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# Haloperidol-Induced Decrements in Force and Duration of Rats' Tongue Movements During Licking Are Attenuated by Concomitant Anticholinergic Treatment

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FOWLER, S. C. AND S. DAS. *Haloperidol-induced decrements in force and duration of rats' tongue movements during licking are attenuated by concomitant anticholinergic treatment.* PHARMACOL BIOCHEM BEHAV **49**(4) 813–817, 1994.– To investigate the hypothesis that haloperidol's impairment of tongue protrusion in rats is Parkinson-like, the effects of centrally active scopolamine hydrochloride (0.1 or 0.2 mg/kg, SC) were evaluated in 36 rats that were also administered haloperidol (0.06, 0.12, or 0.24 mg/kg, IP). Rats were trained to lick water from a force-sensing disk, and the peak force and duration of each tongue contact were recorded along with the number of licks emitted in a 2-min session. Scopolamine hydrochloride significantly reversed haloperidol-induced deficits observed for peak force, duration, and number of licks. When given alone, scopolamine hydrochloride decreased peak force and duration. Fourier methods showed that the basic rhythm of licking was slowed by scopolamine hydrochloride but not by haloperidol. Taken together, the data suggest that central nervous system dopaminergic-cholinergic interactions importantly modulate tongue dynamics in the rat in a manner consistent with such interactions in neuroleptic-treated human patients.

Haloperidol Scopolamine Neuroleptic Anticholinergic Licking Tongue Fourier analysis Parkinson-like Rat

THE purpose of this study was to ascertain whether or not the alterations in tongue dynamics induced in rats by haloperidol (6) can be ameliorated by concurrent treatment with the muscarinic anticholinergic scopolamine. At low doses, haloperidol, a potent neuroleptic with predominantly dopamine $D_2$ receptor affinity, was observed to reduce number of licks emitted and to decrease peak force and duration of tongue contact with a force-sensing surface from which rats licked drops of water (6). Interest in the tongue as a model effector system for study here derives from the fact that neuroleptic-induced side effects in human patients often involve disruption of tongue movements as seen in pseudo-Parkinsonism or tardive dyskinesia (15). Moreover, anticholinergic amelioration of behavioral disruption occasioned by dopamine receptor-blocking drugs is often taken as evidence of the Parkinson-like nature of a neuroleptic's effects because anticholinergics reduce the symptoms of both idiopathic Parkinson's disease and neuroleptic-induced Parkinsonism (13). In view of these considerations, rats were treated with haloperidol, scopolamine HCl, or these two drugs in combination, and the effects were measured in terms of: 1) the number of licks emitted, 2) the peak force and 3) duration of individual tongue contacts, and 4) the dominant frequency of oscillation of tongue movements.

## METHOD

### *Subjects*

Thirty-six male, Sprague-Dawley rats (Harlan Co., Indianapolis, IN) were the subjects. At the time drug evaluations for this study began, they averaged 400 g in body weight and were 4 months old. All rats had previously received six doses

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of raclopride (maximum dose of 1.2 mg/kg) and six doses of SCH 23390 (maximum dose of 0.24 mg/kg) in a study designed to compare the effects of these two compounds. The last of these prior treatments occurred 13 days before the first haloperidol dose was administered in the current study. Animals were maintained on a restricted watering regimen of 5-min access 30 min after the 2-min experimental session. Measurement of licking performance occurred between 1400 and 1600 h daily during the light portion of the light-dark cycle in the vivarium (lights on 0800–2000 h).

*Apparatus*

The single recording chamber has been described in detail (6). It consisted of a modified Gerbrands rodent operant chamber fitted with a front panel containing a 6 × 6 cm square hole at floor level to which was affixed a 3-cm long transparent plastic enclosure where the lick surface was available through a 12-mm diameter circular hole on the bottom surface of the enclosure. The 18-mm diameter lick disk was position 5 mm below the inside surface of the plastic enclosure. The lick disk was rigidly attached to the shaft of a force transducer (Sanborn Model FTA 100). The natural frequency of the transducer assembly was 160 Hz. Support circuitry limited the high-frequency band pass to 100 Hz, thereby eliminating any natural frequency oscillations. The transducer gain was calibrated to resolve force to 0.2-g equivalent weights. A Labmaster interface recorded the force data at 100 samples/s via a 386-based computer. Illumination in the lick chamber was provided by a single GE 1819, 24 VDC light bulb located on the top center of the left side panel. Water (0.055 ml) was delivered onto the lick disk via 20-ga stainless steel tubing when the computer activated a solenoid valve interposed between the water reservoir and the tubing. The computer program measured the number of licks, peak force, and duration of tongue contact in real time. The force threshold for lick detection was 1.0 g, and the force criterion required for programmed consequences was 4.0 g. The entire force-time record for each 2-min session was stored in RAM and then transferred to hard disk at the end of each session for later Fourier analysis.

*Procedure*

During the initial session of training, the rectangular recession in the front panel and the contact disk were baited with water. As the tongue made 1-g contacts with the disk (as displayed on the computer monitor), the experimenter manually activated the solenoid valve to dispense water onto the disk. Once licking was established in this manner, the computer dispensed the water on a fixed-ratio 1 (FR1) basis for licks of 4 g or more. Then the water drop delivery was gradually shifted from a FR1 to a final FR12 schedule. Sessions lasted 2 min during which rats averaged about 600 licks. By the time the haloperidol observations were made, the rats had experienced about 60 unassisted sessions of licking water from the disk with water drop delivery every 12th lick. Drug testing was carried out every third day with two vehicle sessions separating drug sessions. Three doses of haloperidol (0.06, 0.12, 0.24 mg/kg, IP, 45 min) were given in counterbalanced order. Then these three doses of haloperidol were administered in combination with scopolamine HCl (0.1, 0.2 mg/kg, SC, 1 h). Doses of both drugs were counterbalanced for order of administration (with 2 vehicle days between drug treatments). Effects of the two doses of scopolamine HCl only were then assessed.

*Drugs*

Haloperidol (doses expressed as free base), obtained from Sigma (St. Louis, MO), was dissolved in a vehicle prepared by a drop of lactic acid per 10 ml of saline according to the concentration desired. Also obtained from Sigma, scopolamine (doses expressed as the hydrochloride) was dissolved in saline. For both routes of administration the injection volume was 1 ml/kg.

*Quantitative Analysis*

Effects of drugs and doses were examined in terms of four dependent variables: 1) number of licks in 2 min; 2) lick duration, which is the time the lick force remained above the detection threshold for a single lick; 3) the peak force of each lick event; and 4) the dominant rhythm of licking measured in hertz by spectral analysis (Fourier) techniques as described below. For a detailed discussion of the relationships between and among these measures of lick performance the reader is referred to a previous paper (6). To obtain session measures for lick peak force and lick duration for each rat, the peak force and duration data for individual lick events were compiled into a relative frequency distribution and the median of this distribution was used as the dependent variable, which was then subjected to statistical analysis procedures. Dose-effect data were expressed as a proportion of vehicle control performance, where the vehicle performance was for each subject an average of all the vehicle sessions. Repeated measures two-way (four levels of haloperidol dose × three levels of scopolamine dose) randomized block factorial analyses of variance (ANOVA) procedures were applied to assess treatment effects. Post hoc *t*-tests were used to assess differences between the two scopolamine doses when these were given alone. In the statistical tests, the degrees of freedom were sometimes less than one would expect for 36 rats because one subject died in the course of the experiment and occasionally a data point was lost because of diskette failure, etc. Also, at the two higher doses of haloperidol, some rats made no licks so peak force, duration, and rhythm measurements did not exist for analysis.

The force-time data from each session were subjected to Fourier analysis using the Fourier Perspectives III software available from Alligator Technologies (Carlsbad, CA). Each 120-s session of data was partitioned into 11 successive 10.24-s subepochs, each of which was Fourier transformed into the frequency domain. The 11 resulting power spectra were then ensemble averaged to yield a single power spectrum for each rat each session. The frequency associated with the spectral peak in the 3.5–6.5-Hz region of the spectrum was taken as the dominant rhythm of the lick oscillations. With these methods, the dominant rhythm is a measure of the periodicity of the lick oscillations; therefore, the rhythm of the oscillatory process can be largely independent of the number of licks. For example, two rats could have nearly identical dominant frequencies, even though one of them stops licking halfway through the session and thus emits 50% fewer licks than its cohort. When less than 10 licks were emitted in a burst, as was the case under some drug treatments, the dominant rhythm of licking was not estimated. This latter decision also influenced the degrees of freedom in some of the statistical tests.

## RESULTS

Absolute values for the four dependent variables characterizing tongue dynamics during the vehicle treatment conditions

are shown in Table 1. These data provide an anchor point for the proportion of control values arrayed in Fig. 1 as well as for comparison with previously published results.

*Number of Licks*

The effects of scopolamine HCl alone, haloperidol alone, and these two drugs in combination on number of licks are shown in the lower left quadrant of Fig. 1. Scopolamine HCl significantly reduced licking, $F(2, 60) = 3.266$, $p = 0.045$, with both doses having effects that were not significantly different from each other, $t(34) = 0.756$, $p > 0.100$. Haloperidol dose-dependently reduced number of licks, $F(3, 90) = 14.130$, $p < 0.001$. As suggested by the plotted data, the two drugs significantly interacted, $F(6, 180) = 20.711$, $p < 0.001$, such that both doses of scopolamine HCl substantially restored licking decreased by the two highest doses of haloperidol. A post hoc simple effects ANOVA indicated that 0.1 mg/kg scopolamine HCl produced more licking in the presence of haloperidol than the higher dose of scopolamine HCl, $F(1, 31) = 13.528$, $p = 0.001$.

*Peak Force*

Tongue contact force dose-dependently decreased after the rats were treated with scopolamine HCl, $F(2, 60) = 3.449$, $p = 0.038$; the 0.2-mg/kg dose of scopolamine had an effect significantly greater than the 0.1-mg/kg dose, $t(34) = 2.370$, $p = 0.024$. Haloperidol significantly decreased peak force of tongue contact, $F(3, 90) = 5.117$, $p = 0.003$. Consistent with the data shown in Fig. 1 (upper left quadrant), the two drugs interacted significantly, $F(6, 180) = 4.221$, $p = 0.001$. At the 0.1-mg/kg dose of scopolamine HCl, haloperidol's force-reducing effects were markedly attenuated, as portrayed graphically and confirmed by a comparison of haloperidol only vs. 0.1-mg/kg scopolamine HCl, $F(1, 31) = 6.027$, $p = 0.020$. Scopolamine HCl 0.2 mg/kg plus haloperidol across the three doses did not significantly antagonize haloperidol's effect on peak force of tongue contact, $F(1, 34) = 0.047$, $p > 0.100$.

*Duration*

The duration of tongue contacts with the lick surface (Fig. 1, upper right) was shortened by scopolamine HCl, $F(2, 58) = 3.195$, $p = 0.048$, but differences between scopolamine doses were not significant, $t(33) = 1.236$, $p > 0.100$. The main effect for haloperidol dose was not significant, $F(3, 87) = 2.449$, $p = 0.069$, owing to the nature of the significant interaction term, $F(6, 174) = 4.289$, $p < 0.001$. The primary effect of haloperidol when given alone was to decrease duration about equally below control levels at each dose: at the 0.06-mg/kg dose, $t(35) = 3.880$, $p < 0.001$; at the 0.12-mg/kg dose, $t(35) = 6.585$, $p < 0.001$; and at the 0.24-mg/kg dose, $t(35) = 7.451$, $p < 0.001$. Amelioration of the haloperidol-induced decrease in duration was significant at the 0.1-mg/kg dose of scopolamine HCl, $F(1, 31) = 12.649$, $p <= 0.001$. Despite the graphic separation evident in Fig. 1, antagonism of haloperidol's effects by the 0.2-mg/kg dose of scopolamine HCl was not significant, $F(1, 34) = 2.212$, $p > 0.100$.

*Dominant Rhythm of Licking*

As shown in Fig. 1 (lower right), the basic rhythm of licking was not significantly affected by haloperidol, $F(3, 54) = 2.290$, $p = 0.089$. On the other hand, the main effect for scopolamine HCl indicated a significant slowing of lick rhythm, $F(2, 36) = 22.532$, $p < 0.001$. The graphic representation of the data suggested that, compared to being given alone, scopolamine HCl may have had a greater slowing effect in the presence of haloperidol. This is supported by the fact that whereas scopolamine at the 0.1-mg/kg dose was not significantly different from vehicle, $t(32) = 0.840$, $p = 0.407$, scopolamine 0.1 mg/kg plus haloperidol produced a slowing of rhythm compared to haloperidol alone, $F(1, 20) = 21.745$, $p < 0.001$.

## DISCUSSION

The data on tongue dynamics during licking given in Table 1 agree closely with those previously gathered with the same methods (6) or similar methods (5,16,17). The mean 5.31-Hz frequency of oscillation of licking as determined here by the Fourier analyses accords well with several reports using somewhat different methods to estimate this basic rhythm (7,10,14).

The finding that haloperidol reduced number of licks and decreased peak force and duration of tongue contact with the disk is congruent with the literature (6). However, the previously observed, slight but statistically significant, slowing of the lick rhythm by haloperidol was not replicated in the current study, although the effect of the highest dose of haloperidol was in the expected direction. This lack of replication may have been the result of some of the more sensitive rats being deleted from the repeated measures ANOVA because they did not respond at the higher doses. In either this or the

TABLE 1

DESCRIPTIVE STATISTICS OF THE TONGUE DYNAMICS

| | Dependent Variable | | | |
|---|---|---|---|---|
| Statistic | No. of Licks in 2 min | Peak Force of Licks (g) | Duration of Licks (s) | Dominant Rhythm (Hz) |
| Minimum | 522.1 | 4.72 | 0.057 | 4.73 |
| Maximum | 673.8 | 12.01 | 0.094 | 5.73 |
| Mean | 616.6 | 8.36 | 0.071 | 5.31 |
| Median | 616.4 | 7.82 | 0.070 | 5.30 |
| SEM | 5.4 | 0.31 | 0.001 | 0.04 |

Values are for 36 rats trained to lick water from a force-sensing disk (see the text under Quantitative Analysis for further explanation).

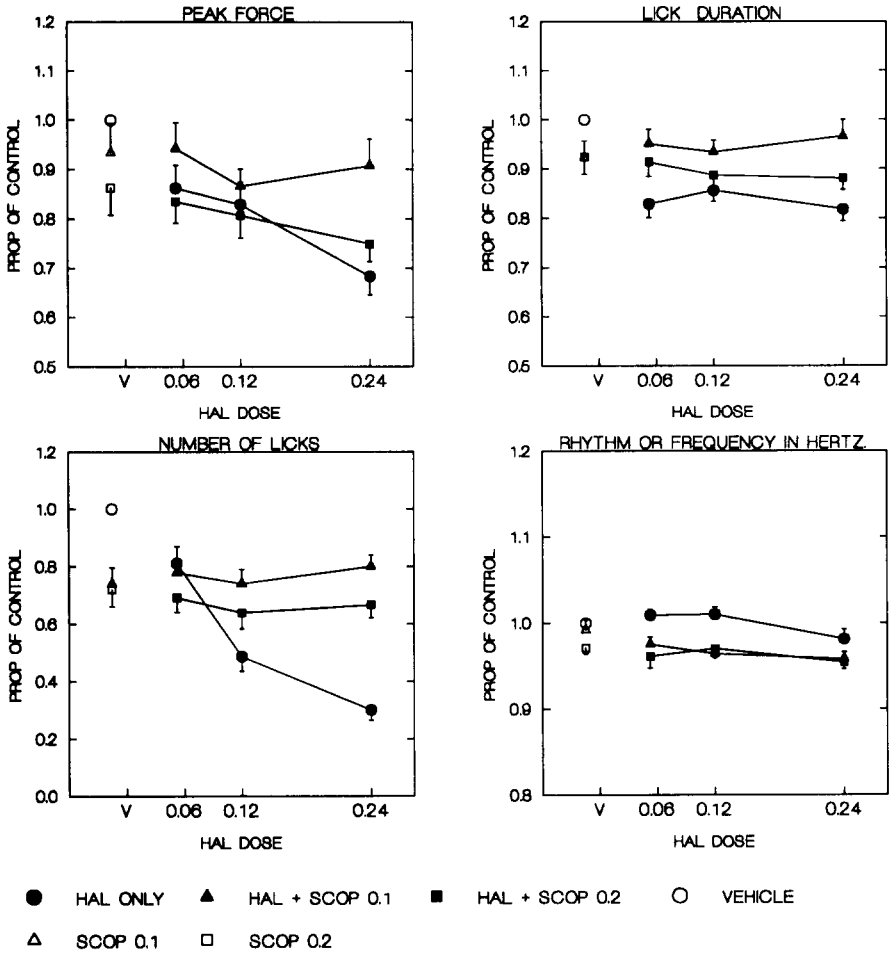


FIG. 1. Proportional effects of haloperidol and haloperidol plus scopolamine HCl on the indicated measures of tongue dynamics as rats licked drops of water from a force-sensing disk. Unfilled symbols designate haloperidol vehicle only or haloperidol vehicle with scopolamine HCl or scopolamine methyl nitrate. Brackets indicate 1 SEM; when a bracket is not shown the SEM was smaller than the data symbol.

cited paper, the effect of haloperidol on lick rhythm was quite small compared to its robust decremental effects on the other measures of licking performance.

Even though scopolamine HCl had disruptive effects on licking that were in the same direction as those induced by haloperidol, when the two drugs were coadministered the disruptive effects were not additive. Instead, the centrally active anticholinergic, particularly at the 0.1-mg/kg dose, markedly attenuated haloperidol's interference with licking. This result is consistent with the interpretation that haloperidol's disruption of licking behavior was a Parkinson-like effect (6). In addition, these data both reassert the importance of a proper dopaminergic/cholinergic balance for the expression of normal behavioral output [e.g., (13)] and extend this concept to rat tongue usage. The results also support the idea that the amplitude modulation (peak force variation) of licking is centrally mediated in part by neostriatal circuits where dopamine-acetylcholine interactions are prominent [e.g., (1)].

The fact that scopolamine HCl alone significantly diminished peak force and duration of licking may also be related to a dopamine/acetylcholine imbalance (11,12), but in this case the disequilibrium resulted from a relative dopamine excess instead of deficiency. Muscarinic cholinergic agonists, such as pilocarpine, were reported to induce pronounced orolingual dyskinesias, including chewing movements and tongue protrusions not directed at any object (11). Although the anticholinergic effects witnessed here for peak force and duration may be the result of neostriatal events, the slowing of the lick rhythm by scopolamine HCl (Fig. 1, lower right quadrant) seems more likely to be the result of scopolamine's interference with excitatory cholinergic influences on the hypoglossal nucleus (8). Dopamine does not appear to be a direct modulator of neurons in the hypoglossal nucleus (3), but these neurons do receive strong noradrenergic (3) and serotonergic influences (2,4,9). We currently have no explanation for why the scopolamine/haloperidol combination produced a greater slowing of rhythm than scopolamine alone.

When scopolamine HCl and haloperidol were given together, licking rhythm was slowed significantly, even though these same drug/dose combinations resulted in improved performance relative to that witnessed for the effects of haloperidol only on number of licks and on peak force and duration

of individual licks. Taken together, these results and the discussion in the foregoing paragraph suggest that the oscillation frequency of licking may be controlled separately from the initiation of licking (number of licks) and from the amplitude modulation of licking (peak force). These latter aspects of the lick performance were responsive to $D_2$ dopamine receptor antagonism and to the muscarinic modulation of this blockade, and therefore are probably reflective of processes homologous to those seen in neuroleptic-induced motor side effects in human patients.

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