Genetic and Environmental Variability in Lick Rates of Mice

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HOROWITZ, G. P., F. K. STEPHAN, J. C. SMITH AND G. WHITNEY. Genetic and environmental variability in lick rates of mice. PHYSIOL BEHAV. 19(4)493–496, 1977. The hypothesis of lick rate invariance was tested by comparing rates of licking among two inbred strains of mice and their F₁ hybrids across two different fluid regimens. DBA mice licked significantly faster than C57 mice, while the lick rate of both reciprocal F₁ groups was intermediate between that of the two parental strains. This ordered relationship held when either water or a morphine solution was the available fluid. C57 mice, but not others, also displayed transient changes in lick rate with changes in fluid regimens. Thus, rates of licking among mice are neither genetically nor environmentally invariant.

Lick rates Genetic factors Taste

SINCE Stellar and Hill [9] reported that rats lick at a rate of 6–7 counts per second, or not at all, much attention has been devoted to developing and testing the hypothesis of lick rate invariance in a number of mammalian species [2]. There are two central aspects of this hypothesis. The first is the invariance of lick rates within subjects across a variety of experimental and developmental conditions. It has been shown that interlick intervals do not vary significantly when albino rats are exposed to different levels of deprivation, solutions of varying degrees of palatability, or when they are made polydipsic by surgical manipulations [4]. Similar results have also been reported for cats [7]. In both rats [8] and cats [7], age does not seem to be a critical variable affecting lick rates. However, contrary to the predictions of the invariance hypothesis, significant differences in the local lick rates of rats as a function of sex, time of day and number of test sessions have been reported [3].

The second aspect is a lack of variability in lick rate among individuals within a given species, when the experimental condition is held constant. This aspect is reflected in the suggestion that the rate of 6–7 licks per second reported for rats [9] is not an average value, but rather a constant that describes the licking behavior of all rats [6]. Individual differences in fluid ingestion arise, according to this view, not from differences in burst rates of licking, but from differences in the duration and number of bursts.

The apparent invariance of lick rates within species and across experimental conditions is of interest for several reasons. For example, it has been suggested that licking is an on-off phenomenon, possibly under relatively simple, and therefore identifiable, neural control [4]. Furthermore, it has been suggested that the invariance within species together with differences in lick rates among species may provide a useful behavioral index of phylogenetic relationships [7]. Invariance of lick rates is also an important consideration for response-based reinforcement theory [6].

The present study was designed to investigate both the effects of palatability of the ingested solution and the influence of genetic factors on local lick rates in mice. The animals tested were part of an ongoing experiment to evaluate the effects of ingested morphine on several behaviors, including lick rates. At the concentration used in this study, morphine is bitter to humans and is avoided by mice in a two-bottle choice situation [5]. Differences in lick rates between inbred strains of mice that are greater than differences within strains would suggest genetic factors that influence this behavior. These differences would support the existence of within species variability.

METHOD

Animals

The 36 animals were 9 male mice each of two inbred strains (C57BL/6J and DBA/2J) and their derived reciprocal hybrids (C × D, D × C). All animals were raised in our laboratory, weaned at 21 days of age and housed as same-sex littersmates until the beginning of the experiment. In both rearing and experimental conditions, the subjects were exposed to a 12 hr light – 12 hr dark photoperiod, with the light period beginning at 0730 each morning. Food was available ad lib throughout the experiment.

Apparatus

The experimental cages were standard stainless steel mouse cages (10 × 24 × 13 cm) with floor and front of

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FIG. 1. Mean peak interlick intervals (ILI) are shown for four strains of mice across days of three experimental conditions. Standard errors around data points range from 0–3 msec. These mean peak ILI's correspond to mean lick rates from about 7.5, 9.2 and 10.5 licks per second for C57, F1 and DBA mice, respectively.

Hardware cloth. Food was available from a hopper located on the back wall of the cage. Each unit included a drinking spout mounted on the front of the cage. Solutions were available from an inverted 25 ml graduated cylinder attached to the drinking spout. An infrared light emitting diode and a transducer were positioned so that each lick interrupted the infrared beam resulting in a switch closure. A PDP–8e computer was used to measure and record the intervals from each lick onset to onset. In this report, these intervals are referred to as interlick intervals (ILI), and a frequency distribution of these intervals from 0–390 msec was plotted with a class interval size of 10 msecs. An onset to onset interval greater than 390 msec was considered a pause, and was excluded from subsequent analysis (cf. [4]). The reciprocal of these intervals represents the rate of licking.

Procedure

Drinking was monitored 20 hr each day starting at 1400 hr. From 1000 hr–1400 hr each day, the routine maintenance was performed. Fluid intake was measured to the nearest 0.1 ml daily.

During a ten day baseline period, drinking tubes were filled with tap water. Following this baseline period, the water in each drinking tube was replaced with 0.375 mg/ml (w/v) morphine sulfate in aqueous solution, starting at 1400 hr. The same procedure was followed until 10 days of morphine treatment was completed (treatment phase). Finally, following this prolonged morphine exposure, all animals were again given water in their drinking tubes for a period of 5 days (withdrawal phase).

RESULTS AND DISCUSSION

The analysis of modes of the interlick interval histograms (peak ILI) showed clear differences among the genotypes of mice tested. Figure 1 shows the daily mean peak ILI's for the four strains of mice under baseline, treatment and withdrawal conditions. A mean peak ILI was calculated for each mouse under each of the three conditions, and these values were subjected to a repeated
measure analysis of variance. As is evident in Fig. 1, there was a significant strain difference when peak ILI's were examined, $F(3,32) = 112.75, p<0.01$. Orthogonal comparisons revealed that C57 mice were significantly different from DBA mice, $F(1,32) = 325.41, p<0.01$, and that there was no difference between the reciprocal hybrids $F(1,32) = 1$. The combined hybrids differed significantly from the theoretical midparent value, $F(1,32) = 12.70, p<0.01$, indicating incomplete dominance in the direction of the fast licking parental strain. Subsequent post hoc Tukey B comparisons [10] confirmed that the hybrids were significantly different from both parental strains ($p<0.01$ for each comparison).

The analysis of mean peak ILI's did not reveal significant effects of either experimental condition or the genotype $\times$ condition interaction. However, since scores from individual animals were expressed as mean peak ILI's for each experimental condition, the analysis might not have been sensitive to changes in lick rates during the transition from one experimental condition to another. In order to examine this possibility, scores from mice of each genotype were analyzed for changes from Day 10 to Day 11 (baseline to treatment) and from Day 20 to 21 (treatment to withdrawal). No significant changes were evident during either transition for mice of the DBA, D $\times$ C or C $\times$ D strains. However, C57 mice displayed orderly and significant changes in peak ILI's, increasing their lick rate from the last day of baseline to the first day of morphine treatment, $t(8) = 3.50, p<0.01$, and decreasing their lick rate from the last day of treatment to the first day of withdrawal, $t(7) = -1.92, p<0.05$. These results suggest that there may be a transient genotype-specific sensitivity to changes in ingested solutions as reflected in local lick rates. It should be noted that injections of morphine cause a more pronounced hyperactivity in C57 mice than in the other genotypes included in this study [1]. The observed increase in lick rate from Day 10 to Day 11 in C57 mice may be due to central stimulating properties of the drug, rather than changes in the palatability of drinking solutions. This suggestion is supported by the fact that fluid ingestion decreased from the last day of baseline to the first day of treatment for C57 mice, even though lick rate increased over the same period. In other experiments conducted in our laboratory, the lick rate of C57 mice did not change when water was replaced with saccharin, while mean fluid intake went from 7.3 ml of water to 14.1 ml of saccharin. This finding is in agreement with previous findings for rats [4], and supports the suggestion that duration and number of drinking bouts may be more important than burst lick rates in determining fluid intake.

Figure 2 represents the ILI distribution of one C57 and one DBA mouse on the last day of water baseline. Each point is expressed as the percent of the peak ILI responses. The standard deviations of these distributions are similar to...
those previously reported for rats when a cut-off criterion of 200 msec is applied (97.3% of the ILI's for the DBA animal and 98.4% of those of the C57 are less than 200 msec). When only these ILI's are considered, the DBA mouse has a mean ILI of 100.9 msec and a standard deviation of 15.5, while the mean (± standard deviation) ILI for the animal of the C57 strain is 129.4 ± 18.3 msec. Although these means are different from those reported previously for rats, the standard deviations are remarkably similar for both species (cf. [4]). As in the study employing rats as test animals, there are hints of harmonic ILI's which become more noticeable with increased fluid intake (e.g., saccharin). The nature of these harmonics has not been established, but they may indicate that the animal is swallowing.

The demonstration of clear genetic differences among inbred and hybrid mice in local lick rates is strong evidence against the invariance of lick rates within the species tested in this study. Systematic changes in the peak ILI for C57 mice, corresponding to changes in ingested solutions, suggest a need for a reexamination of lick rates across various solutions using subjects of diverse genetic background.

The intermediate values exhibited by reciprocal hybrids is interesting, especially with reference to the possibility that local lick rate is under relatively simple neural control [4]. Further genetic analysis could prove fruitful in clarifying the nature of the genetic system or systems influencing this possibly simple mechanism.

REFERENCES