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NEUROCHEMICAL CHANGES CORRELATED WITH BEHAVIOR MAINTAINED UNDER FIXED-INTERVAL AND FIXED-RATIO SCHEDULES OF REINFORCEMENT

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Key pecking of 4 pigeons was maintained under a multiple 3-min fixed-interval, 30-response fixed-ratio schedule of food presentation. Only one schedule was in effect during an experimental session, and each was correlated with a different keylight stimulus and location (left vs. right). The different schedule components alternated across days or weeks. Cerebrospinal fluid was collected from chronically implanted intracerebroventricular cannulae following sessions with the different schedules, as well as following sessions in which reinforcement was withheld (extinction), when response-independent food was delivered, and when the experimental chamber was dark and there were no scheduled events. Metabolites of the neurotransmitters serotonin, norepinephrine, and dopamine were assayed in cerebrospinal fluid using high-performance liquid chromatography with electrochemical detection. Compared to the fixed-ratio condition, responding maintained under the fixed-interval schedule resulted in consistently higher levels of the serotonin metabolite 5-hydroxyindoleacetic acid and of the dopamine metabolite homovanillic acid in all pigeons. Levels of 3-methoxy-4-hydroxyphenylethylene glycol, a metabolite of norepinephrine, and dihydroxyphenylacetic acid, another dopamine metabolite, were also higher in 3 of the 4 pigeons following exposure to the fixed-interval schedules when compared to levels of these metabolites after exposure to the fixed-ratio schedule. Extinction of fixed-ratio responding resulted in large increases in 5-hydroxyindoleacetic acid compared to levels of this metabolite under the fixed-ratio schedule, whereas this serotonin metabolite decreased during extinction of responding under the fixed-interval schedule. Control procedures suggested that the neurochemical changes were not related to the rate of responding but were a function of the specific experimental conditions. Distinctive neurochemical changes that accompany schedule-controlled responding show the sensitivity of the neurochemical environment to behavioral contingencies and demonstrate further the profound impact that such contingencies have on biobehavioral processes.

Key words: schedule-controlled responding, neurochemistry, fixed-interval schedules, fixed-ratio schedules, behavioral neurochemistry, response-independent reinforcement, extinction, key peck, pigeons

Schedules of reinforcement have been shown repeatedly to be of fundamental importance in determining the temporal and intensive structure of behavior. Schedule-controlled rates and patterns of responding also contribute significantly in determining the behavioral effects of other variables such as noxious stimuli and drugs (Kelleher & Morse, 1968; Morse & Kelleher, 1970, 1977). Drugs such as amphetamine and pentobarbital have been shown to produce different effects depending on whether responding is maintained by fixed-ratio (FR) or fixed-interval (FI) schedules of reinforcement (Dews, 1955, 1958).

Nearly 20 years ago, Seiden, Sparber, and their colleagues (e.g., see reviews by Seiden, MacPhail, & Oglesby, 1975, and by Sparber, 1975) initiated experiments designed to examine neurochemical changes correlated with different types of schedule-controlled behavior. Studies by Seiden's group used drugs such as alpha-methyltyrosine, which blocks the synthesis of catecholamines, to determine whether performances under FI or FR schedules were differentially affected and also whether catecholamine content in the brains of rats responding under these schedules was different. Schoenfeld and Seiden (1969), for example, found that alpha-methyltyrosine produced large decreases in responding maintained at high rates under FR schedules, whereas lower rates of responding maintained under FI schedules were reduced to a lesser extent. Despite the different effects of alpha-methyltyrosine on responding under FI and FR sched-

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ules, catecholamine contents in the brains of rats that had responded under the different schedules were similar.

Schoenfeld and Seiden (1969) and Lewy and Seiden (1972) also used tritiated norepinephrine ([^{3}H]NE) to study the activity of this neurotransmitter in different regions of the brain in rats responding under a variable-interval (VI) schedule of water reinforcement. Compared to a control group of rats that was deprived of water but not trained under the VI schedule, [^{3}H]NE activity in certain areas of the brain increased after responding under the VI schedule. Thus, neurochemical activity was modified subsequent to responding under a schedule of reinforcement and, although the specific factors responsible for this effect (e.g., activity involved in responding, history of intermittent reinforcement, etc.) were not clear, it was suggested that schedule-related neurochemical changes might contribute to the differential effects of drugs found under different schedules of reinforcement (Lewy & Seiden, 1972).

Sparber and his colleagues (e.g., Sparber & Tilson, 1972; Tilson & Sparber, 1970, 1972) employed a different procedure to examine neurochemical changes correlated with schedule-controlled behavior. Using a push–pull cannula technique developed by Gaddum (1961), these investigators perfused the lateral ventricle of rats responding under various FR schedules, as well as under other procedures, with radiolabeled NE. The push–pull cannula in these studies was constructed from two pieces of concentric tubing with the inner tubing extending beyond the larger outside tubing. As the perfusion medium was infused through the inner cannula, it mixed with fluid in the extracellular space that was withdrawn at the same rate from the outer tubing and collected for later analysis. These efforts were the first attempt to measure simultaneously ongoing operant behavior and neurochemistry. Sparber and his colleagues found that [^{3}H]NE activity, as measured by elevated levels in the perfusate, increased in rats responding under an FR schedule when reinforcement was discontinued during extinction and also found neurochemical changes in the presence of stimuli correlated previously with the presentation of electric shock.

Although providing valuable information about behaviorally induced neurochemical changes, the techniques used by Seiden and Sparber had certain limitations. Experiments measuring turnover and metabolism necessitated that the rat be sacrificed to obtain this information. Repeated measures on the same subject, therefore, were not possible, nor could the same subject be examined under multiple conditions. Although the push–pull perfusion procedure permits the collection of perfusates from the brains of behaving animals under different experimental conditions, because of tissue damage surrounding the perfusion site, the longevity of this technique is quite short, thereby precluding the collection of neurochemical information over a more extended period of time (cf. reviews by Barrett, in press; Barrett & Nader, 1990).

The present experiments were conducted in an effort to collect neurochemical information from behaving animals over a lengthy period of time and in an individual subject exposed to multiple experimental conditions. A guide cannula, stereotaxically and chronically positioned in the third ventricle of the pigeon brain, was used to collect cerebrospinal fluid (CSF) after the pigeon was exposed to each component of a multiple FI FR schedule. So that samples could be collected under each schedule under stable conditions, components alternated on a daily or weekly basis. The longevity of the preparation not only allowed CSF collection following each schedule but also permitted the collection of neurochemical data in the same pigeon under conditions of extinction and conditions of response-independent food presentation, as well as when there were no scheduled events and the pigeons were kept in a darkened chamber.

METHOD

Subjects

Four White Carneau pigeons, obtained from the Palmetto Pigeon Plant and maintained at 85% of their free-feeding body weights, were housed singly in a temperature- and humidity-controlled vivarium (lights on from 7:00 a.m. to 8:00 p.m.). Weights were controlled by postsession supplemental feeding with Purina® Pigeon Checkers; pigeons had continuous access to crushed oyster shells and water in the home cage.

Apparatus

Experiments were conducted in a chamber that contained two response keys (Gerbrands)

mounted on the front aluminum wall. The keys (2 cm diameter) were 10 cm apart and 23 cm from the floor and could be transilluminated with different colors. A peck on either key that exceeded 0.5 N was counted as a response. Centered directly below the keys was an opening through which mixed grain could be presented from a solenoid-operated feeder. The opening was enlarged to 5 cm by 15 cm to allow the pigeon to insert its head safely and easily when the cannula's protective cap was in place. The three other walls and ceiling of the chamber were constructed of Plexiglas; the total interior dimensions were 22 cm by 27 cm by 31 cm. The experimental chamber was located inside a larger acoustically insulated shell that contained a speaker, through which white noise was presented, and a ventilating fan.

Behavioral Procedure

Pigeons were initially trained to peck a key that was transilluminated with a white light using the method of successive approximations (Ferster, 1953). Shaping was conducted in a chamber that differed from that used in the experiment in that it contained only a single key. Each peck on the key initially produced 4-s access to grain. Over the course of 3 to 5 days, the response requirement on the key was gradually raised to 30. Subsequently, sessions were conducted in the two-key chamber. When illuminated, the left key was amber and responses produced food according to an FR 30-response schedule. When the right key was illuminated red, the first response after 3 min produced food (FI schedule). The different components, each correlated with the different keylight colors and location, alternated in a mixed sequence, initially on a daily basis. When CSF samples were collected, schedules alternated weekly or every 2 weeks. Sessions terminated after 30 (FR) or 10 (FI) food presentations. Sessions were conducted 5 days per week.

CSF samples were collected weekly under each schedule condition and under additional conditions described below. At least triplicate determinations were made under each schedule. Once samples were collected under the FR and FI schedules, two samples were collected during sessions under which either the FR or FI keylight colors were illuminated but key pecking did not produce food (extinction). Session duration was based on the average session time for each of the two schedules (approximately 40 min for the FI and 20 min for the FR). Samples were also collected after pigeons were placed in the experimental chamber and there were no scheduled events (darkened chamber condition). Again, the duration of the session was comparable to that of the different schedule conditions when food delivery was in effect. Finally, food was delivered response independently, either every 3 min or every 25 s to approximate the interreinforcement intervals under the FI and FR schedules, respectively (response-independent condition). This procedure was designed to examine the possible neurochemical effects of food presentation and eating in the absence of responding. During the darkened chamber condition and during response-independent food presentation, no keylight was present and pecking did not occur. These conditions were in effect for a single session only; otherwise either the FI or FR schedule was in effect on other days.

Surgical Procedure

After responding stabilized under both schedules, as indicated by the absence of any systematic trends over a 5-day period, the pigeons were implanted with chronic indwelling intracerebroventricular guide cannulae. The pigeons were anesthetized with a combination of 10.0 mg/kg sodium pentobarbital and 20.0 mg/kg ketamine HCl, both administered into the pectoral muscle. The feathers on the head were trimmed and the pigeon placed in a stereotaxic apparatus. A hole was drilled through the skull at the following coordinates: lateral = +1.8; anterior–posterior = +6.8, relative to stereotaxic zero. A stainless steel guide cannula (Plastic Products) was lowered until there was a precipitous drop of fluid in a 15-cm length of tubing, attached to the cannula, that was filled with phosphate-buffered saline solution. The sudden drop in the fluid-filled tube was taken as an indication that the guide cannula had punctured the ventricle. The cannula was firmly secured with dental cement surrounding the guide cannula and four stainless steel screws fixed to the skull. The pigeon was allowed to recover for 4 to 7 days before being returned to the experimental chamber. Implantation of the guide cannula produced no disruptive effects on either rate or patterns of responding in any of the pigeons. During all times when CSF was not being collected, the guide cannula was protected by a cap consisting of a stylet

attached to a Teflon cap (Plastic Products) threaded down to protect the guide tube from contaminants.

Collection of CSF

CSF was collected immediately after the completion of the session. The dummy cap was removed and a push–pull cannula assembly (Plastic Products) was threaded onto the guide tube. The push–pull assembly was cut so that the push tube extended into the ventricle 1.0 mm beyond the tip of the cannula. CSF was withdrawn from the actual "push" side of the assembly, while the normal "pull" side was vented to atmosphere. CSF was withdrawn into silicone tubing connected to a Gilson Minipuls pump calibrated to withdraw CSF at a rate of approximately 1.0 μL/min. Samples were collected for 10 to 20 min; the CSF was then frozen immediately on dry ice.

Neurochemical Analyses

Neurochemical assays for the metabolites of dopamine (dihydroxyphenylacetic acid [DOPAC] and homovanillic acid [HVA]), serotonin (5-hydroxyindoleacetic acid [5-HIAA]), and norepinephrine (3-methoxy-4-hydroxyphenylethylene glycol [MHPG]) were conducted using high-performance liquid chromatography (HPLC) with electrochemical detection. CSF samples were thawed on wet ice, vortexed, and centrifuged. Five-, 10-, or 20-μL aliquots of CSF were added to 95, 90, or 80 μL, respectively of 2 M acetic acid and 2% ethanol (v/v). Fifty microliters of this mixture were injected onto a reverse-phase Rainin Microsorb C18 5 μ analytical column protected by a 7-μm Rp-18 guard column cartridge (Brownlee Laboratories). A Bioanalytical Systems Model LC-4 amperometric detector, with a TL-5 glassy carbon electrode set at +0.84 V relative to an Ag/AgCl reference electrode, served as the detection system. The mobile phase, driven by a Waters Model 6000 K pump linked to a Waters WISP 712 automatic sample injector, consisted of a 0.15 mM monochloracetic acid buffer (pH 3.0) containing 0.672 mM EDTA, 0.37–0.58 mM sodium octylsulfate, and 2.4–1.2% (v/v) acetonitrile, depending on the condition of the analytical column. The mobile phase was degassed under vacuum and filtered through a 0.22 μm durapore filter. The flow rate of the mobile phase was 2.0 mL/min. The amounts of biogenic amines in each sample were calculated by comparing peak heights, determined by a Waters Data Module (730), with those from standards run the same day. The detection limits of this assay for these compounds ranged from 25 to 50 pg per injection where the detection limit was defined as three times the baseline noise. Each analytical run contained a sample of pooled pigeon CSF with known metabolite levels and replicate samples at the beginning and end of each run to ensure reproducibility and stability of the system.

Data Analysis

Behavioral data are presented as average responses per second under the two schedules for individual pigeons. Neurochemical data are presented as picograms of the metabolites per microliter of CSF.

RESULTS

Behavioral and Neurochemical Effects under FI and FR Schedules

Control performances of all pigeons were similar to those characteristic of responding under these schedules (Figure 1). Rates of responding were higher under the FR schedule, with the average rate of responding on days when samples were collected ranging from 1.54 to 3.96 responses per second across pigeons; average response rates under the FI schedule, also based on days when CSF samples were collected, ranged from 0.70 to 1.13 responses per second. Control response rates for each pigeon are shown in Figure 2 and are also presented in Table 1.

Responding under the FR and FI schedules also resulted in selective differences in neurotransmitter metabolite levels (Figure 2). Levels of MHPG and DOPAC, metabolites of norepinephrine and dopamine, respectively, were slightly higher in 3 of 4 pigeons under the FI schedule compared to the FR. Larger differences occurred with the metabolite of serotonin, 5-HIAA, which was substantially higher when pigeons responded under the FI schedule compared to the FR schedule. Absolute levels of 5-HIAA were also higher than those of MHPG and DOPAC, as were levels of the other dopamine metabolite, HVA. As with the other metabolites, HVA levels were higher under the FI than under the FR schedule.

Chromatographic records of the analysis of CSF using HPLC procedures with electrochemical detection are shown in Figure 1. The chromatographic records are taken from the same sessions depicted by cumulative response records (shown on the left) in which either the FR or FI schedule was in effect. Clear differences in the magnitude of the levels of 5-HIAA and, to a lesser extent, HVA can be seen in the height of the curves related to these compounds when the FI schedule was in effect.

For Pigeons P-329, P-1681, and P-111, response rates during extinction of FI responding increased slightly compared to rates under the FI schedule when food was delivered (Table 1). Response rates during extinction of FR responding did not change substantially from those that occurred when food was delivered, except for Pigeon P-1681 whose rates of responding in extinction decreased by approximately 67%. Changes in metabolite levels under extinction, during exposure to the darkened chamber, and when food was delivered independently of responding are shown in Figures 3 through 6.

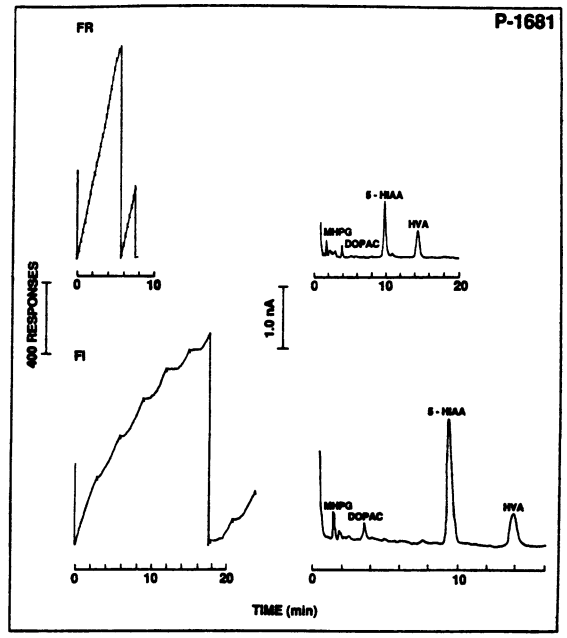

Fig. 1. Representative cumulative response records (left) for P-1681 under the FI and FR schedules taken from the sessions immediately prior to measurement of CSF metabolites. The chromatograms (right) show the elution patterns of the various metabolites under the FR and FI schedules. The *x* axes for the chromatograms differed because the injection volumes differed; the *y* axes remained the same, and the height of each curve corresponds to the quantity of that metabolite in CSF.

Effects on MHPG

Extinction of responding maintained under the FI schedule did not produce consistent or extensive effects on MHPG levels across pigeons (see Figure 3). Extinction of FR responding consistently increased MHPG levels above mean values obtained under the FR schedule in all subjects; however, the increases did not exceed measures of variability obtained under the FR schedule. MHPG levels decreased below those obtained under the FI schedule alone in 3 of the 4 pigeons when placed in the darkened experimental chamber for the usual length of the FI session. This condition increased MHPG levels over those obtained under the FR schedule, also in 3 of 4 pigeons. With the exception of P-329, MHPG levels under the darkened chamber conditions were comparable during weeks when either the FR or FI schedule was otherwise in effect and were comparable regardless of the length of time spent in the chamber under these conditions. Thus, this relatively unperturbed condition may most closely resemble baseline levels of the metabolites uninfluenced by other experimental variables. Exposure to response-independent food at the rate typically obtained under the FR and FI schedules produced levels of MHPG that were very similar to those obtained under the respective response-dependent schedules in all pigeons.

Effects on DOPAC

DOPAC levels did not change systematically or extensively across most experimental manipulations (Figure 4). Levels of this metabolite were slightly lower after extinction of responding under both FR and FI schedules when compared to levels obtained after exposure to the schedules with reinforcement. As with MHPG, levels of DOPAC were comparable under the conditions in which animals were simply placed in the darkened chamber for periods of time approximating the session duration for the FI and FR schedules.

Effects on 5-HIAA

Extinction of responding under the FI schedule decreased levels of 5-HIAA slightly in 3 of the 4 pigeons (Figure 5). In contrast,

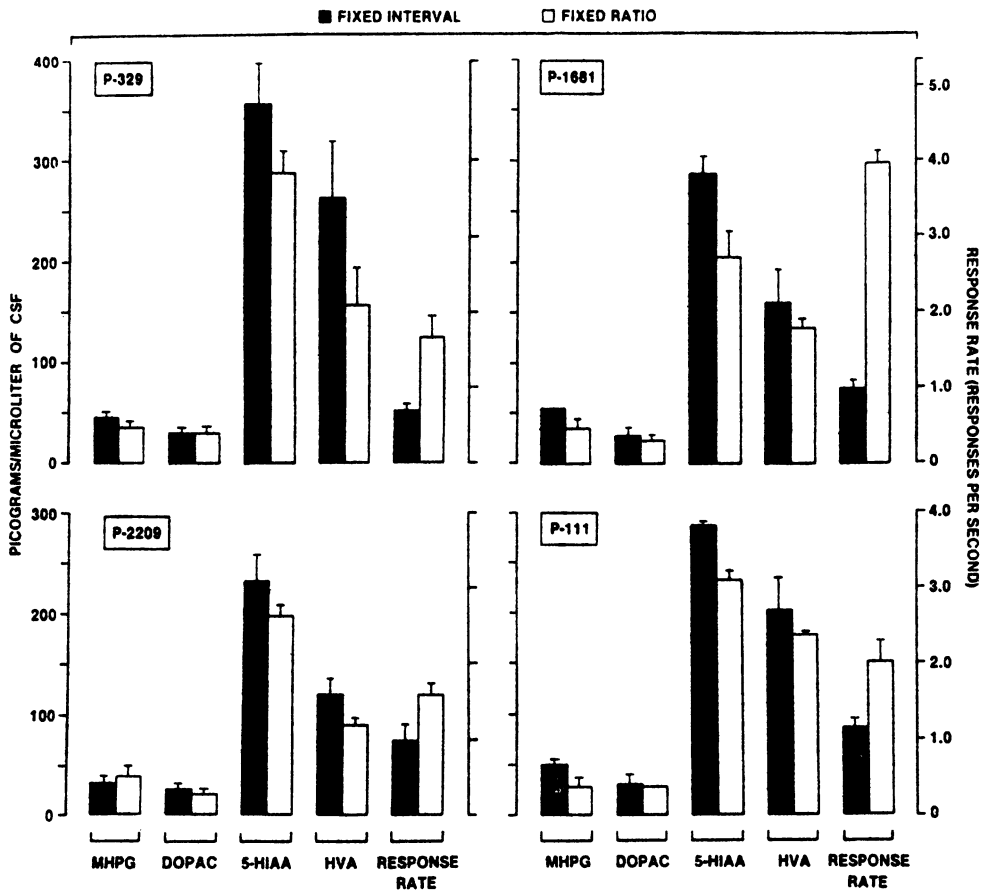

Fig. 2. Changes in neurotransmitter metabolite levels in CSF and response rates under FI and FR schedules for individual pigeons. See text for details and abbreviations. Vertical bars denote +1 *SD* from CSF samples collected and response rates obtained under each schedule condition on two to four occasions.

5-HIAA levels increased during extinction under the FR schedule for all pigeons except P-111 when these levels were compared to those obtained under the FR schedule. As was the case with MHPG and DOPAC, levels of 5-HIAA were comparable across all subjects (with the exception of P-329) during exposure to the darkened chamber. Although delivery of response-independent food did not produce any effects on 5-HIAA when delivered at the

Table 1

Response rates under different conditions. Numbers are average responses per second of two determinations; numbers in parentheses denote range of values.

Subject	FI	FR	FI EXT	FR EXT
P-329	0.70	1.67	1.20	2.06
	(0.69–0.71)	(1.45–1.86)	(1.15–1.25)	(2.01–2.11)
P-1681	1.00	3.96	1.26	1.30
	(0.95–1.05)	(3.83–4.09)	(1.19–1.32)	(0.84–1.76)
P-2209	0.98	1.54	0.49	1.55
	(0.76–1.20)	(1.40–1.67)	(0.45–1.52)	(1.39–1.70)
P-111	1.13	2.00	1.27	2.10
	(0.99–1.26)	(1.90–2.10)	(0.66–1.88)	(1.45–2.76)

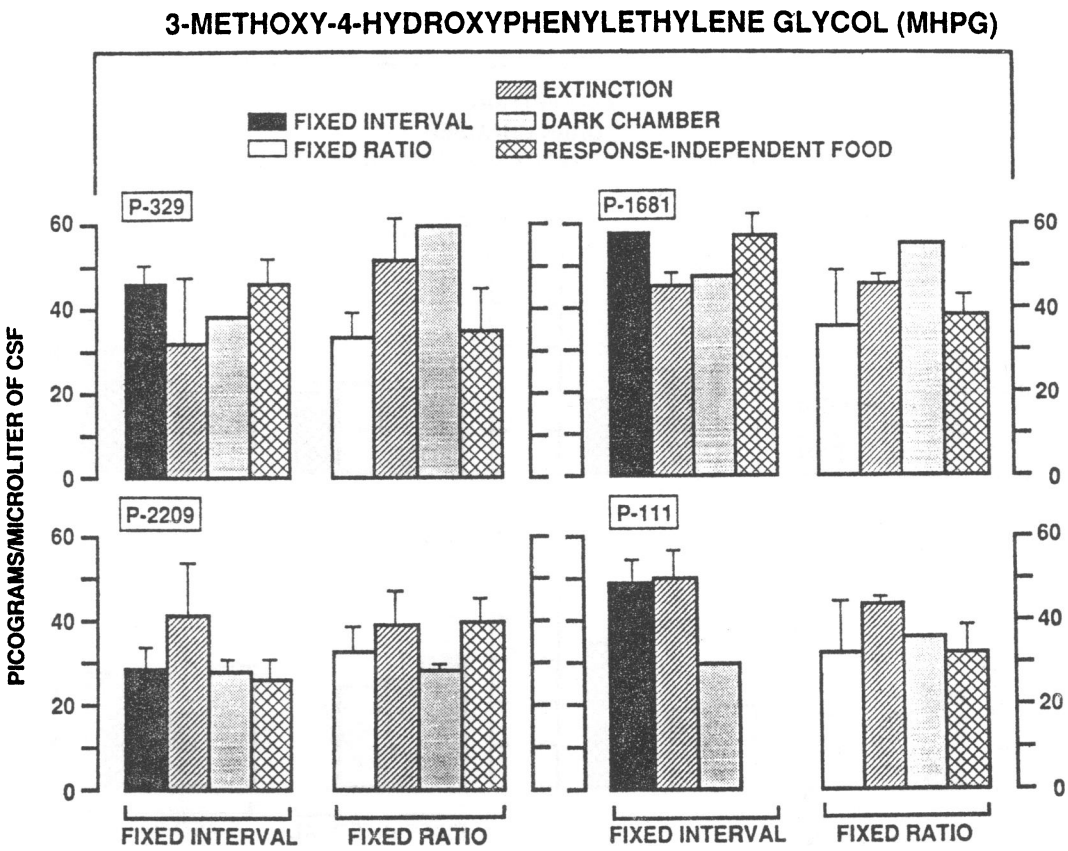

Fig. 3. Changes in MHPG under different experimental conditions. Details as in Figure 2. P-111 was not studied under the response-independent food condition. See texts for details of the schedule conditions.

frequency obtained under the FI schedule, levels of this metabolite increased above those obtained under the FR schedule when food was delivered response independently at the average frequency obtained under the FR schedule.

Effects on HVA

Extinction of responding under the FI schedule produced slight decreases in HVA below those obtained under the FI schedule in all subjects except P-1681 (Figure 6). In contrast, HVA levels increased in all subjects except P-111 during FR extinction when these levels were compared to levels obtained under the FR alone. As was true with the other metabolites, levels of HVA under the darkened chamber procedure were similar in most subjects (except P-329). HVA levels were not systematically affected under the FI response-independent food condition, but uniformly increased above levels obtained under the FR schedule in all subjects. In all instances, these increases produced by the response-independent delivery of food at the frequency obtained under the FR schedule exceeded those obtained under the extinction procedure.

DISCUSSION

Consistent neurochemical differences were obtained in pigeons responding under different schedules of reinforcement. When responding was maintained under the FI schedule, metabolite levels of the neurotransmitters of serotonin, norepinephrine, and dopamine typically were elevated above those obtained when responding was maintained under the FR schedule of reinforcement. The largest and most consistent schedule-related effects occurred with 5-HIAA and HVA. In general, metabolite levels did not appear to be strongly influenced by the rate of responding. In many cases, and particularly under the FR schedule

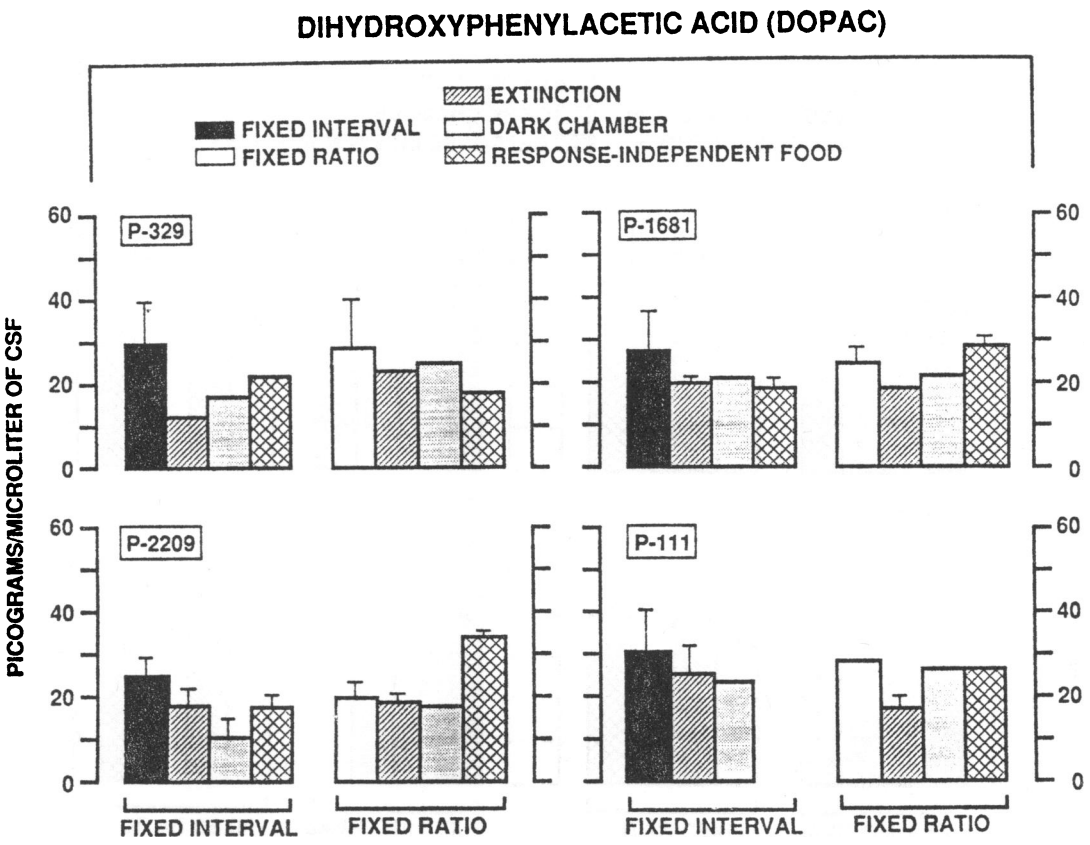

Fig. 4. Changes in DOPAC under the different experimental conditions. Details as in Figure 3.

with 5-HIAA, metabolite levels were comparable under conditions of extinction and response-independent food. Because responding did not occur to the darkened key under the latter condition, it seems that responding or response rate per se is not solely responsible for the differences seen in the metabolite levels. The relationships among these multiple variables are complex, and a complete understanding will require careful parametric analyses.

Different interreinforcement intervals also may have influenced metabolite levels under the FI and FR schedules. This might have been the case with MHPG, because metabolite levels under the response-independent food conditions were comparable to those under the respective schedules. However, with other metabolites, particularly 5-HIAA and HVA, this was not the case; in some cases, despite differences in metabolite levels under the FI or FR schedule conditions, levels under the respective response-independent food conditions were comparable (e.g., P-329 and P-1681 for 5-HIAA, Figure 5) or even in the opposite direction (e.g., P-1681 and P-2209 for HVA, Figure 6). Thus, there does not appear to be a direct and consistent relationship between interreinforcement interval and metabolite levels. As with response rate, however, systematic exploration of these variables is necessary.

The present technique of exposing pigeons to a multiple schedule of reinforcement, with components that alternate over days rather than within a single experimental session, permitted a direct comparison of schedule-related neurochemical differences within the same subject for a period of well over a year. Assays of CSF conducted during the condition in which the pigeons remained in a darkened chamber for the usual lengths of the FR or FI sessions but did not receive food indicated that, under these conditions, metabolite levels were very similar. This suggests that the differences obtained under the schedules do not reflect long-term changes in basal neurotransmitter levels but are, instead, a function of the more recent

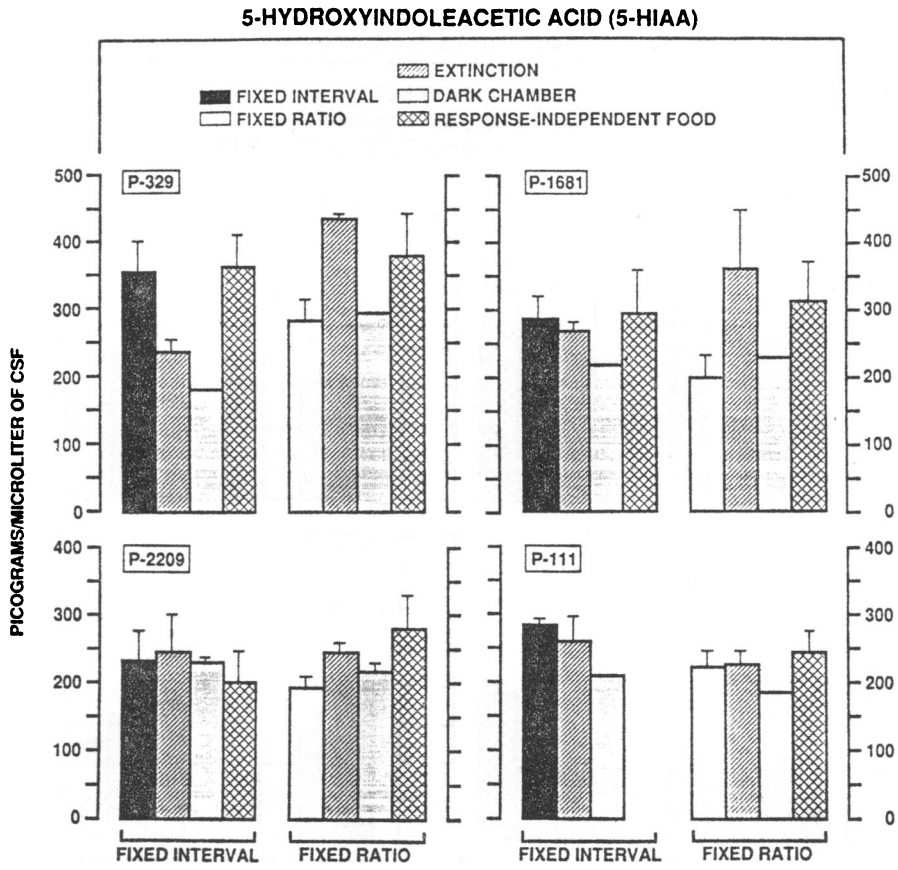

Fig. 5. Changes in 5-HIAA under the different experimental conditions. Details as in Figure 3.

conditions imposed. Whether there are conditions under which behavioral or environmental variables such as prolonged exposure to noxious stimuli can produce long-term changes in neurotransmitter or metabolite levels remain an interesting question for further research.

Although CSF levels of neurotransmitter metabolites were sensitive to the schedule manipulations in the present study, CSF measures of this type reflect an aggregate of neuronal activity in heterogeneous regions of the brain. Thus, it is not possible to obtain measures of neuronal activity in a discrete region of the brain using this technique. Other techniques, such as brain microdialysis, which allow measurement of neurotransmitter activity in smaller, focused regions of the brain will ultimately provide information of this type. However, as with other procedures (such as push–pull perfusion), the length of time over which neuronal activity can be measured repeatedly in a single subject may be limited (Barrett, in press). Thus, a selection of methodologies must, at least at present, be a compromise between several experimental objectives.

One of the metabolites of dopamine, HVA, was more sensitive to schedule effects than was DOPAC. Although the reasons for this difference are not clear, basal HVA levels were higher than those of DOPAC, which may allow a greater range of variation. In addition, these compounds are metabolized differently in the central nervous system and are present in differing amounts in different regions of the brain (Cooper, Bloom, & Roth, 1986). The proximity of neuronal structures close to the ventricle may play a role in the levels present and altered under the present experimental conditions.

Effects obtained with MHPG in the present

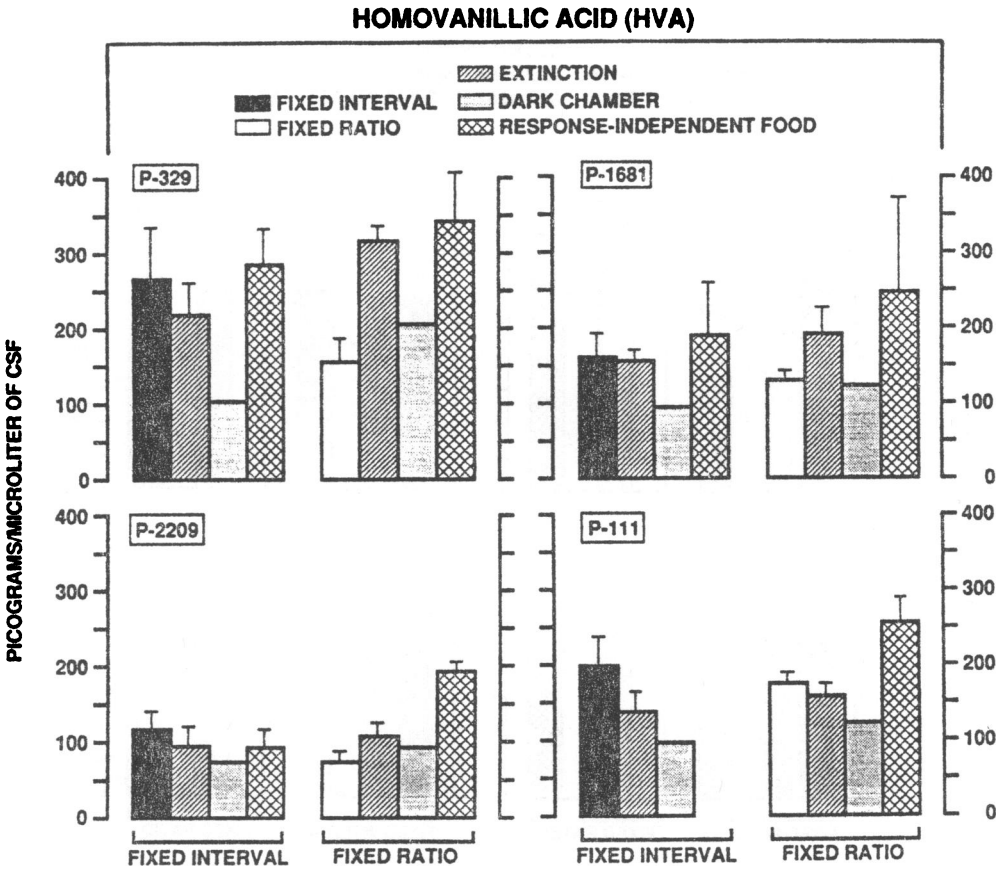

Fig. 6. Changes in HVA under the different experimental conditions. Details as in Figure 3.

study, in which response-independent food delivery yielded changes comparable to those under the respective response-dependent conditions, are similar to those obtained in a previous study by Lewy and Seiden (1972). In that study, NE metabolism was increased in subjects given response-independent food under a variable-time schedule when this group was compared to rats not exposed to the schedule of food delivery. A number of studies have shown that eating can result in neurochemical changes (e.g., Heffner, Hartman, & Seiden, 1980) but the precise role in these changes of scheduled presentation of food, eating, and of stimuli correlated with food delivery remains to be more fully explored. Thus, it appears that food presentation alone, independently of responding, may play an important role in inducing neurochemical changes. Again, however, evaluation of this possibility warrants systematic analyses over a wide range of parameters.

The present study has shown that schedules of reinforcement can produce distinctively different neurochemical changes in the same subject. Schedules of reinforcement can do so without apparently altering the steady state of the system, although, as mentioned previously, it remains to be determined whether other procedures (e.g., punishment) will produce more enduring neurochemical changes. It is clear from the present study that behavioral contingencies operating under schedules of reinforcement can result in distinctive neurochemical changes. It is well known that the schedule of reinforcement can play a significant role in determining the behavioral effects of drugs (Kelleher & Morse, 1968; McKearney & Barrett, 1978) and that drugs interact with neurotransmitter systems shown in the present experiment to be responsive to different schedules of reinforcement. Intensive study of the dynamic interplay between behavior, neurochemistry, and drug action promises to yield

valuable information at all levels of analysis. The results of the present experiment add to a growing body of literature pointing to the importance of behavioral processes in modifying the neurochemical milieu (cf. Barrett & Nader, 1990; Dworkin & Smith, 1989). In contrast to most previous work, in which neurochemical measures were obtained on a single occasion and results were compared across groups, the methods of the present approach permit long-term analyses of single subjects under several conditions, including an evaluation of the effects of previous behavioral history (Barrett, 1977; Barrett & Nader, 1990; Barrett & Witkin, 1986). This allows measurement of the dynamic interactions between behavior and neurochemistry envisioned some years ago by Skinner (1938), who wrote: ". . . a science of the nervous system will some day start from the direct observation of neural processes and frame its concepts and laws accordingly" (p. 422).

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