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Lick Sensors as Tools in Behavioral and Neuroscience Research

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WEIJNEN, J. A. W. M. *Lick sensors as tools in behavioral and neuroscience research.* PHYSIOL BEHAV **46**(6) 923–928, 1989.—Lick sensors can be valuable tools in behavioral and neuroscience research on licking and drinking behavior. The focus of this discussion is the recording of licking in the rat. However, comments on the application of these sensors to the measuring of fluid intake are included as well. Lick sensors should be used with adequate precautions. Some constraint on the access of the animal to the drinking tube is necessary for the adequate recording of each single lick. Published drawbacks to the use of electrically operated lick sensors are discussed, and reduced to realistic proportions. With these latter sensors one can obtain behavioral and electrophysiological data that are directly related to the time of making and breaking contact of the tongue with the fluid that is drunk.

Drinkometer Electrophysiological recording Lick sensor Rat Water intake

SINCE the publication of "An Electronic Drinkometer" (11) devices for the registration of licking or drinking in rodents have become routine tools in laboratory experiments involving fluid intake. They register aspects of tongue movement in the licking animal. Lick sensor is, therefore, a more appropriate name than drinkometer, as drinking is not measured directly.

It is good practice in research to carefully analyze the behavior of the tools that are used. They must accurately execute their task, without contributing to the final result that is being obtained. If more than one type of instrument is available for the same task, the best one should be selected.

COMPARING DIFFERENT TYPES OF SENSORS

Several methods for the measurement of licking and drinking in the rat have been described, with comments on their relative merits (5, 8, 22). The operation of many of these devices involves the passage of a small current through the animal, upon contacting the drinking tube with its tongue. The possibility exists that this electrical current affects licking behavior, or interferes with concurrent electrophysiological measurement; this will be discussed later. For that reason Dunn and Fray (5) selected two other types of sensors for a comparative study. Their principles of operation were not dependent on current flow through the animal. One design was based on tongue movement detection by a photocell, the other one on a capacitative principle. To determine which of the two instruments provided the better measure of drinking, the authors analyzed data consisting of licks recorded and volume drunk, from taste aversion experiments. These experiments themselves were not discussed. Scatter diagrams of the data, expressed as total number of licks versus volume drunk, were presented. Lines of best fit (least squares method) were calculated, and an elaborate statistical analysis of the results was presented. In the discussion the authors concluded that ". . . both . . . devices give an unbiased measure of licking and that this measure correlates significantly with the volume of fluid ingested . . . ." It was also concluded that the photocell device provided a more accurate and reliable measure than the capacitative device. Indeed, the scatter diagram of the results obtained with this latter instrument showed considerable variability. The analysis of the data, however, raised doubts about the performance of both devices. The straight lines that were fitted through the diagrams did not travel through the origin of the graphs, leaving for both experiments on the average 321 and 484 licks, respectively, unaccounted for with regard to fluid intake. It was argued that possibly not every lick (actually 20 to 25% of the total number of licks) had resulted in water intake. Inspection of the scatter diagrams shows clearly that one can but conclude that the number of licks cannot be converted into an accurate estimate of the volume of water ingested by individual animals, in spite of the significant statistical correlation between the number of licks and the total fluid intake that was calculated over the entire experiment.

Are these instruments that bad? Not necessarily so, if adequate precautions are taken. The bad performance of the capacitative sensor might have been partly due to ". . . contact between the rat and the spout being maintained between licks on some occasions," as the authors stated. This problem is frequently encountered if animals have free access to the watering tube, as was the case in the experiment involving the capacitative sensor. The usual solution to this problem is recessing the tube to a convenient distance behind a hole in the wall. This measure might at the same time have prevented spurious contacts of the rats with the drinking tube that did not involve the tongue (e.g., with paws, nose, etc.). In the experiment involving the use of photocell drinkometers, the access to the watering tube was restricted to the tongue. Care was taken to position this tube at an optimal distance behind the hole in the wall. The scatter diagram of the data obtained with this device showed orderly results. It is therefore possible that the main variable in the study of the two drinkometers was the difference in access to the watering tube, and not a difference in drinkometer design.

The data obtained with the photocell device can be further analyzed. The line of best fit through these data, a straight line, showed that the volume per lick strongly varied with the fluid intake that was measured over a 5-min period: 2.3 μl/lick at a total water intake of 1 ml, and 7.5 μl/lick at 10 ml (approximate values estimated from the illustration). Drawing an eye-fitted curve through the data, instead of a straight line, would normalize the low lick volume at an intake of 1 ml to some extent: 3.8 μl instead of 2.3 μl/lick. There might be an explanation for the relationship between the volume/lick and total fluid intake in the reported data that could account for a bias to small lick volumes at lower total intake values. The data were obtained during the first 5 min of 15-min trials, taken from taste aversion experiments. The aversion might have expressed itself in both a smaller volume/lick and in a lower total intake, compared to normal ingestion parameters obtained with, presumably, nonaversive fluids in the same experiment.

Estimating the volume of water that is ingested from the number of licks, is, however, a problem. The volume per lick is not a constant factor. Many situational variables have been described; within-session trends have also been reported (1, 9, 19, 28). Lick sensors can be used for measuring fluid intake if they are coupled to fluid dispensers that provide an exact volume of fluid per lick (12). Alternatively, one can measure the time spent licking, if the fluid is made available through a licking-operated pump that supplies the fluid at a constant flow rate (26). A disadvantage of these two methods is that the animal cannot influence the lick volume or the volume per second, respectively, any more. Other methods of measuring fluid intake were discussed by Dunn and Fray (5), or can be found elsewhere in the literature [e.g., (2, 3, 7, 14, 20)].

In conclusion, no definite statement can be made, as yet, about the relative merits of the two devices that were used for the sensing of licking in the drinking rat. A further comparative investigation of instruments for the recording of licking seems justified. Good sensors should accurately register tongue-fluid contact duration for each single lick. This enables the experimenter to investigate microbehavioral changes in response to altered conditions, as for example in taste aversion studies. Care should be taken that only contacts of the tongue with the fluid or with the drinking tube are measured. The operation of the sensor and the drinking situation itself should not interfere with licking or drinking.

For a comparative study of lick sensors, the same licking/drinking situation should be used with the different sensors. If possible, these sensors should be employed simultaneously. Devices that are operated by a small current passing through the animal during tongue contact with the drinking tube should not be discarded too soon from this "competition." For this purpose, their use and limitations in their application will be discussed.

ELECTRICALLY OPERATED LICK SENSORS

The principle of operation of these devices is simple. Upon contacting the drinking tube with its tongue, the animal completes the input circuit of the sensor. A small, subthreshold current passes through the animal. This current is amplified and used for output purposes: Closing a relay and/or changing a logical level, for the duration of the contact. For an extensive discussion of this kind of sensor, and of the behavioral consequences of suprathreshold electrical tongue stimulation, see Weijnen (22,23). Main conclusions and recommendations:

1) Positively reinforcing effects of lick-contingent electrical stimulation of the tongue, without concomitant water intake, have been observed in rats at current intensities as low as 0.5 to 1 μA. Behavioral effects at current levels lower than 0.5 μA (DC) have not been demonstrated under these, so-called, "dry-licking" conditions. The threshold intensity in a water-licking situation is not firmly established. Rats can discriminate between water and water with concomitant tongue stimulation at 5 μA; negative results were obtained with a 1-μA current.

2) Long and/or shielded input leads should be avoided, as capacitative discharge of the voltage, which develops over the open input circuit of the instrument, may lead to a suprathreshold transient current through the animal, upon making tongue contact with the tube. This may affect the animal and can corrupt electrophysiological recording. For the same reason the voltage used to generate the sensing current should be low (e.g., 6 to 7.5 V). In this context it will be clear that one should avoid adding a capacitor to the input circuit of the sensor to reduce the sensitivity to stray AC voltages, as was recently recommended (6). It is better to put the high-impedance part of the sensor circuit and the test chamber, in a shielded environment (see Fig. 1).

3) A good electrical contact of the animal with the metal floor of the test chamber is required for reliable operation of these sensors; regular cleaning of the floor is advised. A stainless steel plate ensures a better contact than the bars of a grid floor.

An important advantage of electrically operated sensors over photocell instruments is that they sense direct contact with the drinking tube. A well-chosen position of the drinking tube, relative to a properly shaped opening in the wall of the test chamber, ensures that tongue-tube contact coincides with tongue-fluid contact. As an extra precaution electrically isolated drinking tubes could be employed. Used this way, these sensors can register responses that are time-locked to the sensory consequences of contact with the fluid, with millisecond precision. This is, for example, of interest in the study of changes in reaction time to tongue contact with the fluid following conditioned taste aversion. The sensor output can also be used as a trigger for the averaging of evoked potentials, electromyographic activity of tongue and jaw muscles, etc. Photocell sensors are at a slight disadvantage with respect to the above-mentioned points, as they register the interruption of a light beam with the tongue, and not tongue contact wih the fluid or tube. Changes in the posture of the animal, relative to the drinking tube, can influence the duration of the beam interruption, without affecting contact time to the same extent. Drops of fluid, adhering to the tip of the drinking tube, can interfere with proper functioning of photocell sensors (8). It may also be difficult, wih these sensors, to avoid the registration of aborted licks, or of tongue movements that do not result in contact with the drinking tube.

The adequate recording of each lick with electrically operated sensors requires the same precautions as with other sensors. Descriptions of methods of recessing the drinking tube can be found in the literature (5, 16, 22). The required restraint on the access to the drinking tube should be limited to a minimum; otherwise the lick rate and volume/lick can be affected significantly. The lick rate decreases if the distance of the rat to the watering tube is increased. Changing this distance is, in fact, probably the most effective way of manipulating lick rate in the range of 5 to 7.5 Hz. The rate of fluid lapping can be influenced in a similar way, to values as low as 4 Hz (15). The shape of the hole in the wall of the test chamber (Fig. 1) ensures a stable position of the head of the rat relative to the drinking tube. A sliding panel, at the outer side of the hole, will facilitate the timing of drinking sessions. The zygomatic arches of an adult rat effectively limit the penetration of a drinking animal into the hole, to its mouth, without restricting the movement of the lower jaw. Further stabilization of the rat can be achieved by fixing a horizontal strip against the wall, approximately 5 mm under the

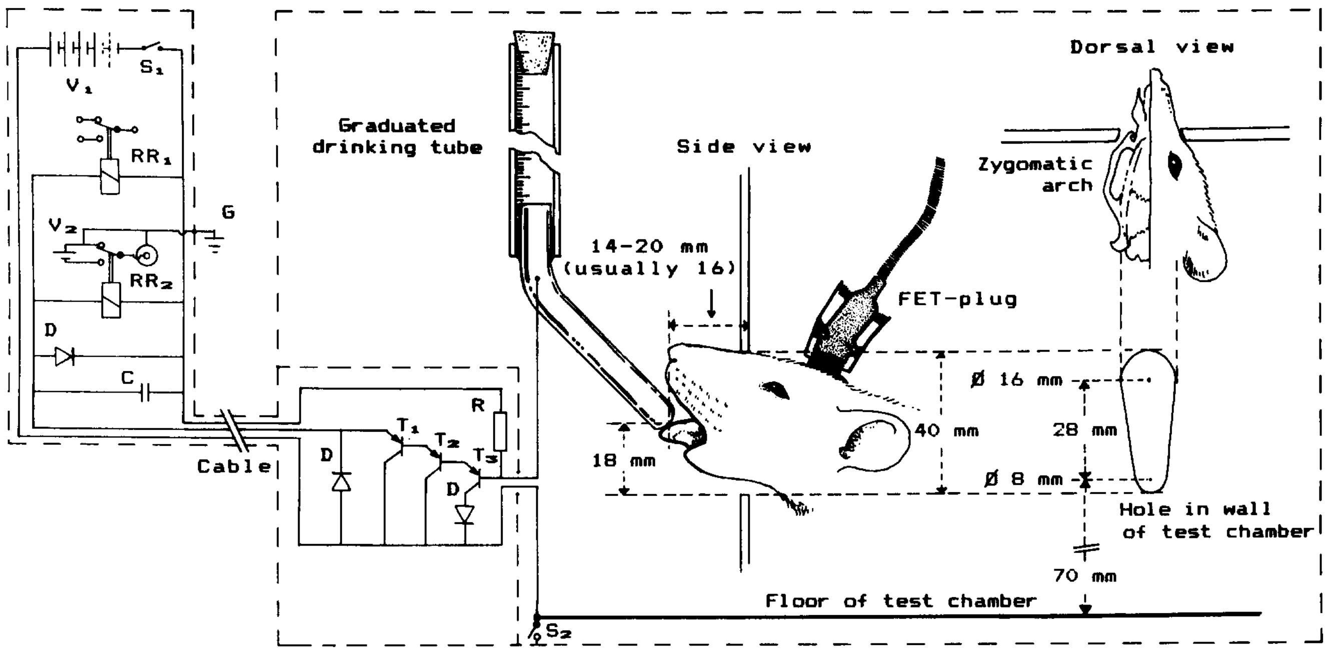

FIG. 1. Lick sensor circuit and drinking environment diagram. C: 470 pF, D: silicon diode, FET-plug: head-mounted electrophysiological recording assembly (25), G: ground, R: 75 Mohm, RR_1: reed relay (for counting, etc.), RR_2: reed relay supplying logical output, S_1: power supply switch, S_2: switch to ground the floor of the test chamber (N.B.: floor—and other parts of the test chamber that are within reaching distance of the animal—should be electrically isolated from ground during electrophysiological recording, when the skull of the rat is grounded by the recording assembly), V_1: 6 or 7.5 V, V_2: 1.5 V, $T_{(1-3)}$: BC-159 or modern equivalent transistors (BC-559), selected for a high h_{FE} at 1 μA to ensure optimal performance. Shielding of the equipment is important for electrophysiological purposes, but is also needed to protect the high-impedance part of the sensor that is sensitive to artefacts.

drinking hole. Most animals will use this strip as a front paw support. If a metal strip is used, it should be connected to the lick sensor, in the same way as the floor of the test chamber. A ground rod, on which an animal can place its paw during drinking, has been described earlier. It was used for the monitoring of drinking behavior of mice in standard plastic cages (4).

In our laboratory we use electrically operated lick sensors according to the design presented in Fig. 1. These sensors have proven to be extremely reliable, and do not need maintenance or tuning. Both logical output (1.5 V) and normally open and closed contacts of a relay are available for registration purposes. The use of reed relays ensures an output that follows the duration of the contact of the tongue with the drinking tube with a time shift smaller than 1 msec. Some sensors produce time shifts up to 10 msec (22) or more (10). We use the logical output of the sensor primarily for computer analysis of licking and of licking-related events.

After recording the logic output signal of the sensor on an instrumentation recorder, these data can be subjected to computer analysis. We measure the duration of each tongue contact with the drinking tube and of each interval between two successive contacts, in milliseconds, or with higher precision, if necessary. These data are stored sequentially. The interlick interval is not measured separately, but calculated by adding a contact duration to the subsequent intercontact interval. Figure 2 shows the relationship between tongue movement and the measurements derived from the output of the lick sensor. In this simple illustration no account is taken of altered tongue movements that occur in the interlick interval during swallowing. Swallowing takes place after about every 5 to 8 licks, and extends the intercontact interval for approximately 20 msec (26).

Figure 3 gives an example of the distribution of the contact durations, intercontact intervals, and interlick intervals that can be registered during a drinking session. Note the absence of contact durations exceeding 150 msec, indicating that, indeed, single licks were registered. To this end, the animal was kept at such a distance from the drinking tube that maintenance of contact between tongue and tube, between successive licks, was prevented. If this distance would have been too great, subsidiary peaks in the intercontact and in the interlick interval distributions would have occurred, due to missed contacts (22). These subsidiary peaks may show up at the modal duration or interval value, increased with multiples of the modal interlick interval.

Alternative versions of our sensor have been equipped with a retriggerable timer. Set at 250 msec, the timer gives a continuous output, as long as the rat licks steadily at a rate exceeding 4 Hz. This output can be used to measure time spent licking (22) and/or to drive a pump that supplies water to the drinking tube at a constant rate (26). Subsequent computer analysis of the recorded signal can be undertaken to calculate, and plot, water-intake versus time curves.

Reported Drawbacks of Electrically Operated Lick Sensors

Various disadvantages have been ascribed to the use of electrically operated lick sensors. For this reason Dunn and Fray selected other designs for their study. The main disadvantages would be:

1) The passage of the electrical current through the body of the animal may interfere with taste discrimination: for example, a choice between water and a very mildly bitter quinine solution (17).

2) Electrically operated lick sensors may introduce artifacts in electrophysiological recordings or interfere with electrical stimulation techniques (17,18).

3) Suprathreshold currents can markedly influence the behavior of the animal. The aversive effects of electrical stimulation are

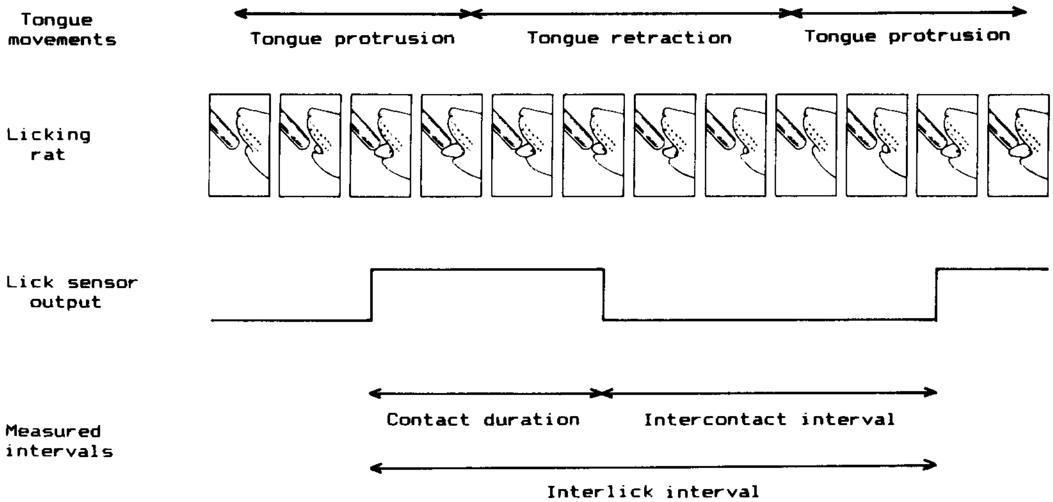

FIG. 2. The relationship of the output of the lick sensor to the movements of the tongue.

well known, but also positively reinforcing properties have been reported. The reinforcing effect of electrical tongue stimulation in thirsty rats was discovered when a commerically available sensor was used that passed a suprathreshold current through the animal (21,23). The same current can have positive effects at the level of the tongue, and evoke negative reactions as a result of stimulation of the nose, lips, or paws of the rat (23).

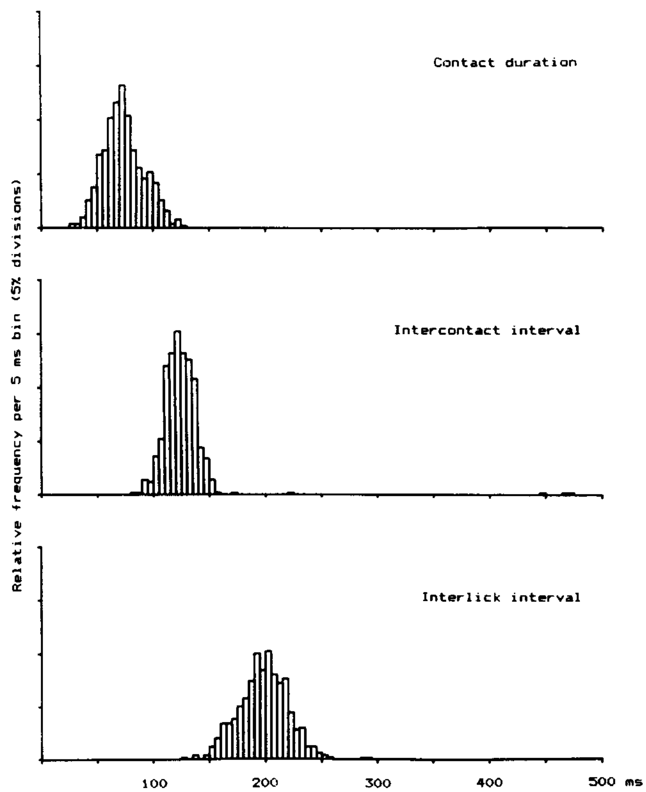

FIG. 3. Computer analysis of data recorded with an electrically operated lick sensor. The first 500 licks of a drinking session were used. The watering tube was put at 16 mm behind the wall of the test cage (see Fig. 1). Simultaneously with the recording of licking, the electromyographic activity was measured for both a tongue-protruder (genioglossus) and a jaw-opener (anterior digastric) muscle. The activity of the former muscle is presented in Fig. 5.

4) Electrically operated lick sensors cannot be safely used within a microwave field (13).

We have tried to replicate the findings concerning the sensor-taste interaction, without success. Proving that the very low currents employed by well-designed sensors have no effect whatsoever in taste experiments is, however, a formidable task.

In our laboratory sensors of the design shown in Fig. 1 are used during the registration of lick-synchronous brain and muscle potentials. The sensor output provides trigger moments for computer averaging of the differentially amplified data. Figure 4 shows that artifact-free recordings of both field potentials and multiple unit activity can be obtained. A clear separation of the multiple unit activity, around the moment of breaking the contact of the tongue with the drinking tube, into two separate bursts was obtained by recessing the tube to 18 mm (see Fig. 1). Even the electrophysiological measurement of tongue or jaw muscle activity does not exclude the use of electrically operated lick sensors, as is shown in Fig. 5 [see also (26)]. Occasionally, sensor artifacts show up—as transients—at the moment of making or breaking contact with the drinking tube; but these are time-locked events of short duration, that are easily detected in the averaged results when the leading edge and, subsequently, the trailing edge of the lick signal are used for triggering purposes. If such an artifact is detected, one should confine the averaging of the licking-related potentials to a time epoch that does not contain a transient at the other edge of the lick signal, as this will be spread out in the average and might not be recognized as an artifact. Monopolar registrations that are referenced against ground pose serious problems. Interference with electrical stimulating techniques can be minimized by disconnecting the sensor for the duration of the stimulation.

Concerning the third point of criticism, suprathreshold currents can indeed influence behavior. This can sometimes be turned into an advantage. Suprathreshold currents can, for example, be paired with lithium chloride poisoning, to induce conditioned aversion (poison avoidance) to electrical stimulation of the tongue in rats (23). Another advantage of electrically operated lick sensors is the ease of further developments of these instruments. Weijnen and

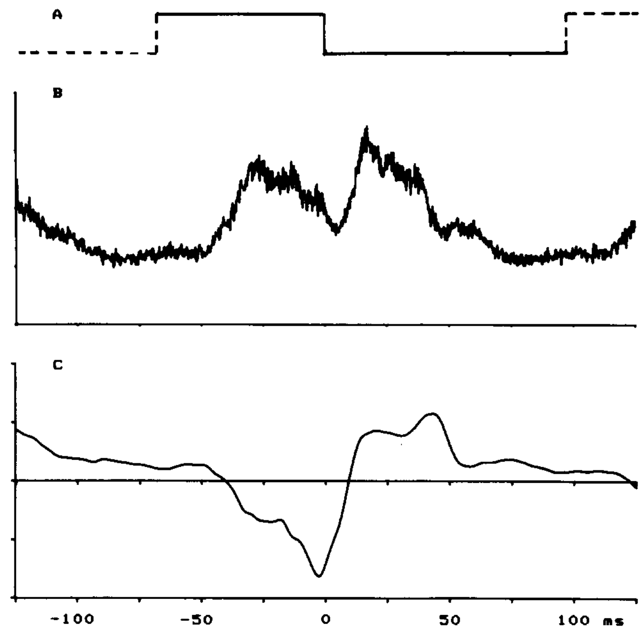

FIG. 4. Tongue retraction-related multiple unit activity (B) and field potentials (C), recorded from the dorsal part of the motor nucleus of the tongue (nucleus hypoglossus). A moveable bipolar electrode, made of teflon-insulated stainless steel wires (diameter 75 μm), was employed. Peri-event averages are shown with the moment of breaking of tongue contact with the drinking tube as trigger signal, as illustrated by the sensor output (A). The data were digitally filtered. The multiple unit activity average was calculated after high pass filtering (−3 dB at 300 Hz) and rectifying of the raw data. The average of the field potentials was obtained after low pass filtering (−3 dB at 135 Hz). (Unpublished data, Wouters and Weijnen, 1986.)

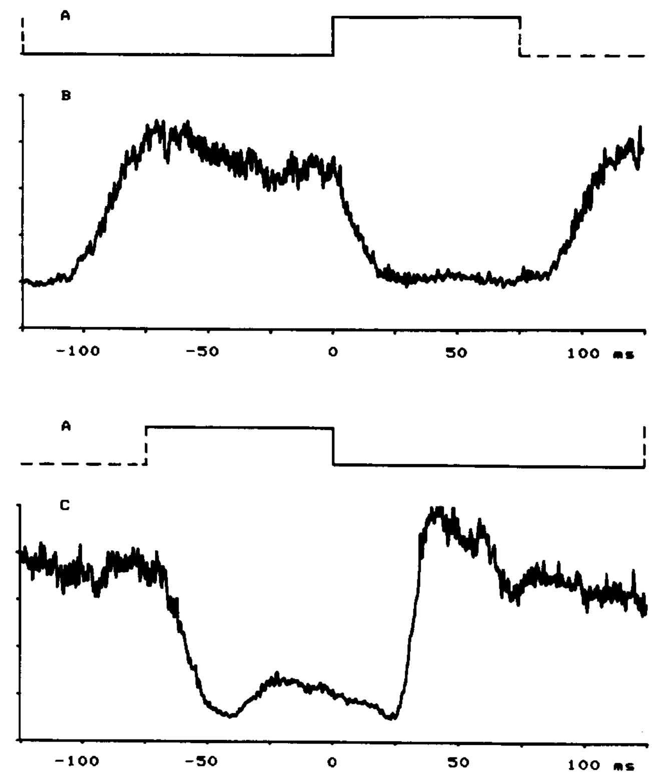

FIG. 5. Electromyographic activity of the genioglossus muscle—a tongue protruder—in the drinking rat. Bipolar nichrome wires (diameter 25 μm) were implanted 10 days before the recording session. Peri-event averages are shown of the digitally high pass-filtered (−3 dB at 140 Hz) and rectified data. As triggering signal for the computer averaging of the data served, respectively, the moment of making (B) and of breaking (C) tongue contact with the drinking tube, as illustrated by the sensor output (A). A sharp onset of activity of the genioglossus occurred during the intercontact interval. The activity of the muscle declined rapidly when the tongue contacted the drinking tube. In this experiment, tongue protrusion started approximately 25 msec after breaking of tongue-tube contact. A behavioral analysis of the licking data from this experiment was presented in Fig. 3. (Unpublished data, Weijnen and VanKempen, 1987.)

Brozek (in preparation) have upgraded such a sensor to a licking-operated electrical tongue stimulator for the study of cortical evoked potentials, that are generated by anodic and cathodic stimulation of the tongue (24). This instrument made it possible to study brain potentials that are time-locked to electrical tongue stimulation with millisecond precision.

The incompatibility of electrically operated sensors with the presence of microwaves is a clear limitation in their use; but this problem is only met in few experiments.

After considering these arguments we must conclude that, in spite of some remaining problems with specific applications, electrically operated lick sensors can be valuable tools in collecting behavioral and electrophysiological data that are directly related to the time of making and breaking contact of the tongue with the fluid that is drunk.

DISCUSSION

The choice of the type of lick sensor that will be used in a particular experiment will depend strongly on the specific requirements that need to be met. For event counting or registration purposes one just needs a sensor that produces a standard pulse as output for each lick. Higher demands are made on instruments that are supposed to supply accurate measurement of tongue contact, and duration of this contact, with the fluid or the drinking tube.

Many sensors are commercially available, and new designs are regularly published. Good use of them requires that access to the drinking tube be restricted to the tongue, otherwise spurious contacts are measured and there is also a high risk of maintaining contact between licks. This latter condition leads to a reduction of the number of licks recorded. Care should be taken that the access restriction does not affect licking or drinking. The adequate registration of licking can become difficult under demanding conditions; for example, during operant licking tasks ('dry licking'). Regular observation of animal behavior has learned that the incidence of responses made by nose, teeth, or paw contacts can increase dramatically under these conditions. We have encountered the same problem in attempts to slow down the lick frequency of rats during continuous drinking. Success was only achieved after making lick-contingent water delivery conditional to simultaneous interruption of a light beam that was aimed at the nose of the drinking animal. This procedure effectively suppressed all extralingual contacts.

It is important to remember that the measurement of tongue contact with the fluid or drinking tube does not provide precise information on the start of tongue protrusion or retraction, as these events take place during the intercontact interval and during the contact with the tube, respectively (see Fig. 2). An array of photosensors might be necessary to obtain registration of the onset of tongue retraction (27). A more sophisticated way to get this information would be the registration of the electromyogram of tongue muscles, but this is not a suitable method for routine experiments.

It will be clear that lick sensors can provide accurate data on licking and can be used for the programming of the direct consequences of the response and for the registration of licking-related potentials. They do not necessarily directly supply reliable information on the volume of fluid intake.

A direct comparison between different types of lick sensors has yet to be made, in order to reveal the merits of particular designs. Critical remarks in the literature, concerning the use of sensors that pass a small electrical current through the animal, have been reduced to realistic proportions.

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