)

effect did not reach the statistically significant level ($F(2,75) = 0.13$; $F(6,75) = 0.10$, respectively), suggesting that AMPH caused similar augmentation of startle in rats receiving sham or adrenal operations. Post-hoc tests showed that only at the dose of 5.0 mg/kg AMPH significantly enhanced startle in each group (all ps < .05), but the effect of 1.0 or 3.0 mg/kg failed to reach statistical significance. There was no significant Block effect ($F(11,825) < 1$), but the Block× Drug interaction was significant ($F(33,825) = 1.85$, $p < .01$), indicating that the enhancement became greater as the session progressed. However, the Block×Group and three-way interactions were not significant (both $Fs < 1$).

In view of the above findings, we further investigated whether ADX or ADMX would attenuate the sensitization effect induced by repeated injections of AMPH. Groups of sham operated, ADMX and ADX rats were subjected to the sensitization procedure as described in the Method section. In the midst of startle sessions during the induction phase, half of rats received saline for 7 days (sham: n = 9; ADMX: n = 6; ADX: n = 7), and the other half received 5.0 mg/kg of AMPH (sham: n = 9; ADMX: n = 8; ADX: n = 10). After one or two days of withdrawal, they were challenged with saline and 3.0 mg/kg AMPH in two consecutive days to evaluate the sensitization effect. The induction phase data are shown in Figure 7. They were analyzed by a three-way mixed-design ANOVA with Drug and Surgery as two between-subject variables and Day as the within-subject variable. The analysis revealed a significant main effect

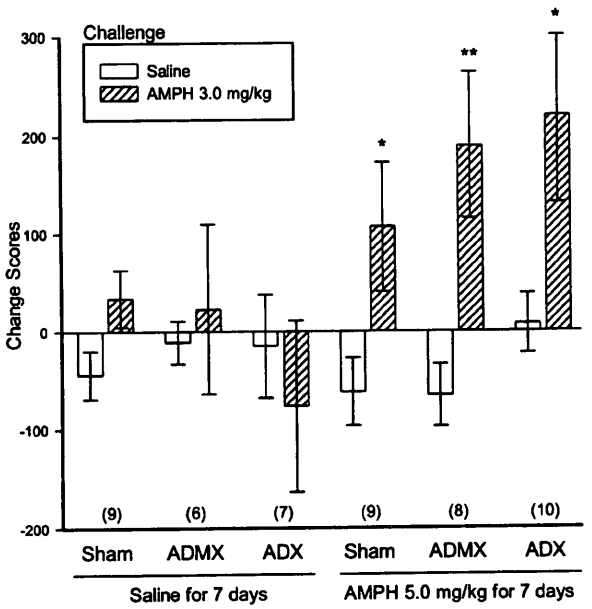

Fig. 8. ADX and ADMX failed to disrupt AMPH induced sensitization. (*p < .05, different from the scores under saline challenge in the correspondent group)

of Drug ($F(1,43) = 21.46$, $p < .001$). The Surgery and Day main effects as well as all interaction effects did not reach statistical significance (all $Fs < 1$).

Results shown in Figure 8 indicate that repeated injections of AMPH induced sensitization in the acoustic response, and such sensitization was prevented neither by ADX nor by ADMX. A preliminary analysis indicated that all effects involving the Block

factor were not significant, therefore the data were collapsed for all blocks and analyzed by a three-way mixed-design ANOVA with Chronic Injection and Surgery as two between-subject variables and Challenge as the within-subject variable. The main effects of Chronic Injection and Challenge were significant ($F(1,43) = 4.37$, $p < .05$; $F(1,43) = 18.63$, $p < .001$), and so was their interaction ($F(1,43) = 8.05$, $p < .01$). The Surgery main effect and all other interactions did not reach statistical significance (all $Fs < 1$). Post-hoc tests revealed that for the various operated groups of rats receiving repeated injections of saline in the induction phase, there was no sign of sensitization in response to a challenge dose of AMPH (all $ps > .05$). In contrast, all rats receiving AMPH in the induction phase showed significant sensitization no matter they were in the SHAM, ADMX, or ADX group ($p < .05$, $p<.01$, $p < .05$; respectively).

Experiment IV: Lack of Effect of Icv Infusion of α-helical CRF_{9-41} on Expression of Sensitization

The above findings led to further pursue on whether the AMPH-induced sensitization in startle involves increased release of CRF. Three groups of rats implanted with icv cannula were subjected to the procedures of inducing sensitization. Group 1 (n = 5) received saline during the induction phase whereas Group 2 (n = 5) and Group 3 (n = 16) received 5.0 mg/kg AMPH during the induction phase. Figure 9 shows that 5.0 mg/kg AMPH enhanced acoustic startle during the induction phase. The data were analyzed by a two-way mixed-design ANOVA with Drug as the between-subject variable and Day as the within-subject variable. The Drug effect was significant ($F(1,24) = 4.18$, $p < .05$), but the Day effect and Drug×Day interaction were not (both $Fs < 1$).

At the expression phase, Group 1 and Group 2 received icv infusion of vehicle, whereas Group 3 received icv infusion of 50.0 μg of α-helical CRF_{9-41}. After drug challenge, rats received additional 240 trials for two hours. Their averaged startle change scores were calculated for each hour. Figure 10 shows that in rats treated with saline in the induction phase and icv infusion of vehicle in the expression phase, AMPH challenge unexpectedly suppressed startle, chronic injections of AMPH attenuated this suppressing effect. Infusion of the CRF antagonist did not block this attenuation of suppressing effect, actually it tended to render the enhancing effect of AMPH on startle more, rather than less, prominent. Preliminary inspection of the results showed different pattern of effects on the first and second hours, thus data was analyzed by a three-way mixed-design ANOVA with Group as the between-subject variable and Challenge and Hour as two within-subject variables. The main effect was significant for Group ($F(2,23) = 7.01$, $p < .01$), but not for Challenge ($F(1,23) <$

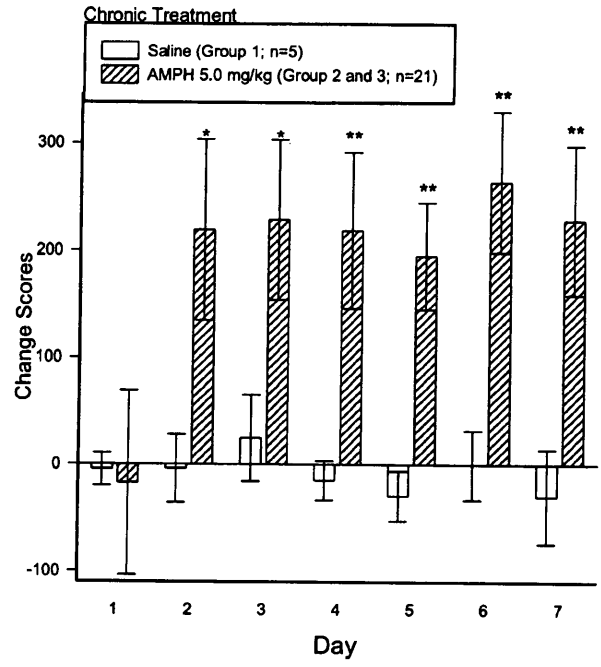

Fig. 9. AMPH (5.0 mg/kg) significantly enhanced startle in the induction phase in Experiment IV. (*p < .05, **p < .01, different from the score of Group 1)

1). The Hour main effect approached statistical significance ($F(1,23) = 3.91$, $.05 < p < .06$). There were also significant Group×Drug and Drug×Hour interaction effects ($F(2,23) = 5.12$, $p < .01$; $F(1,23) = 7.46$, $p < .01$, respectively). Post-hoc tests showed that over the total two-hour testing period, AMPH challenge elicited lower change scores than saline challenge for Group 1, which received chronic saline injections and icv infusion of vehicle during challenge. The change scores under AMPH challenge in Groups 2 and 3, which received chronic AMPH injections, were higher than those in Group 1, but Groups 2 and 3 did not differ from each other. The data were further analyzed on an hour by hour basis. For the first hour, the scores under AMPH challenge were significantly higher than those under saline challenge in Group 3 ($p < .01$), but not in Groups. 1 and 2. However, the change scores under AMPH challenge in Groups 2 and 3 were higher than those in Group 1 ($p < .05$, $p < .001$, respectively). For the second hour, the scores under AMPH challenge were significantly lower than saline challenge in Group 1 ($p < .05$), but no such difference was significant in Groups 2 and 3. In addition, the change scores under AMPH challenge of Group 3 were higher than the correspondent scores of Group 1 ($p < .05$).

To explore whether a clearer pattern would emerge by reducing in-group variability, the data of Groups 1, 2 and 3 were divided into high responder and low responder subgroups (as defined by change scores above or below the group median, respectively). The results are depicted in Figure 11. In the low

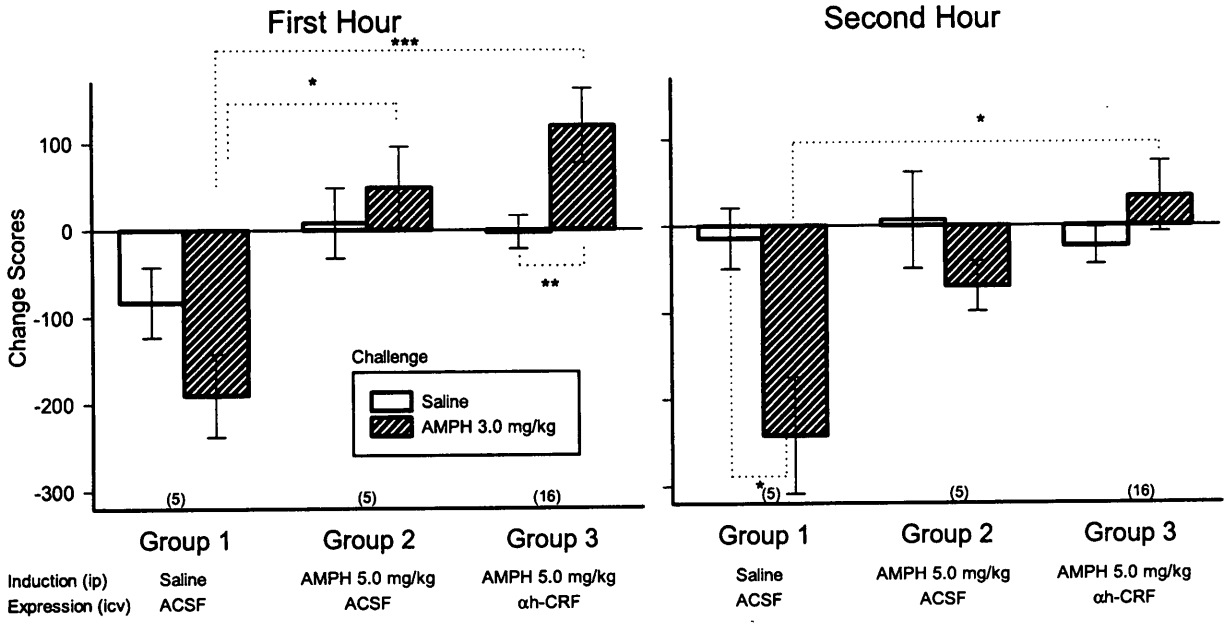

Fig. 10. Intracerebroventricular infusion of α-helical CRF_{9-41} did not prevent the rat from expressing AMPH-induced sensitization of acoustic startle. During the induction phase, Group 1 received ip injection of saline whereas Group 2 and Group 3 received AMPH 5.0 mg/kg. At the challenge phase, Group 1 and Group 2 received icv infusion of vehicle, whereas Group 3 received icv infusion of 50.0 μg of α-helical CRF_{9-41} (α-CRF_{9-41}). The left panel represents the mean change score during the first hour of the expression phase, and the right panel represents the data for the second hour. (*p < .05, **p < .01, ***p < .001, significant score difference between two groups was indicated by dotted lines)

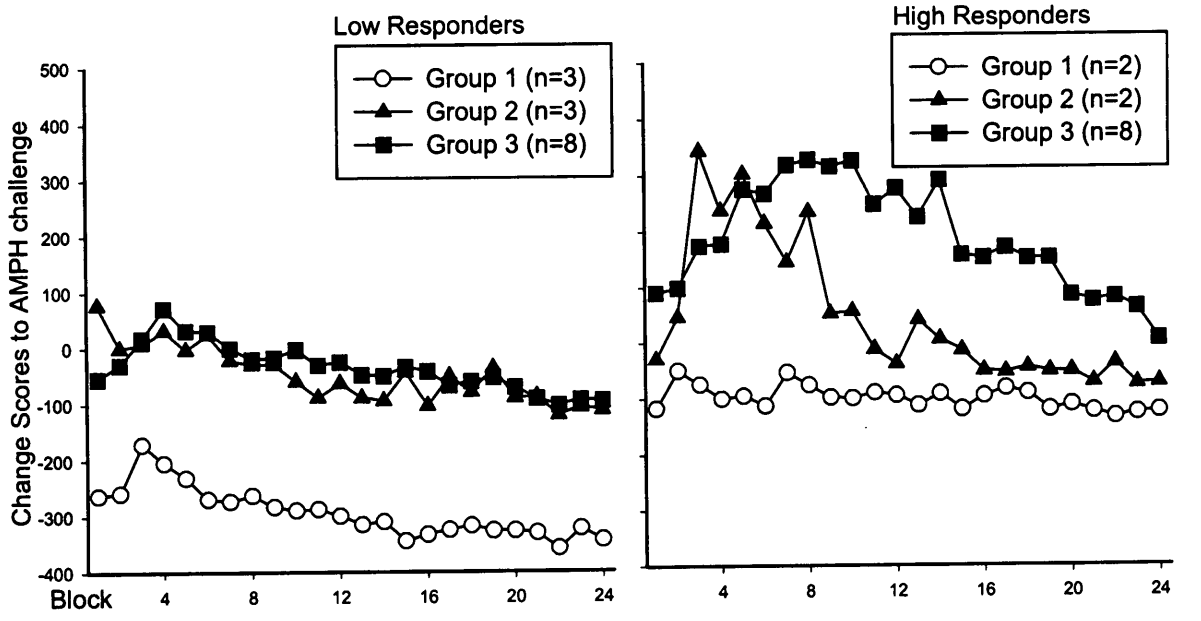

Fig. 11. Time course of startle responses to AMPH challenge in Experiment IV. According to their change scores below or above the median, rats in each group were divided into low responder (left panel) and high responder (right panel) subgroups, respectively. AMPH challenge (3.0 mg/kg) caused a suppression effect in low responders, and chronic treatments of 5.0 mg/kg AMPH ameliorate this suppression. AMPH challenge caused no apparent effect in high responders, and chronic AMPH treatments made the rat more sensitized to this challenge. The effects of AMPH challenge were not altered by icv infusion of α-helical CRF_{9-41} before the challenge. (Groups 1, 2, and 3 as defined in Fig. 10)

responder subgroups, 3.0 mg/kg AMPH suppressed startle to a level lower than the pre-injection baseline in rats treated with chronic saline, and this suppression was abolished in rats treated with chronic AMPH. In the high responder subgroups, 3.0 mg/kg AMPH caused no significantly higher startle than the baseline in rats treated with chronic saline. The chronic AMPH treatment sensitized the rat to 3.0 mg/kg AMPH,

replicating the findings of previous experiment. In both low and high responder subgroups, α-helical CRF_{9-41} did not abolish the effect induced by the chronic AMPH treatment.

Discussion

Results of Experiment I replicated the findings that acute injection of AMPH enhanced acoustic startle in rats at a dose range comparable to that of a previous study (11). Such an enhancing effect became apparent 10 min after the AMPH injection, and remained undiminished for the rest of a test session. As a matter of fact, it could last for more than 2 hours according to our unpublished observation. The high startle score observed in this study could not have been due to inadvertent activation of the sensor by stereotyped movement of a sensitized rat, because when an acute injection of AMPH at a dose inducing intensive stereotypy was administered, negligible scores could be recorded by the vibration sensor in the absence of any startle-eliciting stimulus (unpublished observation).

Results of Experiment II demonstrated a robust sensitization effect induced by AMPH to enhance acoustic startle in rats. Augmentation of the AMPH effect was evident in two manners. First, during the induction phase, the saline-injected rats showed progressive startle habituation either within or between the daily sessions over a 7-day period. In contrast, rats receiving 5.0 mg/kg of AMPH persistently showed enhanced acoustic startle and no sign of habituation over the period. The lack of progressive elevation of startle during this period may be due to a ceiling effect. Second, in the challenge phase, previous exposure of AMPH facilitated an otherwise subthreshold effect of 3.0 mg/kg AMPH, suggesting that rats became more sensitive to a low dose of the drug. These results extended what has been demonstrated in mice by Kokinidis and colleagues (26-28).

Studies have reported evidence that more robust sensitization developed only after a long abstinence period. For example, chronic AMPH administration enhanced rates of local cerebral glucose utilization when challenged one week or one month after withdrawal, but caused only mild effects if challenged two days after the pretreatment (21). It was also reported that sensitized stereotyped behaviors did not express until two weeks after discontinuation of AMPH treatment (47). The present study showed that sensitization persisted one month after withdrawal from induction. However, with the present injection regimen, AMPH induced significant sensitization of acoustic startle in rats without a long withdrawal period. This rapid development of sensitization could be somehow related to the intrinsic properties of the affected system--the acoustic startle response. Alternatively, the high dose regimen used in this study may accelerate the development of sensitization. It should be noted that under certain conditions, for example, when a single shot of 3.0 mg/kg AMPH was given, AMPH-induced sensitization in acoustic startle took more than two weeks to become fully apparent (Chen & Liang, in preparation). Factors affecting the progress rate of plastic changes underlying drug-induced sensitization should be pursued in the future.

Substantial evidence shows that corticosteroid plays a role in various forms of neural plasticity related to learning and memory (44, 45), aging (70), stress-induced atrophy of hippocampus (41) as well as drug- or stress-induced sensitization of locomotor behavior (13, 55, 56). Results from Experiment III find that eliminating adrenal corticosteroid by ADX did not block the acute or chronic effects of AMPH on acoustic startle: The ADX rats showed responses to AMPH similar to those of the sham operated controls. Our findings are consistent with a suggestion that adrenal cortical hormones circulating in the blood are not necessary for development of behavioral sensitization (2). On the other hand, a study did show that adrenalectomy blocked sensitization induced by AMPH in a prepulse inhibition of startle paradigm (71), although another study failed to reproduce such sensitization (15). Thus, subtle differences in the dosing regimen and behavioral paradigm may contribute to the discrepant findings on the role of corticosteroid in induction/expression of the psychostimulant sensitization effect. Peripheral epinephrine is critical for the action of AMPH on experience-dependent neural plasticity as attested by removal of the adrenal medulla, which is the major source of peripheral epinephrine, attenuating AMPH-induced memory enhancement (43). However, this action may not contribute much to the neural plasticity underlying sensitization, because sensitization persisted after either ADX or ADMX.

Eliminating corticosteroid from the periphery increases release of CRF, and acute injections of AMPH also elevates the brain CRF level (66). In view of the evidence that CRF is involved in AMPH-induced sensitization of locomotor or stereotypy behavior (4, 6, 7, 61), elevated CRF after ADX may have enhanced startle and thus counteracted an otherwise apparent blockade of sensitization effects by elimination of corticosteroid. Results from Experiment IV are not consistent with this conjecture by showing that in the presence of α-helical CRF_{9-41} a challenge still elicited augmented startle responses in rats pretreated with chronic injections of AMPH. In rats pretreated with chronic injections of saline, the icv infusion procedure switched an otherwise subthreshold startle enhancing effect of 3.0 mg/kg AMPH

into a suppression effect, as shown in Group 1 of the experiment. Such an effect was largely contributed by 3 low responders to AMPH (see Figure 11). The exact reason for this reversal is unknown but may be due to complicated interactive influences between the icv infusion stress and AMPH on acoustic startle. Chronic AMPH injections lifted the suppression (see low responders of Group 2), and this effect was not blocked by α-helical CRF_{9-41} (Group 3). For the high responders, a genuine sensitization effect was found by comparing Group 1 and Group 2, and this sensitization was even slightly enhanced by α-helical CRF_{9-41} (the high responders of Group 3). It appears unlikely that increased CRF release contributes to the expressed sensitization response, although other doses of α-helical CRF_{9-41} and alternative infusion procedures should be tested. The present results by themselves could not rule out a role of CRF in induction of sensitization, however, other data showed that in enhancing acoustic startle, repeated icv infusion of CRF for six days led to tolerance rather than sensitization (Liang, unpublished findings).

Early and late phases of sensitization in locomotion were shown to be mediated, respectively, by transient and persistent forms of plasticity in the nervous system (18, 68). Likewise, in acoustic startle, different mechanisms may also be engaged on the basis of whether short- or long-term sensitization is accrued. Chronic administration of psychostimulants induced long-lasting sensitization of the HPA axis with a delayed rather than immediate onset (63). The possibility that corticosterone and/or CRF mediates only long-term sensitization of the acoustic startle could not be refuted by the present results in which sensitization was assessed shortly after chronic exposure. However, Schmidt and his colleagues demonstrated that long-term locomotor sensitization induced by stress or drugs was not correlated to activity of the HPA axis after three weeks of withdrawal (64) and suggested that long-term sensitization induced by drug is also independent of the HPA activity.

In conclusion, the present study demonstrated in rats a robust sensitization of the acoustic startle responses after intermittent injections of AMPH. The effect appeared shortly after termination of chronic treatments. Further, this quickly developed sensitization effect is not altered by adrenalectomy, adrenal demedullation, or CRF antagonism, suggesting that the HPA activity might not be highly involved in this type of sensitization. While more research is needed to completely exclude a role of corticosteroid, epinephrine, or CRF in AMPH-induced sensitization of acoustic startle, the present effect may nonetheless involve other actions of AMPH or stress. AMPH causes marked release of central catecholamines including dopamine and norepinephrine. Yohimbine, an α_2 antagonist, which may enhance the central noradrenergic tone by blocking inhibition of the locus coeruleus mediated through autoreceptors, exerts an excitatory effect on acoustic startle, which was also impervious to adrenalectomy (23). The central noradrenergic function is extensively implicated in behavioral plasticity (31, 32, 37), and may work either conjunctively or independently with the HPA axis. Future research should address the contribution of altered central noradrenergic or other functions to the AMPH-induced sensitization effect.

Acknowledgements

This research was supported by grants NSC87-2413-H-002-02-G9 and NSC88-2413-H-002-018 from the National Science Council of Republic of China to K. C. Liang. These data were presented, in part, at the Annual Meeting for the Chinese Psychological Association, Taipei, Taiwan, 2000. The authors would like to thank Mr. Wu-Yen Yu for his excellent service to the psychobiology laboratory and those who took care of the animals. We also like to express our appreciation to Tsu-Wei Wang and Chang-Chi Hsieh for their assistance in some of the experiments and to Dr. Sigmund Hsiao for his useful suggestions.

References

1. Antelman, S.M., Eichler, A.J., Black, C.A. and Kocan, D. Interchangeability of stress and amphetamine in sensitization. *Science* 207: 329-331, 1980.
2. Badiani, A., Morano, M.I., Akil, H. and Robinson, T.E. Circulating adrenal hormones are not necessary for the development of sensitization to the psychomotor activating effects of amphetamine. *Brain Res.* 673: 13-24, 1995.
3. Bennett, C., Liang, K.C. and McGaugh, J.L. Depletion of adrenal catecholamines alters the amnestic effect of amygdala stimulation. *Behav. Brain Res.* 15: 83-91, 1985.
4. Cador, M., Cole, B.J., Koob, G.F., Stinus, L. and Le Moal, M. Central administration of corticotropin releasing factor induces long-term sensitization to d-amphetamine. *Brain Res.* 606: 181-186, 1993.
5. Chen, D.Y., Ho, S.H. and Liang, K.C. Startle responses to electric shocks: measurement of shock sensitivity and effects of morphine, buspirone and brain lesions. *Chin. J. Physiol.* 43: 35-47, 2000.
6. Cole, B.J., Cador, M., Stinus, L., Rivier, C., Rivier, J., Vale, W., Le Moal, M. and Koob, G.F. Critical role of the hypothalamic pituitary adrenal axis in amphetamine-induced sensitization of behavior. *Life Sci.* 47: 1715-1720, 1990.
7. Cole, B.J., Cador, M., Stinus, L., Rivier, J., Vale, W., Koob, G.F. and Le Moal, M. Central administration of a CRF antagonist blocks the development of stress-induced behavioral sensitization. *Brain Res.* 512: 343-346, 1990.
8. Davis, M. Neurochemical modulation of sensory-motor reactivity: acoustic and tactile startle reflexes. *Neurosci. Biobehav. Rev.* 4: 241-263, 1980.
9. Davis, M. The role of the amygdala in fear and anxiety. *Annu. Rev. Neurosci.* 15: 353-375, 1992.
10. Davis, M., Gendelman, D.S., Tischler, M.D. and Gendelman, P.M. A primary acoustic startle circuit: lesion and stimulation studies. *J.*

Neurosci. 2: 791-805, 1982.
11. Davis, M., Svensson, T.H. and Aghajanian, G.K. Effects of d- and l-amphetamine on habituation and sensitization of the acoustic startle response in rats. *Psychopharmacologia* 43: 1-11, 1975.
12. De Vries, T.J., Schoffelmeer, A.N., Tjon, G.H., Nestby, P., Mulder, A.H. and Vanderschuren, L.J. Mifepristone prevents the expression of long-term behavioural sensitization to amphetamine. *Eur. J. Pharmacol.* 307: 3-4, 1996.
13. Deroche, V., Piazza, P.V., Casolini, P., Le Moal, M. and Simon, H. Sensitization to the psychomotor effects of amphetamine and morphine induced by food restriction depends on corticosterone secretion. *Brain Res.* 611: 352-356, 1993.
14. Deroche, V., Piazza, P.V., Maccari, S., Le Moal, M. and Simon, H. Repeated corticosterone administration sensitizes the locomotor response to amphetamine. *Brain Res.* 584: 309-313, 1992.
15. Druhan, J.P., Geyer, M.A. and Valentino, R.J. Lack of sensitization to the effects of d-amphetamine and apomorphine on sensorimotor gating in rats. *Psychopharmacol.* 135: 296-304, 1998.
16. Dykman, R.A., Ackerman, P.T. and Newton, J.E. Posttraumatic stress disorder: a sensitization reaction. *Integrative Physiological & Behavioral Science* 32: 9-18, 1997.
17. Echols, S.D. Circling of mice bearing unilateral striatal lesions: development of increased response to d-amphetamine. *Life Sci.* 21: 563-568, 1977.
18. Heidbreder, C.A., Thompson, A.C. and Shippenberg, T.S. Role of extracellular dopamine in the initiation and long-term expression of behavioral sensitization to cocaine. *J. Pharmacol. Exp. Ther.* 278: 490-450, 1996.
19. Henry, C., Guegan, G., Cador, M., Arnauld, E., Arsaut, J., Le Moal, M. and Demotes-Mainard, J. Prenatal stress in rats facilitates amphetamine-induced sensitization and induces long-lasting changes in dopamine receptors in the nucleus accumbens. *Brain Res.* 685: 179-186, 1995.
20. Horger, B.A., Shelton, K. and Schenk, S. Preexposure sensitizes rats to the rewarding effects of cocaine. *Pharmacol. Biochem. Behav.* 37: 707-711, 1990.
21. Huang, Y.H., Tsai, S.J., Wang, Y.C., Yu, M.F., Yang, Y.C. and Sim, C.B. Differential development of the enhanced metabolic response during amphetamine sensitization. *Neuropsychobiol.* 36: 177-181, 1997.
22. Kalivas, P.W. and Stewart, J. Dopamine transmission in the initiation and expression of drug and stress-induced sensitization of motor activity. *Brain Res. Rev.* 16: 223-244, 1991.
23. Kehne, J.H. and Davis, M. Central noradrenergic involvement in yohimbine excitation of acoustic startle: effects of DSP4 and 6-OHDA. *Brain Res.* 330: 31-41, 1985.
24. Kehne, J.H. and Sorenson, C.A. Effects of pimozide and phenoxybenzamine pretreatments on amphetamine and apomorphine potentiation of the acoustic startle response in rats. *Psychopharmacol.* 58: 137-144, 1978.
25. Kirk, R.E. Experimental design: procedures for the behavioral sciences. Brooks/Cole Publishing Company, Pacific Grove, CA, 1995.
26. Kokkinidis, L. Effects of chronic intermittent and continuous amphetamine administration on acoustic startle. *Pharmacol. Biochem. Behav.* 20: 367-371, 1984.
27. Kokkinidis, L. and Anisman, H. Involvement of norepinephrine in startle arousal after acute and chronic d-amphetamine administration. *Psychopharmacol.* 59: 285-292, 1978.
28. Kokkinidis, L. and MacNeill, E.P. Potentiation of d-amphetamine and L-dopa-induced acoustic startle activity after long-term exposure to amphetamine. *Psychopharmacol.* 78: 331-335, 1982.
29. Kreek, M.J. and Koob, G.F. Drug dependence: stress and dysregulation of brain reward systems. *Drug and Alcohol Dependence* 51: 23-47, 1998.
30. Lang, P.J., Bradley, M.M. and Cuthbert, B.N. Emotion, attention and the startle reflex. *Psychol. Rev.* 97: 377-395, 1990.
31. Lee, E.H.Y., Lee, C.P., Wang, H.I. and Lin, W.R. Hippocampal CRF, NE, and NMDA system interactions in memory processing in the rat. *Synapse* 14: 144-153, 1993.
32. Lee, E.H.Y. and Ma, Y.L. Amphetamine enhances memory retention and facilitates norepinephrine release from the hippocampus in rats. *Brain Res. Bull.* 37: 411-416, 1995.
33. Lee, Y., Schulkin, J. and Davis, M. Effect of corticosterone on the enhancement of the acoustic startle reflex by corticotropin releasing factor (CRF). *Brain Res.* 666: 93-98, 1994.
34. Lett, B.T. Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. *Psychopharmacol.* 98: 357-362, 1989.
35. Leyton, M. and Stewart, J. Preexposure to foot-shock sensitizes the locomotor response to subsequent systemic morphine and intranucleus accumbens amphetamine. *Pharmacol. Biochem. Behav.* 37: 303-310, 1990.
36. Liang, K.C. Pretraining infusion of DSP-4 into the amygdala impaired retention in the inhibitory avoidance task: involvement of norepinephrine but not serotonin in memory facilitation. *Chin. J. Physiol.* 41: 223-233, 1998.
37. Liang, K.C., Chen, H.C. and Chen, D.Y. Posttraining infusion of norepinephrine and corticotropin releasing factor into the bed nucleus of the stria terminalis enhanced retention in an inhibitory avoidance task. *Chin. J. Physiol.* 44: 33-43, 2001.
38. Liang, K.C., Chen, L.L. and Huang, T.E. The role of amygdala norepinephrine in memory formation: involvement in the memory enhancing effect of peripheral epinephrine. *Chin. J. Physiol.* 38: 81-91, 1995.
39. Liang, K.C., Juler, R.G. and McGaugh, J.L. Modulating effects of posttraining epinephrine on memory: involvement of the amygdala noradrenergic system. *Brain Res.* 368: 125-133, 1986.
40. Liang, K.C., Melia, K.R., Miserendino, M.J., Falls, W.A., Campeau, S. and Davis, M. Corticotropin-releasing factor: long-lasting facilitation of the acoustic startle reflex. *J. Neurosci.* 12: 2303-2312, 1992.
41. Magarinos, A.M. and McEwen, B.S. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neurosci.* 69: 89-98, 1995.
42. Magos, L. Persistence of the effect of amphetamine on stereotyped activity on rats. *Eur. J. Pharmacol.* 6: 200-201, 1969.
43. Martinez, J.L., Vasquez, B.J., Rigter, H., Messing, R.B., Jensen, R. A., Liang, K.C. and McGaugh, J.L. Attenuation of amphetamine-induced enhancement of learning by adrenal demedullation. *Brain Res.* 195: 433-443, 1980.
44. McEwen, B.S. Corticosteroids and hippocampal plasticity. *Ann. N. Y. Acad. Sci.* 746: 134-142, 1994.
45. McGaugh, J.L. Hormonal influence on memory. *Annu. Rev. Psychol.* 34: 297-323, 1983.
46. Mittleman, G., Jones, G.H. and Robbins, T.W. Sensitization of amphetamine-stereotypy reduces plasma corticosterone: implications for stereotypy as a coping response. *Behavioral and Neural Biology* 56: 170-182, 1991.
47. Paulson, P.E., Camp, D.M. and Robinson, T.E. Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats. *Psychopharmacol.* 103: 480-492, 1991.
48. Pauly, J.R., Robinson, S.F. and Collins, A.C. Chronic corticosterone administration enhances behavioral sensitization to amphetamine in mice. *Brain Res.* 620: 195-202, 1993.
49. Pelton, G.H., Lee, Y. and Davis, M. Repeated stress also sensitizes the excitatory effects of CRF on the acoustic startle reflex. *Brain Res.* 778: 381-387, 1997.
50. Piazza, P.V., Deminiere, J.M., Le Moal, M. and Simon, H. Factors that predict individual vulnerability to amphetamine self-administration. *Science* 245: 1511-1513, 1989.

51. Piazza, P.V., Deminiere, J.M., Le Moal, M. and Simon, H. Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. *Brain Res.* 514: 22-26, 1990.
52. Post, R.M. Sensitization and kindling perspectives for the course of affective illness: toward a new treatment with the anticonvulsant carbamazepine. *Pharmacopsychiatry* 23: 3-17, 1990.
53. Post, R.M. and Weiss, S.R.B. Sensitization and kindling phenomena in mood, anxiety, and obsessive-compulsive disorders: the role of serotonergic mechanisms in illness progression. *Biol. Psychiatry* 44: 193-206, 1998.
54. Post, R.M., Weiss, S.R.B. and Smith, M.A. Sensitization and kindling: Implications for the evolving neural substrates of post-traumatic stress disorder. In M.J. Friedman, Charney, D.S. and Deutch, A.Y. (Eds.), Neurobiological and clinical consequences of stress: From normal adaptation to PTSD. Philadelphia: Lippincott-Raven Publishers, pp. 203-224, 1995.
55. Rivet, J.M., Stinus, L., Le Moal, M. and Mormede, P. Behavioral sensitization to amphetamine is dependent on corticosteroid receptor activation. *Brain Res.* 498: 149-153, 1989.
56. Roberts, A.J., Lessov, C.N. and Phillips, T.J. Critical role for glucocorticoid receptors in stress- and ethanol-induced locomotor sensitization. *J. Pharmacol. Exp. Ther.* 275: 790-797, 1995.
57. Robinson, T.E. Behavioral sensitization: characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female rats. *Psychopharmacol.* 84: 466-475, 1984.
58. Robinson, T.E. Persistent sensitizing effects of drugs on brain dopamine systems and behavior: implications for addiction and relapse. In S.G. Korenman and Barchas, J.D. (Eds.), Biological Basis of Substance Abuse. New York: Oxford University Press, pp. 373-402, 1993.
59. Robinson, T.E., Becker, J.B. and Presty, S.K. Long-term facilitation of amphetamine-induced rotational behavior and striatal dopamine release produced by a single exposure to amphetamine: sex differences. *Brain Res.* 253: 231-241, 1982.
60. Robinson, T.E. and Berridge, K.C. The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Res. Rev.* 18: 247-291, 1993.
61. Sarnyai, Z., Hohn, J., G., S. and Penke, B. Critical role of endogenous corticotropin-releasing factor (CRF) in the mediation of the behavioral action of cocaine in rats. *Life Sci.* 51: 2019-2024, 1992.
62. Schmidt, E.D., Schoffelmeer, A.N., De Vries, T.J., Wardeh, G., Dogterom, G., Bol, J.G., Binnekade, R. and Tilders, F.J. A single administration of interleukin-1 or amphetamine induces long-lasting increases in evoked noradrenaline release in the hypothalamus and sensitization of ACTH and corticosterone responses in rats. *Eur. J. Neurosci.*: 1923-1930, 2001.
63. Schmidt, E.D., Tilders, F.J., Janszen, A.W., Binnekade, R., De Vries, T.J. and Schoffelmeer, A.N. Intermittent cocaine exposure causes delayed and long-lasting sensitization of cocaine-induced ACTH secretion in rats. *Eur. J. Pharmacol.* 285: 317-321, 1995.
64. Schmidt, E.D., Tilders, F.J.H., Binnekade, R., Schoffelmeer, A.N. M. and De Vries, T.J. Stressor- or drug-induced sensitization of the corticosterone response is not critically involved in the long-term expression of behavioural sensitization to amphetamine. *Neurosci.* 92: 343-352, 1999.
65. Segal, D.S. and Mandell, A.J. Long-term administration of d-amphetamine: progressive augmentation of motor activity and stereotypy. *Pharmacol. Biochem. Behav.* 2: 249-255, 1974.
66. Swerdlow, N.R., Koob, G.F., Cador, M., Lorang, M. and Hauger, R. L. Pituitary-adrenal axis responses to acute amphetamine in the rat. *Pharmacol. Biochem. Behav.* 45: 629-637, 1993.
67. Wise, R.A. and Bozarth, M.A. A psychomotor stimulant theory of addiction. *Psychol. Rev.* 94: 469-492, 1987.
68. Wolf, M.E., White, F.J., Nassar, R., Brooderson, R.J. and Khansa, M.R. Differential development of autoreceptor subsensitivity and enhanced dopamine release during amphetamine sensitization. *J. Pharmacol. Exp. Ther.* 264: 249-255, 1993.
69. Yehuda, R. and Antelman, S.M. Criteria for rationally evaluating animal models of posttraumatic stress disorder. *Biol. Psychiatry* 33: 479-486, 1993.
70. Yen, S.S. and Laughlin, G.A. Aging and the adrenal cortex. *Exp. Gerontol.* 33: 897-910, 1998.
71. Zhang, J., Engel, J.A., Soderpalm, B. and Svensson, L. Repeated administration of amphetamine induces sensitisation to its disruptive effect on prepulse inhibition in the rat. *Psychopharmacol.* 135: 401-406, 1998.