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Effects of Neuroleptics on Rate and Duration of Operant Versus Reflexive Licking in Rats

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GRAMLING, S. E. AND S. C. FOWLER. *Effects of neuroleptics on rate and duration of operant versus reflexive licking in rats.* PHARMACOL BIOCHEM BEHAV 22(4) 541–545, 1985.—Operant conditioning techniques were used to train one group of nine rats to lick a dry horizontal metal disk on a fixed ratio 15 schedule with water reinforcement delivered at a different location. Another group of seven rats licked reflexively from a water reservoir positioned with the same spatial arrangements as the metal disk. The distance the rats' rongues traversed (10 mm) to contact the licking surface was the same in both the operant and reflexive lick conditions. The effects of three neuroleptics, haloperidol (0.06, 0.12, 0.24, 1.0, 2.0 mg/kg), chlorpromazine (0.5, 1.0, 2.0 mg/kg) and clozapine (2.5, 5.0, 7.5 mg/kg) on average lick rate and median lick duration were assessed for both groups. Dose related decreases in average lick rate were observed in both groups of rats as a function of dose of each of these neuroleptics. Moreover, operant lick rates were proportionately more affected by neuroleptic treatment than were reflexive lick rates. The dose-response effect for the duration variable was different for the two lick conditions in that reflexive lick duration was lengthened as dose increased, whereas operant lick duration was lengthened only at the lower doses of these drugs. The differential effect of these neuroleptics on operant vs. reflexive licking suggests that neuroleptics attenuate selectively those responses that require relatively more conditioning to acquire. These results may be analogous to the initiation deficit that has been suggested to account for neuroleptics' selective attenuation of avoidance, while leaving relatively intact the escape response in escape/avoidance procedures.

Haloperidol Chlorpromazine Clozapine Operant licking Lick duration Rats

IN the conditioned active avoidance paradigm animals treated with neuroleptics will perform an instrumental response (e.g., run to a "safe" compartment) in order to escape an aversive stimulus at doses that attenuate or abolish these same responses to a warning stimulus [6]. Since the response requirements for escape and avoidance are the same, a motor impairment interpretation of neuroleptics' "anti-avoidance" effects would seem to be ruled out [4]. Recent investigations suggest that an associative deficit interpretation of neuroleptics' anti-avoidance effects is also inadequate (e.g., [1,2]). Another possible explanation for the anti-avoidance effects of neuroleptics suggests that neurolpetics selectively attenuate the organisms ability to initiate learned, instrumental, "voluntary" behavior compared to more automatic reflexive behaviors [3, 9, 16]. Specifically, neuroleptics are thought to attenuate selectively the animal's ability to initiate the conditioned avoidance response (CAR) but not the unconditioned (escape) response [9]. One implication of this literature is that the "conditionedness" of the response is important in determining the extent of neuroleptic disruption of performance. Likewise, the ability of neuroleptics to reduce appetitively-motivated responding may also be governed by the extent to which such behaviors are learned or "conditioned" [10]. However, there are currently few methods which test animals with appetitive reinforcers in a procedure analogous to the escape/avoidance paradigm (i.e., identical response requirements) to determine if the instrumental response is more impaired than its reflexive counterpart. Such a procedure is clearly needed, however, if one is to evaluate the "conditionedness" idea with an appetitive task.

The present research attempts to separate, at least partially, the motor effects of neuroleptics from their other putative effects by proposing the rat's licking behavior as an appetitive analogue of the escape/avoidance paradigm. The lick response is well suited for this purpose in that it is a response which ordinarily occurs in the absence of any explicit associative conditioning procedures (i.e., a thirsty rat licking reflexively from a reservoir), and yet it is amenable to modification by conditioning procedures (i.e., exemplified by a rat licking a dry metal disk for a water reinforcer elsewhere in the chamber). The reflexive/operant lick procedure seems to parallel some of the important features of the escape/avoidance procedure in that there are nearly identical response requirements in the two tasks but different amounts of conditioning are required to express the response. Any neuroleptic-induced impairment observed in the operant lick condition beyond that observed in the reflexive condition could not be due to motor impairment per se, and would seem to increase the generality of the idea that neurolpetics

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attenuate most readily those responses that require relatively more conditioning to acquire.

METHOD

Subjects

Twenty male Sprague-Dawley rats (Holtzman Co.) ranging between 275 and 325 grams in ad lib weight served as subjects. Rats were maintained on water deprivation in individual home cages that provided continuous access to food. To keep body weight nearly constant (80% of ad lib weight) animals in the operant lick condition received approximately 6 min daily access to water in their home cages 1 hr subsequent to the experimental session. Animals in the reflexive lick condition consumed their daily ration during the experimental session. During the course of the experiment four rats died, one in the operant condition and three in the reflexive condition, from apparent chronic respiratory disorders.

Apparatus

The apparatus consisted of two simultaneously operative experimental chambers measuring 23 cm long, 20 cm wide, and 19 cm high. Each chamber was fitted with a grid floor composed of 6.5 mm diameter rods running parallel to the front of the chamber. The front panels of the chambers were made of aluminum and the remaining sides and top were Plexiglas. Illumination was provided by a single small lightbulb located on the top center of the front panel.

A 5.5 cm circular opening was centered in the front panel 11 mm above the floor and permitted head entry into a cylindrical recession that extended 3.5 cm from the front panel of the chamber wall. A 1.5 cm circular opening in the cylindrical recession was positioned parallel to the grid floor 1.2 cm from the front panel and permitted tongue access to the licking surface beneath. The lick surface for the operant lick condition consisted of a dry horizontal disk 18 mm in diameter and was located 10 mm beneath the circular opening in the cylindrical recession. Water reinforcement (0.05 ml) was delivered by a solenoid valve into a brass cup mounted on the lower right front panel, 8.5 cm from the circular opening.

In the reflexive lick condition a reservoir filled with tap water was located in the same position as the operant disk. The fluid level in the reservoir was carefully raised to 10 mm beneath the cylindrical recession prior to each rat's session. During the course of a session, the fluid level dropped less than 1 mm. Rats in both conditions wore "Elizabethan" collars to prevent spurious recordings of nose, jowl, and forelimb contacts by limiting the extent of head or limb entry into the cylindrical recession. The collars were made of Teflon (2 mm thick and 6.0 cm in diameter) and permitted relatively unencumbered movement in the recording chambers.

Programming of contingencies and recording of data were accomplished with a laboratory computer (PDP 8/e) and associated peripherals. The system recorded the duration of individual licks to the nearest 0.01 sec. The contact circuits used to record licking passed less than 1.5 microamps through the rat.

Procedure

Rats were assigned to either an operant or reflexive lick condition. In the operant lick condition rats first received magazine training, wherein reinforcement delivery was paired with a distinctive solenoid click on a variable time basis. Following magazine training, head entry into the cylindrical recession was manually shaped. Tongue extension through the 1.5 cm circular opening in the cylindrical recession to the flat surface beneath was accomplished by first "baiting" the disk with a drop of water. When all rats were reliably licking the dry disk surface (no baiting was involved) on a continuous reinforcement schedule the ratio was gradually raised to a fixed ratio 15 (FR 15).

Rats in the reflexive lick condition were simply placed in the chamber and given access to the water reservoir. The cylindrical recession was "baited" with water on the first day in order to speed the initiation of licking through the 1.5 cm opening that provided access to the water reservoir. Baseline data were collected for both groups of rats in daily seven minute sessions for 10 days prior to beginning the acute neuroleptic dosing regime. The 5-day dosing cycle consisted of 3 no-injection days, 1 injection control day, and 1 drug evaluation day. Occasionally, the time between drug assessments was more than five days, though never less, and the complete cycle always preceded the drug assessment day.

The neuroleptics used in the present study varied on a continuum of motor side effect liability with haloperidol (HAL) high, chlorpromazine (CPZ) medium and clozapine (CLOZ) low on this clinical dimension. The dosages administered were; HAL: 0.06, 0.12, 0.24, 1.0, 2.0 mg/kg, IP, 2 hr before testing; CPZ: 0.5, 1.0, 2.0 mg/kg, IP, 1 hr before testing; CLOZ 2.5, 5.0, 7.5 mg/kg, IP, 1 hr before testing. The drugs were prepared for injection as follows: HAL (supplied by Janssen) was mixed in a solution of methylparaben-prophylparaben, lactic acid, and sterile water; CPZ (supplied by Smith, Kline, and French as the salt) was dissolved in 0.9% saline immediately before injection; CLOZ (supplied by Sandoz) was dissolved immediately before injection in 0.9% saline with sufficient 0.1 N HCl to achieve solution. Two determinations at each dose of each drug were taken for each rat.

Drug effects were characterized by the dependent measures, average rate and median lick duration. Median lick duration is a measure of the amount of time the rat's tongue is in contact with the water and is based on the distribution of individual lick durations throughout each rat's session. In the lever press situation the response duration measure has provided behavioral information about drug effects nonredundant with the rate of response measure [19]. These dependent measures were expressed as a proportion of the previous day's value in order to equate for differences in the baseline response rates between operant and reflexive licking. Before statistical analyses were undertaken an average was taken of the two determinations of each drug dose for each rat. Data were not included in the duration analysis if an animal made less than five licks in a session.

RESULTS

t-Tests for independent groups were first calculated on the control rate and duration data to determine the predrug differences between reflexive and operant licking on these measures. There were large differences between groups on the average rate measure, $t(14)=28.6$, $p<0.0001$. The means (and standard errors of the mean) for reflexive and operant lick rate were 3.30 (+0.09) resp/sec and 0.71 (+0.04) resp/sec, respectively. However, there were no significant differences between groups for the baseline median lick duration data, $t(14)=0.561$, $p>0.2$. Reflexive and operant me-

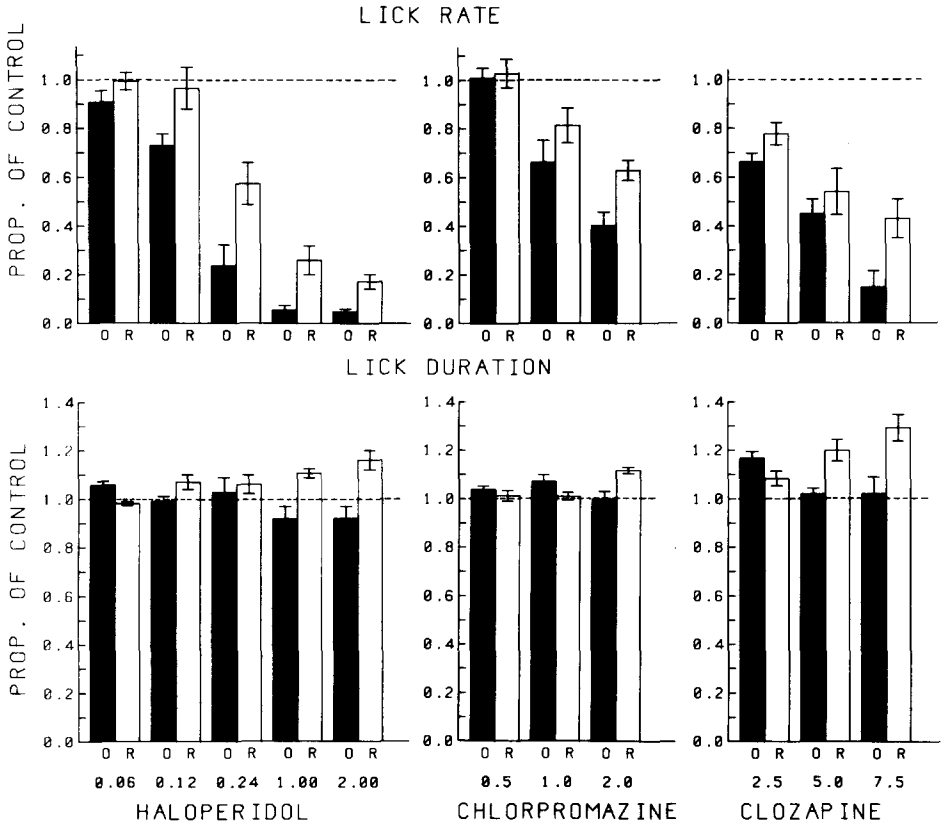

FIG. 1. Average lick rate (upper axes) and median lick duration (lower axes) for the indicated drugs and doses. Darkened bars represent the operant lick condition and open bars represent the reflexive lick condition. The data were expressed as a proportion of the previous days' injection control value where 1.0 (dotted horizontal line) reflects no change from control. Vertical brackets indicate ±1 SEM.

dian lick durations were, respectively, 0.075 (+0.003) sec and 0.072 (+0.003) sec.

Figure 1 shows that HAL, CPZ, and CLOZ each produced dose related decreases in both operant and reflexive licking on the measure of average rate (upper row of axes). Moreover, operant licking (darkened bars) was more affected (i.e., showed proportionately greater rate reductions) than reflexive licking for all three of these neuroleptics.

When the HAL data were entered into a split-plot factorial analysis of variance (SPF-ANOVA), the repeated measures dose effect was significant, $F(4,56)=114.47$, $p<0.0001$, indicating that increasing doses of HAL produced progressively less responding regardless of lick condition. The between-groups comparison (operant vs. reflexive) for this same analysis was also significant, $F(1,14)=18.01$, $p<0.001$, verifying the visual impression of Fig. 1 that rate of operant licking was more impaired by HAL than was reflexive licking. Similarly, the repeated measures dose effect on rate for CPZ was significant, $F(2,28)=36.18$, $p<0.0001$. The tendency for operant licking to show greater rate reductions than reflexive licking under CPZ, though not as pronounced as with HAL, was significant, $F(1,14)=4.94$, $p<0.05$. CLOZ's dose dependent rate reducing effects on licking also yielded a significant repeated measures dose effect, $F(2,28)=25.94$, $p<0.0001$. The group effect was significant, $F(1,14)=6.67$, $p<0.05$, indicating that CLOZ, too, produced greater rate reducing effects on operant licking relative to reflexive licking. Dose-by-group interaction effects were not significant for any of the three drugs.

Between-group comparisons by means of SPF-ANOVAs could not be performed on the lick duration measure in a routine manner, since operant licking was greatly reduced or eliminated at the higher doses of these drugs (i.e., no responding results in an unacceptably high level of missing data on this measure, whereas with the average rate measure a zero value is a legitimate data point). Figure 1 (lower set of axes) indicates, however, that in the reflexive lick condition (open bars) these three neuroleptics lengthened the amount of time the tongue was in contact with the water in a dose-related manner. When the effects of HAL, CPZ, and CLOZ on reflexive lick duration data were analyzed in repeated measures ANOVAs, the dose effects for reflexive lick duration were significant, $F(4,24)=4.39$, $p<0.01$; $F(2,12)=12.35$, $p<0.01$; $F(2,12)=15.49$, $p<0.0005$, respectively, for each drug.

As can be seen in Fig. 1, the dose effect on operant lick duration was different from that observed for reflexive licking. The lowest dose of each of these drugs produced a significant lengthening of operant lick duration compared to saline values (by t-tests for correlated groups; HAL 0.06 mg/kg: $t(8)=3.450$, $p<0.001$; CPZ 0.5 mg/kg: $t(8)=2.370$, $p<0.05$; CPZ 1.0 mg/kg: $t(8)=2.79$, $p<0.05$; CLOZ 2.5 mg/kg: $t(8)=6.2$, $p<0.001$). However, at higher doses the duration of

the operant lick response was not significantly different from control values. The large SEMs in the operant group at the higher doses were due in part to the small number of animals which performed sufficiently to characterize the effect of neuroleptic treatment on operant lick duration.

DISCUSSION

Licking is a biologically primitive, reflexive response with a relatively invariant, stereotypic topography [7,20]. In view of the lick response's invariance, its duration has been proposed as a sensitive index of drug effects on the motoric aspects of licking [11,12]. The dose-related lengthening of reflexive lick duration observed in the present study suggests that the decreases in reflexive lick rate may be attributable, at least in part, to a neuroleptic-induced motor impairment. A similar lengthening effect on the duration measure has been reported following pimozide treatment with nondeprived rats licking a sucrose solution where the spatial arrangements were nearly identical to the present experiment [11]. Moreover, though not measured in the present study, neuroleptic treatment has also been reported to increase the time between licks [11,14], suggesting again a general slowing of the lick response as a result of neuroleptic treatment.

The dose-related lengthening of reflexive lick durations observed in the present study can be considered analogous to the motor deficit exhibited by rats treated with neuroleptics escaping an electric shock in the escape/avoidance paradigm. In a simple escape procedure, for example, the performance of rats receiving 1.2 mg/kg CPZ was significantly slower than saline controls in escaping shock in a straight alley runway, and substantial avoidance deficits were observed at this, and lower doses as well [15]. In the same study [15] rats dosed with phenobarbital (40 mg/kg) exhibited a slowing of the escape response similar to that observed in the CPZ treated animals; however, the phenobarbital treated rats, unlike the CPZ rats, did not fail to avoid the shock in response to a warning stimulus. Thus, though measurable motor deficits can be detected in the escape/avoidance procedures, these motor effects of neuroleptics do not seem to account for the anti-avoidance action of neuroleptics (e.g., [4,6]). Similarly, the proportionately greater rate reductions observed in the operant lick condition relative to the reflexive lick condition is probably not attributable to the lengthening effect of neuroleptics on the duration of the lick response. That operant lick duration was little affected or even shortened at doses that produced the largest decrements in lick rate suggests that the decreases in operant lick rate were not simply due to a further slowing of operant licking. Since the kinetic requirements of the lick response were identical in the two lick conditions, the differential effect of these neuroleptics on operant licking relative to reflexive licking suggests a non-motor explanation.

One might speculate that the different dose effects observed for the operant vs. reflexive lick duration reflect a difficulty in the operant group with the reinitiation of the lick response. Specifically, since the operant contingency involved movement between the operandum and the water cup, the operant rats were required to initiate repeatedly the lick response. If one of the effects of neuroleptics is to retard initiation of instrumental responses, then the proportionately greater effects of these neuroleptics on operant rate would be expected. Presumably, however, some part of the rate decrease observed in operant licking is due to the same motor impairment as that exhibited by the reflexive lickers, particularly where similar lengthening effects were observed on the duration measure. Nonetheless, it seems clear that neuroleptic treatment affects the tendency, as well as the ability, to lick in an operant context.

The results of the lick procedure presented here suggest that the explicit operant contingency, or the "conditionedness" of the response is an important determinant of neurolpetics' effects on behavior maintained by positive as well as negative reinforcers. In the escape/avoidance procedure the inability to initiate voluntary motor movements (i.e., initiate the avoidance response) has been attributed by some authors to a "subtle motor deficit" (e.g., [9]), rather than to the conditionedness of the response per se. Though subtle motor deficits are apparent in both the escape/avoidance procedure and the reflexive/operant lick procedure, it is possible that the deficit in initiation is a dissociative effect, not a motor one. Dissociative effects as used here means that responses which require more associative complexity for their occurrence are more easily uncoupled, or dissociated, from the stimulus cues which occasion them.

Neuroleptic treatment has been reported to have little effect on discrimination accuracy (e.g., [17,18]), but perhaps neuroleptics reduce the eliciting power of discriminative stimuli (independently of discriminability) whose behavior-controlling effects have been established by conditioning procedures. This view is not incompatible with the concept that stimulus efficacy may modulate the extent of neuroleptic disruption of ongoing behavior [5]; however, the dissociative idea being expressed here further implies a neuroleptic-induced deficit in response selection.

These speculations are tempered by the current absence of data to support neuroleptic specificity in producing differential effects on operant versus reflexive licking. Caution is further warranted in that the differential effect of these neuroleptics on operant compared to reflexive licking may be due to differences in their baseline rates of responding, rather than to the type of response per se. Though this interpretation cannot be ruled out, it is important to note that in the present study high rates (reflexive licking) were proportionately *less* affected by neuroleptic treatment than low rates (operant licking), a pattern not typically observed with rate dependent drug effects of neuroleptics [21].

One might also note that the use of separate groups of rats in the lick procedure precludes a complete analogy with the escape/avoidance procedure. Since the operant contingency involved the animals' moving between the operandum and the reinforcer dispenser and these additional motor movements were not required in the reflexive lick condition, the proportionately greater rate decreases observed in the operant lick condition might be due to a neuroleptic impairment of these additional locomotor movements. Some authors [8] have argued convincingly that neuroleptics promote static postural "inertia" and therefore it is possible that the locomotor component was an important determinant of neuroleptic effects on operant rate. At the higher doses of these drugs some of the animals in the operant lick condition did not respond at all and of those animals that did respond, some ceased before ever receiving the first reinforcer. Therefore, in these cases movement to the dispenser was not the key variable in cessation of responding. Moreover, where the response requirements were identical in both groups (i.e., the distance to the licking surface) the operant lick durations were affected differently than the reflexive lick durations suggesting that the effect of neuroleptic treatment was more than just a locomotor impairment.

The lick procedure would seem then to be a useful tool in separating neuroleptics' motor effects from their other putative behavioral effects. These results suggest that the explicit operant contingency or the conditionedness of the response may be an important determinant of the extent of neuroleptic disruption of ongoing behavior. This contention is congruent with the observations that animals treated with neuroleptics demonstrate the ability to learn and perform accurately discrimination tasks when the response demands of the task are minimized [18]. Moreover, both pretraining and intermittent retraining are reported to attenuate pimozide-induced avoidance deficits [3], suggesting again that the associative concomitants of the response are important determinants of the extent of neuroleptics' behavioral effects.

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