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A low-cost solution to measure mouse licking in an electrophysiological setup with a standard analog-to-digital converter

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Abstract

Licking behavior in rodents is widely used to determine fluid consumption in various behavioral contexts and is a typical example of rhythmic movement controlled by internal pattern-generating mechanisms. The measurement of licking behavior by commercially available instruments is based on either tongue protrusion interrupting a light beam or on an electrical signal generated by the tongue touching a metal spout. We report here that licking behavior can be measured with high temporal precision by simply connecting a metal sipper tube to the input of a standard analog/digital (A/D) converter and connecting the animal to ground (via a metal cage floor). The signal produced by a single lick consists of a 100–800 mV dc voltage step, which reflects the metal-to-water junction potential and persists for the duration of the tongue–spout contact. This method does not produce any significant electrical artifacts and can be combined with electrophysiological measurements of single unit activity from neurons involved in the control of the licking behavior.

Keywords: Licking; Central pattern generator; Awake mouse; Electrophysiology; Lickometer; Cerebellum

1. Introduction

Fluid licking in rodents is a rhythmic behavior that is probably driven by a central pattern generator (CPG): it is a highly stereotyped behavior characterized by repetitive tongue and jaw movements, which are in turn under the control of one or more rhythmically active neural networks (for review, see Travers et al., 1997). Monitoring licking activity is also a very useful method for rating the dose-related behavioral effects of acute or chronic drug treatment (e.g. Genn et al., 2003; Hsiao and Spencer, 1983; Peachey et al., 1976).

Licking in rodents has typically been measured with "lickometers" utilizing either electrical, optical or force sensors (for review, see Weijnen, 1998). Electric sensors are most commonly used, and typically depend on a high-frequency ac contact circuit. Commercially available lickometers designed for use with rats and mice come at a cost of a few hundred to several thousand dollars, and vary widely in application, from those used to monitor licks from a single spout/bottle to those capable of switching between multiple spouts/bottles (Glendinning et al., 2002; Hill and Stellar, 1951; Smith, 2001). Here we describe a method that allows the reliable and temporally precise measurement of the licking behavior at virtually no cost, provided that a standard analog-to-digital converter is available. An advantage of our method is that it is easily combined with electrophysiological measurement of neuronal spike activity during licking behavior. A standard stainless steel sipper tube attached to a small drinking bottle is used, occupying minimal space and compatible with almost any existing in vivo recording setup. Most importantly, lick contact produces no significant electrical artifact during extracellular recording of single-unit brain activity.

2. Material and methods

2.1. Animals

Adult male and female mice (>60 days old) from three common inbred strains (C57BL/6J, DBA/2J, BALB/cByJ) were individually housed in plastic shoebox cages in a temperature and humidity-controlled vivarium on a 12:12 h light-dark cycle. Animals were treated according to a protocol approved by the

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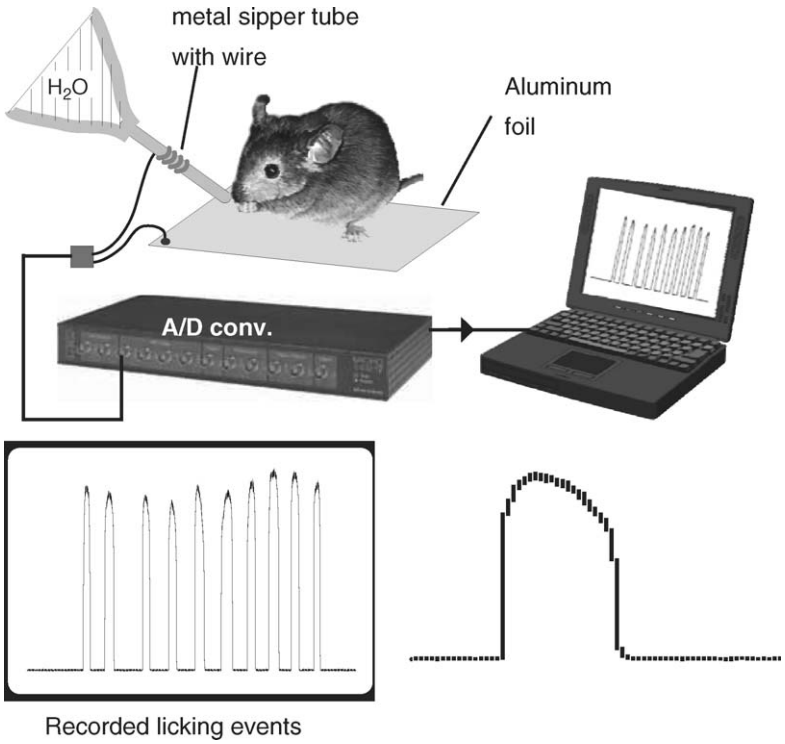

Fig. 1. Experimental setup to measure lick events with a standard analog/digital (A/D) converter. Water was delivered through a metal sipper tube that had bare copper wire wrapped around it. The bottom of the mouse cage was covered with aluminum foil. The input of the A/D converter was connected to the sipper tube wire and the ground was connected to the aluminum foil. Each lick closed the electrical circuit for the duration of the tongue–sipper tube contact and the junction potential between the metal sipper tube and the water or the mouse's saliva could be recorded. Junction potentials could reach amplitudes of 1 V. Baseline noise was typically <5 mV. Lower right panel illustrates one typical licking event. Note that the rise-time is much faster than the fall-time.

University of Tennessee Health Science Institutional Center Animal Care and Use Committee. Ad libitum water was removed from the cages of mice approximately 23 h prior to training or testing in the lickometer. During training or testing mice received all their daily fluid in the lickometer.

2.2. Lickometer and recording of licking behavior

During a session, mice were placed in a 14 cm × 14 cm × 7.6 cm opaque plastic chamber with a 9 mm × 25 mm opening on one wall for sipper tube access. The stainless steel sipper tube was approximately 10 cm long and curved, with a 3 mm orifice, and was connected via a # 0 sized neoprene stopper to a 60 ml plastic bottle (Fig. 1). The lickometer cannot distinguish between a tongue lick and a paw touching the spout. However, the chamber opening and the position of the spout enable a contact of the spout with the tongue only during drinking (for drinking setup configuration, see Vajnerova et al., 2003). Water-restricted mice were given two 20 min training sessions per day (separated by ∼4 h) for 3–5 days before testing began.

During recording of licking behavior, the chamber bottom was covered with aluminum foil and the water spout was connected to an A/D converter.

The central pin (core) of a BNC input connector of either a CED 1401 or a Digidata 1322A A/D converter was connected to the sipper tube and the grounded housing (shield) of the BNC was connected to the aluminum foil (Fig. 1). A positive voltage step of 100–800 mV with rise times <1 ms could be measured whenever the mouse's tongue touched the steel sipper tube (Fig. 2). The voltage signal did not need amplification but could be directly acquired using standard A/D interfaces. We have used the *power*CED 1401 (Cambridge Electronic Design, Cambridge, UK) with an input range of ±5 V, and the Digidata 1322A (Axon Instruments, Foster City, CA) with an input range of ±10 V. Both interfaces have input resistances of 1 MΩ and were equipped with 16 bit A/D converters resulting in a voltage resolution of 0.15 mV (CED) and 0.3 mV (Digidata).

2.3. Electrophysiological single unit recording during licking behavior

Mice used in electrophysiology experiments were given water ad libitum in the 24 h period prior to surgery. One hemisphere of the cerebellum was exposed, and a recording chamber was secured over the opening with dental acrylic. A metal post was then cemented to the skull with dental acrylic and allowed the mouse's head to be fixed during simultaneous recordings of cerebellar Purkinje cell spike activity and licking behavior. Mice recovered for 3–5 days after surgery. Ad libitum access to water was removed from the cages of the mice ca. 23 h prior to electrophysiological recordings. Recordings were performed with platinum–tungsten electrodes mounted in a micromanipulator (Thomas Recording, Germany). Electrodes were lowered into the cerebellum until stable single unit activity was recorded.

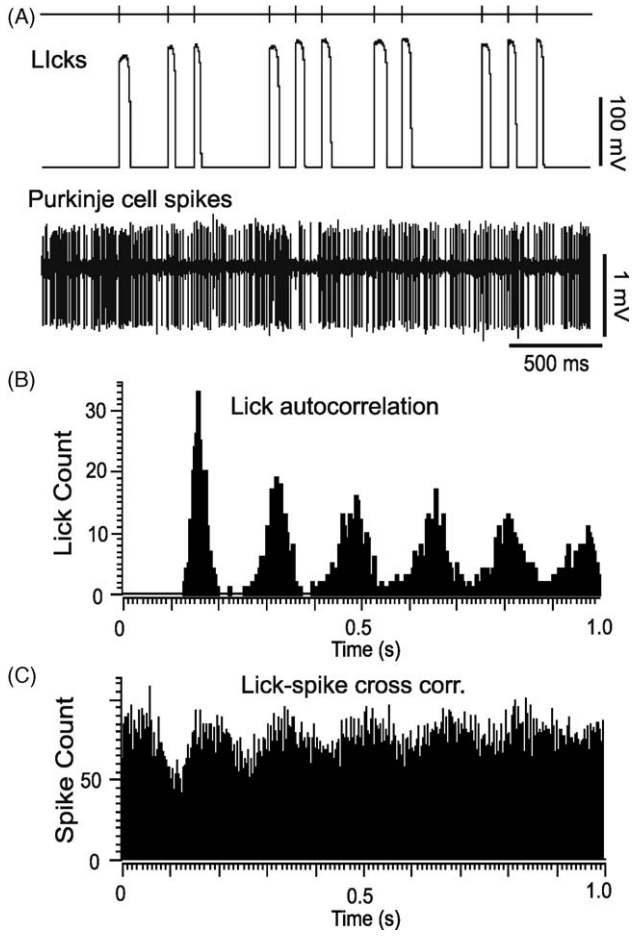

Fig. 2. Simultaneous recording of licking behavior and single unit Purkinje cell spike activity. (A) Upper trace: onset of lick events extracted from the raw voltage traces below with a fixed threshold set to 80 mV. Middle trace: raw data traces of lick related junction potentials digitized at 2 kHz. Bottom trace: simultaneously recorded spike activity of a single unit Purkinje cell in Crus I of the right cerebellar hemisphere of a C57BL/6J mouse (sampling rate: 25 kHz). This unit's spiking activity was modulated by the rhythmic licking behavior as shown by the lick–spike cross-correlation analysis in (C). (B) Autocorrelogram of the lick events shows the temporally precise rhythmicity of the behavior at a periodicity of around 10 Hz. Zero on the time axis marks the time of lick onset in both (B) and (C). Bin width: 10 ms. (C) Cross-correlation of lick-onsets and spike activity shows the Purkinje cell's modulation of spike firing with the licking behavior. The main component of the cell's lick related activity change is a reduction in spontaneous firing preceding lick onset. Bin width: 1 ms.

The sipper tube was then moved close (∼2 mm) to the mouse's mouth, and the mouse started to lick water. Spike and lick signals were digitized using a CED *power*1401 (CED, Cambridge, UK) and stored on computer hard disk. Data analysis was performed off-line using the Spike2 software (CED, Cambridge, UK). Licks were detected by a threshold set to voltage values between 10 and 20% of the peak junction potential. The junction potential rise-time was less than 1 ms. Lick-onset time could therefore be determined with millisecond precision irrespective of the precise threshold setting. The junction potential fall-time, however was about 10-times slower than the rise-time. We think that the relatively longer decay time of the licking signal might be caused by the bridge of fluid that forms between the tongue and the spout. This bridge gradually becomes thinner upon the tongue's retraction thereby prolonging contact but via a rapidly shrinking contact surface. Therefore, it will be difficult to accurately determine the time at which the tongue has left the spout. In this study, we have defined the lick duration as the time spent above 10–20% of the peak amplitude. To compare our method with an already established method, we also recorded licking signals using an MS-160 lickometer (DiLog Instruments, Tallahassee, FL). The lickometer apparatus consists of a Plexiglas cage (29 cm long × 14.5 cm wide × 23 cm high) with a metal wire mesh floor, clear walls on the side, and a front metal wall containing a slot. In front of the cage, a sliding rack that can hold up to 16 tubes is positioned so that the spout on each tube lines up with the open slot in the front of the cage once it moves into place. A sliding metal door blocks this slot to prevent the rat's access to the spout between solution presentations. The lickometer measures the number of licks by passing a current of <60 nA through the animal each time its tongue contacts the drinking tube. Data were stored on a computer for later analysis. For the purpose of this direct comparison we connected the two poles of an A/D converter to one of the metal spouts and to the wire mesh cage bottom of the MS-160 lickometer. The output of the MS-160 was disconnected from its computer (that holds the lick count soft- and hardware) and connected to a different input of the A/D converter. We were therefore able to simultaneously record the junction potential signal and the MS-160 signal while a mouse licked water.

3. Results

Junction potentials occur wherever dissimilar conductors are in contact. The voltage generated by the contact of the mouse tongue with the spout is due to the metal–water junction potential for the following reasons: (i) connecting the BNC core and shield directly with each other, i.e. short-circuiting the input to ground or via resistors of different magnitude, reduced the 60 Hz cycle noise in the acquired signal but produced no voltage change; (ii) connecting both poles of the BNC via a resistor of 1.5 MΩ produced no voltage change; (iii) a voltage change (100–1000 mV) was produced when the core and shield of the BNC cable were connected to two stainless steel probes immersed in tap water, de-ionized water or saline. This result indicates that a junction potential develops at the metal–liquid junctions.

The duration of the voltage step corresponded to the duration of contact between the spout and the aluminum foil. The connection could be established even by poorly conducting materials like de-ionized water. In control experiments, voltage signals were generated whenever the spout was connected to the aluminum foil with either a drop of water, a finger or a wet paper towel (data not shown). During licking behavior, the mouse establishes an electrical contact that persists for as long as the tongue touches the spout. Hence, the junction potential signal can be used to measure both the timing of licking activity as well as the duration of spout contact for each individual lick.

An example of how this recording method was successfully combined with electrophysiological recording is shown

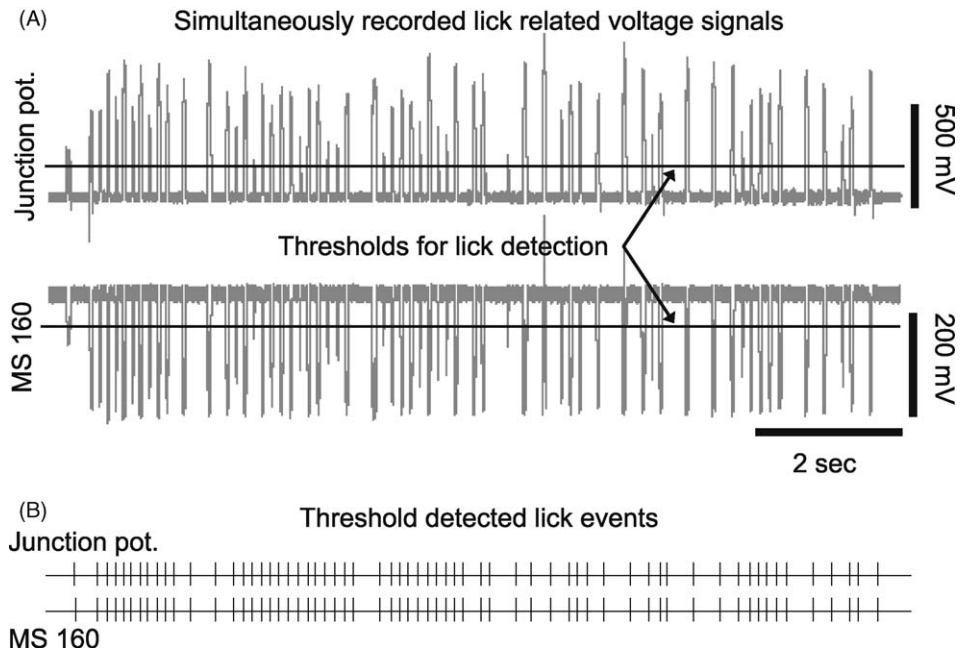

Fig. 3. A train of licks simultaneously recorded with the junction potential method and with a commercial lickometer (MS-160, Dilog Instruments, FL). The input of an A/D converter was connected to the water bottle spout and the ground to the metal cage wall of the MS-160 rig. The BNC output of the MS-160 was connected to a different channel of the same A/D converter and both voltage signals were simultaneously recorded while a mouse (BALB/cByJ female) licked water. (A) The junction potential method produced a positive voltage deflection, whereas the MS-160 produced a drop in voltage from 3 V dc signal. Fluctuations in amplitude are most likely due to differences in surface area of contact between the spout and the tongue. In control experiments using finger contact signal amplitude increased with increasing skin area contacting the spout. Connecting the A/D converter to the MS-160 considerably increased noise and amplitude fluctuations in licking related voltage signals. Signal-to-noise ratios for each method used by itself are significantly better (cf. Fig. 2). Licking events were detected using fixed voltage thresholds indicated by the solid lines. (B) Licking events detected by threshold crossings in the MS-160 and the junction potential signals are identical in timing and number.

in Fig. 2. It is important to note that the junction potential method described here either did not produce any detectable electrical artifacts in the spike signal recordings, or produced small artifacts that did not interfere with spike detection. The size of the artifact could be reduced to zero by improving the mouse–aluminum foil contact. That was achieved by moistening the aluminum foil with tap water. Fig. 2 shows the spike activity of a Purkinje cell in Crus I of the right cerebellar hemisphere of a C57BL/6J mouse recorded during licking. This neuron did not show oscillatory spike activity during rest (data not shown) but clearly modulated its spontaneous firing rate in phase with the licking movement as shown by cross-correlation analysis (Fig. 2C). Other cells recorded in the same area showed similar behavior ($n > 10$) but had different phase relationships between the rhythmic licking movement and spike rate modulation.

We next investigated whether the licking signals obtained using our method are comparable to those obtained using a commercially available lickometer (MS-160, DiLog Instruments, Tallahassee, FL, see Brot et al., 2000). Licking events detected by threshold crossings in the MS-160 and the junction potential signals were identical in timing and number showing that the performance of the junction potential method is equivalent to that of long time established methods (Fig. 3). These results indicate that the method described here is an accurate, yet inexpensive solution for electrophysiological investigation of the neuronal mechanisms underlying licking behavior.

4. Discussion

Lick sensors are valuable tools to study licking and drinking behavior (Weijnen, 1989). Commercially available lickometers come at a cost of a few to several hundred dollars (e.g., TSE systems, model 2.07, Midland, MI; Columbus Instruments drinkometer, Columbus, OH; Stoelting, model 57450, Wood Dale, IL; Med Associates Inc., model ENV-250, St. Albans, VT; Coulbourn Instruments, model H24-01, Allentown, PA; MS-160 Lickometer, DiLog Instruments, Tallahassee, FL). There are three different types of sensors in use: electrical, optical, and force sensors. They differ in the exact time of activation during the tongue protrusion/retraction cycle and precautions in the use of each type of sensor need to be taken. Weijnen and Mendelson (1977) and Weijnen (1977, 1989, 1998) has published several excellent articles and reviews outlining the advantages and disadvantages of each type of lickometer. One method involves incorporating a miniature strain gauge in the drinking spout so that the pressure of each lick can be recorded (Vrtunski and Wolin, 1974). Movements of the tongue can also be detected when it crosses a light beam mounted in front of the drinking spout (Schoenbaum et al., 2001; Smith et al., 1992).

By far the most common method now in use involves passing an undetectable current (<1 μA) through the drinking spout. When the animal drinks it becomes part of a circuit from the spout to the cage floor, which is grounded, and this change in conductivity is amplified, shaped, and recorded by a computer

(Vajnerova et al., 2003). In this method, the animal tongue contact with the fluid or the fluid delivery device causes a change in voltage or capacitance (Field and Slotnick, 1987; Hill and Stellar, 1951; Mundl and Malmo, 1979; Spector et al., 1990; Weijnen, 1989). Rats and mice appear to be unable to detect direct current in the sub-μA range (<1 μA; Gannon et al., 1992; Weijnen, 1989), so this approach is useful for behavioral experiments. However, even these very low currents may cause large noise artifacts in brain recordings, and thus, these methods alone are not suitable for neurophysiological studies (unpublished observations). Although a modification involving alterations in capacitance and the use of ac currents has been reported to work without causing noise artifacts, this method requires a relatively complex circuit (Mundl and Malmo, 1979). In order to overcome the disadvantages of using electric sensors, Schoenbaum et al. (2001) recently developed an optical method for detecting licking behavior during recording of electrophysiological signals from the brain. This method, however, requires custom preparation of a drinking well and the purchase and adaptation of optical sensor equipment.

The method described here requires only a standard A/D converter, needs no additional equipment and performs as reliably as commercially available lickometers (Fig. 3). We suggest that our method mostly measures the metal-to-liquid junction potential which occurs between the water and the metal sipper tube (Fig. 1). The sipper tube is made of stainless steel, which contains in large part iron that can exist in solution as a doubly positively charged ("ferrous") ion or a triply positively charged ("ferric") ion. Such a system is often called a "redox couple", such as the "ferrous/ferric" couple. In addition to iron, stainless steel contains a minimum of 12% chromium, which makes it rust-resistant. Nevertheless, the chromium in the steel is also oxidized in water to form a thin, invisible layer of chrome-containing oxide, called the passive film. However, the exact nature of the redox reactions generating the metal-to-liquid junction potentials is unknown in our conditions. An additional junction potential most likely occurs at the junction between the animal paw and the aluminum foil. Replacing the aluminum foil with a stainless steel reference electrode inserted in the animal brain resulted in a reduced amplitude of lick related junction potentials (50–150 mV). It is therefore possible that many factors affect the amplitude of the recorded junction potentials such as the area of contact, the humidity of the tongue, the conductivity of the mouse body, and the composition of the metallic probes. Investigation of these factors is beyond the scope of this study.

The mouse closes the electrical circuit whenever the tongue touches the sipper tube or the water. The junction potential is in the order of few hundred millivolts and can be digitized and analyzed without additional amplification. A fixed voltage threshold can be used to reliably detect lick onsets (Fig. 3). This method can be ideally combined with electrophysiological measurement of neuronal spike activity during the licking behavior (Fig. 2) because it produces no or only minor electrical artifacts.

In summary, we have described a simple, yet precise and reliable method to record licking behavior at virtually no cost and with the same performance as that of established commercial devices. An additional advantage of our method is that it can be combined with electrophysiological recordings and – since no additional equipment is needed (other than a wire wrapped around the spout), and space requirements are minimal our method should readily work in most existing in vivo recording setups.

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