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BRIEF COMMUNICATION

Some Effects of Pimozide and of Shifts in Sucrose Concentration on Lick Rate, Duration, and Interlick Interval

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GRAMLING, S. E. AND S. C. FOWLER. *Some effects of pimozide and of shifts in sucrose concentration on lick rate, duration, and interlick interval.* PHARMACOL BIOCHEM BEHAV 25(1) 219–222, 1986.—Multiple dependent measures were employed to characterize the licking behavior of rats exposed to shifts in reward magnitude or injected with pimozide (PIM). Nondeprived rats licked either a 32% (n=14) or 4% (n=15) sucrose solution in daily 10 min sessions. Rats in the 32% condition were then down-shifted to either a 16% (n=7) or 4% (n=7) sucrose solution. Rats in the 4% condition were up-shifted to either 16% (n=7) or 32% (n=8) sucrose solution. The response profiles generated by those rats shifted to a lower reward magnitude were contrasted with either rats shifted from a 32% sucrose solution to a no-reward (plain tap water) condition, or with rats maintained on a 32% sucrose solution and administered the neuroleptic PIM (0.5 mg/kg or 1.0 mg/kg). Rats down-shifted from a 32% to 4% sucrose solution generated response profiles more similar to rats shifted to plain tap water than rats maintained on a 32% sucrose solution and administered PIM. These results suggested that PIM treatment is not functionally equivalent to either a shift to no-reward or to a shift to reduced reward conditions.

Anhedonia Licking Sucrose reward Pimozide Interlick interval Lick duration Rats

THE anhedonia hypothesis of neuroleptic action interprets the apparent similarities in the pattern of responding produced by animals in a no-reward condition and animals administered neuroleptics as a failure in both cases to activate the final common pathway of the neural substrate of reward in the brain (i.e., neuroleptic treatment and extinction procedures are postulated to be functionally equivalent procedures; [13]). A recent study [5] which tested nondeprived rats in an anhedonia paradigm failed to observe similar patterns of responding between rats in a no-reward condition and rats treated with the neuroleptic pimozide (PIM). In the Gramling *et al.* [5] study, rats trained to lick a 32% sucrose solution received either PIM or were exposed to a no-reward (plain tap water) condition. Both PIM treatment and no-reward conditions resulted in rate reductions on the first test day (relative to controls). The composite response profiles based on lick rate, lick duration, and interlick interval (ILI) were distinctly different, however, suggesting that the rate reducing effects of PIM and no-reward were occasioned by somewhat different, rather than identical processes [5].

The revised anhedonia hypothesis emphasizes that neuroleptics blunt (rather than totally block) the response sustaining capacity of reinforcers [7,12]. Therefore, neuroleptic treatment may be more analogous to shifts to lower reward magnitudes than it is to extinction. Recent reports in the anhedonia literature have compared the effects

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of PIM with reduced reward conditions [4,14] although these studies have not provided a microanalysis of licking behavior based upon measures of individual response properties (i.e., ILI and lick duration). Thus, in the present study multiple dependent measures were used to compare the licking behavior of rats shifted to a lower sucrose concentration with the licking behavior of rats which received injections of PIM but experienced no change in sucrose concentration.

Additionally, different groups of rats were shifted from a low sucrose concentration to a higher sucrose concentration in order to obtain additional parametric data on the ILI and lick duration measures. Although there is an extensive literature concerning the effects of shifts in reward magnitude per se on licking (e.g., [3, 9, 10]), these studies (like those directly addressing the anhendonia hypothesis [4,14]) do not include measures of individual response properties of the rats licking behavior. Measures such as lick duration [5, 6, 8], ILI [5,8], and tongue extension [1] have been used to infer motor deficits induced by drug administration [5, 6, 8] and various brain lesions [1]. In that the shift conditions represent a non-motor (presumably) manipulation, a microanalysis of the rats' licking behavior under these conditions may contribute to our understanding of these measures (i.e., lick duration, ILI) in relation to behavioral processes.

METHOD

Subjects

Thirty-two male Sprague-Dawley rats (Holtzman Company) weighing approximately 400 g prior to the experiment were used. The rats were individually housed and given continuous access to food and water in the home cages (i.e., rats were nondeprived). An additional 24 rats from a previously published study [4] were included because data relevant to the present work were not reported at that time. These 24 rats were housed and maintained in the same manner as described for the 32 rats just mentioned.

Apparatus

Four simultaneously-operative experimental chambers [4] were used in the present experiment. A circular opening centered in the front panel permitted head entry into a cylindrical recession. An opening in the floor of the recession permitted tongue access to a sucrose solution reservoir beneath, whose fluid level was 10 mm below the cylindrical recession prior to each rat's session. A contact circuit which passed less than 1.0 microamp through the rat was used to record licking. Each chamber was serviced by a separate microcomputer (Apple II+) which recorded the data with a resolution of 0.01 sec.

Procedure

The 32 rats were randomly assigned to either a 32% (n=16) or 4% (n=16) sucrose solution condition. During the initial 10 minute session, the cylindrical recession providing access to the sucrose solution was baited with a few drops of the appropriate solution to speed the initiation of the lick response. Most of the animals began to lick from the reservoir during the first session. For the rats that did not lick initially, the fluid level was raised to the cylindrical opening and then gradually lowered to 10 mm beneath the recession. Three rats were dropped from the study because they failed to lick consistently; two were from the 32% condition and one was from the 4% condition.

Subsequently, the rats received daily 10-min sessions with the appropriate sucrose solution for 22 consecutive baseline days. On the day after the last day of baseline, the concentrations of sucrose solution were shifted. Animals previously exposed to the 32% solution were randomly assigned to either a 16% (n=7) or 4% (n=7) sucrose solution condition (referred to as 32%-16% and 32%-4%, respectively). Conversely, animals previously exposed to the 4% solution were randomly assigned to either a 16% (n=7) or 32% (n=8) sucrose solution condition (referred to as 4%-16% and 4%-32%, respectively).

The additional 24 rats were exposed to a 32% sucrose solution during baseline in the same manner as that described above. Following baseline these 24 rats were randomly assigned to one of three treatment conditions (n=8) where they were either shifted to a no-reward condition or maintained on a 32% sucrose solution and injected (IP) with one of two doses of PIM. The groups were designated EXT, PIM 0.5 + RWD, and PIM 1.0 + RWD, respectively. Rats in the EXT group received vehicle injections and were exposed to plain tap water on test day. Rats in the PIM 0.5 + RWD and PIM 1.0 + RWD were injected with PIM (0.5 mg/kg and 1.0 mg/kg, respectively) and exposed to a 32% sucrose solution on test day. All injections preceded data collection by four hours. The drug, dose levels, and time since injection were the same as those used by Wise *et al.* [12]. Rats in all conditions were randomly assigned to postshift treatment conditions rather than matched because of the difficulty in matching across multiple dependent measures.

The effects of PIM and shifts in sucrose concentrations were characterized by average lick rate, median lick duration, median ILI of the first mode of the ILI frequency distribution, and the proportion of long pauses in a session. Median lick duration was calculated by taking the median of the frequency distribution of all of the lick durations throughout a rat's session. Unlike the distributions of ILI, the duration distributions are not multimodal [7,11]. Median ILI was calculated by including only intervals less than 0.2 sec. The 0.2 sec cutoff was an empirically derived criterion that fell between the first and second ILI modes for all rats on the last preshift day. This ILI measure includes only the shortest intervals or the fastest licks that the animal exhibits within a session (5–6 licks/sec [1,11]). The proportion of long pauses reflects pauses between bursts of licking and includes only those intervals 0.5 sec or longer.

RESULTS

There was little difference in preshift lick rates between rats in the 32% and 4% sucrose solution conditions. The means (and standard errors of the mean) for baseline lick rates for the 32% condition (n=14) and the 4% condition (n=15) were 1.23 (±0.14) licks/sec and 1.29 (±0.14) licks/sec, respectively. Random assignment of the 32% rats to their respective postshift conditions resulted in significant ($p<0.01$) preshift differences on the rate measure between the 32%-16% and the 32%4% groups. The means (and standard errors of the mean) were 1.56 (±0.16) licks/sec and 0.89 (±0.15) licks/sec, respectively. Preshift differences between the 4%-32% and 4%-16% groups were minimal.

The effects of shifts in sucrose concentrations were assessed by *t*-tests for related groups. The *t*-tests were calculated between the last preshift session and postshift session for each group on each dependent measure and these results are presented in Table 1. The 32%-4% group exhibited a

TABLE 1

THE EFFECTS OF SHIFTS IN SUCROSE CONCENTRATIONS ON THE INDICATED MEASURES OF LICKING

Dependent Variables	Down-Shift Group 32-4 B	Down-Shift Group 32-4 S	Down-Shift Group 32-16 B	Down-Shift Group 32-16 S
Rate				
Licks/sec	1.56	0.24†	0.89	0.65
(± SEM)	0.16	0.05	0.15	0.10
ILI	10.16	9.72	10.92	11.04
(± SEM)	0.37	0.37	0.47	0.37
Pauses	0.06	0.18†	0.11	0.10
(± SEM)	0.01	0.03	0.02	0.02
Duration	7.76	7.24	7.58	7.71
(± SEM)	0.26	0.11	0.08	0.12
	Up-Shift Group 4-32 B	Up-Shift Group 4-32 S	Up-Shift Group 4-16 B	Up-Shift Group 4-16 S
Rate				
Licks/sec	1.32	1.75	1.26	1.65*
(± SEM)	0.23	0.16	0.17	0.13
ILI	10.43	11.07	11.04	12.43*
(± SEM)	0.26	0.35	0.13	0.45
Pauses	0.07	0.10†	0.06	0.10*
(± SEM)	0.02	0.02	0.01	0.02
Duration	7.41	7.39	7.33	7.65†
(± SEM)	0.23	0.20	0.21	0.19

B = Baseline, S = Shift Day. Statistical comparisons by *t*-tests for related measures: *$p<0.05$, †$p<0.01$.

The unit of measurement was 0.01 sec.

The pauses measure reflects the proportion of interlick intervals 0.5 sec or longer.

TABLE 2

THE EFFECTS OF SHIFTS FROM BASELINE TO EITHER EXTINCTION OR PIMOZIDE CONDITIONS ON THE INDICATED MEASURES OF LICKING

Dependent Variables	EXT B	EXT E	PIM 0.5 + RWD B	PIM 0.5 + RWD Drug	PIM 1.0 + RWD B	PIM 1.0 + RWD Drug
Rate						
Licks/sec	1.16	0.07†	1.22	0.83†	1.48	0.61†
(± SEM)	0.13	0.01	0.16	0.18	0.18	0.17
ILI	10.07	9.67	10.52	11.26†	10.33	12.28†
(± SEM)	0.22	0.19	0.45	0.51	0.45	0.44
Pauses	0.06	0.33†	0.06	0.07	0.04	0.11†
(± SEM)	0.02	0.05	0.01	0.01	0.01	0.01
Duration	7.43	6.38†	7.55	7.61	7.46	7.59
(± SEM)	0.14	0.16	0.21	0.26	0.14	0.08

B = Baseline, E = Extinction Day, Drug = Drug Day.

Statistical analysis by *t*-tests for related measures: *$p<0.05$, †$p<0.01$.

Lick duration and ILI are measured in units of 0.01 secs.

The pauses measure reflects the proportion of interlick intervals 0.5 sec or longer.

significant decrease in lick rate and a significant increase in the proportion of long pauses on the first postshift day. Lick duration tended toward shorter times but the effect was not significant, and median ILI was little affected by the down-shift to the 4% sucrose solution. Rats in the 32%-16% did not exhibit significant changes on any of these dependent measures, though there was a tendency toward lower lick rates.

Both groups of rats shifted to higher sucrose solution concentrations exhibited an increase in lick rate in the shift session. However, the effect was significant only in the group shifted from a 4% to a 16% solution. Comparisons between preshift and shift conditions also revealed that the proportion of long pauses significantly increased in both the 4%-32% group and the 4%-16% group. The increase in lick rate and the increase in the proportion of long pauses observed in the rats shifted from a 4% to a 16% solution was accompanied by a significant lengthening of lick duration and a significant lengthening of median ILI. A similar, though non-significant trend towards longer times on the ILI measure was observed in rats shifted from 4% to a 32% solution.

Table 2 presents the results for the PIM 0.5 + RWD, PIM 1.0 + RWD, and EXT rats from the Gramling *et al.* [5] study. Related groups *t*-tests were calculated between the last day of baseline and treatment session on each of the dependent measures. Rats maintained on a 32% sucrose solution and then exposed to either drug (0.5 mg/kg or 1.0 mg/kg PIM) or no-reward (control injections plus plain tap water) conditions exhibited a significant decrease in lick rate and an increase in the proportion of long pauses (EXT and PIM 1.0 + RWD only). Rats shifted to an extinction condition exhibited significantly shorter times on the lick duration measure though ILI was not significantly affected by the shift to plain tap water. Conversely, rats treated with PIM showed little change on the duration measure but exhibited significantly longer times on the ILI measure on their first day of drug exposure.

DISCUSSION

The response profile generated by rats shifted from a 32% sucrose solution to a 4% sucrose solution more closely resembled the response profile generated by rats shifted from a 32% sucrose solution to plain tap water (extinction) than the response profile generated by PIM treated rats. Though no-reward, down-shift (32%-4%), and PIM (1.0 mg/kg) conditions all resulted in a decrease in lick rate and an increase in the proportion of long pauses, they differed on the ILI and lick duration measures. Rats in the down-shift conditions (top of Table 1) did not exhibit a lengthening of the ILI measure which characterized the PIM treated animals (see ILI in Table 2). On the measure lick duration there was a trend toward shorter times by rats in the 32%-4% condition, whereas rats in the PIM condition were little affected and rats in the no-reward condition exhibited significantly shorter times on the postshift day. These data suggest that the effects of PIM can be distinguished from the effects of no-reward and reduced reward when measures of individual response properties are included.

The increase in the ILI, lick duration, and pauses measures exhibited by rats in the up-shift groups (bottom of Table 1) suggest that changes in these measures do not necessarily reflect either motor processes (ILI, lick duration) or reward processes (pauses) as had been previously speculated [5]. These findings are important because they suggest that, like the dependent measure average rate, the additional dependent measures used in this and previous studies are not necessarily selectively affected by any one experimental manipulation (i.e., none of the measures used in this study are "pure" measures of either reward or motor processes). The controversy concerning the extent to which the rate reducing effects of neuroleptics are attributable to either reward processes or motor processes remains unresolved, however, expanding the class of dependent variables furthers the development of more precise drug-behavior classification techniques.

The up-shift data also suggest that the relationship between the magnitude of shift in sucrose concentration and the magnitude of the effect on the dependent measures was non-monotonic (since significant results were obtained when sucrose concentration was shifted from 4% to 16% but not when the sucrose concentration was shifted from 4% to 32%). The inclusion of additional increments along the continuum of reward magnitude shifts (e.g., 4%-8%; 4%-24% etc.) would clarify this issue. The limited parametric data regarding the effects of reward magnitude shifts on these dependent measures and the absence of direct comparisons between groups suggests that caution is warranted in interpreting these results.

Nevertheless, the multiple dependent measures in the present study provided a more complete response profile which differentiated the effects of no-reward or reduced reward from the effects of PIM. These results are contrary to the predictions made by the anhedonia hypothesis which posits that the patterns of responding produced by extinction procedures (and presumably reduced reward conditions) and PIM treatment should be qualitatively, if not quantitatively, similar [13]. The anhedonia hypothesis maintains that both extinction procedures and neuroleptic treatment exert their behavioral effects by a failure to activate the final common pathway of the reinforcement substrate in the brain [13] and therefore similarities in the patterns of responding produced by rats treated with neuroleptics and rats exposed to extinction procedures are considered critical evidence for neuroleptics' putative effects. The data from the present experiment are incongruent with the anhedonia hypothesis since PIM treatment produced patterns of responding qualitatively different from no-reward and reduced reward conditions.

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