

¹ lickcalc: Easy analysis of lick microstructure in experiments of rodent ingestive behaviour

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⁶ Summary

⁷ Lick microstructure is a term used in behavioural neuroscience to describe the information that
⁸ can be obtained from a detailed examination of rodent drinking behaviour. Rather than simply
⁹ recording total intake (volume consumed), lick microstructure examines how licks are grouped,
¹⁰ and the spacing of these groups of licks. This type of analysis can provide important insights
¹¹ into why an animal is drinking, for example, whether it is influenced by taste or affected
¹² by consequences of consumption (e.g., feeling “full”). The simplicity of using lickcalc,
¹³ a browser-based application with a simple interface, will make microstructural analysis accessible
¹⁴ to any researchers who wish to employ it while providing sophisticated analyses with high
¹⁵ scientific value.

Statement of need

¹⁶ lickcalc is a software suite that performs microstructural lick analysis on timestamps of lick
¹⁷ onsets and/or offsets. Microstructural analysis was first described in Davis & Smith (1992)
¹⁸ and has since then been used to understand diverse phenomena. In-depth reviews on many
¹⁹ of these, and microstructural parameters used to study them, are available (Johnson, 2018;
²⁰ Naneix et al., 2020; Smith, 2001). Briefly, although much of the foundational work on drinking
²¹ microstructure was on licking for nutritive solutions (e.g., sucrose solutions), microstructural
²² analysis can also be used to study intake of water (McKay & Daniels, 2013; Santollo et
²³ al., 2021), ethanol (Patwell et al., 2021), and other tastants such as non-caloric artificial
²⁴ sweeteners, sodium and quinine (Lin et al., 2012; Spector & St. John, 1998; Verharen et al.,
²⁵ 2019). Lick microstructure has been used to shed light on, for example, how licking is affected
²⁶ by neuropeptides (McKay & Daniels, 2013), enzymes in the mouth (Chometton et al., 2022),
²⁷ ovarian hormones (Santollo et al., 2021), nutrient restriction (Naneix et al., 2020), response
²⁸ to alcohol (Patwell et al., 2021), and diet (Johnson, 2012). The number of lick bouts over
²⁹ a session are thought to reflect post-ingestive feedback from the consumed fluid, whereas the
³⁰ number of licks in a bout are thought to reflect palatability of the solution.
³¹

³² Lick microstructure can provide nuanced information about why an animal is drinking. Often,
³³ changes in microstructure are accompanied by changes in total intake, but this is not always
³⁴ the case: sometimes, equal intake will be achieved by quite different licking patterns that
³⁵ indicate changes in orosensory and post-ingestive feedback (Johnson et al., 2010; Volcko et al.,
³⁶ 2020). Analyzing lick microstructure is therefore highly valuable when trying to understand
³⁷ how a manipulation, X, affects appetite; if X causes an animal to feel more sated after
³⁸ drinking, that may lead to a different interpretation than if X were to reduce the palatability
³⁹ of the solution. Because of the value of microstructural data, many labs habitually record
⁴⁰ and analyze it. There are many others, however, that have not yet begun collecting and/or
⁴¹ analyzing these data. Investing in lickometers can be costly, but there are an increasing number
⁴² of cost-effective alternatives to commercial products. As such, several open-source lickometer

43 designs are now available (e.g. Frie & Khokhar (2024); Monfared et al. (2024); Petersen et al.
44 (2024); Silva et al. (2024); Raymond et al. (2018)).

45 Recording individual licks with high temporal resolution is necessary for microstructural analysis
46 of drinking behavior, but another barrier to reporting microstructure is its analysis. This
47 problem is now easily solved by lickcalc. lickcalc does not require any special software
48 or coding knowledge: all the user has to do is load a file with timestamps of lick onsets
49 (and, ideally, offsets) into the application and lickcalc will generate detailed microstructural
50 analysis, with a high degree of user control over key parameters. Resulting data provide values
51 for number of licks, number of bursts, and burst size (among others) – the values that are
52 often reported and used to draw inferences about postigestive and orosensory feedback of
53 the solution. But importantly, several plots are also displayed that show information that
54 helps with quality control of the data and challenges the user to think critically about which
55 parameters they have chosen. In short, lickcalc makes microstructural analysis accessible
56 to any with appropriate data, while providing detailed information needed for appropriate
57 parameter selection and quality control.

58 Key features

59 lickcalc has several features that make it exceptionally user-friendly while providing sophisticated
60 and detailed microstructural analysis. Some of these features include:

- 61 ■ **Ease of use:** Files of various formats can simply be dragged into the lickcalc software
62 to trigger analyses. Parameters can be set manually using sliders, and results exported
63 to Excel with the push of a button.
- 64 ■ **Flexibility:** The user sets key parameters appropriate for their experimental setup and
65 data. Data can be analysed across the whole-session, within different epochs, or based
66 on a trial structure.
- 67 ■ **Customization:** By using the configuration file, users can change default settings to
68 match their preferences and avoid manually changing settings for each file loaded.
- 69 ■ **Results compilation:** Data from multiple sessions and/or individuals can be exported into
70 a single Excel file, which streamlines analysis. A batch mode is also included allowing
71 multiple files to be analysed simultaneously.
- 72 ■ **Detail of analysis:** One of the benefits of using lickcalc is the level of detail it provides.
73 In addition to the properties often reported (e.g., burst number, burst size), lickcalc
74 computes and displays attributes of the data that are important in establishing the quality
75 of the data and determining appropriate parameters for its microstructural analysis. Four
76 charts are:
 - 77 1) *intraburst lick frequency*, or how often certain interlick intervals within a burst of
78 licking occur. While a rodent is licking, its tongue makes rhythmic protrusions that
79 are under the control of a central pattern generator (Travers et al., 1997). Rats
80 typically lick 6–7 times per second (Davis & Smith, 1992), while mice lick at a
81 slightly higher rate (Johnson et al., 2010). In addition to these species differences,
82 there are also strain differences (Johnson et al., 2010; St. John et al., 2017).
83 Because intraburst lick rate is under the control of the central pattern generator, it
84 should remain relatively stable across mice and conditions (unless a manipulation is
85 expected to cause changes in the central pattern generator). A typical chart for
86 a mouse might show a sharp peak around an intra-burst ILI of ~129 ms, which
87 corresponds to a lick rate of 7.75 Hz. Much smaller peaks are often present at the
88 harmonics of the intra-burst ILI (e.g., a primary peak at 129 ms will have smaller
89 peaks at 258 ms, 387 ms, and so on), often because of “missed licks” in which the
90 mouse attempts to lick but its tongue misses the spout. A large number of these,
91 or other differences from the expected pattern of results, may indicate problems

- 92 with the experimental setup (e.g., if the animal fails to reach the spout frequently,
 93 then perhaps the spout is too far away).
- 94 2) *lick length* is only available when lick offsets are included in the data file. As with
 95 intraburst lick frequency, lick length should show little variability and the graph
 96 will have a sharp peak. Occasionally a lickometer will register longer licks than
 97 normal. A common cause of this is formation of a fluid bridge between the tongue
 98 and the spout during periods of high frequency licking. This can be prevented by
 99 moving the bottle further from the animal. In addition, other causes are if a rodent
 100 grabs the spout with its paws, or if a fluid droplet hangs between the spout and
 101 the cage and thus completes the electrical circuit. Concerns about data quality may
 102 be warranted with increasing number and duration of long licks. `lickcalc` displays
 103 both the number and maximum duration of licks above the threshold that the user
 104 has set. There is also an option to remove these problematic licks from the dataset.
- 105 3) *burst frequency*, or how often certain burst sizes occurred. This is informative
 106 because burst size, by virtue of being a mean (mean licks per burst), does not
 107 take into account potentially relevant information about the distribution of licks in
 108 each burst. For example, a burst size of 80 could result from bursts all containing
 109 between 70 and 90 licks, or from many single licks and one or two bursts with a lot
 110 of licks. The latter case might raise some questions about how reliable the burst
 111 size value is. Although single licks occur, they can also be caused by non-tongue
 112 contact with the lickometer. Changing the minimum licks/burst parameter can
 113 filter out some of these suspect “licks.”
- 114 4) *Weibull probability*. The Weibull analysis, as described in Davis (1996), uses a
 115 mathematical equation to fit data to a survival function. Although used by some
 116 (Aja et al., 2001; Moran et al., 1998; Spector & St. John, 1998), it is still relatively
 117 rare to find Weibull probabilities in microstructural analyses. The Weibull function
 118 can be used on several aspects of data, such as lick rate across a session, but in
 119 the `lickcalc` program the Weibull probability is calculated for burst size. It plots
 120 the probability that, given n licks, the mouse will continue to lick. This makes it
 121 sensitive to the licks per burst parameter that is set by the user. The Weibull α
 122 and β values reflect the slope and shape parameters, respectively. Slope (α) has
 123 been shown to vary with palatability.

124 Design and usage

125 `lickcalc` is hosted by UiT The Arctic University of Norway and can be accessed at lickcalc.uit.no.
 126 Alternatively, it can be installed locally following instructions in the repository. To use `lickcalc`,
 127 the user drags a file into the application, changes file format if necessary, and indicates which
 128 column contains the lick onsets (and, if applicable, the lick offsets). A plot is automatically
 129 generated that displays a histogram of licks across the session. Session length defaults to the
 130 time of the last lick but can be manually changed, or set in the optional config file. Session
 131 length can be set in seconds, minutes, or hours. The bin size (licks per unit of time) can be
 132 changed manually or in the config file. The user can toggle between the default histogram and
 133 a plot showing cumulative licks.

134 A microstructural analysis is, in essence, a division of individual licks into groups of licks. To
 135 perform this grouping, the user must set several parameters. One of these is the inter-lick
 136 interval (ILI), which is the minimum amount of time licks must be separated by in order to be
 137 considered separate groups. Early studies identified ILIs of 251–500 ms as separating “bursts”
 138 of licking, and pauses of >500 ms as separating “clusters” of licking (i.e., a cluster of licks is
 139 made up of several bursts of licking). Others have argued that an ILI threshold of 1 s provides
 140 better separation of lick bursts (Spector & St. John, 1998). In `lickcalc`, the user can set the
 141 ILI to any value (values between 250 ms and 3 s provided by default but can be adjusted using

the config file). Another parameter that needs to be decided prior to the lick analysis is the minimum number of licks per burst. `lickcalc` allows between 1 and 5 licks by default. The appropriate number of minimum licks per burst may vary depending on experimental set up, and the likelihood that a single lick represents a lick rather than, for example, a paw touching the spout. Finally, in `lickcalc`, the user must set a “long-lick threshold” between 0.1 and 1 s. This parameter is only available when lick offset is included. Licks that are longer than the set threshold are counted as “long” and may indicate a problem (e.g., fluid bridges or a mouse holding the spout with its paws) rather than a true lick. The user can decide whether to remove “long licks” or not. All of these parameters can be set manually or through the config file. Four plots are generated (see *Key Features* section above), and tables are displayed showing values of several properties: total licks, intraburst frequency, number of long licks, maximum duration of long licks, number of bursts, mean licks per burst, Weibull α , Weibull β , and Weibull r^2 .

To save these data, the user has two options. The first is to export a single Excel file in which the user sets the animal ID and chooses which data to export. These data allow the user to recreate the plots displayed in `lickcalc` or perform further analyses. The second option for saving the data is to add the loaded data to the *Results Summary* table. The results in this table remain even as new data files are loaded, so the data from many sessions (and/or individual animals) can be exported into a single Excel file. In addition to the data from the whole session, the user can choose to divide the session into epochs, or to examine only the first n bursts, or perform a trial-based analysis (e.g., for Davis rig experiments). Each of these analysis epochs can be added to the table. The table contains the data and the analysis parameters (e.g., minimum burst size) used to generate them. Finally, a batch process feature is available allowing multiple files to be analysed using the same parameters.

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