

# A novel method for detecting licking behavior during recording of electrophysiological signals from the brain

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## Abstract

We have developed a novel method for detecting licking at a fluid well that is compatible with behavioral neurophysiology. This method uses off-the-shelf fiber optic technology to introduce a light beam through the fluid–air interface of a fluid bolus in a well. A self-adjusting optical sensor detects licking as disturbances in the amplified light surface within the interface when the fluid is disturbed. The proper configuration of fluid well and fiber optics will reliably detect licking and introduce no artifacts into simultaneous high-impedance recordings of extracellular neural activity. This method is also compatible with delivery of multiple fluids to the same well. Unlike present methods of detecting licking in neurophysiological experiments, our approach does not involve the passage of current or capacitance changes in which the animal forms part of a circuit, nor does it require movement of the licking apparatus or any other response beyond the actual licking of the fluid. As a result, noise artifacts in the unit recordings do not occur, and the sensor is highly resistant to artifacts caused by exploration or licking at the fluid well in the absence of liquid. We present neural recording data from units in the nucleus accumbens demonstrating these properties of the lick detector. © 2001 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Licking is a useful measure of liquid consumption in behavioral experiments in animals. There are several published methods in existence that can be used to detect licking. However, based on our review of the literature and our own experience, we found these methods to be incompatible with or difficult to adapt to high-impedance recording of single unit neural activity. One method in use involves making the animal part of an electronic circuit so that contact with the fluid or the fluid delivery device causes a change in voltage or capacitance (Hill and Stellar, 1951; Mundl and Malmo, 1979; Field and Slotnick, 1987; Weijnen, 1989; Spector et al., 1990). Rats appear to be unable to detect direct current in the sub- $\mu$ A range ( $< 1 \mu$ A; Weijnen, 1989), so this approach is useful for behavioral experiments. However, even these very low currents cause large noise

artifacts in brain recordings, and thus, these methods alone are not suitable for neurophysiological studies (Schoenbaum, unpublished observations). Although a modification involving alterations in capacitance and the use of AC currents has been reported to work without causing the noise artifacts that we observe, this method requires a relatively complex circuit (Mundl and Malmo, 1979). Moreover, all of these methods, including ones that utilize an AC current and filtering to reduce recording artifacts, incorporate the relatively high resistance of the rat as part of the detector circuit which means that they are highly susceptible to triggering by non-lick events, as any current pathway of similar or lower resistance from the fluid delivery site to ground will simulate licking. Such pathways can be caused by traces of urine, feces or dirt on the training apparatus where contacted by the animal (Field and Slotnick, 1987; Schoenbaum, unpublished observations). Although this problem is manageable when a spout is used to deliver fluid, we have found it to be unavoidable using a fluid well design due to the ease with which a fluid well is contaminated.

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Other approaches to lick detection of which we are aware detect disruption of a photobeam reflected across the region of fluid delivery (Whishaw and Tompkins, 1988) or movement of a magnet attached to the fluid delivery mechanism during licking (Kay and Laurent, 1999, see Weinhoffer et al., 1993 regarding Hall effect transducers). Although these systems would not be expected to interfere with electrophysiological recording, they typically involve a drinking tube for fluid delivery, which would be difficult to adapt to experiments that require the delivery of multiple fluids. They also appear to have the potential to be triggered by close proximity exploration of the fluid or fluid delivery site in the absence of actual licking. In other words, unlike the electronic circuits described above, these methods do not require actual contact between the rat and the fluid. For example, we use an additional photobeam that passes just above our fluid delivery well ( $< 1$  mm) to detect proximity to the well prior to fluid delivery. The rats in our experiments easily trigger this detector without licking the fluid.

To address these shortcomings, we have developed a lick detector based on simple off-the-shelf fiber optic technology that combines the advantages of the photodetector and electrical methods described above without the disadvantages. This method is relatively easy to implement and interface with computerized detection and control methods, is adaptable to different fluid well designs, causes no artifacts in brain recordings, and is resistant to artifacts caused by exploration in or around the fluid well. Here we describe the components and design of the fluid well, the fiber optic placement and the computer interface, and also show neurophysiological data to illustrate the operation of the sensor during recording from rat nucleus accumbens.

## 2. Methods

### 2.1. Apparatus

The fluid well assembly was mounted on one wall of an operant chamber used for electrophysiological recording (see Schoenbaum, 2000 for details of the chamber). The fluid well consisted of a bowl-shaped depression in a polycarbonate ledge, which was attached to a fluid line for water delivery (Fig. 1A). The well depression was machined with a 1/4 inch ball mill to a diameter of 6 mm and a depth of 2.25 mm, and the interior surface was polished to a smooth finish using 400 grit lapping compound. Fluid entered the well through a 2 mm diameter opening centered at the bottom of the bowl-shaped depression. This opening extended vertically 9 mm into the polycarbonate ledge before meeting the fluid delivery line, which entered

perpendicularly through the ledge from outside the chamber. The fluid delivery line measured 1.5 mm in diameter and was connected to a water line outside of the training chamber. The rim of the well was tapped with two openings  $\sim 1$  mm in diameter, centered 1 mm below the top edge of the well, for the fiber optic cables used to detect licking. Two small diameter (0.5 mm OD fiber; 1 mm OD with sheaths) plastic fiber optic cables (Banner Engineering, Minneapolis, MN) were inserted into these lines but did not enter the well itself. Once inserted in these lines, the fiber optic cables formed a watertight seal that prevented fluid from leaking out of the well through these openings.

The fiber optic cables exited the polycarbonate ledge outside of the training chamber, where they were attached to a D12DAB6FP AC-coupled plastic fiber optic emitter/sensor from Banner Engineering (Fig. 1B). This off-the-shelf unit costs approximately \$130 plus \$20 for the fiber optic cables and is specifically designed to respond reliably to small changes in intensity of a 680 nm light signal and is equipped with an automatic gain control feedback system that adjusts the intensity of the emitter to compensate for changing conditions related to environmental contamination. This property allows the system to self-adjust to the gradual accumulation of dust, feces, urine and other contaminants that occur in the behavioral environment, which ensures that the sensitivity of the unit remains constant. The unit operates using 10–30 V DC power, and has a response time of 50  $\mu$ s. The sensitivity of the sensor can be adjusted through an externally located setscrew with a 15-turn range (Fig. 1B). When triggered by a sudden disruption of light input from the emitter, the sensor emits a single output pulse, the duration of which can be adjusted from 1 to 70 ms through a second setscrew on the sensor (Fig. 1B). The unit is also fitted with LED's to indicate power status, sensing of the emitter beam, and output. In our system, the unit was powered with 24 V DC, and the sensitivity was adjusted so that disruption caused by filling the fluid well was minimized (see below). We used an output pulse of 10 ms duration. Output from the sensor activated an optically isolated Crydom input module (IDC5D) mounted on a Potter and Brumfield relay panel (2IO-24) (both available from Newark Electronics, Hanover, MD), which converted the output to a TTL signal (Fig. 1C). We fed the TTL pulse into an input/output board mounted in a PC running neurophysiological data collection and experiment control software (Datawave Technologies, Longmont, CO). TTL output is convenient since these signals are by definition of appropriate voltage to be detected as high or low transitions by computer systems. Input registers in the Datawave recording system can be easily programmed to detect these changes as task events, and inexpensive I/O cards are available from commercial vendors that can be placed in any PC

to provide similar detection capabilities. Alternatively, output from the detector can be run directly into an A/D converter and sampled like any other signal.

Fluid delivery was accomplished by activation of a large-bore stainless steel solenoid valve (CS-2325 Clip-pard, Minuteman Controls, Wakefield, MA) attached to the water line. To achieve tight control of solenoid activation time (and thus of the volume of fluid deliv-

ery), a computer was programmed to deliver a TTL signal to an optically isolated Potter and Brumfield output relay (ODC-5, Newark Electronics) mounted on the relay panel, which activated the solenoid. We found that a solenoid activation time of 80 ms delivered a 0.05 ml water bolus to the fluid well, although the activation time necessary to achieve a bolus of this size varied somewhat with different water line designs and fluid

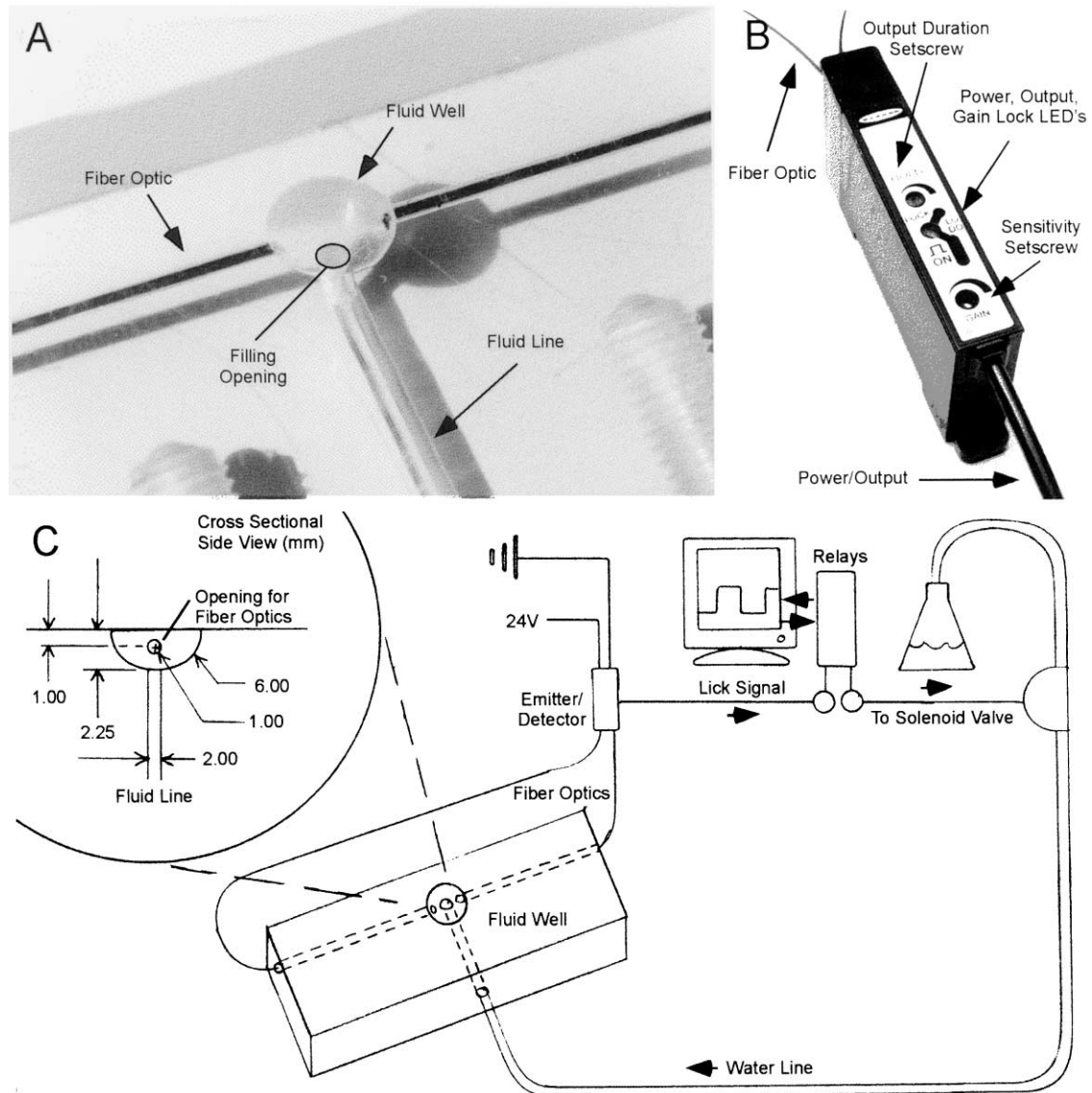


Fig. 1. Photographs and schematics of fluid delivery well and lick detector. (A) Photograph of the fluid well. The polycarbonate ledge shown is mounted on a wall plate and inserted into our behavioral training chambers. The fluid well is a bowl-shaped depression that is created using a ball mill. Fluid enters the well from outside the chamber through a line drilled into the block and terminating in the bottom of the well (see inset). Fiber optic cables are positioned in openings on either side of the well just below the rim, where the fluid–air interface is located when the well is filled. The well design illustrated is large enough to contain a fluid bolus of 0.05 ml; (B) Photograph of the emitter/detector that connects to the fiber optic cables. One light indicates that a stable signal has been established by the automatic gain control circuit, and a second light indicates when this signal is disturbed, causing an output pulse. The device comes equipped with setscrews to allow adjustment of the gain or sensitivity (15 turn) and also the duration of the output pulse (1–70 ms); (C) Schematic of the well shown in A and its connections with other components of the system. The drawing gives exact dimensions of the well block and well and also the locations and size of the lines that enter the well. Correct placement of these openings is crucial to minimize the filling signal and ensure proper operation of the detector for the desired fluid level. The emitter/sensor in our system was powered with 24 V DC, and the output converted to TTL signals and interfaced with our data acquisition computer through an optically isolated relay switch.

viscosities (e.g. water vs. sucrose). This volume of fluid filled the well to a level just over the fiber optic cables, such that the photobeam traveled within the fluid–air interface. Thus, any significant perturbation caused by contact with the fluid surface would disrupt the photobeam and trigger the sensor.

## 2.2. Behavioral and electrophysiological methods

Water-deprived rats were placed in the training chamber and trials were conducted in which an experimenter manually triggered fluid delivery to the well. Rat behavior was observed through a low-light video camera mounted in the training chamber. Flags indicating approach to the fluid well assembly (A), licking (L), and exit from the fluid well assembly (X) were entered through the computer keyboard into the data record by an experimenter observing the rat on a video monitor. Sessions were designed to test whether licking could be reliably detected, whether it could be discriminated from exploratory behavior, and whether neural recordings were disrupted by the operation of the lick detector. To these ends, each session included both fluid delivery followed by bouts of licking and exploration of the well in the absence of fluid delivery. Each session typically consisted of 10 trials of fluid delivery and lasted approximately 5–10 min.

Recordings of extracellular activity from the nucleus accumbens were obtained using a driveable bundle of 10 NiCrFe microwires, with a diameter of 25  $\mu\text{m}$  (see Schoenbaum, 2000 for further details of the recording apparatus). Activity on each microwire was passed through a high-impedance JFET preamplifier headstage, and then bandpass filtered at 300–3000 Hz and amplified differentially at 5000X using a Lynx-8 amplifier (Neuralynx Digital Amplifiers from Datawave Technologies). Neural data were acquired and digitized using the Enhanced Discovery Data Acquisition System (Datawave Technologies), along with signals from the lick detector and an output monitoring signal from the solenoid valve controlling water delivery. These three channels were digitized continuously at 20 kHz so that unit activity, licking and any associated artifacts in the neural data could be examined in detail. The data from each session were examined at high resolution using Datawave Data Manager software (Fig. 2).

## 3. Results

Data were reviewed from five sessions in which unit data was acquired and licking was detected. Neural data showed no evidence of artifact in any of these sessions. Fig. 2 illustrates data from one of these sessions. Unit activity from a microwire located in the nucleus accumbens was recorded differentially against a

second microwire that showed no unit activity. Although the units were not discriminated, there appear to be two or three different amplitude waveforms evident in the extracellular recording (Fig. 2 insets). These waveforms were the only signals observed above the background noise during this session.

Fig. 2A–C show data in successive periods during the recording session, chosen to illustrate the operation of the lick detector during filling of the well, during licking, and during exploration of the fluid well in the absence of fluid. Data are presented in temporal order during the session with gaps where sections were removed for brevity. Fig. 2A begins with three sections of data during filling of the fluid well. Note that the lick detector is activated briefly during filling. Because the detector works by directing a photobeam along the fluid–air interface, the well cannot be filled rapidly without perturbations in the fluid disrupting the stability of the beam. However, by locating the fluid line at the bottom of the well rather than on the side, and by polishing the well to remove uneven surfaces left from the drill used to tap the well, we were able to minimize such perturbations during filling. By these methods, and by using a large-bore, rapid onset/offset valve, we were able to reduce the disturbance due to well filling to the short and reproducible signal in the lick detector illustrated in the first three data sections in Fig. 2A. Note that these signals occur primarily when the valve is open and end abruptly when the valve closes. We were unable to trigger the lick detector by even moderately aggressive vibration or movement of the well block or the behavioral chamber with the sensitivity setting adjusted as shown in Fig. 2. Thus, once the fluid well is filled, the lick detector appears resistant to triggering caused by movement and vibration that does not directly impact the fluid–air interface. Note that the neural signal did not appear to be affected by the output from the lick detector. Moreover, it is possible (although we did not test it) that the filling signal could be further reduced or even eliminated by using a slower rate of fluid delivery.

Fig. 2A also shows data during a bout of licking. The rat approached the well ('A'), but the detector did not register licking until the experimenter observed licking behavior ('L'). Licking was rapid at the start of the bout and then diminished. Although the behavioral flags for licking ('L') and the lick detector signals did not synchronize perfectly, there appears to be equal lag both at the start of licking and at the end of licking, suggesting that this lack of synchronization was due to the reaction time of the observer entering the keyboard strokes. Inspection of the neural data (see insets) indicates that operation of the lick detector occurred without introducing any noise or artifacts into the neural recording.

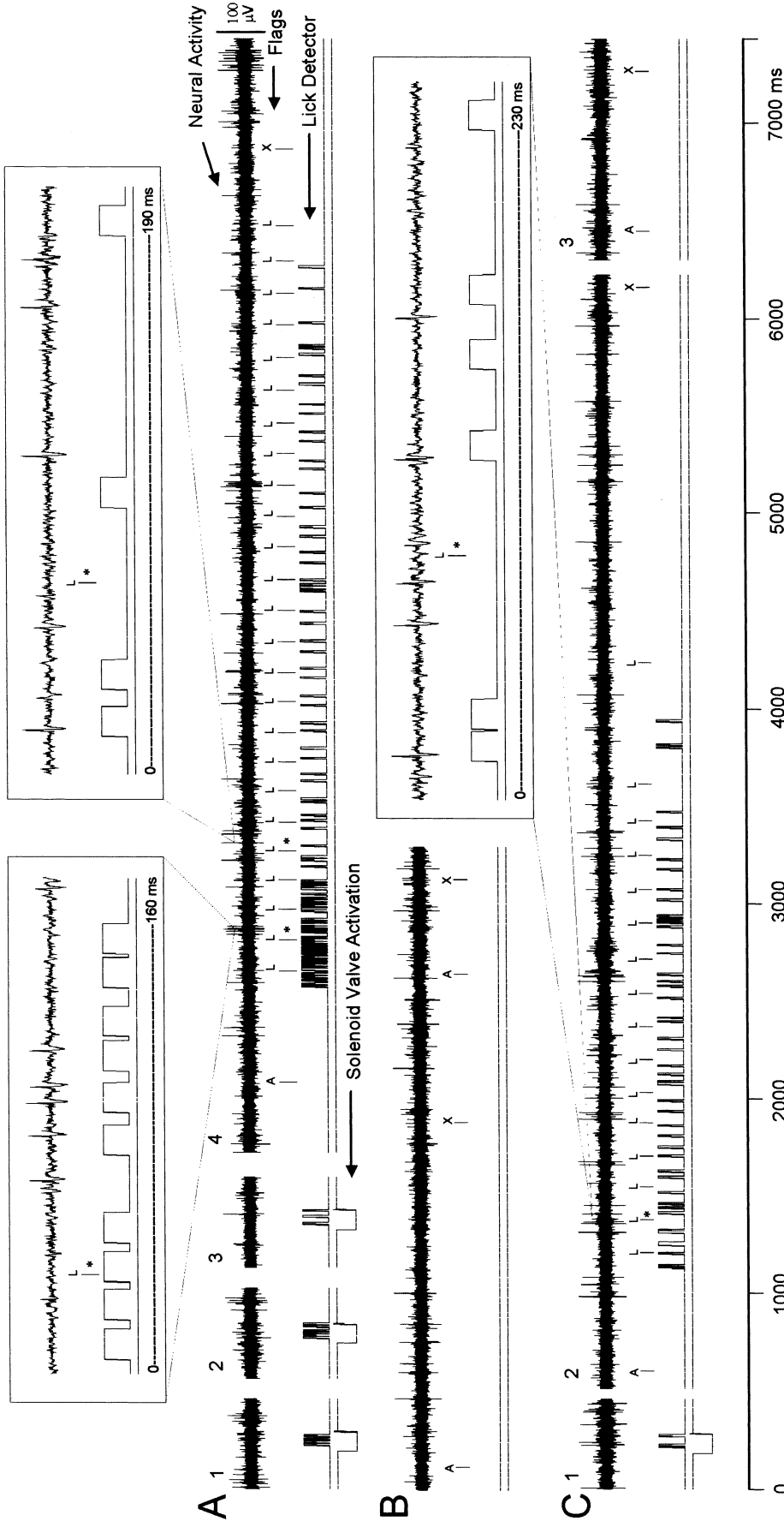


Fig. 2. Detection of licking during recording of unit activity in nucleus accumbens. Three traces are shown in each panel. The top trace shows neural activity recorded differentially from a pair of microwires located in nucleus accumbens. The second trace shows 10 ms TTL pulses indicating disruption of the fiber optic light beam located at the fluid–air interface in the fluid well (Fig. 1). Event flags are located above this trace in each panel. ‘A’ indicates the rat’s approach to the fluid well, ‘L’ indicates licking, ‘X’ indicates exit from the fluid well area. These flags were entered manually through the keyboard by an experimenter observing the rat by video camera, and thus they exhibit some lag time corresponding to the reaction time of the observer. The bottom trace shows activation of the solenoid valve controlling fluid delivery. Insets show neural activity and lick detector activation in specific regions of the data at a higher resolution. (A) This panel begins with three examples of fluid delivery (solenoid valve activation) (1–3). Note that solenoid valve activation (which results in filling of the fluid well) coincides with a short, repeatable signal in the lick detector. The third example of fluid delivery is followed by an example of approach and licking behavior (4). No noise artifacts were observed in the neural data during operation of the circuit, as indicated by the enlarged insets showing neural activity at higher resolution; (B) This panel illustrates the resistance of the detector to artifacts caused by proximity of the rat to an unfilled well. The rat approached and exited the well twice in the data shown, investigating the well by sniffing above it. The well was empty of fluid during this period. The lick detector, which is located below the rim of the well, emitted no signals during this exploratory behavior; (C) This panel further illustrates the fluid delivery signal (1); a bout of licking at a filled well (2); and exploration of the well in the absence of fluid (3). No artifacts were observed in the neural data as a result of licking, and no lick signals were observed during exploration of the unfilled well.

Fig. 2B shows the operation of the lick detector while the rat explored the well in the absence of fluid. The rat approached the well ('A') and investigated for several seconds, exited ('X'), and then returned briefly ('A'). Exploration of the fluid well consisted of a variety of behaviors, but typically included physical contact with the ledge containing the well and whisker movement in close proximity to the well itself. These behaviors were very similar to those observed during fluid consumption, yet, as the figure shows, no signals were observed from the lick detector as a result of these activities. Although any actual entry into the well that breaks the photobeam can cause a single signal from the detector (for example, inserting a fingertip into the unfilled well will trigger it), in practice, the design is highly resistant to triggering by non-licking events for several reasons. First, the photobeam produced by the fiber optics is very narrow, so it would only be disturbed by a very localized and specific interaction (although this is only true when there is no fluid in the well — when the beam travels along the fluid–air interface the effective area that can register disturbances is amplified by the fluid surface). Second, the beam is recessed below the surface of the well, so explorations level with the surface will not disrupt the detector (although when fluid is in the well, the beam is 'bent' along the fluid–air interface and can register above or below its normal range, again amplifying the effective area).

Fig. 2C shows filling of the well followed by a bout of licking the fluid, and subsequent exploration in the absence of fluid. Note again that the filling signal was of short duration and was reliably confined to the time of solenoid activation. Following filling, the rat approached the well ('A') and initiated licking ~ 500 ms later. The lick detector was not activated during the approach period but only when licking was observed. Again there was a 100–200 ms time lag in keyboard entries for licking ('L'), and this lag occurred both at the start and the end of licking. In this trial, the rat stopped briefly and then licked twice more at the end of the trial as indicated by the lick keystrokes ('L') entered manually by an observer. This pattern is reflected in the output of the lick detector, where there is a short pause in the output from ~ 3500 to 3800 ms. Note that the rat remained in the vicinity of the well for 2 s after the last lick keystroke ('L'), but that the lick detector registered no further events. As shown in the inset, neural recordings were unaffected by operation of the lick detector.

#### 4. Discussion

Here we have described a method for detecting licking in rats during recording of high impedance single unit neural activity. This method uses fiber optic tech-

nology to introduce a light beam into the interface between the surface of a fluid bolus and the air above, and a self-adjusting sensor that responds to disturbances in this light surface to detect licking. This method has several advantages over existing methods of which we are aware. First, it uses off-the-shelf technology, which, although moderately expensive (~\$150), includes self-contained hardware for adjustment of sensitivity and output signal, and is easily interfaced with data collection systems either directly or through a cheap input relay.

Second, the small size of the fiber optics, combined with the amplification of the effective area of detection by passage of the photobeam along the fluid–air interface, would allow this system to be adapted to a wide range of fluid well sizes. For example, although our design is sized for rats as experimental subjects, the fluid well could be scaled down to accommodate mice. This would be difficult to accomplish using a conventional photoemitter and detector. A perusal of the Newark Electronics catalog revealed that the smallest photoemitter available has an effective diameter several times larger than our fiber optic system and a significantly larger housing, which would render it difficult to place in the side of any fluid well designed for rats or mice.

A third advantage of our system is that the detector is resistant to triggering by non-lick events, due to the small size and recessed placement of the fiber optic photobeam. We have found this feature to be important, as it ensures that detector activation is tightly coupled to licking. Using the lick detector in our system, we have found that rats can trigger a second photobeam placed only slightly above the fluid well in the absence of licking, suggesting that a subject's close proximity to the fluid is not a reliable indicator of licking.

A fourth advantage is that our detector is compatible with delivery of multiple fluids to the same well. We have adapted the lick detector to a fluid well design used in a number of recent studies (Schoenbaum et al., 1999, 2000) that allows three different solutions to be delivered rather than one. This fluid well is similar to the one pictured in Fig. 1a except that it has additional fluid lines in the same plane but offset and angled from the one pictured. These lines connect to the main fluid line under the well (see Schoenbaum et al., 2000 for photo). This design prevents the rat from identifying a solution by its spatial location, which is crucial in any attempt to study learning of associations between neutral cues and reinforcers. Delivery of multiple fluids in combination with a vacuum drain would be difficult to implement using a drinking tube design.

Finally, unlike electrical methods, this detector does not require the animal to serve as a component of the detector circuit. The majority of these methods are

incompatible with brain recording because they introduce artifacts into the neural data. In addition, all such methods are susceptible to being falsely triggered by non-lick contact near the fluid delivery site. This problem is particularly pronounced when using a well to deliver multiple fluids rather than a single fluid delivery spout that can perhaps be more easily isolated. In our experience, the surface of the polycarbonate block containing the fluid well is easily soiled, which leads to spurious triggering by any contact between the animal and the polycarbonate block due to high resistance pathways between the contact point and the circuit located in the actual well. Thus, for example, contact with these pathways through a paw placed near the well imitates licking. Our detector design avoids both the neural artifact produced by most electric detection methods and the problem of false triggering inherent in these designs. As a result it combines the advantages of both the electrical and conventional photobeam methods of detection, while minimizing their disadvantages.

The primary shortcoming of this system was the presence of a signal in the lick detector resulting from the transition of the light beam from air into the fluid bolus during filling of the well. As one might expect, the timing of the filling signal is clearly related to the passage of the fluid interface across the ends of the optic cables rather than opening or closing of the solenoid, and once the cable ends are submerged, further filling of the well does not disrupt the light beam. In practice this signal reveals when the fluid–air interface reaches the level of the fiber optics in the well.

Although it was possible to eliminate this signal by decreasing the sensitivity of the detector, in practice this resulted in detection of only vigorous licking. In order to minimize this signal, several important steps can be taken. The timing of the signal is related to the speed of filling, and the duration of the signal is related to the amount of turbulence in the fluid bolus during filling. Our design attempted to maximize filling speed while minimizing filling turbulence. To accomplish this we found that several design features were important. First, turbulence seemed to be minimized by use of a ball mill in forming the well. Second, the well surface was polished to a smooth surface. Third, the fluid entry line was located in the precise center of the bottom of the well rather than being offset to the side, and the fluid line entered through this opening after traveling for vertically for some distance below the well. These features insured that fluid would enter the well vertically with no lateral momentum. Finally, the speed of fluid entry was adjusted to be as rapid as possible without causing any overshoot above the well. This was accomplished by first tapping the entry hole into the well to a relatively large diameter and then adjusting the fluid flow rate by increasing or decreasing the length and diameter of the tubing that connected the fluid

reservoir to the fluid line in the polycarbonate block. With some testing, we were able to achieve the performance shown in Fig. 2 in which a limited, repeatable filling artifact is present in the context of robust detection of licking.

It is worth noting that despite this potential drawback, the presence of this signal can actually be useful, in that it serves to verify that the sensor is functioning properly. If no artifact is observed on filling of the well, then the sensor is either not able to detect the fluid–air interface or the surface of the bolus did not reach the level of the fiber optics in the fluid well. During data acquisition, a software filter that ignores signals on the lick detector during filling of the well and shortly thereafter can easily eliminate this filling artifact. We have found that experienced rats — trained to receive quinine and sucrose from the same well — do not lick prior to filling and typically have a licking latency of > 150 ms after fluid delivery, so this filtering strategy works well in this context. An alternative approach would be to fill the well before the animal approaches or to enclose or cover the well during filling. For training in which these approaches are not adequate we are currently using a vacuum-activated, low profile cover made from aluminum that slides over the top of the well. This cover allows a response to be registered by the photobeam above the well but prevents access to the actual well until after fluid is delivered (information available on request).

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