William R. Schafer

Research Statement: a narrative on your scientific career in past, present and future

The relationship between genes, neurons and behaviour is a fundamental problem in neuroscience requiring an understanding of how specific gene products act within the context of neuronal circuitry to integrate sensory information, pattern motor outputs, and control behavioural states. I have been using the nematode *C. elegans*, with its small and completely mapped neuronal connectome, to identify and study fundamental principles of nervous system function at the molecular and circuit levels.

In the course of this work, my group has made important technological, experimental and conceptual contributions to understanding the genetic and neural basis of behaviour. We have pioneered methodologies, in particular optogenetic neuroimaging and high-content behavioural phenotyping, that have transformed studies of neural circuits in genetically-tractable organisms and have been widely influential throughout neuroscience. We have used these approaches to make significant discoveries on the molecular and cellular mechanisms of sensory transduction, the organisational principles of neural connectomes and the roles of monoamine and peptide neuromodulation in neuronal microcircuits. The impact of this work has been recognised through my election to the Royal Society, the Academy of Medical Sciences, and EMBO.

- **Short CV**

Education

1982 - 1986	Bachelor of Arts in Biology (summa cum laude), Harvard University
1986 - 1991	PhD in Biochemistry, University of California, Berkeley

Positions and Employment

1991 - 1992	Postgraduate researcher, Jasper Rine lab, University of California, Berkeley
1992 - 1995	Postdoctoral researcher, Cynthia Kenyon lab, University of California, San Francisco
1995 - 2002	Assistant Professor, Division of Biology, University of California, San Diego
2002 - 2006	Associate Professor, Division of Biology, University of California, San Diego
2003 - 2005	Vice-chair of Neurobiology, Division of Biology, University of California, San Diego
2006 - 2007	Adjunct Professor, Division of Biology, University of California, San Diego
2006 - present	Programme Leader, MRC Laboratory of Molecular Biology, Cambridge
2019 - present	Full Professor (part time), Department of Biology, KU Leuven

Other Appointments

2007 - present	Bye-fellow, Downing College, Cambridge University
2008	Visiting Professor, Ecole Normale Superieure, Paris
2014	Visiting Lecturer, Seoul National University
2019	Visiting Miller Professor, University of California, Berkeley

Selected Awards and Honors

1993	John Belling Prize (best thesis in Genetics, 1988-1993)
1996	Beckman Young Investigator Award
1998	Klingenstein Fellow in Neuroscience
2001	Presidential Early Career Award for Scientists and Engineers (USA)
2009	Elected Member of the European Molecular Biology Organization (EMBO)
2018	Elected Fellow of the Academy of Medical Sciences (UK)
2020	Elected Fellow of the Royal Society

Mentoring of Students and Postdocs

Since starting my research group in 1995, I have trained a total of 25 postdocs, 24 PhD students, and 11 Masters students. Three PhD students from the lab (Rex Kerr, 2002; Katie Kindt, 2006; and Marios Chatzigeorgiou, 2011) and one Masters student (Majdulin Istiban, 2022) received the prize for outstanding student in their year. Just over half the postdocs and PhDs have gone on to independent group/faculty positions after leaving the lab; others have followed research or administrative careers in industry or academia. Trainees who have achieved notable success include:

Postdocs: Alexander Gottschalk (Professor, Goethe-Universität Frankfurt), Massimo Hilliard (Professor, QBI Brisbane), Henrik Bringmann (Professor, Technische-Universität, Dresden), Andre Brown (Reader, Imperial College), Yee Lian Chew (Senior Lecturer, Flinders University), Yi-quan Tang (Professor, Fudan University), Julia Morud (University Lecturer, University of Gothenburg), Iris Hardege (Group Leader, Cambridge University)

PhDs: Laura Waggoner (Professor, Miramar College), Katie Kindt (Investigator/Section Chief, NIH), Marios Chatzigeorgiou (Group Leader, Sars Centre, Bergen), Marina Ezcurra (Senior Lecturer, Univ Kent), Eviatar Yemini (Assistant Professor, UMass Medical School), Barry Bentley (Reader, Cardiff Metropolitan Univ).

Masters: Laura Anne Lowery (Professor, Boston University), Christian Frøkjær-Jensen (Assistant Professor, King Abdullah University).

- **Career Path**

I began my scientific career as a yeast geneticist and biochemist. As a student, I discovered that Ras proteins and yeast mating factors are isoprenylated and demonstrated the importance of this modification for membrane trafficking (Schafer et al., *Science* 245: 379). I subsequently developed an assay for the farnesyltransferase enzyme and identified the gene for one of the enzyme subunits (Schafer et al., *Science* 249**:** 1133). As a postdoc, I changed my research focus to the molecular and neural basis of behavior in the nematode *C. elegans*. I have continued this work as an independent PI, first at the University of California, San Diego and, since 2006, as a group leader at the MRC Laboratory of Molecular Biology in Cambridge. In 2019 I established a research program as part-time professor at KU Leuven, where I have expanded these studies of the neural basis of behaviour to other organisms, including parasitic nematodes and cephalopods.

Since establishing my research group, I have pursued a diverse array of experimental approaches to investigate fundamental questions in neuroscience. In particular, my lab was the first to establish the feasibility of imaging neural activity in vivo with genetically-encoded optical indicators, a methodology now widely used throughout neuroscience. We were the first to successfully use optogenetic sensors, specifically genetically-encoded calcium indicators (GECIs), to monitor excitable cell activity (both muscles and individual neurons) in an animal (Kerr et al., *Neuron* 26: 583). We subsequently used this approach to make fundamental discoveries regarding molecular and circuit mechanisms of behaviour. For example, using simultaneous optical recordings from identified chemosensory neurons (Suzuki et al., *Nature* 454: 114), we discovered that the circuit for appetitive salt taste uses both on- and off-cells to sensitively measure the time derivative of sodium and chloride, allowing navigation through a concentration gradient. We also used optogenetic imaging to elucidate conserved sensory transduction mechanisms, for example showing for the first time that TRPA channels mediate mechanosensation in vivo (Kindt et al., *Nat Neurosci* 10: 568). We also used optogenetic imaging to study TMC channels (Chatzigeorgiou, *Nature* 494: 95), homologues to the mechanotransducers in the human ear. In particular, we showed that ankyrin, linked to TMC through the deafness gene CIB2, functions as the long-sought gating spring that confers sensitivity to mechanical force (Tang et al., *Neuron* 107: 112)

A second major innovation pioneered by my group has been the development of automated and quantitative methods for behavioural phenotyping. In *C. elegans*, as in many other organisms,

behavioural assays have historically been carried out by real time observation, based on endpoint measurements and often subjective and imprecise. More than 20 years ago, we developed methods for automated tracking and probabilistic modeling of egg-laying behaviour, demonstrating that nematodes fluctuate between alternative behavioural states modulated by serotonin and neuropeptides (Waggoner et al., *Neuron* 21: 203). We then applied this approach to more complex locomotor behaviors, relating quantitative postural and motor dynamic features to specific neurons and genes. After developing methods for image segmentation, feature extraction, and unsupervised analysis, we recorded and phenotyped a dataset of hundreds of nervous system mutants (Brown et al., *PNAS* 110: 791, Yemini et al., *Nat Meth* 10: 877, revealing novel behaviours not previously known from years of observer-based research and identifying new phenotypes for nearly 100 previously uncharacterised genes. We also used high-content behavioural analysis to test the hypothesis that control theory can predict neural function based on connectomic network topology (Yan et al., *Nature* 550: 519), validating a theoretical framework with the potential for probing much larger connectomes, including the human brain.

A recent focus of our current work has been to understand networks of neuromodulatory signaling in the brain. Neueuromodulation in *C. elegans* has been a long-standing interest; indeed as a postdoc I was the first to identify a role for dopamine in *C. elegans*, showing that it inhibits both egg-laying and locomotion (Schafer and Kenyon, *Nature*). My lab subsequently identified roles for monoamines (Kindt et al., *Neuron* 55: 662), neuropeptides (Ezcurra et al. *J Neurosci* 36: 3157), and non-canonical neuromodulators (Hardege et al, *PNAS* 119: e2201783119) in arousal, learning, and context-dependent modulation of sensory circuits. Notably, we found that neuromodulatory signaling forms extrasynaptic "wireless" networks, with afferent and efferent branches along with central integrating nodes (Bentley et al., *Plos Comp Biol* 12: e1005283; Chew et al., *Neuron* 99: 1233). By integrating biochemical, transcriptomic and anatomical data we generated a comprehensive map of peptidergic signaling in *C. elegans*, the first such neuropeptide connectome in any animal. This wireless network exhibits a structure and topology that differs in fundamental ways from wired synaptic connectomes, highlighting organizational principles that may apply to larger nervous systems.

Five publications (last 5 years; complete list at https://orcid.org/0000-0002-6676-8034)

Ripoll-Sánchez L, Watteyne J, Sun H, Fernandez R, Taylor SR, Weinreb A, Bentley B, Hammarlund M, Miller DM III, Hobert O, Beets I, Vértes PE, **Schafer WR** (2023) "The neuropeptidergic connectome of *C. elegans*" *Neuron* 111: 3570–3589.
We mapped the neuropeptide signaling network of the entire C. elegans nervous system at single-neuron resolution, the first such wireless connectome in any animal. The organization and topology of this network serves as a prototype for understanding neuroendocrine signaling in larger brains.

Hardege, I, Morud J, Yu J, Wilson TS, Schroeder FC, **Schafer WR** (2022) "Neuronally produced betaine acts via a novel ligand gated ion channel to control behavioural states." *PNAS* 119: e2201783119
This study showed that betaine, a choline metabolite of unknown function in the brain, is released from C. elegans neurons in vivo and act as a neuromodulator to control behavioural states.

Morud J, Hardege I, Liu H, Wu T, Choi MK, Basu S, Zhang Y, **Schafer WR** (2021) Deorphanisation of novel biogenic amine-gated ion channels identifies a new serotonin receptor for learning *Curr Biol* 31: 4282-4292
We identified and characterised five novel monoamine-gated ion channels and demonstrated that one of them, the serotonin receptor LGC-50, is critical for learned avoidance of pathogenic bacteria.

Walker DS, **Schafer WR** (2020) “Distinct roles for innexin gap junctions and hemichannels in mechanosensation” *elife* 9: e50597
This paper showed that nematode innexins, homologues of mammalian pannexins, function specifically in mechanosensation.

Tang YQ, Lee SA, Rahman M, Vanapalli SA, Lu H, **Schafer WR** (2020) Ankyrin is an intracellular tether for TMC mechanotransduction channels *Neuron* 107: 112-125

This study showed that ankyrin interact through adapter proteins with TMC channels, and is likely the long-sought gating spring for the mechanotransduction complex that functions in human hearing.

Other scientific output and impact

Reviewing

Since 2021 I have served on the UK Neuroscience and Mental Health Board, which reviews all neuroscience-related Project Grants, Research Grants, and New Investigator Awards for the Medical Research Council.

I also serve (since 2021) on the Royal Society Research Appointments Panel Bii, which reviews applications for University Research Fellowships for starting PIs. We review all biomedically-related applications, from longlisting to interview stage. I am also a member of Royal Society Sectional Committee 8, which evaluates candidate fellows in the area of multicellular biology.

As an EMBO Member (since 2010) I regularly carry out reviews and applicant interviews for EMBO Postdoctoral Fellowships, and have carried out ad hoc grant reveiws for national funders from the UK, USA, France, Germany, Canada and Greece.

Conference Organisation

I have served on the organising committee for the International *C. elegans* Meeting in 2017 and 2019, and on the advisory committee in 2021. I also served as organiser for the following meetings:
Connectome to Behaviour: Modelling C. elegans at Cellular Resolution (Royal Society, 2018)
Molecules and Mechanisms in Magneto-, Thermo- and Mechanosenation (Titisee Conference, 2016)
EMBO Conference: *C. elegans* Neurobiology (EMBL, 2012)

Leadership and Collaborations

I have a long history of research collaboration, including both ad-hoc interactions (of 99 research articles published by my group, 86 involved collaboration with another lab) and formal joint projects.

In particular, I established and led two international collaborative projects:

Functional analysis of sensory circuits (2001-2004) supported by HFSP, co-PIs Cori Bargmann (Rockefeller), Ikue Mori (Nagoya), Paolo Bazzicalupo (Naples)

Machine vision analysis of behavioural phenotypes (2002-2015) supported by NIH, co-PIs Paul Sternberg (Caltech), Pamela Cosman (UCSD).

I also participated as joint PI in an NIH-funded collaboration to map The neuropeptidergic connectome of *C. elegans* (2018-2023), other PIs Oliver Hobert (Columbia), Isabel Beets (Leuven)

Research projects (awarded in last 5 years)

Cellular and molecular mechanisms of behaviour
Medical Research Council (UK) MC-A023-5PB91 (07/01/21-08/01/26) Role: PI
Research into the mechanisms of behaviour at the molecular and neural circuit levels.

Functional characterization of novel ligand-gated ion channels
FWO G079521N (01/01/2021-31/12/2026) Role: Co-promoter, with Liliane Schoofs
The physiology and function of novel ligand-gated ion channels in nematodes and flatworms

Novel mechanisms of fast neurotransmission in the octopus brain
KU Leuven BOF C14/16/049 01/10/2021-30/09/2025 Role: Co-promoter, with Eve Seuntjens
The role of novel dopamine-gated cation channels in the octopus nervous system

Neuropeptides and nictation
FWO G050825N (01/01/2025-31/12/2028) Role: Co-promoter, with Liesbet Temmerman
The role of neuropeptides in controlling nictation and other dauer-specific behaviours