

thanielsz et al.¹⁰ found also higher thyroxine content in sera of 3 fetal lambs in comparison to maternal values. Relatively high and stable values of thyroxine in sera of 7 bovine fetuses were also described¹², but they were not compared to values of mothers.

In our study considerably higher thyroid hormone values in fetal sera than in maternal samples were demonstrated. We have found that also triiodothyronine was present in fetal sera in relatively high concentration

during the last trimester of pregnancy. These results suggest that thyroid gland has high activity in utero, and that maternal and fetal thyroid hormone pools are relatively independent of one another. Significant formation of triiodothyronine in bovine fetuses is supposed.

¹² P. W. NATHANIELSZ, R. S. COMLINE, M. SILVER and A. L. THOMAS, *J. Endocr.* 67, 71 (1974).

PRO EXPERIMENTIS

The Underwater Electro-Olfactogram: a Tool for the Study of the Sense of Smell of Marine Fishes¹

W. L. SILVER, J. CAPRIO, JOAN F. BLACKWELL and D. TUCKER

Department of Biological Science, Biol. Unit I, Florida State University, Tallahassee (Florida 32306, USA), 20 February 1976.

Summary. Recording the olfactory receptor activity of marine fishes presents problems due to the shunting of the electrical signals by the highly conductive sea water, which results in significant signal loss. By recording the large signal-to-noise ratio D. C. potentials using the underwater electro-olfactogram (EOG), we were able to study olfactory receptor properties of freshwater and marine fishes in a comparable manner.

The underwater electro-olfactogram (EOG)², a slow (DC) potential change recorded in the water above the surface of the olfactory mucosa in response to chemical stimulation, has been used to study olfactory receptor responses of freshwater fishes^{3,4}. A single report⁵ exists concerning EOG responses from a marine species, the Atlantic hagfish (*Myxine glutinosa*, class Agnatha), to amino acids, recently shown to be effective attractants for some marine fishes⁶ and potent chemical stimuli in freshwater teleosts⁷⁻¹⁰. The electrical responses from the excised olfactory organ of the hagfish are characterized by positive-going potential changes. We report here, negative EOG recordings from in vivo preparations of 2 classes of marine fishes: Chondrichthyes, the Atlantic stingray (*Dasyatis sabina*) and Osteichthyes, the sea catfish (*Arius felis*).

Recording the olfactory receptor activity of marine fishes presents problems due to the shunting of the electrical signals by the highly conductive sea water, which results in significant signal loss. Olfactory neural responses have been successfully recorded (AC) in freshwater teleosts^{3,4,7,8} with metal filled glass capillary electrodes, tip plated with Pt-black¹¹, placed against the surface of the olfactory mucosa. We tried this method on marine fishes, but were unsuccessful due to the shunting of the

receptor action potentials. By recording the larger signal-to-noise-ratio DC-potentials, we were able to study olfactory receptor properties of both freshwater and marine fishes in a comparable manner.

Freshly caught specimens from the Gulf of Mexico were immobilized (stingray: MS-222, tricaine-methane sulphonate; catfish: Flaxedil, gallamine triethiodide) and positioned in plexiglass containers with aerated sea water containing MS-222 perfusing the gills throughout the experiments. Stimuli were diluted to at least 50% (stingray) and 25% (catfish) of their applied concentrations as determined by photodensitometry of dye solutions. The EOG was recorded with calomel electrodes via Ringer-agar filled capillary pipettes, amplified by a direct-coupled amplifier, and displayed on a pen recorder.

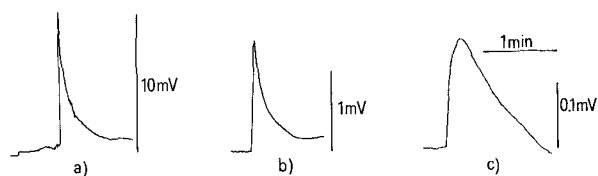


Fig. 1. EOG responses to 1.0 mM L-alanine. a) freshwater catfish; b) sea catfish; c) stingray. The small response magnitude of the sea catfish is due to shunting by the highly conductive sea water; that of the stingray is due to shunting and possibly an insufficient flow of sea water into its large olfactory capsule, which might also account for the long time course of the response. Rising edges of records have been retouched for clarity.

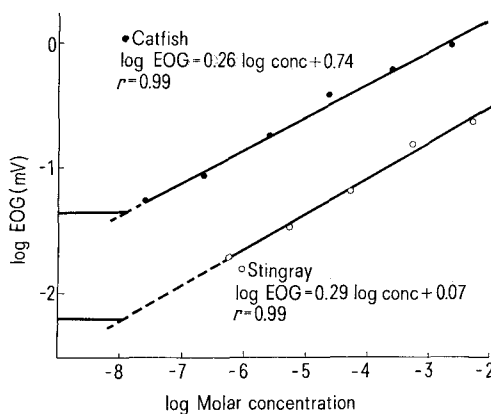


Fig. 2. Stingray (○) L-alanine, and sea catfish (●) L-cysteine, response-concentration curves in a log-log plot. Peak EOG responses are plotted as a function of the estimated concentrations delivered by the stimulator. Thresholds are determined by fitting a straight line to the response values and intersecting it with the control response, i.e. that obtained with the stimulus adjusted for zero concentration. The slight electrophysiological responses to the control stimulus are due primarily to chemical contamination^{8,18}.

Responses recorded by the active electrode positioned in the flowing seawater in the olfactory capsule above the receptors were negative in polarity relative to the reference electrode placed in the mouth (stingray) or against the cranial skin above the surface of the water (catfish). The records obtained are similar to the EOGs recorded in other vertebrates^{2, 12, 13}. However, due to the shunting problem the response magnitudes are small, averaging 0.2 mV for the ray and 2.0 mV for the sea catfish for the most potent amino acid stimuli tested at 1.0 mM.; similarly recorded responses for the freshwater channel catfish (*Ictalurus punctatus*) averaged 10 mV. Figure 1 shows EOG responses to applied concentrations of 1.0 mM L-alanine for the freshwater catfish, the sea catfish, and the stingray.

Figure 2 illustrates response-concentration curves for both the stingray and the sea catfish. The relationship between the EOG and stimulus concentration can best be described by power functions with exponents (slopes) ranging between 0.233 and 0.398 (average = 0.315 ± 0.05) for the ray and 0.191 and 0.333 (average = 0.250 ± 0.05) for the catfish. Estimated electrophysiological thresholds are between $10^{-6.5} M$ and $10^{-8} M$ for both species, which are similar to the values obtained for freshwater catfish^{4, 8}.

The EOG is a simple method for studying the response properties of vertebrate olfactory receptors. Caution must be observed in equating the EOG with the neural response, even though it has been suggested that the EOG is the population average of receptor potentials responsible for the initiation of nerve impulses^{13, 14}. The underwater and air EOGs of the turtle pottled against simultaneously recorded responses of receptor neurons reveal a non-linear relationship which differs quantitatively for different odorants¹⁵⁻¹⁷. However, in the freshwater catfish, the EOG and olfactory neural responses exhibit similar thresholds and phasic-tonic response characteristics⁴. Also, the relationships between the EOG or neural

response and the stimulus concentration are both described by power functions, although the EOG function has a somewhat higher slope⁴.

The underwater EOG preparations of stingray and sea catfish are accomplished with minimum difficulty. There is easy access to the olfactory receptors with little or no surgery required, thus sparing the animal unnecessary stress. We conclude that the underwater EOG is a promising method for the study of the sense of smell of marine fishes.

- ¹ This work was supported by grants from the National Institutes of Health, Nos. NS-08814 and NS-05258.
- ² D. TUCKER and T. SHIBUYA, Cold Spring Harb. Symp. quant. Biol. 30, 207 (1965).
- ³ D. TUCKER and N. SUZUKI, in *Olfaction and Taste* (Ed. D. SCHNEIDER; Wissenschaftliche Verlagsgesellschaft MBH, Stuttgart, Germany 1971), vol. 4, p. 121.
- ⁴ J. CAPRIO and D. TUCKER, Soc. Neurosci. Abstr. 1, 15 (1975).
- ⁵ K. B. DØVING and K. HOLMBERG, Acta physiol. scand. 91, 430 (1974).
- ⁶ A. M. SUTTERLIN, J. Fish. Res. Bd. Can. 32, 729 (1975).
- ⁷ A. M. SUTTERLIN and N. SUTTERLIN, J. Fish. Res. Bd. Can. 28, 565 (1971).
- ⁸ N. SUZUKI and D. TUCKER, Comp. Biochem. Physiol. 40A, 399 (1971).
- ⁹ J. CAPRIO, Comp. Biochem. Physiol. 52A, 247 (1975).
- ¹⁰ E. E. LITTLE, Soc., Neurosci. Abstr. 1, 9 (1975).
- ¹¹ R. C. GESTELAND, B. HOWLAND, J. Y. LETTVIN and W. H. PITTS, Proc. Inst. Radio Engrs. 47, 1856 (1959).
- ¹² T. SHIBUYA, Japan. J. Physiol. 10, 317 (1960).
- ¹³ D. OTTOSON, in *Handbook of Sensory Physiology* (Ed. L. M. BEIDLER; Springer, New York 1971), vol. 4, p. 95.
- ¹⁴ H. DAVIS, Physiol. Rev. 41, 391 (1961).
- ¹⁵ N. SUZUKI and D. TUCKER, Fedn. Proc. Fedn. Am. Socs. exp. Biol. 29, 521 (1970).
- ¹⁶ D. TUCKER, Fedn. Proc., Fedn. Am. Socs. exp. Biol. 25, 572 (1971).
- ¹⁷ D. TUCKER, Proc. Int. Union Physiol. Sci. 9, 572 (1971).
- ¹⁸ J. RASH, C. GEHRKE, R. ZUMWALT, K. JUO, K. KVENVOLDEN and D. STALLING, J. Chromat. Sci., 10, 444 (1972).

Eine Methode zur drahtlosen Messung der Atemfrequenz an Säuglingen A Method for Wireless Telemetry of Breath-Frequency of Babies

B. WOLF

Institut für Biologie II, Lehrstuhl für Zellbiologie der Universität, Schänzlestrasse 9-15, D-78 Freiburg im Breisgau (Bundesrepublik Deutschland, BRD), 6. November 1975.

Summary. A device is introduced which allows a continuous wireless registration of breath frequency of babies.

Angst und Spannungszustände führen allgemein zu einer Erhöhung der Atemfrequenz, wobei Angstzustände nicht die Tiefe, sondern lediglich die Frequenz des Einatmens verändern. Die Veränderung zeigt sich schon wesentlich früher als vergleichsweise die der Herzschlagfrequenz oder des Hautwiderstandes¹. Diese frühzeitige Veränderung macht die Atemfrequenzmessung deshalb so interessant, weil man dadurch von Umstimmungen im Organismus in Kenntnis gesetzt wird, bevor sich irgendeine erkennbare Reaktion am Menschen zeigt oder andersweitig feststellen lässt. Herkömmliche Verfahren sind gerade wegen ihres apparativen Aufwandes bei psychophysischen Messungen erwähnter Art an Säuglingen sehr ungünstig. Er wird in seiner Bewegungsfreiheit eingeschränkt und damit in seinem natürlichen Verhalten zu sehr gestört, als dass eine rückwirkungsfreie Messung noch möglich ist. Letzteres sollte jedoch Voraussetzung für jede

sinnvolle psychophysische und medizinische Messtechnik sein. Derartige Untersuchungen benötigen daher kleine und leichte Sender, wobei einerseits der elektronische Aufwand, Empfindlichkeit und Genauigkeit, andererseits Betriebsdauer und Reichweite in einem günstigen Verhältnis zueinander stehen sollen². Grosse Reichweiten verlangen relativ hohe HF-Leistungen und bedingen hohen Stromverbrauch; Empfindlichkeit und hohe Genauigkeit erfordern hohen elektronischen Aufwand. Diese Probleme lassen sich beim heutigen Stand der Integrationstechnik zwar lösen, doch schliessen die Kosten und Herstellungszeiten der Fotomasken diesen Weg für

- ¹ S. EPSTEIN, in *Neuropsychologie der Angst* (Urban und Schwarzenberg, Wien (1972)).
- ² W. H. KO and M. R. NEUMANN, Science 156, 351 (1967).