Physiology & Behavior, Vol. 60, No. 5, pp. 1277–1283, 1996

0031-9384/96 $15.00 + .00

ELSEVIER

SSDI 0031-9384(96)00252-I

# TongueTwister: An Integrated Program for Analyzing Lickometer Data

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Received 8 February 1996

HOUPT, T. A. AND S. P. FRANKMANN. *TongueTwister: An integrated program for analyzing lickometer data.* PHYSIOL BEHAV **60**(5) 1277–1283, 1996.—The analysis of lickometer data is often rendered prohibitively tedious by the large volume of data generated by the typical experiment. TongueTwister is an integrated program for the rapid and automatic analysis, presentation, and summary of long- and medium-access data collected by lickometers or of brief-access data collected by multi-bottle lickometers such as the DiLog Instruments MS80. The program was written in C++ for Macintosh® computers, and analyzes data collected by MS-DOS PCs. It takes advantage of the Macintosh® user interface to provide quick and convenient output from all the files of a single experimental session, and to export the data to third-party statistical software or other documents. It can batch-process data files by automatically opening and analyzing all the files in a directory; thus, the user can employ directories as a simple database for organizing experimental groups. When a lickometer data file is opened, a textual summary, a raster plot of the lick pattern, the cumulative licks, the lick rate, a histogram of inter-lick intervals, and a breakdown of the session by fractions are automatically calculated and displayed. When an MS80 brief-access file is opened, the lick pattern for each tube presentation and a textual summary of the mean values derived for each tube are automatically displayed. If a directory of files is opened, the mean values derived across all the individual files are calculated and graphed. Analysis parameters can be tailored to the investigator's liking. Tables or graphs can be saved to disk, or copied and pasted into other Macintosh® programs for additional analysis. The program may also be used for general-purpose analysis of periodic event records. *Copyright © 1996 Elsevier Science Inc.*

Licking Taste Time series analysis Computer

THE study of ingestive behavior has been characterized by measurements that quantify food intake, amount of work performed (i.e., bar pressing) (1), speed of running (23) and other measures of motivated appetitive behavior. These measures infer the motivated behavior of ingestion by measuring correlates of ingestion, but do not measure the actual oromotor behavior of ingestion (13). As such, these measures are limited in their value for understanding the moment-to-moment changes in the decision-making processes of the animal to ingest or not to ingest during a meal. To better understand the instantaneous behavior of the animal, the actual orofacial movements that underlie the behavior of ingestion must be studied. In many cases, this calls for studying licking by the rat: the rhythmic protrusion and retraction of the tongue that results in the delivery of liquids to the mouth for sensory evaluation, followed by rejection or delivery to the digestive tract by swallowing.

The number of investigators who use the measurement of licking to understand the behavior of ingestion in the rat has increased markedly in recent years (10). The two most commonly used techniques to measure licking are electric contact (each contact of the rat's tongue with the metal sipper tube momentarily closes an electric circuit) and photobeam break (each protrusion of the tongue momentarily breaks a photoelectric beam). Both take advantage of the motor movement of the rat's tongue during licking, in which the tongue momentarily protrudes from the oral cavity while making contact with the drinking spout.

Early investigators were troubled by the potential of the measurement procedure to interfere with the behavior (i.e., by electric current detection or positioning of the photo beam) (21). Electrical currents below the ability of the rat to detect are now used (60 nanoamp) (21) and sophisticated lickometers that amplify and record the time of each lick with ms resolution in a computer data file are commercially available (DiLog Instruments, Tallahassee, FL).

Organized bouts of licking within the lickometer data are detected using burst and cluster criteria that have gained wide acceptability. The criteria arise from the observation that rats lick at a rate of 6–7 licks/second (2,9,18). This ''local'' rate of licking is governed by a central motor pattern generator and represents the maximal rate of licking that a rat can sustain. This observation is supported first by measurement of licks, in which 90–95% of the licks occur with this frequency (3), second by neurophysiological data demonstrating a central pattern generator for this frequency (8,22), and third by electromyographic

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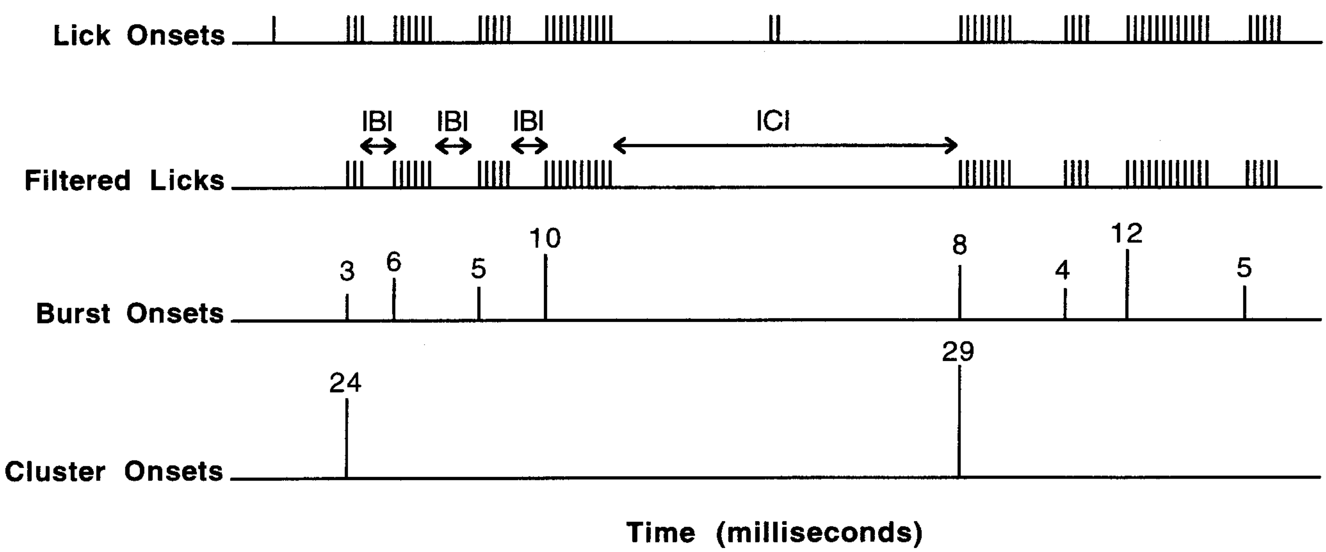



FIG. 1. Examples of time series derived from lickometer data by TongueTwister. From top to bottom: Lick onsets are recorded in ".RAW" and ".RIF" files with ms resolution. Isolated single or double licks are filtered out as potential artifacts. Burst onsets are derived from groups of licks that are separated by a minimal interburst interval (IBI) criteria. The burst onsets are recorded as an onset time, and an amplitude equal to the number of licks within the burst. Cluster onsets are derived from groups of licks separated by an intercluster interval, and recorded with an onset time and amplitude (number of licks). Other time series, such as ILI intervals, cumulative licks, lick rate, etc. are similarly derived.

data (19). When rats lick at a fixed rate of 6–7 licks/s, an interlick interval (ILI) of 0.16 s is generated (3,4). This ILI represents not only the fixed local rate but, also, the maximal rate. Any modulation of the rate of licking must be achieved by increasing the number of pauses and, thus, decreasing the number of licks/s on average. That is to say, when the rat licks without pause, it does so at the rate of 6–7 licks/s, and this rate appears not to be increased for a given configuration of the licking tube. It can only be decreased and only by stopping licking. Thus, while repeated ILIs of 0.16 s occur in long uninterrupted sequences, ILIs of longer duration occur as single events that interrupt or punctuate the long strings of the minimum ILI, and may reflect pauses in the attention of the rat to the behavior of licking.

These longer interruptions can be organized into at least 2 useful subunits of pauses in successive licks: 1. Bursts of licks with pauses of greater than 0.25 but less than 0.5 ms (approximately one missed lick); and 2. Clusters of licks with pauses of greater than 0.50 ms (5). These units generate the additional measures of the interburst interval (IBI) and the intercluster interval (ICI).

TABLE 1

TIME SERIES USED BY TONGUE TWISTER

| |
|---|
| Lick onsets |
| Filtered lick onsets |
| Bursts |
| Clusters |
| Interlick intervals |
| Interburst intervals |
| Intercluster intervals |
| Cumulative licks |
| Lick rate (licks per unit time) |

Even after reducing the raw data of licks into bouts using these criteria, the task of analyzing and displaying lickometer data is time-consuming. The analysis of lickometer data lies between a purely descriptive narrative of the behavior of a single rat, and an analysis based on discreet time sampling of the intake of a group of rats, which allows statistical organization of the data. Although the capacity of the hardware for monitoring licking continues to expand, and theoretical insights into the structure of lickometer data continue to evolve, the day-to-day analysis of the data remains a rate-limiting factor for experimentation.

The program described here, named TongueTwister, analyzes and graphically displays licking data, using widely accepted criteria for organizing the patterns of episodes of licking. The TongueTwister program (TT) offers investigators the ability to display the data in many formats rapidly and automatically. Analysis parameters can be tailored by the user. The results, as well as the raw data, are exportable to other programs for incorporation into presentations or for statistical analysis. TT removes a major bottleneck in lickometer experimentation by relieving the investigator of the difficulties of analyzing and graphing the data in separate steps.[2]

## PROGRAM DESCRIPTION

### *Licking as Time-Series Data*

The time series of licks represents a train of neural pulses generated by the hypoglossal nucleus to the muscles of the tongue and mouth. The neural network underlying ingestive behavior controls proximal liquid intake primarily by modulating the temporal characteristics of these time series (e.g., frequency of licks, bouts of licks, etc.), although the absolute

[2] Copies of the compiled program and documentation are available to academic researchers upon request, or via the World Wide Web at http://bourne.med.cornell.edu

TABLE 2
VARIABLES CALCULATED BY TONGUE TWISTER

| Variable | Description |
|---|---|
| Latency | time between start of test session and first lick |
| Total number of licks | number of licks occurring during test session |
| Number of bursts | number of groups of 3 or more consecutive licks, separated by $<$ burst-interval criteria (250 ms default) |
| Mean burst size | mean number of licks per burst |
| Number of clusters | number of groups of consecutive licks, separated by $<$ cluster-interval criteria (500 ms default) |
| Mean cluster size | mean number of licks per cluster |
| Total lick duration | time between first lick and last lick of test session |
| Total lick time | sum of all ILIs $<$ burst-interval criteria |
| Mean ILI | mean duration of all ILIs $<$ burst-interval criteria |
| Mean IBI | mean duration of all ILIs $>$ burst-interval and ILIs $<$ cluster-interval criteria |
| Mean ICI | mean duration of all ILIs $>$ cluster-interval criteria |
| Percent ILI | percent of total test-session time composed of ILIs $<$ burst-interval criteria |
| Percent IBI | percent of total test-session time composed of IBIs |
| Percent ICI | percent of total test-session time composed of ICIs |

intake per lick depends on many variables, such as the amplitude (i.e., force) of licking (11,12) or the configuration of the tube. TT analyzes lickometer data by transforming the times of individual licks into several time series (Fig. 1).

TT reads data files that list time stamps of each lick onset to ms accuracy. The series of lick onsets forms the basis for further transformations. As an option, the raw lick onsets can be filtered to remove isolated licks. Because rats tend to lick in bursts of 3

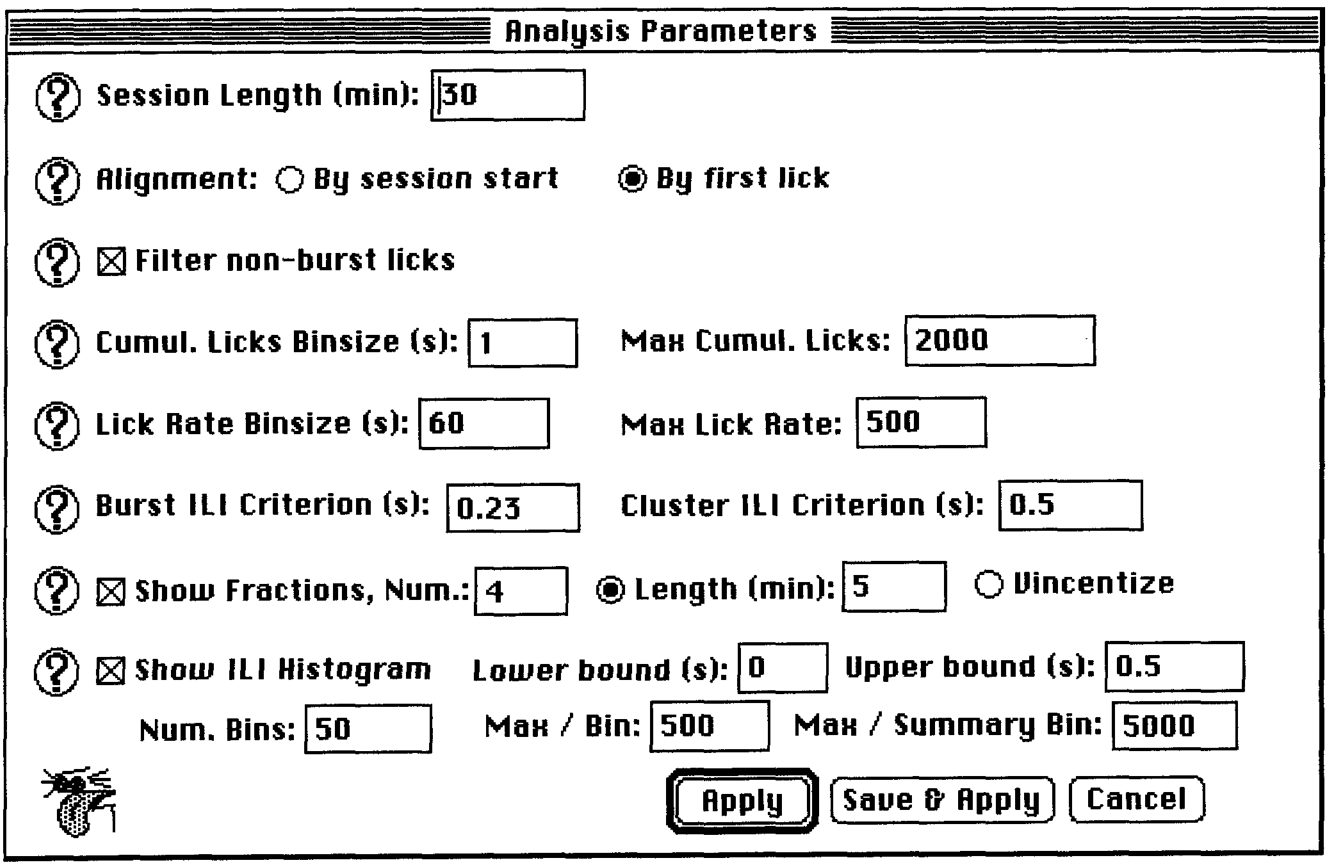
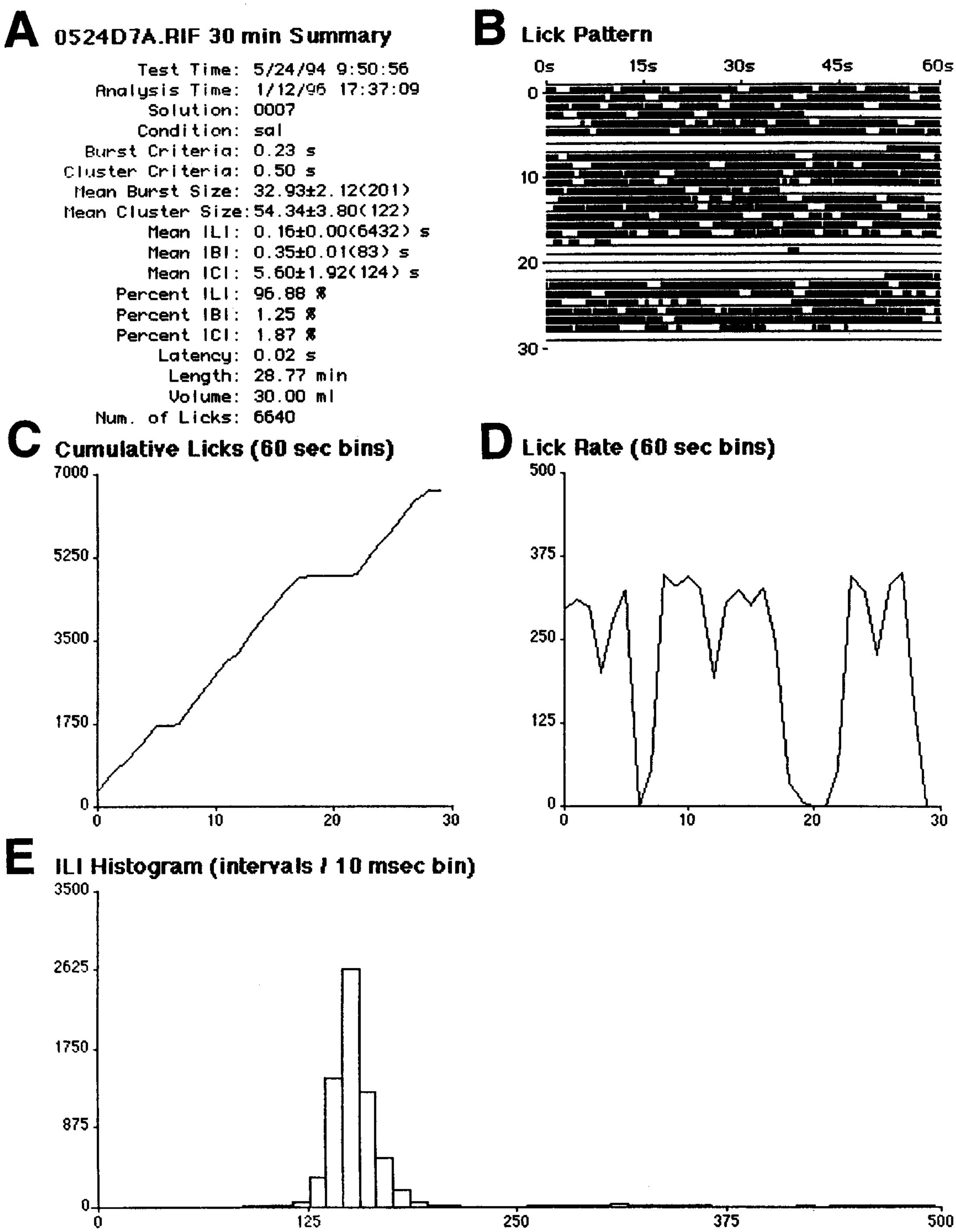



FIG. 2. The analysis parameters dialog box, by which the user can specify the criteria and graphing parameters TongueTwister uses. For example, the minimum and maximum criteria for the duration of interburst and intercluster intervals can be redefined here. The values for $x$- and $y$-axes of the graphs can also be set. Clicking on the question-mark buttons opens help windows that describe the various parameters and their application.




**F** 5 min Fractions (aligned at first lick)

| | Licks | Lcks/Brst | Num | Lcks/Clstr | Num | ILI,s | IBI,s | ICI,s |
|---|---|---|---|---|---|---|---|---|
| 1 | 1392 | 41.32 | 34 | 49.31 | 29 | 0.162 | 0.374 | 0.374 |
| 2 | 1060 | 50.81 | 21 | 74.64 | 14 | 0.155 | 0.358 | 0.358 |
| 3 | 1491 | 31.19 | 47 | 62.56 | 25 | 0.156 | 0.339 | 0.339 |
| 4 | 916 | 22.22 | 41 | 37.09 | 22 | 0.155 | 0.354 | 0.354 |
| 5 | 721 | 40.72 | 18 | 61.33 | 12 | 0.152 | 0.354 | 0.354 |
| 6 | 1060 | 25.90 | 40 | 51.95 | 20 | 0.153 | 0.337 | 0.337 |

or more consecutive licks, isolated single or double licks may represent equipment or movement artifacts and, therefore, may be discarded at the discretion of the investigator.

The time stamps could represent any series of behavioral events such as lick offset, bite onset for chewing, vocalizations for squeaking, etc.; but the DiLog systems, like most lickometer systems, record the time of initial tongue contact with the drinking spout. Thus, TT does not take into account the amplitude or duration of individual licks. These variables may be important under some circumstances, but would require a modified data acquisition program.

Seven time series are derived form the lick onset time series (Table 1). These time series are calculated when the lickometer data file is first opened and are, thus, all available for plotting or analyzing immediately. By employing the time series as the basic data object, the program can display or analyze any or all of the series using the same set of time-series analysis routines. Thus, x-y graphs, raster plots, histograms, cumulative plots, or text files for each of the different time series are all generated by a generic set of routines that are coded only once for a generic time-series data structure. This allows for the addition of other analysis routines that will automatically be applicable to all time series, and adding other time series that can take advantage of all the analysis routines.

Furthermore, by putting derived variables (such as bursts and clusters) into time series with ms accuracy for each datum, the time series can be rapidly binned into larger time units for display or analysis (e.g., bursts per min, or clusters per quarter of the test session.)

Some of the time series consist of time stamps of the event occurrence only, such as the series of lick onset times. Other time series contain evenly binned numbers, such as the cumulative number of licks per unit time. The bout-derived time series (bursts and clusters and intervals), however, contain paired data with both the time of bout onset and the size of the bout. Thus, if the first lick of a burst of 6 licks occurred at 5.2 s, the burst would be represented by the paired data point 5.2, 6. Likewise, an interlick interval between 2 licks at 5.2 and 5.35 seconds would be coded as 5.2, 0.15.

The bout-derived time series are therefore encoded in the same way that hormonal pulse data are often recorded, with a time stamp of pulse onset paired with pulse amplitude or duration (20). This allows for a standard representation of behavioral events in time series that can then be consistently analyzed without ambiguity. Although mathematically satisfying, however, this encoding scheme can lead to counter-intuitive results. For example, if a rat begins a cluster of licking that lasts 5 min within the first min of the test session, the apparent size of the clusters within min 1 of the session will be 5 min. The strength of this scheme is that the program does not need to decide whether to exclude a 5-min cluster from 1-min analyses, or to include just the first min and exclude the remaining 4 min. TongueTwister analyzes whole cluster onsets and sizes, not arbitrarily divided fractions of clusters.

This approach assumes that the underlying neural network generating licking behavior is a pulse generator comparable to the hypophyseal endocrine system that generates discrete pulses (20). For the purposes of analysis, a bout of licking (burst or cluster) is considered a discrete event with duration. The timing of the bouts is characterized by bout onset. Burst and cluster analysis of licking behavior seems to support the assumption that licking is modulated during a meal at the level of burst and cluster size and timing (5). It is possible that future research will render this quantal characterization of licking untenable.

*Derived Variables*

In addition to the time series themselves, a set of descriptive variables are calculated to summarize the test session of licking (Table 2). These variables include session-specific values, such as the latency of the rat to lick, and the overall mean values of the time series described above.

The mean values can also be calculated for arbitrary fractions or intervals of a lickometer test session. The whole test session can be divided into any number of fractions (quarters, fifths, etc.) and the mean values listed in Table 2 are calculated separately for each fraction. The mean values can also be calculated for intervals defined by a start time and an interval duration. For example, the licking can be summarized separately for the first 5 min of a test session in 1-min intervals. This allows direct comparison of the first min of licking, which is correlated with the rat's initial sensory evaluation of the liquid, with subsequent minutes that reveal postingestive modulation of the initial gustatory response (4).

*Analysis Parameters*

TT employs a number of criteria and parameters when analyzing and graphing data. For example, minimal interlick interval criteria are used to distinguish separate bursts and clusters. Standard default values are built into TT (e.g., 0.25 s minimum duration for an interburst interval), but the default analysis and graphing parameters can be changed by the user and saved between program runs (Fig. 2). The most important value that the user must define is the length of the test session for 1- or 2-bottle lickometer tests, because this information is not stored in the ".RIF" or ".RAW" lickometer data files. By retaining the user-defined values between test sessions, the user has to set up the format and analysis parameters for a set of experiments only one time.

*Lickometer File Analysis*

TT can read 2 types of lickometer files: continuous lickometer test sessions and brief-access test sessions. For the first type of lickometer file, TT can read the ".RAW" or ".RIF" files generated by the Dilog Instruments lickometer system, or it can import ASCII text files containing time-stamped lick onsets generated by other lickometer systems. The Macintosh® computer supports the direct reading of MS-DOS disks, so no conversion is required to read disks generated by PC systems. The continuous test sessions usually consist of ad lib 1- or 2-bottle tests lasting from a few min to a few h. (Although TT can accommodate lick data containing up to 4 billion licks spanning test sessions of up to 45 days duration (given enough computer mem-

FIG. 3. An example of the output generated automatically by TongueTwister from a 1-bottle lickometer test session (a food-deprived rat drinking 0007 liquid diet). (A). Descriptive summary statistics of the whole test session. (B). Raster plot of test session. Each horizontal line represents a consecutive minute of the test session. Vertical deflections from the horizontal line represent single licks (which appear merged into solid blocks of lick clusters). (C). Cumulative licks across the 30-min test session plotted with 1-min resolution. (D). Number of licks per 60-s bin across the test session. (E). Histogram of number of interlick intervals in 10-ms bins between 0 and 500 ms. (F). Descriptive statistics for licking within each of the 6 5-min blocks across the session.

**0603DR16.MS8 Tubes**

| # | Conc. | Solution | Pres | Licks | Lat,s | Dur,s | Time,s | #Bur | BurSize | #Cls | ClsSize | ILI,s | IBI,s |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.60 | MNaCl | 1/1 | 170.00 | 29.55 | 55.60 | 23.68 | 12.00 | 14.08 | 11.00 | 15.36 | 0.15 | 0.32 |
| 2 | 0.50 | MNaCl | 1/1 | 123.00 | 2.67 | 55.78 | 15.80 | 14.00 | 8.57 | 13.00 | 9.23 | 0.15 | 0.30 |
| 3 | 0.40 | MNaCl | 1/1 | 154.00 | 5.92 | 58.51 | 19.99 | 15.00 | 10.13 | 14.00 | 10.93 | 0.14 | 0.36 |
| 4 | 0.30 | MNaCl | 1/1 | 360.00 | 20.41 | 59.58 | 57.04 | 5.00 | 71.80 | 5.00 | 71.80 | 0.16 | --0-- |
| 5 | 0.20 | MNaCl | 1/1 | 232.00 | 1.44 | 59.25 | 33.71 | 10.00 | 23.00 | 8.00 | 28.75 | 0.15 | 0.32 |
| 6 | 0.15 | MNaCl | 1/1 | 192.00 | 1.40 | 57.71 | 28.27 | 8.00 | 23.88 | 6.00 | 31.83 | 0.15 | 0.32 |
| 7 | 0.08 | MNaCl | 1/1 | 183.00 | 9.05 | 50.34 | 26.21 | 11.00 | 16.36 | 9.00 | 20.22 | 0.15 | 0.40 |
| 8 | 0.05 | MNaCl | 1/1 | 96.00 | 1.96 | 32.61 | 13.12 | 7.00 | 13.29 | 4.00 | 23.25 | 0.15 | 0.43 |

**0603DR16.MS8 Lick Pattern (60s wait, 60s access)**

| # | Tube | Conc. | Solution | Licks |
|---|---|---|---|---|
| 1 | 1 | 0.60 | MNaCl | 170 |
| 2 | 2 | 0.50 | MNaCl | 123 |
| 3 | 3 | 0.40 | MNaCl | 154 |
| 4 | 4 | 0.30 | MNaCl | 360 |
| 5 | 5 | 0.20 | MNaCl | 232 |
| 6 | 6 | 0.15 | MNaCl | 192 |
| 7 | 7 | 0.08 | MNaCl | 183 |
| 8 | 8 | 0.05 | MNaCl | 96 |

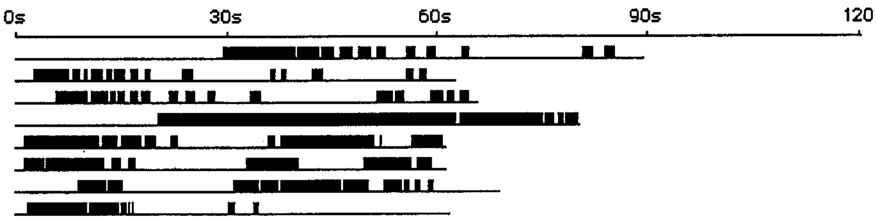



FIG. 4. Example of the output generated from a MS80 brief-access data file (a sodium-depleted rat given 60-s access to each of 8 NaCl solutions presented in order of descending concentration). Top, a summary table of mean values for each solution averaged across presentations. (In this example, only one presentation was made for each solution.) Bottom, raster-plot of licking during each presentation of the solutions. The horizontal access represents the maximum time the MS80 shutter could have been open (120 s = 60-s maximum latency plus 60-s access from first lick, in this example). For each presentation, a horizontal line is drawn indicating the actual time the shutter was open and the tube was available for licking. The vertical deflections from the horizontal line indicate individual licks.

ory), the current version only supports the display of lick files up to 6 h long.)

TT displays summary statistics and graphs for a lickometer test session file in a single window (Fig. 3). When the lick file window is printed, each file fits on 1 or 2 sheets of standard 8.5" × 11" paper. The default displays include a raster-style plot of individual licks across the test session, cumulative licks per minute, lick rate (licks per min), a histogram of ILIs, and summary statistics calculated by fractions or intervals. All of these graphs are automatically generated when a file is opened, requiring no effort from the user aside from specifying the file. The default plotting and analysis parameters can be changed and then saved for use in the next session.

*Brief-Access Trial Analysis*

The second type of lickometer file that TT can analyze is the brief-access test file. In a brief-access test apparatus, such as the MS80 system marketed by DiLog Instruments, the rat is placed in a test chamber in which a shutter opens and shuts to allow limited access to 1 of 8 drinking tubes mounted in a moveable carriage. The rat is given only a short time (ca 10–30 s) to lick at the drinking spout before the shutter closes, and then another 1 of the 8 solutions is moved into position and the shutter reopened. In this fashion, the rat can be repeatedly exposed to 8 solutions in any order in rapid succession. The licks are collected by computer and later analyzed and summarized to reflect the pattern of licking. The advantage of this method is that a large number of test stimuli can be tested within each session. Furthermore, the short duration of contact with the various solutions reveals the rat's initial evaluation of the solution by minimizing the volume ingested and thus the postingestive consequences (14).

TT can read the ".MS8" files produced by the DiLog MS80; the program could be easily modified to read other brief-access lickometer files, such as that used by the Spector gustometer (16). As with the 1- or 2-bottle lickometer sessions, TT opens a single window for each brief-access file it reads (Fig. 4). For each solution presented, the individual licks during the brief-access period are plotted in a table of raster-plots. The pattern of licks is visualized, which immediately reveals differences in the rat's response to the different solutions. Above the table of individual presentations, the mean summary statistics for all the presentations of each solution are tabulated.

*Batch Analysis*

Although it is convenient to open a single lickometer file and automatically have a summary page of the test session, most investigators study multiple rats within an experiment. Therefore, TT has the capability to "batch process" multiple files. Either single-bottle lickometer files or MS80 brief-access files can be grouped together by placing all the files within a single folder (e.g., all the files in a single experimental treatment group would be put in a single directory). In this way TT uses the Macintosh® operating system as a simple database.

The "Open Folder" command in TT will cause all the files within the folder to be opened at once. A summary window also opens, which presents the average values of the descriptive statistics of all the open files. The time series of the open files are also averaged together, so that the mean cumulative licks, mean lick rate, etc. are plotted within the summary window. For brief-access files, TT graphs the mean values (number of licks, number of bursts, burst size, etc.) for each solution averaged across all the open MS80 files. If the summary window is printed, the individual files that are open can be printed at the same time. This allows the user to print the analyses of all the files in an experimental group with one command.

*Exporting Results*

The graphs and summary statistics of any display in the lickometer files, brief-access files, or summary windows can be selected by clicking on the display. Once selected, they can be copied and pasted into other applications, or saved to disk as separate files for analysis by other statistical programs. Graphs can be saved as either graphic objects or the data represented by the graph can be saved into tab-delimited text files for plotting in other graphing programs.

## DISCUSSION

We have described a program for the Macintosh® that integrates the analysis and display of multiple lickometer files into a single set of operations. TT performs a basic set of time-series analyses on medium length or brief-access lickometer data, and automatically visualizes the results with summary tables and graphs. The TongueTwister program will be particularly useful for investigators who collect lickometer data from a large number of animals every day; because of its convenient user interface and rapidity, TT can be used to generate daily summaries of ongoing experiments. Because TT can export its statistical and graphic results to standard computer files, it will also speed up final analysis.

Although there are many options for improving the current TT program, two particular improvements are apparent:

1. There is increased interest in the long-term analysis of ingestion with data collected at the single-lick level (e.g., 17). As stated above, TT can handle files internally up to 45 days long, but the graphic display of the data is currently limited to only a few h. Smith et al. have recently developed a Windows™ program for the analysis and display of long-term licking patterns that fills this need (15).
2. A more sophisticated analytic function that could be added is the facility to fit decay functions to survivor plots of ILIs, bursts, and clusters. Davis et al. have demonstrated that fitting exponential (4) or Weibull (6) functions to lickometer data can reveal the multiple processes that appear to modulate licking during a meal. Changing the orosensory or postingestive characteristics of the ingested solution, or modulating the substrates of ingestion by surgery (e.g., vagotomy) (6) or pharmacology (e.g., cholecystokinin) (7) alters the parameters of functions fitted to lickometer data in a lawful fashion.

Thus, analysis of lickometer data holds the promise of elucidating the sensory and central processing of food stimuli that control the oromotor behaviors of ingestion. TT consolidates into a single program the algorithms and graphing methods developed by many others over the last few decades for understanding lickometer data. TT makes lickometer analysis fast, convenient, and visual. These attributes, essential for such a data-intensive field, should encourage more comprehensive analysis of lickometer data as a routine matter.

## ACKNOWLEDGEMENTS

We would like to thank Dr. John D. Davis for original development of the approach and algorithms for lickometer data analysis that inspired our program, and Ross Henderson for providing lickometer file formats. Drs. Steve French, Tom Stratford, Brian Hrupka, and Ilene Bernstein provided sample data files during program development and debugging. Dr. Gary Schwartz generously volunteered much advice and many suggestions for improving the program.

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