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Dr Talvinder S. Sihra

PERSONAL DETAILS

Surname:	SIHRA	Forenames:	Talvinder Singh
Date of Birth:	17th July 1960.	Place of Birth:	Nairobi, Kenya
Nationality:	British.	Marital Status:	Married.

Present Position

Senior Lecturer.
Department of Pharmacology, UCL, London, U. K.

Education/ Qualifications

1979-1982: B.Sc. Class 2(i) in Biochemistry and Physiology.
Department of Biochemistry,
University of Sheffield, U.K.

1982-1985: Ph.D. Biochemistry.
Thesis: Mechanisms of Amino-Acid Neurotransmitter Release from Isolated Nerve Terminals.
Department of Biochemistry,
University of Dundee, U.K.

Professional History

1978-1979: Laboratory Technician.
Department of Gastroenterology,
Royal Postgraduate Medical School,
University of London, U.K.

1979-1982: Undergraduate. B.Sc. Biochemistry and Physiology.
Department of Biochemistry, University of Sheffield, U.K.

1982-1985: Postgraduate Studentship (SRC). Ph.D. Biochemistry.
Department of Biochemistry, University of Dundee, U.K.

1985-1986: Postdoctoral Research Assistant (MRC).
Neuroscience Research Group, University of Dundee, U.K.

1986-1989: Postdoctoral Associate (European Exchange Fellowship Program).
Principal Investigator- Professor Paul Greengard,
Laboratory of Molecular and Cellular Neuroscience,
The Rockefeller University, New York City, USA.

1989-1992: MRC Fellow and joint principal investigator
(with Professor David G. Nicholls) on a MRC project grant.
Department of Biochemistry, University of Dundee, U.K.

1993-1997: Wellcome Trust University Award Lecturer.
Departments of Pharmacology and of Protein and Molecular Biology,
Royal Free Hospital School of Medicine, University of London, U.K.

1997-1999: Lecturer.
Department of Pharmacology,
University College London (UCL), London, U.K.

APPOINTMENTS

Editorial Activity From Jan. 2002	Editor of the *British Journal of Pharmacology*.
Peer Reviewing Activity 1990-present	*Ad hoc* reviewer for the *Journal of Neuroscience*, *European Journal of Neuroscience*, *Journal of Neurochemistry*, *Journal of Biological Chemistry* and *Neuroscience*.
	Ad hoc referee for grants applications to the Wellcome Trust, MRC and BBSRC.

External Examiner

Jan. 1996	PhD student Denise Lundy (Supervisor: Dr Gethin Mc Bean), Department of Biochemistry, University College Dublin, Eire.
Sep.1997	PhD student Ramesh Chittajallu (Supervisor: Professor Jeremy Henley), Department of Anatomy, University of Bristol.
Jan. 2001	PhD student Mohammed Rahman (Supervisor: DrYuri Ushkaryov), Department of Biochemistry, Imperial College, London.

AFFILIATIONS

1983-present:	Member of the Biochemical Society.
1995-present:	Member of the Society for Neuroscience.

HONOURS

1994-2000:	Elected member of the Neuroscience Group Committee of the Biochemical Society.
1998-2000:	Elected Secretary for the Neuroscience Group Committee of the Biochemical Society.
2000-present:	Elected Member of Council of the Biochemical Society.
2001-present:	Accredited Member of the Institute of Learning and Teaching in Higher Education (ILT).

GRANTS

Jan. 1990	MRC	£36,450	Regulation of Neurotransmitter Glutamate Release. (Fellowship - three years).
Jan. 1990	MRC	£114,709	The Functional Significance of Intraterminal Protein Phosphorylation in the Regulation of Neurotransmitter Glutamate Release. (Project Grant - three years) Joint with Dr D. G. Nicholls.
Jan. 1993	Wellcome Trust	£271,688	Regulation of Neurotransmitter Release: Modulatory Role of Protein Phosphorylation/ Dephorylation and Cytoskeletal Interactions**.** (University Award with three year project grant).
Mar. 1993	Wellcome Trust	£14,923	Supplement for University Award
Mar. 1993	Wellcome Trust	£40,000	Equipment Grant. Joint with Drs. A. C. Dolphin, E. A. Barnard, E. S. Debnam and C. D. Richards.
1994/6/8	Peter Samuel Royal Free Fund	£8,042	Regulation of Neurotransmitter Glutamate Release (1994, £2,633); The Role of Phospholipids in the Regulation of Neurotransmitter Release from Isolated Nerve Terminals (1996, £2,709); Regulation of Neurotransmitter Release from Isolated Nerve Terminals: Role of MAP-kinases (1998, £2,700).
Mar. 1997	Wellcome Trust	£129,922	Modulation of Synaptic Function through Calcineurin-Mediated Protein Dephosphorylation in NG108-15 cells. (Project grant - two years).
Oct. 1997	Wellcome Trust	£252,464	Examination of Protein-Protein Interactions Involving Ion Channels and Anchoring, Synaptic and Signalling Proteins. Joint with Drs. A.C. Dolphin, N. S. Berrow, S. J. Moss and G. W. J. Moss.
Mar. 1999	Wellcome Trust	£36,176	Modulation of Synaptic Function through Calcineurin-Mediated Protein Dephosphorylation in NG108-15 cells. (Supplementary one year).
Oct. 2000	Wellcome Trust	£108,122	Calcineurin-Mediated Regulation of High Voltage – Activated Ca^{2+}-Channels and Stimulus-Secretion Coupling (Project grant – two years).
Oct. 2000	BBSRC	£204,784	Mitogen-Activated Protein Kinase Signalling in Presynaptic Nerve Terminals. (Project grant – three years).

INVITED TALKS

Mar. 1986	Workshop on the "Use and Misuse of Nerve-Ending Preparations" (Organiser: Robert V. Dorman), 17th American Society of Neurochemistry, Montreal, Canada.
Mar. 1986	"Windows in Science Program" (Organiser: Dr David Terrian), United States Air Force and European Office of Aerospace Research and Development, Brooks Air Force Base, Texas, USA.
Jun. 1992	Department of Pharmacology, University of Edinburgh, U.K.
Jul. 1990/91/92/93/94	Invited Lecturer/Instructor, Neuroscience Course, Marine Biological Laboratory, Woods Hole, Massachusetts, USA.
Dec. 1992	Biochemical Society Meeting, London, U.K . Neurochemical Group Colloquium on "Neurotransmitter Release" (Organisers: Professors R. D. Burgoyne and A. C. Dolphin),
Mar. 1995	Department of Biochemistry, University of Leeds, U.K.
Mar. 1997	SmithKlineBeecham Pharmaceuticals, Harlow, Essex, U.K.
Jun. 1999	Department of Pharmacology, University of Birmingham, U.K.
Jan. 2001	Department of Neuroscience, Guys and St Thomas' Hospital School of Medicine, University of London, U.K.
Jun. 2001	Eisai Pharmaceuticals, London, U.K.
Feb. 2002	Department of Cell Physiology and Pharmacology, University of Leicester, U.K.
Feb. 2002	Department of Physiology, University of Liverpool, U.K.

MEETING ORGANISATION

Apr. 1999	Organiser of the two-day colloquium on "Membrane Signalling Complexes" at the 668th Biochemical Society Meeting held at the University of Glasgow.

ACADEMIC SUPERVISION

Research Staff	1990-1991:	Research assistant Anne Barrie (joint with Professor David Nicholls). Currently a postdoctoral fellow at the University of Glasgow.
	1993-present:	Research Technician Tamsin Piper. Now tenured at UCL.
	1997-2000:	Postdoctoral Assistant Dr J. R. Burley. Now Senior Scientific Officer with CENES Pharmaceuticals, Cambridge, U.K.
	1998:	Postdoctoral Associate Dr J. N. Jovanovic. Continuing in academic research at UCL.
	1999-2000:	Dr Su-Jane Wang, Wellcome Trust Travelling Fellow. Now an Assistant Professor at the Fu-Jen Catholic University, Taiwan.
	2000-present	Postdoctoral Assistants, Dr Seraphina Idowu and Dr Michael Postlethwaite.
Graduate (PhD) students	1991-1993:	Eleanor Coffey (joint with Professor David Nicholls). Obtained a PhD and now a group leader at the University of Turku, Finland.
	1993-1996:	Michael Perkinton. Obtained a PhD and is currently a postdoctoral fellow at the Institute of Psychiatry, University of London.
	1999-present:	Jennifer Davies. Current PhD student on a MRC Studentship.
Undergraduate (BSc, MB.BS) students	1990-1992:	Two project students in Biochemistry: Gary Lawrence and Wayne Wilson. Both continued to PhD studentships.
	1995-1996:	Project and Wellcome Trust Summer Fellowship student Victoria Coutinho. Continued to PhD studentship.
	1997-present	Project students: Marie De Souza (1997, continued with Medical School), Shwetha Rao (1998, entered Dental School), Amutha Anpananthar (2000, continued with Medical School).
Research Visitors/ Collaborators	1994:	Dr Elena Lukyanetz from A.A. Bogomoletz University, Kiev (for three months).
	1995:	Dr Claudia Wiedemann from Friedrich-Miecher Instutut, Basel (for two months on two occasions).
	1997:	Dr Jasmina Jovanovic from the Rockefeller University, New York (for two months).

TEACHING ACTIVITY

Undergraduate Teaching

Royal Free Hospital School of Medicine (1993-1996) &
UCL - Royal Free Campus (1997-present):

1993-2001: Preclinical teaching, 2nd Year Part IV MB.BS.

- Lectures (7hr): Blood Sugar Control (1hr), Opioid Analgesics (1hr), Antimicrobial Drugs (3 x 1hr), Anticancer Drugs (1hr) and Antiviral Drugs (1hr).
- Practicals (24hr): Local Anaesthetics (4 x 3hr), Atenolol and Nitrites (4 x 3hr).
- Tutorials (18 x 1hr).

1993-1994: Intercalated BSc Neuroscience Lectures (2hr).

1997-present: MRCPsych Part II Lecture Course (3hr).

UCL - Gower Street Campus:

1998-2001: Preclinical teaching, 2nd Year Part IV MB.BS.

- Lectures: Antimicrobial Drugs (2 x 1hr).
- Organiser of Special Studies Module:- 'Ca^{2+}: Friend Sometime Foe' (5hr).

1998-present: BSc Pharmacology

- Lectures (2hr): Antimicrobial Drugs (2 x 1hr).
- Undergraduate student laboratory-projects supervision.

2001-present: BSc Physiology/Pharmacology: Admissions and Course Tutor to all years.

1998-present: MSc Neuroscience Lectures ('Synaptic Release Proteins') (2hr) and Tutorials/Journal Clubs (2hr).

2000-present: MB.BS. ("New Curriculum")

- Lectures (2hr): Antimicrobial Drugs (2 x 1hr).
- Drug Action Rotations/Practicals (18hr): Problem Solving and Data Handling (6 x 3hr).
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Postgraduate Course Instruction

Jul. 1990-94: Neurobiology Course (Biochemistry Section-three weeks) at the Marine Biological Laboratory, Woods Hole, Massachusetts, USA.
Seminars and hands-on teaching of laboratory techniques to postgraduates, postdoctoral fellows and group leaders.
Directors: Drs. Irwin Levitan and Leonard Kaczmarek.

ENABLING ACTIVITY

Internal Administrative

Organisation of In-Course Assessments (Dec., Mar. and Jun.) for Part IV MB.BS. Pharmacology, Royal Free Hospital School of Medicine (RFHSM) (1995-2001).

Member of the MB.BS. Admissions Advisory Committee, RFHSM (1997-2001).

Member of Interviewing Committees for UCAS applications for MB.BS., RFHSM and UCL (1993-present).

Departmental co-coordinator for Teaching Quality Assessment (TQA) exercise for RFHSM (1999).

Member of the Genetic Modification Safety Committee, Royal Free Hospital School of Medicine (1996-present).

Course Tutor for BSc Physiology and Pharmacology (current).

UCAS Admissions Tutor for BSc Physiology and Pharmacology (current).

External Administrative

Former Secretary of the Neuroscience Group Committee of the Biochemical Society.

Elected member of Council of the Biochemical Society.

FORMATIVE ACTIVITY

Courses Attended

Dec. 1986	Advanced Course on Signal Transduction (one week). Federation of European Biochemistry Societies (FEBS), Spetses, Greece.
Dec. 1993:	Participation in Workshop on 'Effective Supervision of PhD Students' (one day). University of London.
Feb. 1994:	Attendance at the 'TIPS' course on 'Effective Teaching Techniques' (four days). Medical Education Unit, University of London.
Nov. 1998:	The Animals (Scientific Procedures) Act 1986: Home Office Regulations Course for Animal Users and Project License Holders (three days). University College London.
Dec. 1998:	"Brush Up Your Teaching Techniques" (one day). University of London.
Mar. 2000:	"Teaching and Learning; The Way Forward." Innovative computer and internet-based methods for teaching (two days). Education and Information Support Division (EISD), UCL. "Innovative Teaching Methods Workshop (one day)."
May 2001:	Learning and Teaching Support Network (**ltsn**), Centre for Bioscience, University of Leeds, U.K.
Sep. 2000:	Participation in the Physiological Society, "Microelectrode Techniques Workshop" (two weeks). Marine Laboratories, Plymouth, U.K.

RESEARCH ACTIVITY

1978-1979

Effects of perfused bile salts and cimetidine (histamine antagonist) administration on gastric, biliary and pancreatic secretion in man.

Undergraduate Studies 1979-1982

Amino-acid uptake by rat soleus muscle; kinetic characterisation of neutral amino-acid uptake in amino-acid deficient animals.

Graduate Studies 1982-1986

Mechanisms of amino-acid neurotransmitter release from isolated nerve terminals. Bioenergetic characterisation of the isolated nerve-terminal (synaptosomal) release model and demonstration of the functional compartmentation of the neurotransmitters GABA and glutamate. Ca^{2+}-dependent, phasic release was shown to occur by the exocytosis of vesicular pools of transmitter whereas a Ca^{2+}-independent pool of transmitter was shown to efflux more slowly by the thermodynamic reversal of Na^{+}-dependent/electrogenic transporters.

Postdoctoral Studies 1986-1992

(1) Vesicular localisation of amino-acid neurotransmitters and the role of protein phosphorylation in the modulation of the neurotransmitter release. The vesicular localisation of L-glutamate was confirmed using purifed small synaptic vesicles (SSV) to demonstrate concentrative transport. Transport was shown to be electrogenic, utilising an inside-positive membrane potential generated by a vesicular H^{+}-ATPase.

(2) The role of protein phosphorylation in the controlling SSV dynamics in nerve terminals was investigated. Phosphorylation-dependent translocation of the SSV-associated protein, synapsin I, was shown; this implicating the protein as a key player in the organisation of SSVs at nerve terminal active-sites and thereby in the modulation of neurotransmitter release. Supporting this hypothesis, introduction of an auto-activated form of Ca^{2+}/calmodulin dependent kinase II into synaptosomes led to an increase the synapsin I phosphorylation and consequent facilitation of glutamate and norepinephrine release.

(3) The role of protein phosphorylation in the regulation of nerve terminal excitability was examined. Protein kinase C mediated protein phosphorylation was shown to enhance the 4-aminopyridine evoked membrane depolarisation of synaptosomes (this likely occurring by the inhibition of a K^{+}-conductance) and cause a consequent increase in Ca^{2+}-entry, and thereby, a facilitation of glutamate release.

Current Research 1992-present

The interests of my laboratory centre round the mechanisms by which neurotransmitter release is regulated at central nervous system (CNS) synapses.

1) Presynaptic receptors, through ionotropic and metabotropic mechanisms, represent fundamental means for regulating neurotransmitter release. One of my primary interests is to identify and characterize presynaptic receptors that modulate the release of the neurotransmitters glutamate and GABA. The model system we have used for these studies is the isolated nerve terminal preparation (synaptosomes). Nerve terminal depolarization leads to Ca^{2+}-influx and exocytosis, followed by endocytosis and recycling of transmitter containing small synaptic vesicles (SSVs). To delineate the loci at which presynaptic receptor activation impinge, we use membrane potential-sensitive dyes to assay nerve terminal excitability and depolarization, fura-2 to monitor Ca^{2+}- influx and on-line enzymatic assays or HPLC to determine the release of glutamate and GABA by the exocytosis of SSVs. Post-translational modification of the proteins involved in the cascade of events leading to neurotransmitter release offers a powerful means of mediating presynaptic plasticity. Thus, one way that presynaptic receptor activation can potentially modulate the properties of proteins involved in neurotransmitter release is through the stimulation of second messenger cascades that lead to protein phosphorylation or dephosphorylation. We ascertain presynaptic receptor-mediated activation of specific protein kinases and phosphatases by labelling synaptosomal ATP pools with ^{32}P-orthophosphate and determining the phosphorylation states of identified intraterminal substrates for these enzymes. More recently, in collaboration with Professor Paul Greengard, we have developed the use of phosphorylation-state specific antibodies to the synapsins to look at presynaptic signalling cascades instrumental in the modulation of neurotransmitter release through regulation of phosphorylation-dependent interactions between SSVs-synapsins-actin.

Current Research 1992-present (continued)

2) Another major focus of my laboratory is to determine the role of specific protein kinases and phosphatases in the cascade of events leading to SSV exocytosis and endocytosis. For these studies, we have taken the approach of altering the expression of the enzyme of interest in neuronal cell lines and primary cell cultures that are amenable to molecular biological procedures. Currently, we are evaluating the effects of altered expression of the major Ca^{2+}-dependent protein phosphatase, protein phosphatase 2B (calcineurin, CN) in the neuroblastoma x glioma cell-line, NG108-15. Thus we have transfected NG108-15 cells with expression vectors containing sequences of CN (CN-A subunit) cDNA in sense and anti-sense orientations, to thereby overexpress or denude CN respectively. Stable, individual clones are now allowing us to characterise the specific role of the enzyme in: (a) Controlling voltage-dependent Ca^{2+}-influx. (b) The exocytotic/endocytotic cycling of SSVs, and regulation thereof by the phosphoproteins and CN substrates, synapsin I and dynamin. We are examining the effect of CN overexpression or diminution on VDCC activity using whole-cell patch-clamping of wild-type and mutant NG108-15 cells. In parallel experiments, the effects of altered CN-expression on Ca^{2+}-influx is being examined by Ca^{2+}-imaging (using fura-2 as the probe) of single cerebellar granule cell neurons (CGCN) in primary culture. CGCNs as a glutamatergic release model is also amenable to the assessment the role of CN-mediated dephosphorylation of SSV-associated proteins at the active zone, both, by looking at glutamate release per se, and through the use of the SSV probe FM1-43 to image endocytotic/exocytotic events at the single cell level. To facilitate the molecular biological manipulation of presynaptic protein expression in primary cell cultures, we are developing the Semliki Forest viral vector system for the delivery of bicistronic constructs which support co-expression of both the gene of interest and green fluorescent protein as a transfection reporter.

One of the key issues pertaining to signalling molecules like protein kinases and phosphatases is the means by which they are intracellularly targeted and compartmentalized. There is increasing evidence to suggest that protein kinases and phosphatases may indeed be targeted by "anchoring proteins" to specific locations within the neuron and so form signalling units/complexes in the proximity of cognate substrates. We are investigating the role played by one such anchoring protein, AKAP-79 in the physiological signalling mediated by CN. During our studies looking at mechanisms underlying the temporal and spatial regulation of CN function, we have found that, frequenin/NCS-1, a myristoylated membrane targetted Ca^{2+} sensor (and known enhancer neurotransmitter release), interacts with CN and invokes the upregulation of low-voltage activated Ca^{2+} channels into NG108-15 cells prior to their activation. However then, following depolarization, Ca^{2+}/NCS-1 appears to inhibit high-voltage activated Ca^{2+} currents as a negative feedback control akin to that we have characterized for Ca^{2+}/calmodulin stimulation of CN.

(3) In studies relating to the role of CN in nerve terminals, the phosphorylation of specific CN-sensitive phospho-sites on synapsin I by mitogen-activated protein kinase (MAPK) has revealed a novel presynaptic role for this kinase cascade in the regulation of synapsin I function and neurotransmitter release. Having shown that a brain-derived neurotrophin factor (BDNF)/TrkB/MAPK/synapsin I cascade is involved in the regulation of glutamate and GABA release, we are now elucidating in detail the regulation of nerve terminal function by neurotrophin/MAP-kinase signaling by: (a) Pharmacologically manipulating MAP kinase activity in synaptosomes with neurotrophins and kinase inhibitors, and examining the effect on glutamate release in relation to MAP kinase-mediated phosphorylation and CN-mediated dephosphorylation of synapsin I and dynamin I. (b) Identifying and characterizing the specific G-protein-coupled presynaptic receptor mechanisms that crosstalk to and influence nerve terminal MAP kinase activity and thus regulate glutamate release.

(4) To address the role of lipid phosphorylation in the regulation of neurotransmitter, we are investigating the subcellular localisation of phosphatidylinositol kinases and phosphatases. Thus, we have obtained evidence of a phosphatidylinositol 4-kinase (PI4-K) being associated with SSV, facilitating SSV-priming and thereby supporting glutamate release. Details of the presynaptic kinase cascade leading to the production of phosphatidylinositol 4,5-phosphate (PIP_2), the putative effector in SSV-priming, are being elucidated. Intriguingly, the observation that PI4-K might interact with the Ca^{2+} sensor frequenin/NCS-1, throws up the possibility of potential crosstalk between Ca^{2+}-dependent signalling mediated via CN function (described above) and lipid phosphorylation/phosphorylation cascades involved in SSV priming.