Vomeronasal Chemoreception in Vertebrates

A STUDY OF THE SECOND NOSE

Flehmen in stallion: response to estrous urine — elicited by sniffing of volatiles and or nonvolatiles (courtesy Fay Lindsay[©])

Vomeronasal Chemoreception in Vertebrates

A STUDY OF THE SECOND NOSE

Charles Evans

Glasgow Caledonian University, Scotland

Foreword by:

D. Michael Stoddart

Chief Scientist

Australian National Antarctic Research Expeditions

Imperial College Press

Published by

Imperial College Press
57 Shelton Street
Covent Garden
London WC2H 9HE

Distributed by

World Scientific Publishing Co. Pte. Ltd.

5 Toh Tuck Link, Singapore 596224

USA office: Suite 202, 1060 Main Street, River Edge, NJ 07661

UK office: 57 Shelton Street, Covent Garden, London WC2H 9HE

Library of Congress Cataloging-in-Publication Data

Evans, Charles.

Vomeroneural chemoreception in vertebrates : a study of the second nose / Charles Evans.
p. cm.

Includes bibliographical references and index.

ISBN 1-86094-269-5 (alk. paper)

1. Chemical senses. 2. Jacobson's organ. 3. Pheromones. 4. Pheromones--Receptors. I. Title.

QP455 .E936 2003

573.8'7716--dc21

2002038701

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library.

Copyright © 2003 by Imperial College Press

All rights reserved. This book, or parts thereof, may not be reproduced in any form or by any means, electronic or mechanical, including photocopying, recording or any information storage and retrieval system now known or to be invented, without written permission from the Publisher.

For photocopying of material in this volume, please pay a copying fee through the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, USA. In this case permission to photocopy is not required from the publisher.

Printed in Singapore.

Dedication

to

Robert W. Goy
(1924–1999)

y maestr

ACKNOWLEDGEMENTS

With thanks for illustrations reprinted with permission from the Institutions and Companies given below.

Cover: {front} Library, Trinity College, Dublin, 'The Book of Kells': TCD Mss 58; fol 76v (1. 13-17, end detail, right) entitled "*Blue wolf, tongue extended*".

Amer Assoc Adv Science: 2.1,b; 5.13; **Aust Mammal Socy:** 2.6,a/b; 7.6,a/b; **Brill Acad Publ** 7:2 a/b; 7.4,a; 7.6,c; 7.8; **British Museum Natural History:** 1.3; **Cambridge Univ Pr:** 2.12,a/b; 2.13,a; 4.1; 4.2,c/d; **Cell Pr Inc** 6.5,b; **Chapman & Hall:** 1.4; **CRC Pr Inc:** 3.3; 2.18,a; **Elsevier Science:** 1.2; 2.13,b; 3.2; 4.1; 4.3; 5.4; 5.16,a; 6.5,a; 7.1; 7.6,e; 7.10,a/b. **G Fischer:** 2.2,a; **Glasgow Univ Library:** 2.9,a; 2.10,a; **Glasgow Caledonian Univ Library** 7.9,a; **Greenpeace:** 7.6,g; **Kluwer/Plenum Pr:** 1.2; 2.1,a; 2.19,b/c; 3.2; 4.4,a; P 3.1,A/B; P.4,A; 5(Hd.); 5.4; 5.14,a; 7.6,d; **Masson et Cie:** 7.2,b; **Museum Natl d'Hist Naturel, Paris:** 2.11,a; 5.8,b; 7.4,b; **Natl Acad of Sciences, Wash DC:** 6.5,a; **Nature:** 6(Hd.); 5.5,a; 5.14,b; **New York Acad Sciences:** T.3.1; 4.4,a; 5.9,b; 6.2; 6.4; **Oxford Univ Pr:** P.2,C/2; P.5,1; P.6,A; 2.16,a; 3,b; 5.3,b; 6.5,a; 7.2,c; **Pergamon Pr:** 2.19,a; **Rockefeller Univ Pr:** 5.8,a; **Soc d'Endocrinol:** 4.4,b; **Soc. Study of Reproduction:** 7.10,c; **Smithsonian Inst:** 1(Hd.); 3(Hd.); 7.9,b; **Springer Verlag:** 2.4,b/c; 4.5; **Univ Stellenbosch:** 2(Hd.); **Urban & Fischer Verlag:** 2.4,a/c; **John Wiley/Liss Co:** 2.4,b; 2.7; 2.10; 2.11,b; 4.6,a; P.7.1;

[Hd. = heading; P = plate; T = table].

And for permission to use quotations from their publications:-

Ch.1, Allen & Unwin: 'The Biochemistry of Genetics' (Haldane, 1954); **Ch.4**, Penguin Books: 'A Case of Knives' (McWilliam, 1988);

Ch.6, J. Calder: 'The Motor Show' Plays, vol. V. (Ionescu, 1960); other sources are:- **Ch.7**: Darwin C. (1839) 'The Voyage of the Beagle', J. Murray Lond., and: Oxford English Dictionary (compact edit. vol.I, 1979).

My grateful thanks also to all those who variously helped with laboratory and other investigations, as well as with critical improvements to the presentation.

This page is intentionally left blank

CONTENTS

Foreword	xi
Abbreviations	xv
Introduction	xix
Chapter 1 Evolution	1
1.1 Phylogeny	1
1.2 Distribution	3
1.3 Reptiles	4
1.4 Mammals	6
1.5 Monosmic status	11
Chapter 2 Functional Morphology	13
2.1 Comparative anatomy	15
Chapter 3 Chemosignals	44
3.1 Separation, identification and bioassay	47
3.2 External transmission	52
3.3 Internal transmission	58
Chapter 4 Development	70
4.1 Peripheral, central and non-sensory development	71
4.2 Continuous and delayed neurogenesis	81
4.3 Stimulus access	84
4.4 Neurocrine cell migration	86
4.5 Functional maturation	89
4.6 General	91

Chapter 5	Physiology	94
5.1	Activation of the AOS	94
5.2	Deafferentation (Mammals)	107
5.3	Neuroendocrinology	112
Chapter 6	Molecular Biology	136
6.1	Introduction	136
6.2	Structures	138
6.3	Regional expression	141
6.4	Human VN genes	143
6.5	Chromosomal distribution	145
6.6	Cellular expression	146
Chapter 7	Behaviour	150
7.1	Chemoinvestigation and stimulus uptake	150
7.2	Inter-specific interactions	169
7.3	Socio-sexual interactions	173
Selected Bibliography (Further Reading)		183
References		187
Index		261

FOREWORD

Thanks to the keen eyes of a Danish military surgeon, and the burning of some late-night oil, the world of comparative anatomy came to know of the existence of the vomeronasal organ (VNO), sometimes known as Jacobson's organ, after its discoverer Ludwig Levin Jacobson. Despite almost two centuries of advancement of anatomical knowledge, rather little is known about the organ, even to this day. The eponymously-named organ was thought by Jacobson to be secretory in function but this is now known not to be the case. Its location, in the nasal cavity at the base of the nasal septum where that piece of cartilage joins the vomer bone, suggests it has something to do with the nose and the sense of smell. And so it has. The organ certainly does process molecules plucked from the outside world but the transmission of the information gleaned enters the brain not *via* the olfactory bulb, but *via* a small structure closely appressed to the olfactory bulb, and known as the accessory olfactory bulb. Vomeroneasal nerves thus run directly to the evolutionarily ancient smell-brain, or rhinencephalon, *via* their own pathways. If the sense of smell is, by definition, mediated by the nose and nasal mucosa capable of sensing compounds of high volatility, then the VNO is not part of the sense of smell. But it is part of the wider chemical sensory systems found in the air-breathing vertebrates.

The sense of smell, with which we can enjoy a bursting rosebud on a summer's day, or a fine Cabernet, is a relatively recent evolutionary acquisition. For most of evolutionary time our ancestors did not breathe air and gained information about their surroundings by "tasting" their aquatic environments. The so-called "olfactory rosettes" in the heads of

fishes are well-known to anyone who has cleaned a fish; they exemplify the sort of environmental chemical sensory apparatus that supported animal evolution for many millions of years.

When animals fully rid themselves of the necessity to live in water, and started to breathe air, the VNO flourished along with the nose and true olfactory system. The organ is best-developed in reptiles and mammals and in the latter class is almost universally present. Perhaps the main reason for our rather poor understanding of the organ is because our own species, *Homo sapiens*, is amongst the few that appear to be lacking a VNO. There is some debate about this point, though there is agreement that a VNO starts to develop in the human embryo. While some anatomical traces of the organ can be found in some adults, there is as yet no unequivocal proof that a fully functional organ actually exists in humans.

In this book Charlie Evans seeks to demystify the VNO by bringing together a considerable amount of information about the organ's evolution, functional morphology, development, activation and neuroendocrinology, and the genetic requirements of accessory chemoreception. He deals also with the nature of chemical signals that convey information about one individual to another, and about the behaviours animals express when pumping their VNOs with chemically laden aerosols. It clearly sets what is known about the organ and, perhaps more importantly, where the gaps are in our knowledge. The VNO is attuned to molecules of low volatility that cannot trip the nose, enabling the organ to respond to large molecule chemical compounds that advertise (eg.) the state of sexual readiness of the advertiser. Considerable experimental work with rodents has shown that the organ is able to detect the sexual status of a potential partner, though this does not obviate the role of the principal olfactory system in sexual behaviour. The VNO's function can be regarded as "priming" the individual for reproduction. Much more is yet to be learned about how the organ functions, and in which biological contexts it exerts its influence.

It is to be hoped that this monograph will encourage a new generation of scientists to take up the challenge and add their contributions to

our understanding of this small but important anatomical structure. Chirurgen-major Jacobson would have been delighted that the tiny seed he planted has grown into a substantial tree.

Michael Stoddart
Hobart, November 2002

This page is intentionally left blank

ABBREVIATIONS

AOB	Accessory Olfactory Bulb
AOB-x	Accessory Bulbectomy
AOS	Accessory Olfactory System
APH	Aphrodisin
BNST	Bed Nucleus Stria Terminalis
BT	ButyldihydroThiazole
CNS	Central Nervous System
DB	Dehydro- <i>exo</i> Bevicomin
EOG	ElectroOlfactogram
EVG	ElectroVomeronasogram
F.	Flehmen
GPCR	G-Protein Coupled Receptor
GnRH/LHRH	Gonadotrophin Releasing Hormone/Luteinising Releasing Hormone
JO	Jacobson's Organ
LOT	Lateral Olfactory Tract
MHC	Major Histocompatibility Complex
MOB-x	Bulbectomy
MOE	Main Olfactory Neuroepithelium
MOS	Main Olfactory System
NO	Nitric oxide
N-Pp/N-Pd	Naso-Palatine Papilla/Duct
NT	Terminal Nerve (<i>Nervus terminalis</i>)
OBP	Odourant Binding Protein
OR	Olfactory Receptor (molecule)
ORN	Olfactory Receptor (neurone)
OCAM	Olfactory Cell Adhesion Molecule
SO/MO	Septal Organ (of Masera)
VNd	Vomeroneasal Duct
VNE	Vomeroneasal Neuroepithelium
VMod	Vomeromodulin
VNO	Vomeroneasal Organ
VNOR	Vomeroneasal Olfactory Receptor (molecule)
VNR/r	Vomeroneasal gene/allele

xvi Abbreviations

VRN	Vomeronasal Receptor (Neurone)
VNS	Vomeronasal System
VN-x	Vomeronasalectomy
T	Testosterone
UHF	Ultra-High Frequency

This page is intentionally left blank

Fig. Int.1 Title page of Mémoire by L.L. (Louis) Jacobson (submitted to l'Institut de France in 1812).

INTRODUCTION

The discovery of the structure we now call the vomeronasal organ emerged from comparative anatomical work very much of its time. It was named after the veterinarian and physician Ludwig Levin Jacobson (1783–1843) who first described the gross morphology of the structure in the noses of some 15 genera of mammals, and provided figures showing details of the nerve supply to the organ. The work emphasised the “new” organ’s secretory capabilities, and commented on its connections with those between the mouth and nose. Jacobson initially presented his findings in a monograph to the veterinary audience (Jacobson, 1813). The publication history of his original work (in Danish) is documented by Trotier and Døving (1998). This gives a translation, together with reproductions of his striking drawings of the horse nose. The other domestic and non-domestic mammals he examined and/or dissected were also discussed in a “mémoire” apparently sent to the Académie de France. The facsimile page (Fig. Int.1, opposite) of Jacobson’s submission (dated 12 January, 1812) bears the names of its sponsors: de Lacepede, Tenon and Georges Cuvier under whose name the “Rapport fait à l’Institut, sur un Mémoire de M. Jacobson” appeared (Cuvier, 1812; transl. in Bhatnagar and Reid, 1996). An illustrated version of this text was later re-issued in a sparsely distributed paper, edited by Hollnagel-Jensen and Andreasen (1948). The original manuscript of the mémoire is thought to have been lost (?) from the Parisian archives, but does reside in a Copenhagen library (Trotier and Døving, 1998). Although mentioned, but not considered by Jacobson, the first record of such a nasal structure appeared in a much earlier treatise by Frederick Ruysch (1703). While this shows a nasal septum [Fig. Int.2(a)] in which a probe (D) undoubtedly demonstrates the existence of an aperture and a duct-like “nasal canal” in the human neonate, it does not justify any

priority in the discovery of the organ. The figure legends in Ruysch's commentary do no more than label it (E) as a part of septal anatomy; he makes no functional attribution, nor does he recognise it as a vertebrate characteristic. One other precedent to Jacobson is in a treatise on the "olfactory organ" by Sømmerring (1809, not seen).

The nomenclature of the organ is determined by anatomical convention, regrettably discarding the practice of identification after its describer. Its official title is "*Organ on Vomeronasale Jacobsonii*" but

Fig. Int.2(a) "*Neonatus*" Fig. v from: Fred. Ruysch (1703) *Thesaurus Anatomicus: fasc. III, Tab. 4; [p. 49].* Jans Wæsberg, Amsterdam.

the working name is the "Vomeronasal Organ", occasionally qualified as "Vomeronasal Organ (of Jacobson)". The persistence of the original eponymous label is largely due to the quirk of continued usage amongst researchers on snakes and lizards (e.g. Young, 1993); although mammalian specialists too will occasionally relapse (Jastrow and Oelschlager, 1998). The name itself derives from the apparent plough-like shape of a medio-basal nasal bone: "By bony apposition caudally the U-shaped vomer gradually changes into a Y-shape" (Sandikcioglu *et al.*, 1994).

Following Jacobson, the morphological and histological studies in the 19th century did establish that the distribution of the organ was from amphibia upwards, and that in general its microscopic structure was relatively invariant (Herzfeldt, 1888; von Mihalkovics, 1898). Its presence in humans was confirmed for pre-natal stages, and its occurrence again demonstrated, this time in adults, by the insertion of probes (sounds) into the septum [Fig. Int.2(b)] of cadavers and patients (Gratiolet, 1845; Potiquet, 1891). Much of the subsequent speculation

Fig. Int.2(b) “Cloison des fosses nasales” Fig. 1 from: Potiquet (1891). Du canal de Jacobson. de la possibilité de le reconnaître sur le vivant...etc... *Rev laryngol. d'otol. rhinol.*, Paris, 24, 737–753.

on function did not go beyond Jacobson's conclusions that it could be a sensory organ, possibly with some secretory capabilities. Indeed, most attention was given to the evolutionary and taxonomic importance of the nature of and variants in the cartilage surrounding the organ.

The recent resurgence of interest in smells and scents has considerably expanded information on, if not a full understanding of, the multiple chemoreceptive systems in the nose. New techniques and more systematic

approaches have increasingly been applied to the erstwhile Cinderella area of the chemical senses.

A comparison of two compendia on methodology, Moulton *et al.* (1978) with Spielman and Brand (1995), illustrates the refinements of investigative procedures.

The subject of this book is one of several minor chemoreceptive systems which together merited only limited consideration in previous studies on general olfaction by Halaz (1990) and by Farbman (1992). Over the last decade, the vomeronasal sub-system has emerged as a distinct area of interest within the biology of chemoreception. Although specialist reviews regularly appear on vomerolfaction, a particular study of this system is warranted by the increasing recognition given to accessory olfaction.

Comparative approaches to accessory olfaction have been consistent amongst reptilian specialists (Wright, 1883; Kahmann, 1932; Liu, 1999). Until the 1960s, mammals were not quite so fortunate in their devotees. A notable early exception was another Glaswegian enthusiast, the physician Robert Broom, who studied mostly marsupials (Broom, 1895–1915). This comparative morphological tradition has been sustained by Kratzing (1971–1988), also examining marsupials, Wöhrmann-Repenning (1977–1993) on rodents and carnivores, Bhatnagar *et al.* (1976 – on) with bats, and with the first modern monograph, on a prosimian primate (Schilling, 1970).

The chapters that follow deal with the increasing volume of work on a single, but not independent, part of the chemical senses. The evidence presented comes from the lower and higher vertebrates, with the emphasis given to non-domesticated species wherever possible. The informational range now available fortunately extends from ultrastructure and genome mapping to synaptic probes and ethological detail. Points of difference between main and accessory chemoreception are identified, although, where vomeronasal-specific data are lacking, similarities are assumed unless stated. Comparisons with insect chemoreception have not been attempted, although the intricacies of their pheromonal chemicals and signal functions surpass mere mammalian achievements. Nevertheless, insights into chemical signalling by bony fish have led to some comparisons being drawn (Sorenson *et al.*, 1998).

While the mammals predominate in their integration and representation of the sensory world, their noses still tell the brain directly about its chemical space. To explain the workings of accessory olfaction, we need to trace the path of a signal molecule from the moment it leaves its source until a response occurs in the recipient. The events which occur *en route* will determine the effectiveness of the intended communication.

The perception of a stimulus in the brain due to vomeronasal input does not appear to be accompanied by a conscious sensation. The arousal produced by the arrival of "unconscious odours" is nevertheless of considerable importance for the integration of external and internal signalling. The way in which the body recognises an unknown chemical resembles the way in which the immune system sorts out foreign molecules (antigens) from self-molecules. The correct or defence reaction occurs only to the former, much as the nose detects a message molecule; in both cases a specific and appropriate response results. A large proportion of all genes are devoted to these chemical recognition systems. The proportion allocated to vomeronasal function appears relatively small but no less complex than the major systems.

The accessory olfactory complex emerged from a general chemosensory field in fishes through the segregated cell cluster of amphibians, to the distinct sensory apparatus we now see in reptiles and mammals. A general trend revealed by vomeronasal researches is that of a marked decline within arboreal and aquatic animals, which parallels that seen in general olfaction. In the decoding of the sensory grammar of olfaction, some of the basic rules have been unravelled but our current understanding does not yet approach that of sound and sight.

Accessory olfaction contributes to individual and group survival through its influence on the reproductive processes of the diosmic (or double-smelling) groups. Our understanding of its role in the processing of chemosignals depends on the progress of investigations at all levels of analysis. There are many indications that the secondary system employs mechanisms not shared with primary olfaction, reflecting the divergence of its evolutionary history. The various aspects of its workings are presented in an attempt to synthesise the contributions of diverse disciplines and to reveal the nature of an obscure but intriguing sense.

Such an overview inevitably extends into specialised areas of knowledge and experience. I am most indebted to the colleagues who devoted their precious time in providing expertise and critical comment: Drs. E. Grigorieva, M. Halpern, D. Kelly, C. Mucinat-Caretta, L.E.L. Rasmussen, and B. Schaal — I thank them for their efforts. They are, of course, blameless of any inaccuracies and infelicities that remain. I am also most grateful to those who gave illustrations or allowed me sight of unpublished material: I thank Drs. P. Brennan, E.B. Keverne, M. Perret, C.J. Pfeiffer, 'Bets' Rasmussen, A. Schilling, and H. Schultz for their generosity. I am most grateful to Mike Stoddart for his contribution in writing an admirable Foreword.

If this present offering is to be measured against Bacon's aphorism on the quality of books, then it aspires to be mostly swallowed, some of it chewed and digested — and a little of it just tasted.