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Effects of Acrylamide on Locomotion and Central Monoamine Function in the Rat[1]

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RAFALES, L. S., S. M. LASLEY, R. D. GREENLAND AND T. MANDYBUR. *Effects of acrylamide on locomotion and central monoamine function in the rat.* PHARMACOL BIOCHEM BEHAV 19(4) 635–644, 1983.—Male rats receiving acrylamide (ACR) in their drinking water (100 ppm) for a six-week period displayed increased psychomotor stimulation to d-amphetamine (d-A; 1.0 mg/kg SC) under several conditions of handling and drug administration. Following behavioral tests a subset of the animals was sacrificed at 15, 50, 80 and 120 minutes following d-A and the brains removed and dissected for determinations of regional brain levels of several monoamine neurotransmitters and metabolites. ACR rats had elevated levels of 5-hydroxyindoleacetic acid (5-HIAA) in the striatum, septal area, and thalamus. The effect was most pronounced at 15 minutes post-drug with ACR rats not demonstrating a depression in 5-HIAA levels present in controls. Increases in accumben's dopamine and norepinephrine levels, evident after d-A, were of lesser magnitude in ACR-exposed rats. Decreases in dihydroxyphenylacetic acid and homovanillic acid, also evident after d-A, persisted for a longer duration in ACR-exposed rats. Light and electron microscopy of spinal cord, striatum, nucleus accumbens and thalamus did not reveal morphologic abnormalities. Sciatic nerves showed histopathological changes characteristic of multi-focal dying-back peripheral nerve degeneration. It was concluded that acrylamide's effect on the psychomotor stimulant properties of d-A may be related to changes in a serotonergic inhibitory system.

Acrylamide Regional monoamine levels d-Amphetamine Sciatic nerve Histopathology Locomotor activity

BEHAVIORAL studies have demonstrated an alteration in the normal pharmacological responsiveness to d-amphetamine (d-A) and other DA agonists [1, 22, 32] after either acute or chronic exposure to acrylamide (ACR) in rats. Tilson and Squibb [32] used operant performance (lever pressing) as a behavioral index of d-A's effectiveness. In this procedure d-A causes a suppression in lever pressing as its dose is raised. A single administration of 12.5 mg/kg PO of ACR 24 hr prior to evaluation caused an increase in the suppressive action of d-A. In order to insure that the behavioral suppression was specific to dopaminergic agonists these investigators also administered apomorphine and found that ACR exposure resulted in similar effects as were observed following d-A. However, schedule-controlled responding following chlordiazepoxide and clonidine-drugs with non-dopamine sites of action-was not affected.

Rafales *et al.* [22] showed that ACR could augment the pharmacological effectiveness of d-A by facilitating rather than suppressing a behavioral measure. Capitalizing on the well-known psychomotor stimulant properties of d-A, these researchers measured the spontaneous and drug-induced locomotor activity of animals receiving 100 ppm ACR in drinking water. d-A-induced, but not spontaneous locomotor activity was enhanced after three to six weeks of exposure to ACR (100 ppm in drinking water).

Several studies have indicated that an animal's prior experience with testing and handling procedures [7] and repeated drug administration [27] can affect subsequent drug responsiveness. The behavioral studies described above did not explicitly control for these potentially confounding factors. Therefore one aspect of the current series of studies was to assess the effects of ACR on the psychomotor stimulant properties of d-A in animals receiving different handling and drug histories. Psychomotor stimulation produced by apomorphine was also evaluated for a subset of the animals.

The effects of ACR on pharmacological sensitivity suggest underlying neurochemical changes. Studies on postsynaptic function have shown that ACR increases the binding of ^{3}H spiroperidol [2, 4, 34] as a result of an enhanced receptor affinity and an increased number of binding sites for this DA ligand. However, decreases in receptor binding have also been reported [33]. It is also unclear whether changes in receptor affinity and number represent a toxic alteration since they are seen after acute exposure to low doses of ACR [1]. In addition, changes in receptor binding appear to be relatively nonspecific at higher levels or durations of exposure since they have been reported to occur in a variety of neurotransmitter systems including cholinergic, GABAergic, serotonergic and glycinergic [1]. Finally, changes in receptor function may be secondary to alterations in presynaptic neurotransmission. Previous studies have shown changes in whole brain NE [3,10] and DA [10] follow-

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TABLE 1

Week of Exposure	Study 1*	Study 2*	Study 3†
Before onset	d-A locomotor activity	d-A locomotor activity	d-A locomotor activity
1	no test	d-A locomotor activity	saline locomotor activity
2	no test	d-A locomotor activity	saline locomotor activity
3	no test	d-A locomotor activity	saline locomotor activity
4	no test	d-A locomotor activity	saline locomotor activity
5	no test	d-A locomotor activity	saline locomotor activity
6	d-A locomotor activity	d-A locomotor activity	d-A locomotor activity
7–12	(d-A, sacrificed for neurochemistry Week 7)	(apomorphine challenges Weeks 8 and 12)	(apomorphine challenge Week 8, sacrificed for histopathology Weeks 10 and 11)

*N = 16 treated, 16 control.
†N = 10 treated, 10 control.

ing exposure to ACR. Whole brain DOPAC levels have been reported to increase or not change following ACR exposure [3,18]. These neurochemical changes have been observed only under nondrugged (i.e., basal) conditions.

By perturbing the CNS with a pharmacological probe the dynamic response of several neurotransmitter systems can be observed. This permits an assessment of regulatory responses as neuronal systems respond to the drug probe and then return toward baseline levels. Alterations in neurotransmitter function which are not apparent under basal conditions may become manifest when the CNS is disturbed in this way [19].

This rationale served as the basis for a second aspect of the current study: to examine the effects of ACR on the dynamic response of biogenic amines in CNS neuronal systems to a d-A challenge. These measures were acquired in a group of animals which were also assessed for their behavioral response (psychomotor stimulation) to d-A.

METHOD

Animals and Housing

Male Long Evans hooded rats were obtained from Charles River Laboratories (Wilmington, MA) at 70 days of age and individually housed in polycarbonate rat cages with stainless steel wire tops. Ab-Sorb-Dri® (#ABS-3, Garfield, NJ) was used for bedding. Animals were kept in a windowless, air-filtered, temperature (22±1°C) and humidity (50–60%) controlled room having a 12 hr light:dark cycle with the light phase commencing at 7:00 a.m. Laboratory chow (No. 5001, Ralston Purina Co., St. Louis, MO) and deionized water or ACR solution were provided ad lib throughout the study. At weekly intervals rats were weighed and fluid intake was monitored.

Exposure Protocol

ACR solutions (100 ppm) were prepared from A.C.S. chemical grade acrylamide monomer (No. 8887; Sigma Chemical Co., St. Louis, MO) dissolved in deionized water. Fresh solutions were prepared every seven days. Control animals were maintained on deionized water. Exposure was maintained for seven to twelve weeks (see section below on General Experimental Design).

General Experimental Design

Three individual studies were conducted (see Table 1). In each experiment spontaneous and d-A-induced locomotor activity (1.0 mg/kg SC as sulfate) were assessed for control and ACR-treated rats immediately prior to the onset and after six weeks of exposure to ACR. In each experiment testing was initiated when animals were 90–120 days of age. In Study 1 control (N=16) and ACR-treated (N=16) rats were not evaluated for spontaneous or drug induced locomotor activity during the intervening five-week interval. In Study 2, control (N=16) and ACR-treated (N=16) rats received weekly challenges of d-A with subsequent monitoring of their locomotor activity. In Study 3 a group comprised of 10 control and 10 ACR-treated rats was evaluated for locomotor activity after weekly injections of isotonic saline during the intervening five-week period.

In each test session locomotor activity was monitored for 30 minutes prior to, and for two hours after, injections of d-A or the vehicle, saline. Animals were weighed 30–60 minutes

prior to testing which was initiated 2½ hours after onset of the light portion of the light-dark cycle (i.e., 9:30 a.m.). The apparatus for testing locomotor activity has been previously described [22]. Briefly, it consisted of ten transparent Plexiglas cages positioned below red fluorescent lights and above photocell arrays interfaced to a microcomputer. Photocell interruptions produced by the animal's movements were accumulated in five-minute blocks on magnetic tape. Continuous visual observation was allowed through the use of a closed circuit television camera and monitor. Squads of 6–10 animals were tested within a session with equal representation of control and ACR-treated rats. Cage assignments were counterbalanced such that if small differences in cage sensitivity were present they would be randomly distributed across treatment conditions (cage effects were assessed for 78 non-exposed animals during the baseline period with N=4–9 per cage. Sources of variance associated with cage were found to be non-significant).

Animals in Study 1 were subsequently sacrificed for neurochemistry. Animals in Study 2 received subsequent challenges with two doses of apomorphine HCl, (0.625 and 2.5 mg/kg IP) and animals in Study 3 received one dose of apomorphine (2.5 mg/kg IP) prior to being sacrificed for light and electron microscopy.

Neurochemistry

Animals in Study 1 received a second d-A challenge (1.0 mg/kg SC) after the seventh week of exposure. Rats were randomly designated for sacrifice by decapitation at one of four times following d-A injection (15, 50, 80, or 120 min) and regional brain tissue analyzed for monoamine neurotransmitters and metabolites. An additional five control and five ACR-exposed rats with identical handling histories received injections of isotonic saline and were randomly assigned for sacrifice at the same post-injection time points. Brains were removed, rinsed in ice-cold isotonic saline, and quickly dissected over ice. Nucleus accumbens, corpus striatum, septal area and thalamus were dissected essentially according to Heffner *et al.* [17], frozen on dry ice, weighed, and stored at −70°C until extraction.

Individual brain regions were homogenized and extracted essentially according to Co *et al.* [6] with 25 volume of ice-cold 1.0 N formic acid/acetone (15:85, v/v) containing epinine (N-methyldopamine) as the internal standard. The homogenate was centrifuged briefly at 4°C and the supernatant extracted with heptane/chloroform (8:1, v/v) the organic layer discarded, and the aqueous layer dried under N_2 and stored at −20°C until assay (<1 week).

Samples were thawed and redissolved in mobile phase prior to injection of 20 μl into a liquid chromatograph with a glassy carbon electrochemical detector (TL-8A) and an LC-3 amperometric controller (Bioanalytical Systems, Inc., W. Lafayette, IN). Chromatographic conditions permitted the quantitation of tyrosine (TYR), tryptophan (TRP), norepinephrine (NE), dopamine (DA), serotonin (5-HT), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA). All values were corrected for recovery and expressed as μg/g tissue weight. Lowry [21] protein determinations were performed on aliquots of the tissue from each brain region and the concentrations also expressed as picomoles/mg protein.

Morphological Studies

Following 10–11 weeks of ACR exposure Study 3 animals were sacrificed (ten ACR-exposed and five controls) after being weighed and anesthetized with pentobarbital (40 mg/kg IP). Animals were perfused via the left ventricle of the heart with 500 ml of a washout solution followed by 700 ml of fixative. The washout solution was composed of a mixture of one liter of lactated Ringer's solution, two cc of 40,00 units heparin, and 10 g of sodium nitrate. The fixative employed consisted of a 3% solution of paraformaldehyde, 3% glutaraldehyde and 0.1 M sodium cacodylate buffer at pH 7.2. The brain, spinal cord, bilateral sciatic nerves and lumbar dorsal root ganglia were dissected.

Three brain areas of interest—nucleus accumbens, striatum, thalamus—were dissected utilizing the same procedures as employed in the neurochemical analyses. Tissue fragments were then placed back into fixative for one hour and post-fixed in 1% osmium tetroxide for four hours. They were subsequently rinsed in 0.1 M cacodylate buffer twice for 15 minutes and dehydrated in 30%, 50%, 70%, 95% and 100% ethanol, respectively. Tissue samples were rotated in 50% Spurr plastic and 50% propylene oxide, then placed in 100% Spurr and rotated overnight. The following day, sections were embedded in Spurr and baked at 70°C for eight hours.

The spinal cord was cut into five 1 mm thick sections at each of the three major levels: cervical enlargement, mid-thoracic, and lumbar enlargement. These tissues were then placed in fixative for one hour and post-fixed in 1% osmium tetroxide for three hours. The remaining processing was carried out as previously described for the brain tissue. Left sciatic nerve and dorsal root ganglia were processed in a similar fashion.

Both semi-thin (0.5 μM) and thin (900Å) sections were cut from tissue of brain, spinal cord, dorsal root ganglia and the left sciatic nerve. The right sciatic nerve was dissected free of all connective tissue and a segment approximately 8 mm long cut from the middle portion of the nerve. This segment was then placed in fixative for one hour and post-fixed in 1% osmium tetroxide for three hours. The segment was rinsed in cacodylate buffer twice for 15 minutes and placed in 100% glycerine for at least two days prior to individual fibers being separated and placed on a microscope slide and coverslipped with Permount.

RESULTS

Fluid Consumption and Body Weight

Fluid consumption and body weight during the first six weeks of exposure to ACR were analyzed using two-factor analyses of variance (ANOVA) with exposure condition (ACR, control) as a between-subject source of variance and week of exposure as a repeated measure. Separate ANOVAs were computed for each of the three experiments. Unless otherwise noted, all significance levels for repeated measures have been adjusted using the Geisser-Greenhouse criterion [13] to provide a conservative estimate of the F ratio in the event of correlations among the repeated measures.

Fluid consumption increased over the first six week period of exposure in each of the three experiments, $F(5,150)=7.66$, $p<0.001$; $F(5,150)=4.07$, $p<0.01$; $F(5,90)=4.04$, $p<0.005$; Studies 1, 2 and 3 respectively. However, fluid consumption was not significantly affected by ACR, $F(1,30)=1.17$, $p>0.10$; $F(1,30)<1$, $p>0.10$, $F(1,18)<1$, $p>0.10$, nor was the rate at which fluid consumption increased over the six-week interval affected by ACR-exposure, $F(5,150)=1.28$, $p>0.10$; $F(5,150)=2.47$, $p>0.05$; $F(5,90)=1.01$, $p>0.10$.

Prior to exposure animals in the ACR-exposure group were no different in body weight from their age-matched controls. Over the first six-week period of exposure both control and ACR-exposed rats continued to gain weight, $F(6,180) = 378.8$, $p < 0.001$; $F(6,180) = 293$, $p < 0.001$; $F(6,108) = 151.1$, $p < 0.001$. However, rats exposed to ACR gained at a slower rate than control animals, $F(6,180) = 5.52$, $p < 0.01$; $F(6,180) = 13.22$, $p < 0.001$; $F(6,108) = 2.83$, $p < 0.06$. After six weeks of continuous exposure ACR animals had body weights which were 2.4–3.0% less than controls in the three experiments (significant Newman-Keuls comparisons with $p < 0.05$).

Locomotor Activity

In each study spontaneous locomotor activity was evaluated for the week preceding exposure using a two-factor ANOVA with exposure (ACR, control) as a between subject source of variance and six 5-minute intervals during the session as a repeated measure. Spontaneous locomotor activity was found to be equivalent for control animals and those designated to received ACR later in the study.

Locomotor activity following d-A was also similar in each of the three studies for animals assigned to both exposed and control groups prior to the onset of ACR exposure. Again, no significant main effects for exposure, $F(1,30) = 2.84$, $p > 0.10$; $F(1,30) = 2.57$, $p > 0.10$; $F(1,18) = 2.45$, $p > 0.10$, Studies 1–3, respectively, or interactions between exposure and the time after d-A administration were present, $F(23,690) < 1$, $p > 0.10$; $F(23,690) < 1$, $p > 0.10$; $F(213,414) = 1.74$, $p > 0.10$, Studies 1–3, respectively.

Figure 1 depicts spontaneous and d-A-induced locomotor activity following the first six weeks of ACR-exposure for each of the three experiments. Activity is expressed as the mean change from baseline observations prior to exposure. Two-factor ANOVAs with exposure as a between subject source of variance and time after d-A as a repeated measure were performed on these values. Spontaneous activity during the 30 minutes prior to injection was not significantly affected by ACR-exposure in any of the three studies. In contrast, locomotor activity following d-A was significantly enhanced for those rats exposed to ACR over the initial six week interval. This increased sensitivity to d-A was in marked contrast to control animals who were responding close to baseline levels of activity (Fig. 1). Significant interactions between exposure and the 24 5-minute periods within a session for each study, $F(23,690) = 4.06$, $p < 0.002$; $F(23,690) = 2.96$, $p < 0.01$; $F(23,414) = 3.89$, $p < 0.001$, Studies 1–3, respectively, and post hoc comparisons for each study (Newman-Keuls, $p < 0.05$) indicated that the increased response to d-A occurred during an interval spanning 15–65 minutes after injection.

To assess the contribution of handling conditions (no testing vs. weekly tests) and drug history (weekly d-A challenge vs. saline challenge) activity data from all three studies were pooled and analyzed using a single three-factor ANOVA with exposure and study as between-subject sources of variance and the 24 5-minute intervals post-injection as a repeated measure. A significant exposure, $F(1,78) = 7.54$, $p < 0.01$, and exposure × time interaction, $F(23,1794) = 7.27$, $p < 0.001$, confirmed that the first six weeks of exposure to ACR augmented the psychomotor stimulant properties of d-A, primarily during the first hour of the drug response. Handling and intervening drug history were systematically manipulated across the three studies but did not significantly affect the overall drug response, $F(2,78) < 1$, $p > 0.10$, nor did these manipulations interact with the ACR exposure, $F(2,78) = 1$, $p > 0.10$.

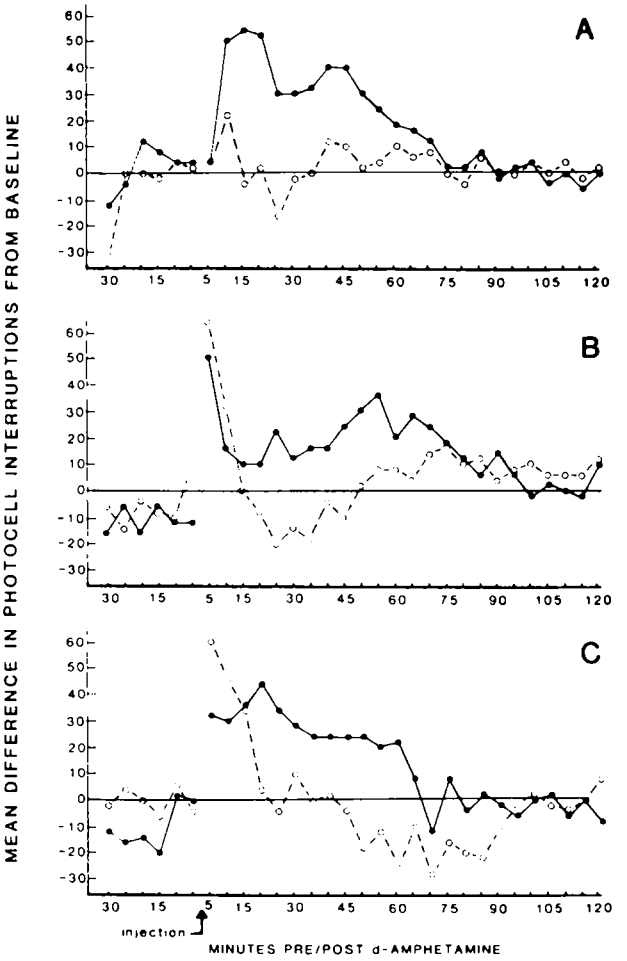


FIG. 1. Changes in spontaneous and d-A-induced locomotor activity after six weeks of acrylamide exposure. (A) Study 1, (B) Study 2, (C) Study 3 (Legends: ●—● acrylamide, — ○ — controls). See text for N's and statistical significance.

The only difference which appeared to reflect handling conditions was the failure of Study 1 rats to show a transient (first 5 minutes post-injection) increased response to the drug injection procedure upon retesting. A significant Study × time interaction, $F(46,1794) = 5.18$, $p < 0.001$, and post hoc comparisons (Newman-Keuls, $p < 0.05$) confirmed that this observation was statistically significant. Additional post hoc comparisons at other times during the session were not significant.

To establish whether the differntial drug response of ACR-exposed rats could be attributed to their moderately reduced body weights (≤3%) as compared to control animals, regression analyses were performed on activity values obtained for the first hour post-drug using orthogonal polynomials [11]. Multiple R values for 4th order polynomials were 0.12, 0.06 and 0.08, for Studies 1, 2, and 3, respectively. Goodness-of-fit tests for polynomials of each degree were all non-significant. These data indicate that the small differences in body weight could not account for the differential response to d-A.

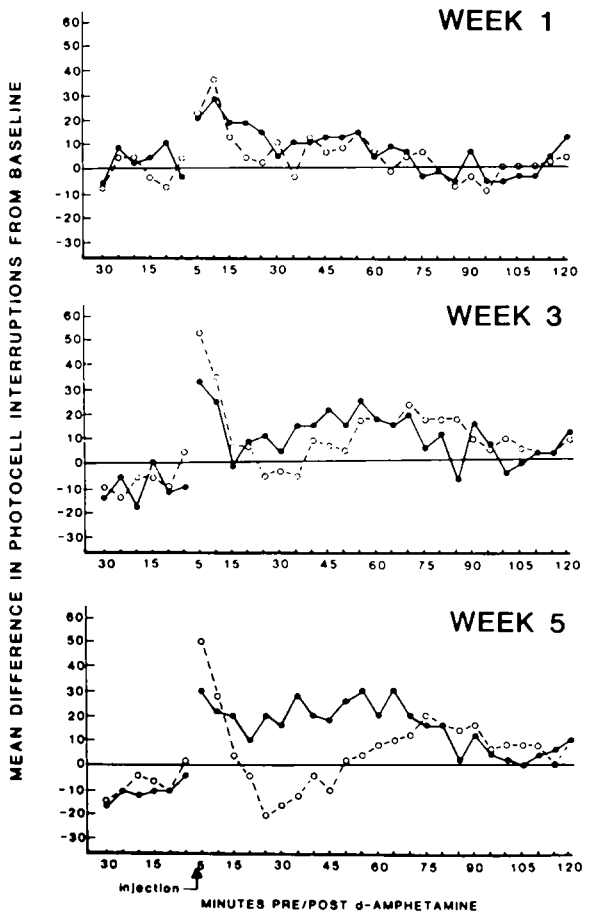


FIG. 2. Changes in spontaneous and d-A-induced locomotor activity after 1, 3 and 5-weeks of acrylamide exposure (Study 2). (Legend: ●—● acrylamide, ○--○ controls). See text for N's and statistical significance.

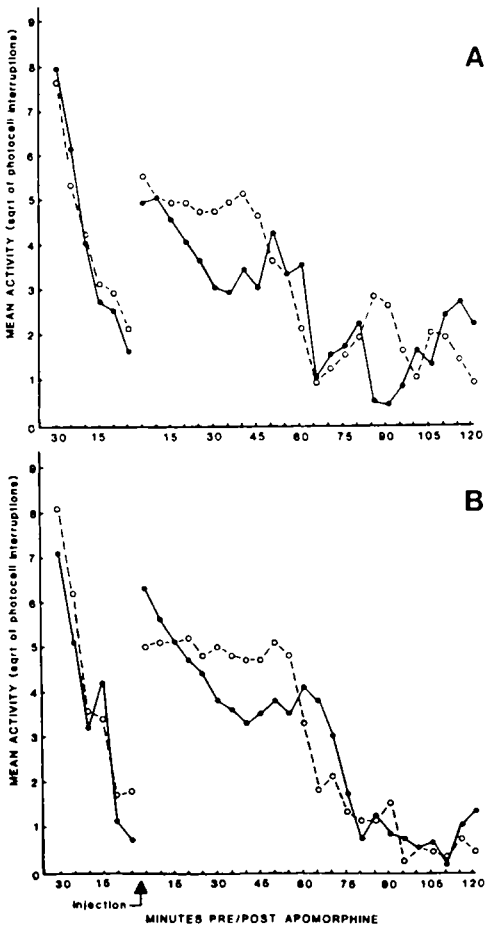


FIG. 3. Mean spontaneous and apomorphine-induced (2.5 mg/kg IP) activity after 8 weeks of exposure. (A) Study 2. (B) Study 3. (Legend ●—● acrylamide, ○—○ controls). See text for N's and statistical significance.

Figure 2 displays the development of the changing pharmacological response to d-A across the first five weeks of ACR exposure for exposed and control rats in Study 2. The enhanced response to d-A is not present during the first week of exposure and appears to develop gradually. This data was analyzed using a three-factor ANOVA with repeated measures for week of exposure and time after d-A. The analysis confirmed an overall enhanced d-A response in ACR-exposed animals, $F(23,690)=2.40$, $p<0.05$, but did not indicate a week by exposure interaction, $F(5,150)<1$, $p>0.10$.

During the same six-week period of exposure ACR-exposed and control animals receiving saline challenges (Study 3) did not differ in their locomotor activity.

Apomorphine Challenges

Figure 3 shows the locomotor activity occurring over a two-hour period following apomorphine HCl (2.5 mg/kg IP) in control rats and animals exposed to ACR for 8 weeks. The pattern of responding is similar for Studies 2 and 3 with ACR-exposed animals showing reduced activity as compared to control animals 15–50 minutes post-injection. These differences were statistically significant for Study 2 only, $F(23,690)=2.14$, $p=0.05$. Intense stereotypy was observed for all animals at the 2.5 mg/kg dose suggesting that a behavioral "ceiling" had been attained. Therefore, a second lower dose of apomorphine (0.625 mg/kg IP) was administered exactly two weeks later to Study 2 animals only. ACR-exposed rats were more responsive to apomorphine at this lower dose for about the first 30 minutes, $F(23,690)=2.40$, $p<0.01$, data not shown.

Neurochemistry

The regional endogenous concentration of the monoamine neurotransmitters, precursors and metabolites in animals given saline injections are presented in Table 2. The values for control animals are in general agreement with several other recent reports [20, 28, 35].

Separate analyses were performed for each region and compound. Each analysis consisted of a two-factor ANOVA with exposure and time after d-A (saline, 15, 50, 80, 120 min) as the between subject sources of variance. Baseline (saline) data were also evaluated separately for each region and compound using single factor ANOVAs.

Analyses reported here were computed on data expressed as $\mu g/g$ tissue. However, it should be noted that duplicate analyses on values expressed as picomoles/mg protein

TABLE 2

REGIONAL ENDOGENOUS CONCENTRATIONS OF NEUROTRANSMITTERS, PRECURSORS, AND METABOLITES (NO DRUG)

Brain Region	Trt	Tyr	NE	DA	DOPAC	HVA	3-MT	TRYP	5-HT	5-HIAA
Nucleus Accumbens	CONT	11.685±0.919	0.906±0.056	5.262 · 0.217	1.250±0.032	0.406 · 0.019	0.259 · 0.065	4.121±0.157	0.871±0.043	0.578±0.041
	ACR	10.625 · 0.621	0.691±0.114	6.275±0.578	1.316±0.060	0.497±0.038	0.364±0.010	4.036 · 0.120	0.920±0.045	0.718±0.045
Septal Area	CONT	10.775±0.746	0.856±0.073	0.760 · 0.067	0.214±0.022	0.110±0.011	N.D.	3.886±0.186	0.682±0.036	0.495±0.024
	ACR	10.211±0.374	0.858±0.062	0.785±0.196	0.255 · 0.041	0.127±0.015	N.D.	3.811±0.150	0.713 · 0.047	0.600 · 0.008*
Corpus Striatum	CONT	12.523 · 0.780	0.123±0.006	11.160 · 0.314	1.475±0.057	0.726±0.044	0.559 · 0.024	4.687 · 0.133	0.677±0.023	0.637 · 0.033
	ACR	10.949±0.327	0.131±0.010	10.130 · 0.434	1.389±0.079	0.742±0.061	0.489 · 0.020	4.048 · 0.253	0.656±0.020	0.737 · 0.036
Thalamus	CONT	11.442±0.845	0.453±0.012	N.D.	N.D.	N.D.	N.D.	4.223±0.274	0.702 · 0.056	0.691±0.040
	ACR	9.999±0.608	0.404±0.025	0.052±0.033	N.D.	N.D.	N.D.	3.888±0.238	0.644 · 0.057	0.776±0.032

Values are expressed as mean μg/g tissue · S.E.M. with N=5 for each determination.
Trt=treatment; N.D. not detectable; CONT=control; ACR=acrylamide. Refer to text for other abbreviations.
*$p<0.01$.

yielded virtually identical results. Post-hoc comparisons were made using the Newman-Keuls procedure ($p<0.05$).

DA System

DA concentrations in the striatum and nucleus accumbens increased following d-A in both control and ACR-exposed rats, $F(4,32)=6.94$, $p<0.001$; $F(4,32)=7.43$, $p<0.001$, respectively. In the nucleus accumbens (Fig. 4) the effects of d-A on DA levels interacted with ACR-exposure, $F(4,32)=2.61$, $p=0.05$. Post-hoc comparisons indicated that DA concentrations were of lesser magnitude for ACR-exposed animals at 80 and 120 minutes post-d-A. Separate F tests on baseline (saline) DA concentrations were non-significant for all regions.

Following d-A DOPAC levels in both ACR and control rats decreased from saline control values in all brain areas except the thalmus (data not shown). These decreases persisted throughout the two-hour period of assessment in the septal area and striatum, $F(4,32)=4.26$, $p<0.01$; $F(4,32)=27.65$, $p<0.001$, respectively. In the accumbens DOPAC levels of control animals returned to baseline levels by 80 minutes post-injection (Fig. 5A). However, DOPAC concentrations for ACR-exposed rats did not recover as rapidly and were significantly less than those of non-exposed controls at 80 and 120 minutes post-injection. These observations were confirmed by the significant interaction term of the ANOVA, $F(4,32)=4.49$, $p<0.01$, and by post hoc comparisons.

Concentrations of HVA showed a similar reduction following d-A administration, although these changes were somewhat delayed compared to changes in DOPAC. Reductions in HVA persisted throughout the 120-minute period of assessment in both the septal area, $F(4,32)-11.54$, $p<0.001$, and striatum, $F(4,32)=8.92$, $p<0.001$. HVA concentrations in the nucleus accumbens (Fig. 5B) showed some return toward saline control values by 120 minutes post-injection. However, levels of HVA for ACR-exposed rats did not return to baseline values and were significantly less than those of non-exposed control animals at 80 and 120 minutes post-injection. An exposure × time interaction, $F(4,32)-3.48$, $p<0.02$, and post-hoc comparisons confirmed these observations which closely paralleled the changes seen for DOPAC in these regions. Separate F-tests on baseline (saline) HVA and DOPAC concentrations were non-significant for all regions.

NE System

NE concentrations were not markedly affected by d-A in the striatum, $F(4,32)-1.36$, $p>0.10$, septal area, $F(4,36)=<1$, $p>0.10$, or thalamus, $F(4,32)=1.52$, $p>0.10$. Only in the nucleus accumbens (Fig. 6) was there a tendency for NE values to increase over the 120 minute period post-injection, $F(4,32)=2.37$, $p<0.08$. NE values for ACR-exposed animals did not increase however, and were generally less than those of non-exposed controls irrespective of the time of drug administration, $F(1,32)=10.18$, $p<0.01$. ACR-exposed rats showed similar decreases in septal NE concentrations, $F(1,32)=8.36$, $p<0.001$, data not shown.

d-A Did not significantly effect TYR levels in any brain region examined. However, for striatum, nucleus accumbens and septal area TYR values of ACR-exposed rats were reduced form those of non-exposed controls (Table 3), irrespective of the time of drug administration.

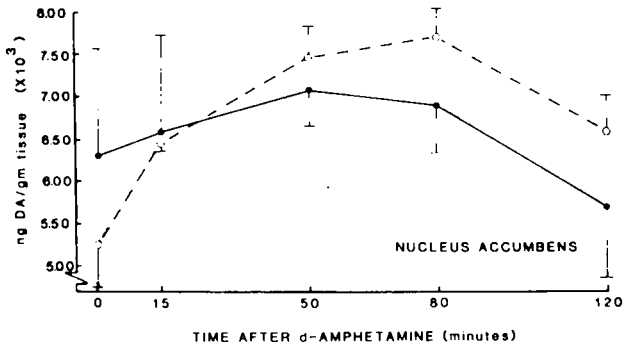


FIG. 4. DA concentrations in the nucleus accumbens for control (○- - -○) and acrylamide-exposed rats (●—●) following saline or d-A injections after 7 weeks of exposure. See text for N's and statistical significance.

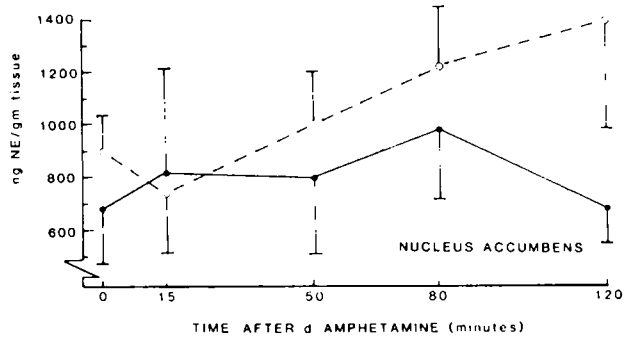
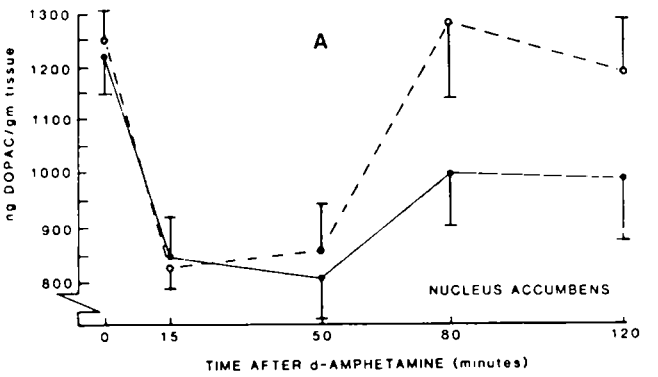


FIG. 6. Norepinephrine concentrations in the nucleus accumbens for control (○- - -○) and acrylamide exposed (●—●) rats following saline or d-A injections. See text for N's and statistical significance.



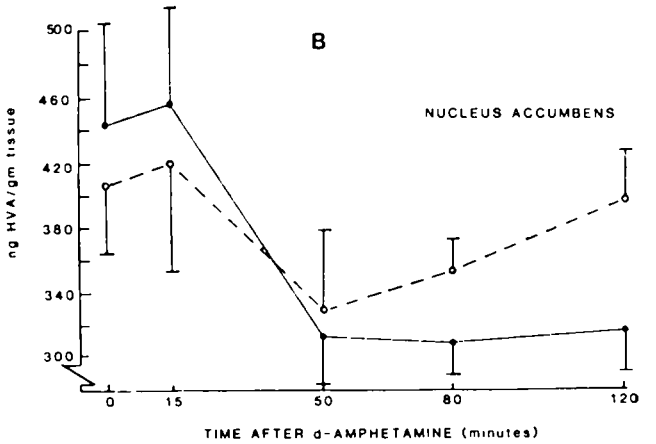


FIG. 5. (A) DOPAC and (B) HVA concentrations in the nucleus accumbens region for control (○- - -○) and acrylamide-exposed (●—●) rats following saline or d-A injections. See text for N's and statistical significance.

5-HT System

Concentrations of 5-HT were generally not affected by the d-A challenge. Only in the septal area was there a significant change in 5-HT levels following drug administration, $F(4,32)=2.89$, $p<0.05$; data not shown. Post hoc comparisons showed that 5-HT levels in the septal area were significantly reduced from baseline values at 80 and 120 minutes post-injection for control and ACR rats. However, ACR-exposed and control animals were equally responsive to d-A, $F(4,32)<1$, $p>0.10$, and no exposure-related effects were present for 5-HT concentrations in the septal area, $F(1,32)<1$, $p>0.10$, or any other region examined.

For striatum, septal area, and thalamus ACR-exposed rats had levels of 5-HIAA greater than control animals (Fig. 7 for thalamus) irrespective of drug challenge or the time at which the drug was administered, $F(1,32)=27.72$, $p<0.001$; $F(1,32)=18.92$, $p<0.001$; $F(1,32)=30.74$, $p<0.001$, for striatum, septal area and thalamus, respectively. A significant exposure × time after d-A interaction, $F(4,32)=2.73$, $p<0.05$, and post hoc comparisons also showed that 5-HIAA levels in the thalamus were transiently reduced 15 minutes post-injection for control but not for ACR-exposed rats (Fig. 7). Similar interactions were evident for 5-HIAA levels in the striatum and septal area, but approached statistical signifiance only for striatal concentrations of 5-HIAA, $F(4,32)=2.42$, $p=0.07$; data not shown.

TABLE 3

MEAN TYROSINE CONCENTRATIONS BY REGION (μg g TISSUE)†

	Striatum	Nucleus Accumbens	Septal Area
ACR	11.69 ± 1.74*	11.16 ± 1.74‡	10.94 ± 1.54§
Control	12.83 ± 1.62	12.77 ± 1.94	12.09 ± 1.79

†Mean ± standard deviation.
*$F(1,32)$ 4.49, $p<0.05$.
‡$F(1,32)=8.70$, $p<0.01$.
§$F(1,32)$ 5.77, $p<0.05$.

d-A Challenges had comparable effects on TRP levels in control and ACR-exposed rats. Significant main effects for time after drug administration, $F(4,32)=5.40$, $p<0.01$; $F(4,32)=5.70$, $p<0.01$, for nucleus accumbens and septal area, respectively, and post hoc comparisons showed TRP concentrations for both groups of animals to be elevated above saline values at 15 and 50 minutes post d-A (data not shown).

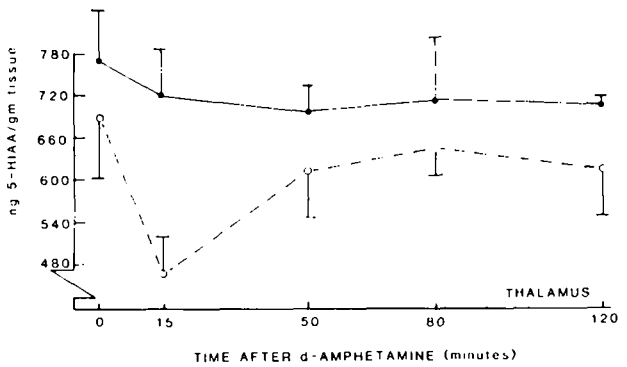


FIG. 7. 5-HIAA concentrations in the thalamus for control (○ - - - ○) and acrylamide-exposed (●—●) rats following saline or d-A injections. See text for N's and statistical significance.

Light and Electron Microscopy and Single-Nerve Fiber Preparation

No pathological findings were evident for any brain region examined or for spinal cord sections or dorsal root ganglia.

Myelin ovoids characteristic of Wallerian-type degeneration were present in approximately 10% of the axons of the sciatic nerve of ACR-exposed rats. A few fibers showed disproportionately thin myelin sheaths consistent with remyelination. A moderate degree of interfascicular edema was also present.

DISCUSSION

Chemical agents such as acrylamide which can modify the response of several interacting neuronal systems have the potential for altering an individual's emotional status, attentional capabilities, and processes by which information is acquired and stored. Untoward effects may only become manifest following periods of stress, in conjunction with aging, or as a result of concommitant exposure to other toxins or drugs. The current study used pharmacological probes to clarify the effects of acrylamide on neurotransmitter function.

We confirmed earlier observations from this laboratory [22] that ACR exposure (100 ppm in drinking water for six weeks) alters the psycho-motor stimulant properties of d-A. After six weeks of continuous exposure to ACR comparable increases in d-A-induced locomotor activity were observed for animals tested and challenged weekly with d-A (Study 2) or saline vehicle (Study 3). This finding obviates prior concern that repeated drug administration, and not ACR exposure per se, could be responsible for the observed changes in response to d-A. Comparable increases in d-A-induced locomotor activity were also observed for animals without repeated experience in the test apparatus or with the injection procedure during the period of ACR exposure (Study 1). Consequently, the effects of ACR on responsiveness to d-A were robust and unaffected by different testing histories.

The observed increases in d-A-induced locomotor activity cannot be attributed to a general increase in arousal or movement, since the spontaneous locomotor activity (i.e., under non-drugged or saline conditions) of control and ACR-exposed animals did not differ at any time. ACR was therefore acting in a more direct manner to alter the psychomotor stimulant properties of d-A.

The augmented drug response appeared to develop over repeated weeks of ACR exposure, suggesting that a critical body burden or an accruing neurochemical alteration was responsible for the observed changes in drug response. This pattern of effects is consistent with that observed previously in this laboratory [22], but is in contrast to the study by Tilson and Squibb [32] in which acutely administered ACR (12.5 mg/kg PO 24 hr pretest) was found to be sufficient to facilitate the suppressive effects of d-A on operant performance. These findings suggest that ACR may have an acute pharmacological mode of action which is distinct from its more chronic toxic effects, and that only operant conditioning procedures may be sensitive to these acute changes.

At a 2.5 mg/kg IP dose of apomorphine the locomotor response of ACR-exposed rats tended to be depressed as compared to that of control animals. This observation is consistent with previous work in which rats pretreated with single doses of 25 or 100 mg/kg of ACR were reported to show less locomotion than controls following a 1.0 mg/kg IP apomorphine HCl challenge [1]. In the current study at a lower dose of apomorphine (0.625 mg/kg IP) ACR-exposed animals showed a modest increase in their locomotor activity as compared to controls. Apomorphine has at best modest psychomotor stimulant properties yet is capable of producing intense stereotypy [9]. These properties may account for the changes in drug response observed in the current study. At the low dose increases in response to apomorphine could still be reflected in the locomotor response of ACR-exposed animals. At the higher dose stereotypy may have interfered with patterns of locomotion such that ACR-induced increases in drug responsiveness caused increases in stereotypy which interfered with locomotion and reduced the measured activity of ACR-exposed rats. Unfortunately, stereotypy was not explicitly quantified in the present experiment, and the basis of this finding must remain speculative.

In the absence of d-A challenge, there were no discernible changes in regional concentrations of DA, DOPAC, HVA, NE, or 5-HT in the current study. Previous studies have reported changes in whole brain NE and DA [3,10]. However, these changes may have been attributable to nutritional deficiencies since sizable reductions in body weight were evident in those studies. Whole brain DOPAC levels have been reported to increase [3] or to remain unchanged [18] following ACR exposure. These contradictory results may be due in part to the variety of dosage regimens employed or the use of whole brain tissue rather than more specific regional samples. It is fair to conclude that none of the previously reported changes in DA or NE systems have been consistently demonstrated.

In contrast, many of the findings of the current study are consistent with the hypothesis that ACR has an effect on serotonergic function. ACR-exposed animals had increased levels of 5-HIAA in three of the four brain regions examined independent of drug administration. This finding is consistent with the increases in whole brain 5-HIAA observed by Farr *et al.* [12]. ACR-exposed animals also did not show the decline in thalamic or striatal 5-HIAA levels exhibited by control animals 15 minutes post-d-A injection. This response in control animals may be the result of an initial d-A--induced 5-HT release with the neurotransmitter feeding back to the raphe cell bodies to reduce the neuronal firing rate (and 5-HIAA production) by a process of self-inhibition. Recent evidence has shown that d-A can inhibit the firing of 5-HT

neurons in this manner [24]. That this response was absent or markedly attenuated in ACR-exposed rats is interesting since 5-HT receptor antagonists also block the inhibitory effects of d-A on 5-HT neurons [24]. 5-HT antagonists facilitate the elicitation of d-A-produced stereotypy [5], and d-A-induced locomotor activity is facilitated by reducing serotonergic function [13]. Analogous increases in activity were observed in ACR-exposed animals. The results of the current investigation are consistent with the hypothesis that ACR antagonizes the activity of CNS neurons which utilize 5-HT for neurotransmission. Subsequent delays in the return to baseline for DOPAC and HVA levels in the accumbens after d-A injections may reflect changes which are secondary to the initial failure of the 5-HT system to respond appropriately.

Body growth rate for ACR-exposed rats was moderately decreased compared to control rates in the current studies. This observation is consistent with that of Gipon *et al.* [15], who administered ACR (20 mg/kg IP) to female rats. Reductions in body weight gain in the present study could not however be correlated with the altered psychomotor stimulant response to d-A or to the observed neurochemical changes seen in ACR-exposed rats. Similarly, Gipon *et al.* [15] found that pair-fed controls did not show the same deficits in motor performance (rotorod balance) as their ACR-exposed counterparts. Therefore, changes in body weight and food intake, while affected by ACR-exposure, cannot account for the observed changes in behavior or neurochemistry.

Signs of Wallerian-type degeneration were evident for the sciatic nerve and were generally consistent with the pattern of degeneration characteristic of a dying-back peripheral neuropathy. However, light and electron microscopy did not reveal abnormal changes for any brain region or spinal cord segment examined. These negative observations are in agreement with earlier studies (cf., [29]) but differ from reports of CNS axonal degeneration [26,29] following ACR exposure. It has also been reported that cell bodies in lumbar dorsal roots show signs of reorganization prior to overt indications of axonal degeneration [30]. This suggests that ACR may have direct effects on CNS neuronal cell bodies. However, in the current study pathological changes were not observed in lumbar dorsal root ganglia at a time when peripheral axonal degeneration in sciatic nerves was clearly apparent.

It is also noteworthy that morphological changes were not detected in the current study in any brain region observed to have neurochemical alterations. This suggests that neurochemical changes may precede gross structural disorganization, and that they can be sensitive indicators of cellular toxicity following exposure to ACR.

In summary the current study used pharmacological probes to clarify the effects of acrylamide on neurotransmitter function. The resultant changes in regional monoamine concentrations and the behavioral responses to dopamine agonists suggested that acrylamide impaired serotonergic neurotransmission. The major findings were that acrylamide: (1) increased the psychomotor stimulant effects of d-A, (2) increased levels of 5-HIAA in the striatum, thalamus and septal area, (3) attenuated the early response (< 15 minute) of forebrain 5-HIAA to d-A, (4) retarded the re-equilibration of DOPAC and HVA concentrations in the accumbens following d-A and (5) blocked the rise in accumben's NE which occurred subsequent to d-A.

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