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Individual Differences in Pavlovian Autoshaping of Lever Pressing in Rats Predict Stress-Induced Corticosterone Release and Mesolimbic Levels of Monoamines

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TOMIE, A., A. S. AGUADO, L. A. POHORECKY AND D. BENJAMIN. *Individual differences in Pavlovian autoshaping of lever pressing in rats predict stress-induced corticosterone release and mesolimbic levels of monoamines.* PHARMACOL BIOCHEM BEHAV **65**(3) 509–517, 2000.—Pavlovian autoshaping CRs are directed and reflexive consummatory responses targeted at objects repeatedly paired with rewarding substances. To evaluate the hypothesis that autoshaping may provide an animal learning model of vulnerability to drug abuse, this study relates individual differences in lever-press autoshaping CR performance in rats to stress-induced corticosterone release and tissue monoamine levels in the mesolimbic dopamine tract. Long–Evans rats (n = 14) were given 20 sessions of Pavlovian autoshaping training wherein the insertion of a retractable lever CS was followed by the response-independent presentation of food US. Large between-subjects differences in lever-press autoshaping CR performance were observed, with group high CR frequency (n = 5) performing many more lever press CRs than group low CR frequency (n = 9). Tail-blood samples were obtained before and after the 20th autoshaping session, then 24 h later the rats were sacrificed and dissection yielded tissue samples of nucleus accumbens (NAC), prefrontal cortex (PFC), caudate putamen (CP), and ventral tegmental area (VTA). Serum levels of postsession corticosterone were elevated in group high CR frequency. HPLC revealed that group high CR frequency had higher tissue levels of dopamine and DOPAC in NAC, lower levels of DOPAC/DA turnover in CP, and lower levels of 5-HIAA and lower 5-HIAA/5-HT turnover in VTA. The neurochemical profile of rats that perform more autoshaping CRs share some features of vulnerability to drug abuse. © 2000 Elsevier Science Inc.

Autoshaping Pavlovian conditioning Lever press Corticosterone Dopamine Serotonin Drug abuse Individual differences Stress Rats

TOMIE (46,47) has noted striking similarities between Pavlovian autoshaping and drug abuse. Pavlovian autoshaping procedures consist of the presentation of a highly localized visual stimulus (conditioned stimulus, CS) followed by the response-independent presentation of a rewarding substance (unconditioned stimulus, US). Repeated CS–US pairings may lead to the acquisition of the Pavlovian autoshaping conditioned response (CR), typically described as a complex sequence of motor responses directed at the CS (2,49). For example, studies reporting lever-press autoshaping in rats have employed procedures wherein the brief insertion of a retractable lever CS precedes each response-independent delivery of the food US. In rats that develop the autoshaping CR, the topography includes lever CS-directed approach responses, followed by grasping, gnawing, and chewing of the lever, typically recorded as Pavlovian lever-press autoshaping CRs [(7,31,35); for review, see (49)].

Autoshaping and drug abuse share in common procedures as well as the behavioral symptoms induced. In both, repeated pairings of a small object CS (i.e., lever or drug-taking implement) with a rewarding substance US (i.e., food or abused drug) leads to CS-directed approach, contact, and manipulation responses, which culminate in the expression of consummatory-like responses directed at the CS. The induced re-

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sponding is highly reflexive, triggered by the CS, difficult to restrain or control (26,47), and exhibits relapse-like effects, including spontaneous recovery and rapid reacquisition (51–53). The induction of autoshaping CRs, therefore, may account for several prominent features of the drug abuse syndrome, and those subjects that readily acquire autoshaping CRs may be particularly vulnerable to drug abuse (46,47).

The autoshaping account of drug abuse predicts that self-administered drugs of abuse will function as effective USs in Pavlovian autoshaping procedures. This has been recently addressed by Carroll and associates, who have engendered lever press autoshaping CRs by pairing of lever CS with intravenous drug self-administration of either amphetamine (5) or cocaine (4,5,16,44). Recent data collected in our laboratory reveal that repeated pairings of a lever CS with a sipper tube of a bottle containing either amphetamine–saccharin solution US or ethanol–saccharin solution US results in the acquisition and maintenance of Pavlovian lever press autoshaping CRs.

Additional recent work in our laboratory suggests that lever press autoshaping induced by pairings of lever CS–food US predicts subsequent ethanol drinking. Rats given lever-press autoshaping procedures were subsequently tested for drinking in the home cage of a solution consisting of 6% ethanol in 0.1% saccharin. The rats that consumed more of the saccharin–ethanol solution were those that had previously performed more lever-press autoshaping CRs (54). There are other data suggesting that ethanol may engender autoshaping CRs. For example, a small object (illumination of a small light above the sipper) can function as an effective signal for ethanol availability, engendering light signal-directed approach responses; moreover, control of approach responding by the light signal is quite robust, even when set in opposition to the location of the ethanol solution itself (13–15).

The present study asks if individual differences in rats in lever-press autoshaping CR performance induced by pairings of lever CS–food US predict individual differences in neurochemical indices proposed to index vulnerability to drug taking. Rats prone to self-administer amphetamine have been reported to exhibit more novelty stress-induced release of corticosterone (23,27,32–34). Individual differences in the pattern of endogenous corticosterone secretion also predicts individual differences in ethanol intake. For example, rats with high basal levels of corticosterone together with an attenuated rise in corticosterone output during stress are predisposed to consume more alcohol in a two-bottle choice test (40), and in adult nonhuman primates, plasma cortisol concentrations are positively correlated with alcohol consumption rate (20). These results are consistent with the finding that adrenalectomy reduces voluntary ethanol intake in rats (12,18,30), and short-term treatment with corticosterone reverses these effects (11,12).

Rats prone to self-administer amphetamine have been reported to exhibit higher levels of dopamine activity in the nucleus accumbens and striatum (27,41), but not in the prefrontal cortex (34,42), and lower levels of serotonergic activity in the mesolimbic dopamine tract (34). In addition, individual differences in the tendency to express nonregulatory ingestive-like responding, which, like autoshaping, is consummatory-like, poorly controlled, and highly variable between subjects, has also been positively correlated with forebrain dopamine (28,29).

In studies of autoshaping, large and reliable between-subjects differences in CR acquisition and asymptotic CR maintenance have been reported in a number of species, including ring doves (1), pigeons (45), and rats (26,48,50). Individual differences in Pavlovian lever-press autoshaping CR performance in rats and their relationship to novelty stress-induced corticosterone release and monoamine levels in mesolimbic dopamine neurons are explored. In the present study, the novel stressor was given immediately prior to the 20th autoshaping session, and consisted of the procedures employed to obtain tail blood samples.

METHOD

Animals

Fourteen adult male Long–Evans (Blue Spruce strain) rats obtained from Harlan–Sprague–Dawley (Almont, NY) weighing approximately 300 g were used. Rats were housed individually in suspended steel cages in a colony room with a 12 L:12 D (on 0200 h) cycle. Rats had continuous access to water in their home cages and were maintained at 80% of their free-feeding body weights by providing supplemental rat chow after each daily session, as needed. Principles of laboratory animal care (ILAR Guide for the Care and Use of Laboratory Animals) were followed.

Apparatus

Autoshaping chambers were four Plexiglas cubicles (23 × 23 × 21 cm) for rats, with stainless steel grid floors, enclosed in sound-attenuating, ventilated outer casings. One house light (GE 1821) was mounted directly above the operant chamber, on the ceiling of the outer hull. The front panel of each chamber was equipped with a retractable lever (BRS/LVE #RRL/005), mounted 8.5 cm above the floor and 7 cm off to the left side of the center line. A food receptacle was mounted on the centerline of the front panel, 3 cm above the floor. Operation of a PDC/PPD pellet dispenser delivered 45 mg food pellets (BioServe, Frenchtown, NJ) into the food receptacle. Masking noise (88 dB, linear scale) was provided by the operation of ventilating exhaust fans mounted on the outer hull. Session events and data collection were controlled by an IBM PC.

Autoshaping Procedures

Rats were run 5–6 days per week between 0900–1200 h (during the light cycle), and received a total of 20 daily sessions of autoshaping. Prior to each autoshaping session, rats were weighed, then, for reasons unrelated to the present study, were given an intraperitoneal injection of 0.9% saline (l ml/kg injection volume), then immediately placed in the autoshaping chamber. Each autoshaping trial consisted of the insertion of the stainless steel lever (CS) into the chamber for 5 s. Withdrawal of the lever was followed immediately by the response-independent operation of the pellet dispenser for 0.70 s, resulting in the delivery of one 45-mg food pellet (US). Each autoshaping session consisted of 25 autoshaping trials, wherein the lever CS and the food US were presented in a paired fashion. The mean interval separating trials was 60 s, with a minimum intertrial interval of 45 s and a maximum intertrial interval of 75 s. The session duration was approximately 30 min. The total number of lever press responses for each subject was recorded on each trial.

Immediately prior to the last (20th) autoshaping session, the rat was manually restrained and a scalpel was used to remove the last 5–10 mm of the tip of the tail. One 100-μl sample of tail blood was collected to assess the presession (basal) level of corticosterone. Immediately following the 20th au-

toshaping session, another 100 microliter sample of tail blood was obtained to assess the postsession (stress) level of corticosterone. For all rats, latency to collect the tail blood samples following the incision was approximately 1–2 min, and systematic group differences in this interval of time were not apparent. Twenty-four hours after the 20th autoshaping session, all rats were sacrificed by rapid decapitation, and dissected tissue samples were stored in liquid nitrogen.

Corticosterone Assay

Blood samples for corticosterone assay were collected in heparinized tubes. Plasma, obtained after centrifugation, was stored at −20°C until assay. Plasma corticosterone was measured by radioimmunoassay (RIA kit, ICN Biomedicals Inc., Los Angeles, CA) using a highly specific corticosterone antiserum with a detection threshold of 0.1 μg/100 ml.

Dissection of the Brain

Animals were sacrificed by rapid decapitation 24 h after the 20th session of autoshaping. The brains were removed, placed on the dorsal surface, and tissue samples of the nucleus accumbens (NAC), prefrontal cortex (PFC), caudate putamen (CP), and ventral tegmental area (VTA) were dissected out, placed in aluminum foil, then stored in liquid nitrogen until assayed according to procedures described elsewhere (10,19). The brains were dissected with the initial coronal slice taken approximately 2.0 mm anterior to the hypothalamus. The next slice was taken directly anterior to the hypothalamus. The CP was then removed from the caudal surface of this slice of brain, based on its distinct morphological appearance. The CP included tissue dorsal to the anterior commissure, ventral to the corpus callosum and medial to the external capsule.

Neurochemical Determinations

Tissue concentrations of dopamine, serotonin, and their metabolites were determined by reverse-phase high-pressure liquid chromatography with electrochemical detection (10). Each tissue sample (30–50 mg) was homogenized in 10 vol (w/v) of 0.4 N perchloric acid and then centrifuged at a temperature of 4°C for 20 min at 15,000 × *g*. The supernatant was assayed on a BAS HPLC system, equipped with a Spectra-Physics model SP8770 dual-piston pump. The sample was delivered through a high-pressure valve, fitted with a 20-μl sample loop, onto a Biophase ODS C-18 reverse-phase column (Bioanalytical Systems, West Lafayette, lN; 5 μm, 250 × 4.6 mm i.d.). The detector (C-4B; BAS) was set at a range of 50 nA for dopamine (10 nA for serotonin) and the sample was oxidized with a +0.72 V potential between the glassy carbon electrode and the Ag/AgCI reference electrode. The filtered and degassed mobile phase consisted of 0.10 M sodium phosphate dibasic and 10% methanol (v/v). The mobile phase was pumped in at a rate of 1.0 ml/min. Quantification was against external standards for dopamine or serotonin. Tissue levels of the following monoamines were determined: dopamine (DA), 3,4-dihydoxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA).

Statistical Analysis

Correlation-regression analyses (SYSTAT) provided Pearson's product-moment correlation coefficients relating each subject's mean CR frequency score during autoshaping sessions 1–10 (autoshaping CR acquisition) or during autoshaping sessions 15–19 (asymptotic autoshaping CR maintenance) to that subject's tissue levels of monoamines in each brain sample (NAC, PFC, CP, VTA) and to that subject's corticosterone levels (presession, postsession) and to changes in corticosterone levels (corticosterone change = postsession minus presession corticosterone). Stepwise multiple regression analyses (SYSTAT) provided models of the neurochemical profile of subjects that performed more autoshaping CRs during autoshaping sessions 1–10 and 15–19. For each subject, for each session, the total number of lever-press CRs was derived (CR frequency). Effects of group (high CR frequency vs. Low CR frequency) on mean CR frequency during autoshaping sessions 1–20 were assessed by two-way repeated-measures analysis of variance using MANOVA (SYSTAT). Effects of group (high CR frequency vs. low CR frequency) on mean tissue levels of monoamines, turnover, and on levels of corticosterone were assessed by one-way analysis of variance (ANOVA, SYSTAT).

RESULTS

To evaluate relationships between autoshaping CR performance and neurochemical indices (corticosterone and monoamine levels), the data were subjected to two types of inferential statistical analyses. First, correlation-regression analyses were employed, using an alpha level of 0.05, to relate individual subject autoshaping CR frequency scores to neurochemical indices. To confirm trends identified by correlation-regression, analysis of variance was employed, using an alpha level of 0.10, to evaluate if rats assigned to groups based on their autoshaping CR performance (high vs. low CR frequency) also differ in neurochemical indices.

Neurochemical Correlates of Autoshaping

Correlation-regression analyses revealed that an individual rat's mean autoshaping CR Frequency during sessions 1–10 (autoshaping acquisition) was significantly positively correlated with postsession corticosterone levels and with changes in corticosterone levels (ps < 0.05) (Table 1). These analyses also revealed that an individual rat's mean autoshaping CR frequency during sessions 1–10 (acquisition) was significantly positively correlated with DA levels in NAC ($p < 0.05$) and significantly negatively correlated with DOPAC/DA turnover ratio in CP ($p < 0.01$). Moreover, an individual rat's mean autoshaping CR frequency during sessions 1–10 (acquisition) was significantly negatively correlated with tissue levels of 5-HIAA in VTA and significantly negatively correlated with 5-HIAA/5-HT turnover ratio in VTA (ps < 0.05) (Table 2). Stepwise multiple regression analysis revealed that three factors accounted for over 87% of the variance in autoshaping performance during sessions 1–10 (acquisition). The neurochemical profile of the rats that performed more autoshaping CRs during sessions 1–10 (i.e., more rapidly acquired the lever-press autoshaping CR) were high in postsession levels of corticosterone, low in DOPAC/DA turnover in CP, and high in DA levels in NAC.

Correlation-regression analyses revealed autoshaping CR frequency during sessions 15–19 (asymptotic autoshaping) was not significantly correlated with presession corticosterone levels, postsession corticosterone levels, or changes in corticosterone levels (Table 1). These analyses revealed autoshaping CR frequency during sessions 15–19 (asymptote) was significantly positively correlated with DA levels in NAC ($p < 0.05$), significantly positively correlated with DOPAC

TABLE 1
CORTICOSTERONE–AUTOSHAPING CORRELATIONS

	Autoshaping Sessions	
Corticosterone	1–10	15–19
Presession	0.132	0.031
Postsession	0.658*	0.283
Change (Post-pre)	0.599*	0.275

Pearson's product-moment correlation coefficients relating mean autoshaping CR frequency scores during autoshaping sessions 1–10 (acquisition) and during autoshaping sessions 15–19 (asymptote) to corticosterone levels. Presession corticosterone levels were determined from samples taken immediately before the 20th autoshaping session. Postsession corticosterone levels were determined from samples taken immediately after the 20th autoshaping session. Post-pre (change) in corticosterone levels was derived by subtracting each subject's presession corticosterone level from that subject's postsessoin corticosterone level (Post-pre). The asterisk (*) indicates that the correlation coefficient is statistically significant at the 0.05 level of confidence.

levels in NAC ($p < 0.01$), and significantly negatively correlated with DOPAC/DA turnover ratio in CP ($p < 0.01$). Moreover, autoshaping CR frequency during sessions 15–19 (asymptote) was significantly negatively correlated with 5-HIAA levels in VTA ($p < 0.05$) (Table 3). Stepwise multiple regression analysis revealed that two factors accounted for over 94% of the variance in autoshaping CR performance during sessions 15–19 (asymptote). The neurochemical profile of the rats that performed more autoshaping CRs during sessions 15–19 (i.e., maintained higher asymptotic levels of lever-press autoshaping CR performance) were low in DOPAC/DA turnover in CP and low in 5-HIAA/5-HT turnover in VTA.

Group Differences in Neurochemistry

Because there were large between-subject differences in the acquisition and maintenance of autoshaping CR performance, subjects were divided into two groups based on their mean CR frequency scores during sessions 1–10. Five subjects (high CR frequency) had mean CR frequency scores during sessions 1–10 greater than or equal to 10.0 (mean = 31.04, SEM = 7.93) and nine subjects (low CR frequency) had scores less than 10.0 (mean = 1.74, SEM = 0.57). Analysis of autoshaping CR frequency during sessions 1–20 (Fig. 1) revealed a significant effect of groups, $F(1, 12) = 71.50$, $p < 0.01$, a significant effect of sessions, $F(19, 228) = 3.03$, $p < 0.01$, and a significant groups by sessions interaction effect, $F(19, 228) = 3.33$, $p < 0.01$.

To validate the significant relationships between autoshaping and neurochemistry that had been documented previously in the correlation-regression analyses, the neurochemical data were evaluated for group differences using analysis of variance techniques with an alpha level of 0.10. These analyses substantiate the statistical significance of many of the functional relationships between autoshaping and neurochemistry that were previously identified by the correlation-regression techniques. ANOVA revealed that the groups did not differ in presession levels of corticosterone; however, the groups did differ significantly in postsession levels of corticosterone, $F(1, 12) = 4.27$, $p < 0.07$, and in change in corticosterone levels (postsession minus presession), $F(1, 12) = 3.56$, $p < 0.08$ (Fig. 2).

TABLE 2
MONOAMINE–AUTOSHAPING CORRELATIONS
AUTOSHAPING SESSIONS 1–10

	Brain Region			
Monoamine	NAC	PFC	CP	VTA
DA	0.671*	−0.417	0.213	−0.022
DOPAC	0.519	−0.322	−0.429	−0.183
DOPAC/DA	0.037	0.294	−0.704†	−0.387
HVA	0.274	−0.299	−0.132	0.031
HVA/DA	−0.171	−0.069	−0.333	−0.134
5-HT	0.164	−0.176	0.031	−0.413
5-HIAA	−0.334	−0.415	−0.250	−0.586*
5-HIAA/5-HT	−0.525	−0.521	−0.408	−0.630*

Pearson's product-moment correlation coefficients relating mean autoshaping CR frequency scores during autoshaping sessions 1–10 (acquisition) to monoamine levels in NAC (nucleus accumbens), PFC (prefrontal cortex), CP (caudate putamen), and VTA (ventral tegmental area). The DOPAC/DA turnover ratio was derived by dividing for each subject the tissue level of DOPAC by the tissue level of DA. The 5-HIAA/5-HT turnover ratio was derived by dividing for each subject the tissue level of 5-HIAA by the tissue level of 5-HT. The asterisk (*) indicates that the correlation coefficient is statistically significant at the 0.05 level of confidence. The dagger (†) indicates that the correlation coefficient is statistically significant at the 0.01 level of confidence.

TABLE 3
MONOAMINE–AUTOSHAPING CORRELATIONS
AUTOSHAPING SESSIONS 15–19

	Brain Region			
Monoamine	NAC	PFC	CP	VTA
DA	0.674*	−0.367	0.335	−0.264
DOPAC	0.732*	−0.307	−0.473	−0.404
DOPAC/DA	0.199	0.159	−0.836*	−0.452
HVA	0.404	−0.295	−0.015	−0.297
HVA/DA	−0.133	−0.173	−0.253	−0.368
5-HT	0.189	−0.276	0.067	−0.574
5-HIAA	−0.196	−0.375	−0.289	−0.624*
5-HIAA/5-HT	−0.413	−0.147	−0.513	−0.461

Pearson's product-moment correlation coefficients relating mean autoshaping CR frequency scores during autoshaping sessions 15–19 (asymptote) to monoamine levels in NAC (nucleus accumbens), PFC (prefrontal cortex), CP (caudate putamen), and VTA (ventral tegmental area). The DOPAC/DA turnover ratio was derived by dividing for each subject the tissue level of DOPAC by the tissue level of DA. The 5-HIAA/5-HT turnover ratio was derived by dividing for each subject the tissue level of 5-HIAA by the tissue level of 5-HT. The asterisk (*) indicates that the correlation coefficient is statistically significant at the 0.05 level of confidence.

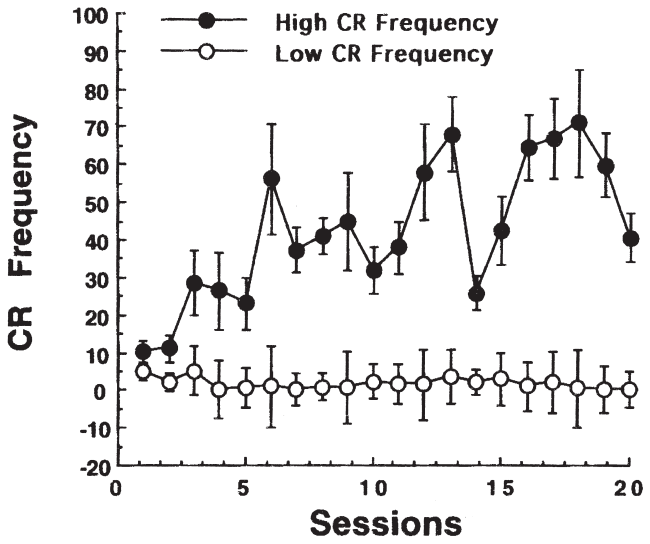


FIG. 1. Mean lever press autoshaping CR frequency scores for groups high CR Frequency and low CR Frequency as a function of autoshaping sessions 1–20. The vertical bars represent the standard errors of the mean (SEM).

Group Differences in Monoamines

Separate analyses were performed to evaluate effects of groups (high CR frequency vs. low CR frequency) on tissue monoamine levels in each of the four brain regions (NAC, PFC, CP, VTA). There were also significant group differences in tissue levels of DA in NAC, $F(1, 10) = 3.50$, $p < 0.10$, and the groups did not differ in DA levels in PFC, CP, or VTA (all ps > 0.20) (Fig. 3, upper panel). There were significant group differences in tissue levels of DOPAC in NAC, $F(1, 10) = 13.23$, $p < 0\ 01$; and the groups did not differ in DOPAC levels in PFC, CP, or VTA (all Fs < 1) (Fig 3, middle panel). There were significant group differences in DOPAC/DA turnover ratio in CP, $F(1, 11) = 4.10$, $p < 0.07$, and the groups did not differ in DOPAC/DA turnover ratio in NAC, PFC, or VTA (all ps < 0.20) (Fig 3, lower panel). ANOVAs revealed no group differences in tissue levels of HVA or in HVA/DA turnover ratio in any of the four brain regions (all Fs < 1) (not shown in figure).

ANOVA revealed no significant group differences in tissue levels of 5-HT in any of the four brain regions (all ps > 0.20) (Fig. 4, upper panel). There were significant group differences in tissue levels of 5-HIAA in VTA, $F(1, 10) = 6.01$, $p < 0.05$, and no significant group differences in tissue levels of 5-HIAA in NAC, PFC, or CP (all ps > 0.10) (Fig. 4, middle panel). There were significant group differences in 5-HIAA/5-HT turnover ratio in VTA, $F(1, 10) = 5.73$, $p < 0.05$, and no significant group differences in 5-HIAA/5-HT turnover ratio in NAC, PFC, or CP (all ps > 0.10) (Fig. 4, lower panel).

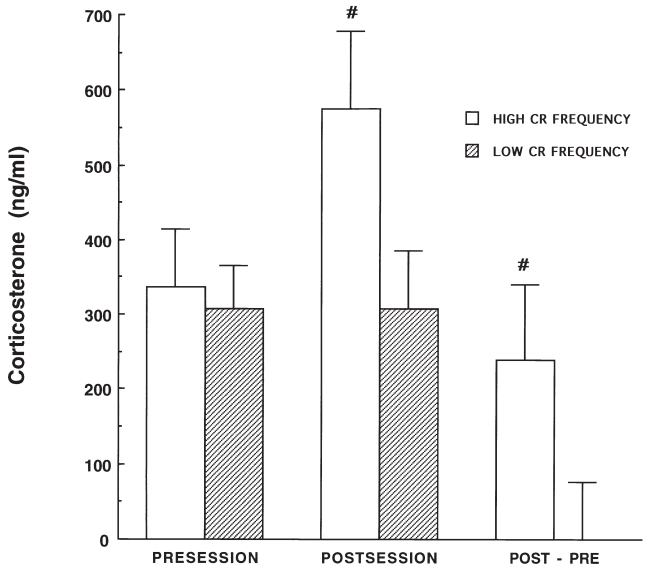


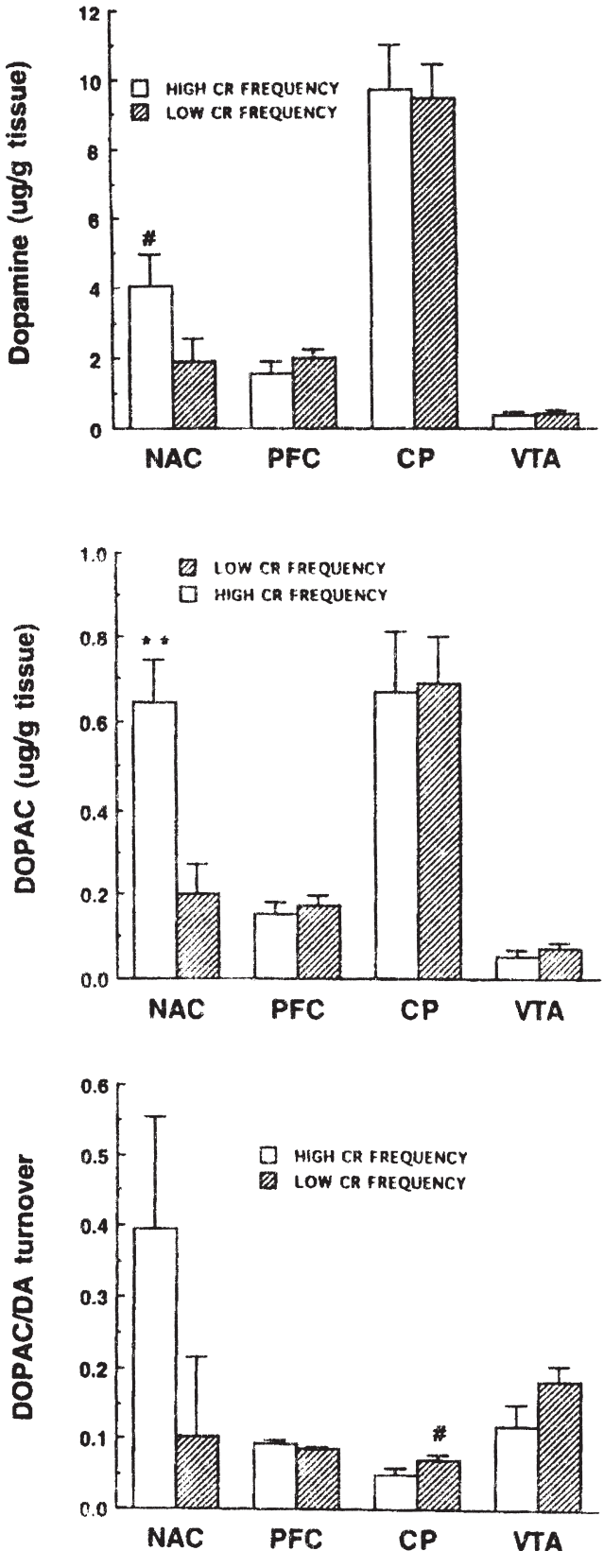
FIG. 2. For groups high CR frequency and low CR frequency, nanograms of corticosterone per milliliter of serum in samples obtained by tail cuts immediately before (PRESESSION) or immediately after (POSTSESSION) the 20th autoshaping session. Change in corticosterone was derived by subtracting for each subject the PRESESSION corticosterone level from the POSTSESSION corticosterone level (POST–PRE). The (#) indicates that the group differences are statistically significant at the 0.10 level of confidence.

DISCUSSION

Rats that more rapidly acquired the lever press autoshaping CR (higher CR frequency during sessions 1–10) showed higher stress-induced corticosterone release, lower DOPAC/DA turnover ratios in CP, and lower tissue levels of 5-HIAA in VTA as well as lower 5-HIAA/5-HT turnover ratios in VTA. A three-factor neurochemical model (high postsession corticosterone, low DOPAC/DA turnover in CP, and high DA in NAC) accounted for over 87% of the variance in autoshaping acquisition. Rats that maintained higher asymptotic levels of lever-press autoshaping CR performance (higher CR frequency during sessions 15–19) showed higher tissue levels of DA and DOPAC in NAC, lower DOPAC/DA turnover ratios in CP, and lower levels of 5-HIAA in VTA. A two-factor neurochemical model (low DOPAC/DA turnover ratio in CP and low 5-HIAA/5-HT turnover ratio in VTA) accounted for over 94% of the variance in asymptotic autoshaping performance.

The neurochemical correlates of acquisition and asymptotic performance are not identical; nevertheless, there is a high degree of congruence in the directions of the correlations relating early and later autoshaping performance and neurochemical indices. For example, stress-induced corticosterone release was positively correlated with CR acquisition and with asymptotic CR performance, but only in the former case was the correlation significant. In addition, 5-HIAA/5-HT turnover is negatively correlated with CR acquisition and asymptotic CR performance, but only in the former case was the correlation significant. On the other hand, tissue levels of DOPAC in NAC were positively correlated with CR acquisition and asymptote, but only in the latter case was the correlation significant.

Pavlovian investigators have distinguished between different stages of the learning process. CR acquisition presumably reflects the learning of the CS–US association, while asymptotic CR performance presumably reflects the tuning or adaptation of the expression of the somatomotor performance (39). These results reveal that the neurochemical correlates of CR acquisition and asymptotic CR performances were, with some degree of latitude, quite similar, and this is not unex-



pected in view of the high degree of stability observed across sessions in between-subjects differences in lever pressing.

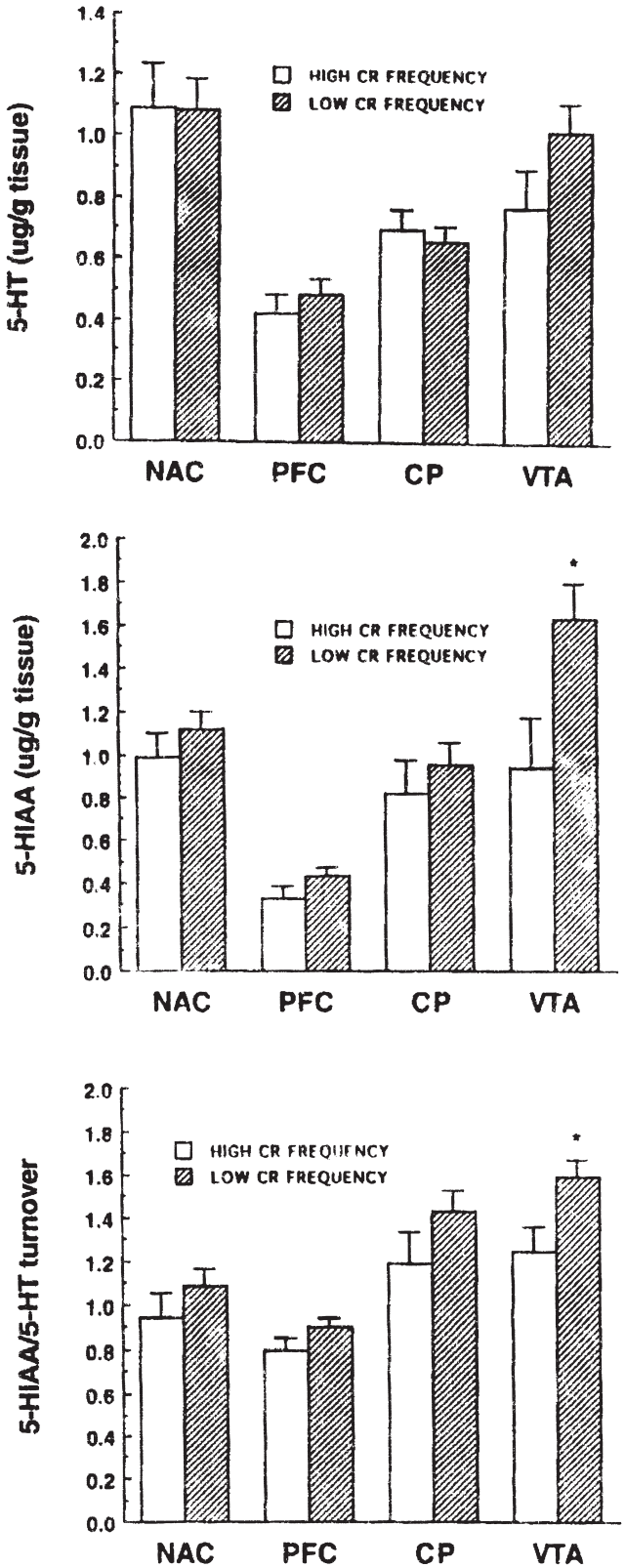
Relationships between autoshaping CR performance and neurochemistry that were identified by correlation-regression analyses were reevaluated using ANOVA. Group high CR frequency yielded higher postsession corticosterone levels and larger changes in corticosterone levels between the presession and postsession assays than did group low CR frequency. Group high CR frequency also provided higher mean tissue levels of DA and DOPAC in NAC, lower mean DOPAC/DA turnover ratios in CP, and lower mean tissue levels of 5-HIAA and lower mean 5-HIAA/5-HT turnover ratios in VTA. Thus, each of the effects identified by correlation/regression analysis was verified by ANOVA.

There are similarities between the neurochemical profile of autoshaping and pathophysiological markers of vulnerability to amphetamine self-administration. Both are positively correlated with stress-induced corticosterone release, increases in indices of DA functioning in NAC, and decreases in indices of 5-HT functioning in VTA. Nevertheless, more detailed analysis reveals that the neurochemical profiles differ in several respects. For example, autoshaping was nonsignificantly negatively correlated with tissue levels of DA and DOPAC in PFC, while Piazza and his associates have reported significantly lower levels of DA activity in PFC in rats vulnerable to amphetamine self-administration (34,42). This discrepancy may be due to the use of food deprivation in the present study. Food deprivation has stress-like effects, raising DA and DOPAC levels in PFC (3), which may, in turn, limit the degree to which the tail-cut stressor could produce still further effects. This would be expected to blunt the range of individual differences and reduce the likelihood of observing correlations.

The use of food deprivation in the present study may also have contributed to the discrepancy between autoshaping and amphetamine self-administration studies in tissue levels of DOPAC in CP. Although autoshaping was unrelated to DOPAC levels in CP, rats prone to self-administer amphetamine show higher tissue levels of DOPAC in CP (34). Biochemical studies have suggested that DA release in the prefrontal cortex exercises an inhibitory control on DA transmission in subcortical structures, including CP (24,25,43). Food deprivation would, therefore, be expected to contribute to a reduction in DOPAC levels in CP [cf. (21)], which may mask this effect of the tail-cut stressor, thereby reducing the range of individual differences and reducing the likelihood of observing correlations.

Overall, lower 5-HT, 5-HIAA, and 5-HIAA/5-HT turnover has been observed in rats high in autoshaping or am-

FIG. 3. (Upper panel): for groups high CR frequency and low CR frequency, mean levels of DA expressed as micrograms of DA per gram of tissue in NAC (nucleus accumbens), PFC (prefrontal cortex), CP (caudate putamen), and VTA (ventral tegmental area). (Middle panel): for groups high CR frequency and low CR frequency, mean levels of DOPAC expressed as micrograms of DOPAC per gram of tissue in the four brain regions. (Bottom panel): for groups high CR Frequency and low CR Frequency, mean DOPAC/DA turnover ratio, derived by dividing for each subject the tissue level of DOPAC by the tissue level of DA in the four brain regions. The vertical bars represent the standard errors of the mean (SEM). The (#) indicates that the group differences are statistically significant at the 0.10 level of confidence. The double asterisk (**) indicates that the group differences are statistically significant at the 0.01 level of confidence.



phetamine self-administration. Tissue levels of 5-HIAA and 5-HIAA/5-HT turnover were significantly negatively correlated with autoshaping, and tissue levels of 5-HT and 5-HIAA in VTA were significantly negatively correlated with amphetamine self-administration (34). This suggests that autoshaping and amphetamine self-administration are more highly expressed by rats with reduced serotonergic activity. This result is consistent with the role ascribed to serotonergic systems in psychomotor stimulant reinforcement and dopamine activation (22).

It is appropriate to consider alternative interpretations of the observed correlational relationships between autoshaping and neurochemical indices. For example, the high CR frequency rats perform more lever press responses, and the motor activity per se may induce release of corticosterone and influence tissue levels of catecholamines (9). Although systematic assessment of gross motor activity were not recorded in this study, obvious differences between groups during the autoshaping sessions was not apparent. Autoshaping investigators have reported some evidence of small but statistically significant negative correlations between measures of gross motor activity and measures of autoshaping in pigeons and ring doves (1), and context-change manipulations that reduce gross motor activity often increase the performance of autoshaping CRs (49). Thus, it seems unlikely that the relationships between autoshaping and neurochemical indices are due to similar and concomitant group differences in gross motor activity per se.

Another possibility is that the high CR frequency rats may be frustrated because lever pressing does not increase the frequency of food presentation. According to this view, the well-documented relationships between frustration effects and corticosterone release (6,8) and frustration effects and tissue catecholamine levels (9) may mediate the effects reported here. On the other hand, corticosterone release is typically observed when reward schedules are changed to extinction (9) or from higher to lower levels of reward (17), and neither of these types of changes were employed in the present study, where food presentations occurred at the end of each trial throughout the study regardless of whether or not the lever pressing response was performed.

Recent work in our laboratory suggests that autoshaping is predictive of ethanol drinking in rats. Rats given lever-press autoshaping procedures were subsequently tested for drinking in the home cage of a solution consisting of 6% ethanol in .1% saccharin. The rats that performed more lever-press autoshaping CRs subsequently drank more of the saccharin–ethanol solution (54). In addition, another recent study reveals that rats that perform more lever-press autoshaping CRs are likely to be more impulsive, as measured by the tendency to chose small immediate rewards rather than larger

FIG. 4. (Upper panel): for groups high CR frequency and low CR frequency, mean levels of 5-HT expressed as micrograms of 5-HT per gram of tissue in NAC (nucleus accumbens), PFC (prefrontal cortex), CP (caudate putamen), and VTA (ventral tegmental area). (Middle panel): for groups high CR frequency and low CR frequency, mean levels of 5-HIAA expressed as micrograms of 5-HIAA per gram of tissue in the four brain regions. (Bottom panel): for groups high CR frequency and low CR frequency, mean 5-HIAA/5-HT turnover ratio, derived by dividing for each subject the tissue level of 5-HIAA by the tissue level of 5-HT in the four brain regions. The vertical bars represent the standard errors of the mean (SEM). The single asterisk (*) indicates that the group differences are statistically significant at the 0.05 level of confidence.

delayed rewards (48). This link between autoshaping and impulsivity is particularly interesting in view of the recent reports linking impulsivity to ethanol consumption (36,38). Specifically, Poulos and his associates have shown that impulsive rats that choose small immediate rewards over larger delayed rewards subsequently consume more ethanol. Their work reveals that impulsivity and ethanol drinking are linked phenomena (37), and provides empirical support for the hypothesis that autoshaping, a form of impulsive responding, is linked to the tendency to consume abused drugs. The results of the present study add to the growing body of evidence suggesting that autoshaping and drug abuse may be related phenomena.

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