The Electroolfactogram: A Review of Its History and Uses

JOHN W. SCOTT AND PAMELA E. SCOTT-JOHNSON
1Department of Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322
2Department of Psychology, Spelman College, Atlanta, Georgia 30314

KEY WORDS   olfactory receptor neurons (ORNs); electroolfactogram (EOG); odor selection and delivery

ABSTRACT   The electroolfactogram (EOG) is a negative electrical potential recorded at the surface of the olfactory epithelium of vertebrates. It represents primarily, if not exclusively, the summed generator potential in the olfactory receptor neurons (ORNs). While a number of studies suggest that secretory or inhibitory events may also contribute to the EOG, these are not well established. This review outlines (1) the cellular and physiological nature of the EOG response; (2) methodological considerations regarding odor selection and delivery, surgical preparation, response descriptions, and analysis; and (3) application of the EOG in human, fish, and insect olfaction and pheromonal responsivity. A number of technical issues associated with EOG recording are also discussed. Microsc. Res. Tech. 58:152–160, 2002.

INTRODUCTION

Electrical activity can be recorded with gross electrodes from the receptor sheet of certain sensory organs. For example, the cochlear microphonic and the electroretinogram have been studied for decades and are still used in the experimental and clinical literature (Chertoff et al., 2000; Flock and Flock, 2000; Holder, 2001). Electrical activity in the olfactory epithelium of dogs in response to odorant presentation was apparently first described by Hosoya and Yoshida (1937). Ottoson (1956) independently studied these responses in the olfactory epithelium of the frog and rabbit. He gave them the name “electroolfactogram” (EOG) and argued that the EOG is a summed generator potential and described ways in which it could be used to study olfactory transduction. The EOG has been used to assess the response of populations of the cells that bear the olfactory receptor proteins, the olfactory receptor neurons (ORNs). In recent years, the EOG has become an important tool for evaluating the effects of genetic manipulations and for studying other properties of the olfactory receptor sheet. For this reason, it is now useful to review some of the properties of this activity with a view to understanding its strengths and limitations. We have also tried to review some of the practical issues in recording EOGs.

According to our current understanding, odorants bind receptor proteins on ciliary membranes projecting from the ORNs into the mucus. These receptors act through second messengers to open ion channels leading to depolarization at the ciliary surface of the ORNs relative to the tissue under the indifferent electrode (reviewed by Getchell, 1986). This is confirmed by direct observation (Firestein and Shepherd, 1991; Lowe and Gold, 1991; Leinders-Zufall et al., 1997). Ottoson concluded that only the surface negative potential reflected the true generator potential and suggested that other electrical events recorded during odor stimulation were artifacts (Ottoson and Shepherd, 1967). We will try to show where some of these issues have been clarified and others remain uncertain.

THE NATURE OF THE EOG

This review primarily concerns electrophysiological measurements of the population response in the olfactory epithelium. In most cases, these measurements have been made as Ottoson (1956) described, that is with direct coupled amplifiers and with recordings from the ciliary surface of the epithelium. In a few cases, electrodes have been placed on the base of the cribriform plate from the cranial side (Thommesen and Døving, 1977) or penetrated through the epithelial sheet after removing the bone (Chaput and Chalonsonnet, 1997; Chaput, 2000; Ezeh et al., 1995; Potter and Chorover, 1976; Scott et al., 1996; Scott-Johnson et al., 2000). In some reports, the population electrical activity has been measured with voltage sensitive dyes (Kent and Mozell, 1992; Kent et al., 1996; Youngentob et al., 1995; Youngentob and Kent, 1995). We will only refer to single cells observed by electrophysiology or calcium imaging where those experiments pertain to particular issues raised in the population recordings.
Several recent reports have utilized the EOG to evaluate the elements of the olfactory transduction process. Some of these have provided insight into the question of which elements generate the EOG. Brunet et al. (1996) observed that knockout of the olfactory cyclic nucleotide gated channel abolished all surface negative EOG responses to a panel of odorants. Wong et al. (2000) disrupted the type III adenyl cyclase gene and found a similar abolition of EOGs. Belluscio et al. (1998) disrupted G_{olf}, which mediates the activation of type III adenyl cyclase. This produced a dramatic reduction of the surface negative EOG in newborn mice and a nearly complete loss of response in 3-week old mice. In all three knockout conditions, the response loss was irrespective of whether the odorants were of the types reported to evoke increases in receptor cell cAMP or IP_{3} (Breer and Boekhoff, 1991). These observations further support Ottoson’s (1956) contention that the EOG results from odorant stimulation but do not completely rule out the possibility that there is a contribution from other cells that are excited indirectly as a consequence of ORN response.

The other cell type that would be a candidate for the electrical source of the EOG is the supporting cell (or sustentacular cell) of the epithelium. Intracellular recordings from the salamander epithelium demonstrated a population of non-spiking cells that had resting potentials more negative than spiking cells (Getchell, 1977; Masukawa et al., 1983; Trotier and MacLeod, 1986). These are thought to be the supporting cells. These cells showed changes in membrane potential during odor responses, but Trotier and MacLeod (1986) report that they always lagged the EOG by at least 150 msec. Trotier (1998) used patch electrodes to record and label frog supporting cells. He found changes in currents and potential during odor stimulation, but no conductance changes. He concluded that these were passive changes reflecting the extracellular changes in potassium concentration previously reported (Khayari et al., 1991). These observations indicate that the supporting cells are not likely to be a major contributor to the EOG. Several investigators have studied manipulations of the ionic environment of the epithelium on the EOG. Among these are studies by Leveteau et al. (1989), who concluded that Ca^{2+} ions contribute to the response and by Ishimaru (1992) who concluded that Ca^{2+} ions suppress the EOG. These are not issues that could be conclusively established by population recordings. Patch clamp studies show that one role of Ca^{2+} entry is to amplify the response by increasing Cl⁻ conductance (reviewed by Frings et al., 2000).

**METHODOLOGICAL CONSIDERATIONS**

**Response Description**

The EOG usually has a rapid rising phase and a somewhat slower decline (Ottoson, 1971). The latency is difficult to estimate in most cases because of problems detecting the exact arrival time of the odorant at the receptor sheet. Ottoson (1971) estimated that latency at around 100 msec for the frog. Van As et al. (1985) estimated latencies of 100–200 msec in salamander. Mackay-Sim and Kesteven (1994) applied the odorant stimulus very close to the epithelium (<1 mm) and estimated a latency of 32 msec for the rat. This latency is in agreement with the lag between electrochemical artifacts recorded with metal electrodes in the rat and the EOG in our preparation (unpublished observations).

The decay of the EOG may reflect mechanisms for clearance of the odorant from the epithelium as well as properties of the receptor cell. With sustained stimulation, the response reaches a steady state as initially described by Ottoson (1956). Ottoson (1956, 1971) also described oscillatory changes in the frog EOG in response to strong odor stimulation. Adrian (1955) had described oscillations in the rabbit epithelial response. Such oscillations are prominent in the salamander (Dorries and Kauer, 2000), where it is speculated that the epithelial oscillations may drive the oscillations often observed in the olfactory bulb. Similar observations have been made for catfish (Parker et al., 2000; Nikonov et al., in press). We have rarely observed oscillations in rat EOG and have seen large oscillatory bulb potentials without observing oscillatory EOGs (Scott and Sherrill, 2001).

Although some reports have detailed response rise times (Scott-Johnson et al., 2000; Van As et al., 1985), most measurements of the EOG have concentrated on the peak amplitude of the transient response. This is in contrast to measurements of summated action potentials from olfactory nerve (Mozell et al., 1984, 1987) where the areas under the response curves have been used as response measures. The very slow response decline (Ottoson, 1956), often extending for seconds, makes integration of EOG records impractical.

**Electrochemical and Other Artifactual Responses**

Mozell (1962) and Müller (1971) suggested that some of the responses seen in the epithelium represent electrochemical reactions between the odorant and electrodes. Getchell (1974) conducted a study of the salamander EOG in which he controlled for electrochemical effects on moist tissues by using the same electrode (a silver-silver chloride gelatin bridge electrode as recommended by Ottoson and Shepherd, 1967) to record successively from olfactory epithelium, extracellular fluid covering leg muscle, and oral epithelium. In this and subsequent reports, certain authors (Getchell, 1974; Getchell and Getchell, 1977; Mackay-Sim and Kesteven, 1994; Mackay-Sim and Shaman, 1984) distinguished between the negative component (V_{EOG(-)}) emphasized by Ottoson as the true EOG and the positive component (V_{EOG(+)}), to be discussed later.

Electrochemical and mechanical artifacts are a significant issue in EOG recording. They can sometimes be recognized by a shorter latency than true physiological responses. We rarely have electrochemical problems while using silver-silver chloride agar bridge electrodes. However, with air flow through an intact nasal cavity when the electrode has penetrated the epithelium from the outside (Ezech et al., 1995; Scott et al. 1996; Scott-Johnson et al., 2000), we have often seen small movements of the tissue that were correlated with baseline shifts. Changes in the humidity of the air stream associated with the odorant are also a potential source of artifacts. Ottoson (1956) observed shifts in potential related to humidity. Because of the extreme sensitivity of the epithelium to drying, it is not practi-
cal to use completely dried air. Therefore, when there is suspicion of artifacts, one reasonable precaution is to try to match the air flow rate and humidity of the stimulus and unstimulated conditions. It is also very easy to draw odorous contaminants into the apparatus (depending on its design). We have found it very important to include a blank stimulus that is as similar to the odorant conditions as possible. This has allowed us to detect and remove contaminants and other artifactual conditions. We have also found that, for purposes of reducing errors due to cross contamination with odorants, it is not desirable to include strong odorant stimuli (or stimulus concentrations) in series with weak stimuli as minor contamination can obscure the response to weak stimuli.

Correspondence With Action Potentials

Mozell (1962) catalogued several conditions under which there was failure of correlation between the EOG and the spike activity recorded in olfactory nerve in response to the same odorant. These included differences in the response durations, differences in the concentration response curves, and differences in the rate of recovery from adaptation. Although Ottoson and Shepherd (1967) suggested that many of these differences might have arisen from a mismatch in the position from which the EOG was recorded and the area of mucosa sampled by the recorded nerve twig, Mozell in most of his subsequent work (Mozell, 1964, 1970; Mozell and Jagodowicz, 1973) has employed summed nerve responses as a measure of receptor neuron response.

There are other indications of mismatch between the EOG and action potentials in the receptor neurons. In some cases, extracellular spikes of receptor neurons decrease in size over the course of response and frequently become lost in the noise (Baylin, 1979; Duchamp-Viret, 2000). Trotier and MacLeod (1983) observed in intracellular recorded salamander ORNs that there is a clear size decrease riding on a strong depolarization, but that small spikes continue. It is not clear from these recordings whether the axon is firing in these cases, but it seems likely that there is a full action potential that is not reflected in the recordings from the soma, since recordings from axons do not appear to show such dramatic response decrements (Getchell and Shepherd, 1978; Gesteland et al., 1965).

Spatial Resolution of the Response

The EOG obviously represents activity in a population of cells, but the exact number of cells participating in the response is difficult to estimate. The space constant of the olfactory epithelium, i.e., the distance over which the voltage (or ability to activate receptor cells) decays to 1/e (~37%) from a point source of current injection, has been estimated to be about 117 μm in the rat (Mackay-Sim and Kesteven, 1994). A smaller number of experiments have used rats or mice fitted with a tracheal cannula to pull air through the nose in a procedure that might be called an artificial sniff. Thommesen and Devin (1977) used this preparation with the electrodes placed on the cribriform plate. Ezeh et al. (1995) and Scott-Johnson et al. (2000) also used an artificial sniff but introduced the electrodes through the epithelium into the lumen until they detected a large negative response to odorous stimuli.

Surgical Preparation

Most of the EOG publications have been for frog, salamander, rat, or mouse preparations, although some work has been on species such as dog (Myers et al., 1984; Myers and Pugh, 1985). For the frog and salamander, the roof of the olfactory sac is generally exposed exposing the floor of the cavity (see descriptions by Ottoson, 1956; Gesteland et al., 1965; Getchell, 1974). Silver-silver chloride electrodes filled with Ring er’s solution in gelatin or agar are usually used for the recordings and silver-silver chloride indifferent electrodes are commonly placed in the mouth. In many instances, the animals were anesthetized or decorticated, although Gesteland et al. (1965) noted that although single unit activity disappeared after death, the EOG could be maintained for several hours after circulation ceased. The rat and mouse preparation in most common use was introduced by Edwards et al. (1987). Animals have been killed by anesthetic overdose or decapitation, and the nasal septum or midline aspect of the epithelium over the endoturbinate bones surgically exposed. This approach allows direct application of odorant to the epithelium and is analogous to the preparation used for frog and salamander experiments. This preparation has subsequently been used for study of spatial distribution of responses (Edwards et al., 1988; Kent et al., 1996; Mackay-Sim and Kesteven, 1994; Scott et al., 1996, 1997, 1999, 2000; Youngentob et al., 1995) and for study of the effects of lectins on the olfactory response (Shirley et al., 1987).

Other Technical Issues in EOG Recording

There are a number of other issues that are of technical importance in recording of the EOG. Gesteland et al. (1965) described the problems of epithelial drying and of thinning of the epithelial mucus after it has been touched by an electrode. For example, sometimes the mucus shrank back from the electrode tip causing changes in resistance or even a sudden break in contact. We have also found that it is important to avoid overheating the tissue. If the temperature is kept cool (~17°C) responses can often be maintained for several hours without significant change in the relative response to different odorants, although the overall response magnitude may decline (Scott and Brierley, 1999). Gesteland et al. (1965) also reported significant
changes in the frog when circulation stopped. Scott et al. (1996) compared anesthetized rats with rats freshly killed by anesthetic overdose or CO₂ and found little difference except some slowing of response kinetics. Most investigations of EOG in rodents have used freshly killed preparations. Notable exceptions were reports by Chaput and Chalansonnet (1997), Chaput (2000), Potter and Chorover (1976).

We have typically normalized results using a standard odorant that evokes responses broadly throughout the epithelium (usually isoamyl acetate, see Scott and Brierley, 1999). This standard stimulus is presented frequently, so that the normalization can take into account sudden changes in response at a particular electrode site. While this step obviously throws away information about absolute response magnitude, it has been particularly useful for investigating relative response across a series of stimuli of different chemical composition. We have also adopted the practice of comparing the responses simultaneously recorded on multiple electrodes as introduced by Mustaparta (1971). Use of multiple electrodes (up to eight) and response normalization has also reduced variation arising from gradual decrease in response magnitude over time and allowed us to compare a large set of stimulus odorants (Scott et al., 2000). Of course, there is a practical limitation to the number of simultaneous electrodes that can be placed. One of the significant advantages of the voltage sensitive dye method is the ability to make simultaneous measurements from many sites, limited only by the field of view and the plane of focus (Youngentob et al., 1995).

COMPLEX EOG PATTERNS AND THE ISSUE OF INHIBITION IN THE OLFACTORY EPITHELIUM

EOG Observations of Positive Waveforms

Several investigators have reported complex waveforms containing surface-positive components (Getchell, 1974, Gesteland et. al, 1965; Takagi and Shibuya, 1960; Takagi et al., 1969; Takagi, 1969). Ottoson and Shepherd (1967) suggested that initial positive waves or apparent positive off responses might arise because the odorant stimulus is unevenly distributed across the epithelium. This view holds that electrodes on tissue that are relatively inactive (either because they are damaged or not strongly stimulated) will record a voltage generated by current returning to the surface from active sites. This voltage would show an opposite sign to that for the variety of waveforms that can be recorded in the olfactory bulb after nonuniform activation of the lateral olfactory tract or olfactory nerve (Shepherd and Haberly, 1970). In our own laboratory, initial positive responses usually occurred where the tissue under the electrode was damaged or when the odor stimulus was not optimally placed over the tissue. In spite of concerns about stimulus-induced artifacts, the consistent correlation of initial positive waveforms with specific odorants (Gesteland et al., 1965; Getchell, 1974) strengthens the case that the phenomenon is real in some instances.

Positive Waveforms as an Indicant of Secretion

Okano and Takagi (1974) observed that odorants evoking positive responses in frog epithelium also produced granular secretion from sustentacular cells of the epithelium. Surface-positive voltages could still be evoked in mice by triethylamine stimulation after knockout of the olfactory cyclic nucleotide gated channel (Brunet et al., 1996) or of C₅₆ (Wong et al., 2000). The surviving surface-positive waveforms were attributed to secretion by analogy to the Okano and Takagi observations. However, the sustentacular cells of the mammalian epithelium are not thought to be capable of granular secretion (Getchell and Getchell, 1992), and the issue of electrogenic secretion processes has not been studied in mammalian olfactory epithelium. Therefore, while secretory action via some other mechanism is a plausible cause of positive EOG waveforms observed in mammals, there is no direct evidence to support it. None of the claims of positive potentials has been documented with more advanced methods for studying field potentials, such as current source density analysis (Aroniadou-Anderjaska et al., 1999; Nicholson and Freeman 1975).

Off-Responses in the EOG and the Issue of Inhibitory Responses

Takagi and Shibuya (1960) described on- and off-responses to odorant presentation in frog EOGs. In these cases, there was an increased negativity when the odorant stimulus was removed. These results were interpreted as evidence of inhibitory interactions in the olfactory epithelium. Ottoson and Shepherd (1967) cautioned that these were likely to be produced by uneven activation of the epithelium. It has been pointed out that there is no evidence of synaptic interaction in the olfactory epithelium (Graziadei, 1971), but there are several ways that odorants might produce inhibitory effects. Inhibitory currents have been studied in toad and rat ORNs with patch clamp recordings (Sanhueza et al., 2000). In some cases, the stimuli for these inhibitory responses were odorant mixtures associated in the literature with induction of IP₃ in olfactory ciliary preparations (Morales et al., 1994, 1997). Inhibitory responses to odorants are well documented in spiny lobster (Ache and Zainizarov, 1995) where inhibition is mediated by a cAMP message and excitation is mediated by an IP₃ message. It is noteworthy that genetic knockout (Wong et al., 2000) or pharmacological blockade of cAMP pathways (Chen et al., 2000) is reported to prevent responses to “IP₃ odors” (Breer and Boekhoff, 1991) in isolated salamander ORNs as well as mouse EOGs.

There is also evidence in newt olfactory receptor neurons that odorants can directly act on cyclic nucleotide gated channels to decrease their conductance (Kawai et al., 1997; Kurahashi et al., 1994). Part of this evidence rests on larger responses and faster rise times for short odorant pulses and for long ones (Kurahashi et al., 1994). While we have seen some evidence of faster rise times in EOG responses with short duration stimuli, we have not seen reliable evidence of increased negative EOGs at the termination of odorant stimuli (unpublished data). It is possible that either a cyclic nucleotide or direct channel action might produce EOG...
off-responses, but we know of no documented cases where the mechanisms of EOG off-responses have been established.

EXAMPLES OF APPLICATION OF THE EOG
Spatial Properties of the EOG

EOG recording has been an attractive way to approach the question of spatial localization of response in the olfactory epithelium because it represents a summation of activity from many cells and is, therefore, relatively stable over time. This approach has inherent problems. We now know that receptor neurons that express a single receptor gene are distributed widely within the epithelium of many species (Ressler et al., 1993; Vassar et al., 1993; Ngai et al., 1993; Marchand et al., 2001). The first systematic investigations of localized EOG response to odorants in the epithelium were performed in frogs (Daval et al., 1970, 1980; Mustaparta, 1971). These reports indicated that there were some odorants that differed in their ability to evoke responses along the anterior-to-posterior axis of the epithelium. However, they made it clear that, because most odorants evoke responses in all parts of the epithelium, more complicated analyses are necessary. The anterior-posterior differences in odor response were confirmed in the salamander by Mackay-Sim et al. (1982) and Mackay-Sim and Shaman (1984), who for the first time showed that the anterior-posterior gradients were independent of stimulus concentration. Spatially organized responses in amphibians were also confirmed by Kent and Mozell (1992) with voltage sensitive dyes, although they found the spatial effect to be stronger in salamander than in frogs. There is a general consensus in this work that water soluble odorants like butanol evoke larger responses in the anterior part of the salamander epithelium, while less soluble odorants like limonene evoke larger responses in the posterior part.

Observations on the spatial distribution of odorant responses in mammals were initiated with the observations of Thommesen and Dævng (1977), who recorded the responses from the dorsal surface of the cribriform plate as described above. They found two groups of odorants with spatially differential responses, and concluded that these observations reflected differences between the dorsal and ventral parts of the nasal cavity (as contrasted with an anterior-to-posterior gradient in frogs or salamanders). Studies of the EOG in the surgically opened rat nose preparation have agreed in finding a non-homogeneous response to odorant stimuli, but have not always agreed on the pattern of that distribution. Mackay-Sim and Kesteven (1994) used a very small odorant applicator to stimulate a confined area around a single recording electrode, which they moved through a series of 16 standard recording positions. Youngentob et al. (1995) studied responses on both the septal and turbinate surfaces with voltage sensitive dyes while using a larger odor tube to uniformly activate the epithelium. Both groups found reliable patterns across animals. Ezeh et al. (1995) and Scott et al. (1996) found regional differences in the intact rat nose that reflected the dorsomedial vs. ventrolateral division suggested by Thommesen and Dævng (1977). They also reported that the odorants preferentially activating the two regions were systematically different, in that polar odorants produced larger responses in the dorsomedial recess of the epithelium.

This work was extended (Scott et al., 1996; Scott and Brierley, 1999) to specifically test the relationship between the response patterns and the gene expression zones as defined in the neonatal rat and mouse by Vassar et al. (1993) and Ressler et al. (1993). Highly polar odorants (for example, aromatic or cyclic terpene ketones and aldehydes) evoked larger responses in the dorsomedial part of the epithelium, while saturated hydrocarbon odorants evoked larger responses ventrolaterally. The correlation with the organization of the expression zones was confirmed for a large sample of odorants at multiple concentrations and was seen across several surfaces of the epithelium. These results agree with previous observations about water solubility of odorants. Interestingly, the recent demonstration of receptor expression zones in salamander (Marchand, 2001) also suggests that those zones correlate with functional response distributions.

Effect of Airflow on Spatial Response Distribution

Most of the EOG studies on spatial factors in the olfactory epithelium have been performed on what we have called the “opened” preparation where odorants are applied directly to the epithelium. Mozell and his colleagues have published a series of summated nerve recordings showing that the tissue of the intact frog nasal cavity is capable of removing more polar odorants from the airstream before they reach the receptor cells (Mozell 1964, 1970; Mozell and Jagodowicz, 1973; Hörnung and Mozell, 1977; Mozell et al., 1991). These observations raise two questions. First, are the differential local responses in the olfactory epithelium produced entirely by this sorption effect? Second, even if there is an intrinsic, differential activity corresponding to gene expression zones, does the sorption of odorants from the air stream contribute to normal sensory processing?

Several of the EOG studies sought to avoid differential sorption in their procedures. Youngentob et al. (1995) directly applied the odorant uniformly over the epithelium. Mackay-Sim and Kesteven (1994) limited the odorant to a very small region just around the electrode. Scott et al. (1997) tested three directions of air application and found that all produces the same pattern of response. Thus, it is a reasonable conclusion that there is an inherent response pattern that follows receptor sensitivity.

EOG and voltage sensitive dye recordings also support the concept that odorant sorption from the airstream is physiologically important (Kent et al., 1996; Scott-Johnson et al., 2000) by comparing the responses seen during normal airflow to those with direct odor application. The modeled velocity of air flow in the rat nose (Kimbell et al., 1997) correlates strikingly with the rat receptor gene expression zones (Vassar et al., 1993), implying that the flow patterns and receptor gene expression zones may have evolved together. ORNs in different regions of the epithelium project axons to circumscribed regions of the olfactory bulb (Astic et al., 1987; Schoenfeld et al., 1994). There are likely several components to the odorant specificity.
seen at the glomerular layer of the olfactory bulb including: the airflow pattern, the intrinsic distribution of receptor types, the broad topographic projection pattern from the receptor zones to the bulb, and the specific segregation of axons from specific ORN types into glomeruli (Mombaerts et al., 1996; Ressler et al., 1994; Vassar et al., 1994). The EOG, as a population measure, is appropriate to study the first two of these variables.

**EOGs in Fish**

The EOG has been an important tool for assessing olfaction in fish. A series of cross-adaptation and mixture studies were used to demonstrate that different classes of amino acids stimulate different groups of receptors in channel catfish (Byrd and Caprio, 1982; Caprio and Byrd, 1984; Caprio et al., 1989). These groupings have been generally confirmed by calcium imaging of the activity of the zebrafish glomerular layer (Friedrich and Korsching, 1997). EOG recordings have also been used to study the pheromonal system in goldfish (Li et al., 1995; Sorensen et al., 1988; 1995).

**EOG in Humans**

It is possible to perform successful EOG recordings in humans (Furukawa et al., 1989). Hummel et al. (1996) have used the EOG to compare the physiological response and subjective ratings of desensitization to odorants by human subjects. Leopold et al. (2000) have used the EOG along with biopsy to estimate the distribution of human olfactory epithelium. Apparently because of the problems of working in the narrow spaces of the nose there are very few reports of clinical use of the EOG in evaluation of anosmia.

**Trigeminal and CO₂ Responses**

It has long been known that odorous substances can also activate the trigeminal nerve (Silver and Moulton, 1982; Tucker, 1963). This literature was recently reviewed by Bryant and Silver (2000). Slow negative potentials can be recorded from the nasal epithelium, apparently including the olfactory epithelium, in response to stimuli, such as CO₂, that are considered to be pure trigeminal irritants (Kratskin et al.; 2000; Thurauf et al., 1991, 1993, 1994). On the other hand, responses to CO₂ have been observed in an olfactory receptor neuron (Getchell and Shepherd, 1978). Coates et al. (1998) have observed carbonic anhydrase activity in a small population of bullfrog olfactory receptor neurons and suggested that they might be responsible for producing such responses. Coates (2001) has also reported carbonic anhydrase activity and EOG-like responses to CO₂ at a number of sites in the rat olfactory epithelium. The issue of whether any of the responses seen in olfactory epithelium to CO₂ and other recognized trigeminal irritants come from trigeminal endings or olfactory receptor neurons is not clearly resolved.

**Vomeronasal Organ**

The vomeronasal organ (Jacobson’s organ) is a structure along the base of the nasal septum of many mammals and reptiles. Its receptor neurons project to an accessory olfactory bulb that is in many ways similar to the main olfactory bulb. Müller (1971) recorded EOG-like surface potentials from the vomeronasal organ (of lizards and mice) to a series of odorant stimuli and found that many, but not all, of the stimuli that evoked responses in the olfactory epithelium were also effective on the vomeronasal organ. Taniguchi et al. (1998) and Inouchi et al. (1993) recorded “electrovomeronasograms” from the garter snake showing a substantially larger response to earthwork electric shock secretions (a behavioral stimulus to these animals) than to a general odorant stimulus (n-amyl acetate). These responses were shown to diminish with bilateral cautery of the vomeronasal nerve, which caused degeneration of the receptor epithelium. Leinders-Zufall et al. (2000) observed surface negative field potentials in mouse vomeronasal organ using low concentrations of chemicals known to affect the onset of puberty when presented to the vomeronasal systems of female mice. These responses were confirmed with extracellular single unit recordings and calcium imaging. Field potentials recordings have also been reported from the human vomeronasal organ (Grosser et al., 2000; Monti-Bloch and Grosser, 1991). This observation has contributed to a controversy about whether humans have a functional vomeronasal system (Meredith, 2001).

**Electroantennogram**

The electroantennogram (EAG) is a potential analogous to the EOG that can be recorded between the tip and the base of insect antennae. During odor responses, the tip of the antenna becomes negative relative to the base. While this technique was described for the study of moths (Boeckh et al., 1965; Schneider, 1969), it has been used to study olfaction in a variety of species, including spiders where some of the chemoreceptors are found on other appendages (Hebets and Chapman, 2000). The EAG has been used to evaluate the response to pheromones across related species of moths (Priesner, 1969). Ziesmann et al. (2000) used the EAG to assess odorous contaminants in laboratory room air. It has been used to assess the rate of development of odorant sensitivity in moths (Schweitzer et al., 1976). Vickers and Baker (1994) used the EAG from excised antennae mounted on intact male moths to track the pheromone plume during flight in a wind tunnel. Recently, the EAG has been used to assess mutants in Drosophila (Acebes and Ferrus, 2000; Albert, 1991; Martin et al., 2001) in ways that are similar to the use of the EOG with mouse mutants.

**CONCLUSION AND CONTINUED USEFULNESS OF THE EOG**

The success of the last decade with patch clamp recording of single olfactory receptor neurons has supplemented the usefulness of the EOG for studying many of the properties of olfactory receptor neurons. However, we have already cited a number of studies in which the EOG was useful for screening gene knockouts and spatial distribution of response. Buiakova et al. (1996) also employed the EOG to test a knockout of the olfactory marker protein (OMP) in mice. They were able to show a significantly slowed time course of the response, which encouraged behavioral studies showing diminished olfactory sensitivity in these animals (Youngentob and Margolis, 1999; Youngentob et al., 2001). This
effort shows some of the limitations of the use of EOG in certain circumstances in that Buiakova et al. (1996) noted that the between animal variation was too large to reliably detect reliable peak voltage differences between wild type and knockout animals. Ivic et al. (2000) have subsequently reproduced the EOG kinetic results in OMP knockout mice in which the OMP gene was replaced by adenoviral infection. That report also employed only time course comparisons using normalized data.

As a measure of activity in a population of ORNs, the EOG continues to be a valuable tool for assessment of the presence or absence of a response, as with several tests of specific knockouts. With appropriate normalization, it can be very useful in determining whether there are differences in the response to different odorants. This has been valuable both in the issue of spatial localization and in the evaluation of receptors carried by viral vectors or genetic manipulation (Zhao and Reed, 2001). Quantitative comparisons of the overall olfactory sensitivity in wild type and manipulated animals are much more difficult, as in the example of Buiakova et al. (1996) discussed above. However, even in that case, the response kinetics were able to provide evidence of dysfunction. We have outlined some precautions and uncertainties that must be kept in mind by the practitioner. We hope that these comments may inspire or guide future researchers using the technique.

ACKNOWLEDGMENTS

The work in our laboratory was supported by National Institute of Deafness and Other Communications Disorders grants DC00145 to P.E.-J. and DC00113 to J.W.S. and by a Research Infrastructure in Minority Institutions Award from the National Center for Research Resources to Spelman College (RR11598). The authors thank Lisa Sherrill for aid in preparation of the manuscript.

REFERENCES

Flock A, Flock B. 2000. Hydrops in the cochlea can be induced by sound as well as by static pressure. Hear Res 150:176–185.


Li W, Sorensen PW, Gallaher DD. 1995. The olfactory system of the migratory adult sea lamprey (Petromyzon marinus) is specifically and acutely sensitive to unique bile acids released by conspecific larvae. J Gen Physiol 105:569–587.


