BIOGRAPHICAL SKETCH

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NAME: Pfaff, Samuel L.

eRA COMMONS USER NAME (credential, e.g., agency login): sampfaff

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Carleton College, Northfield, MN	B.A.	06/1979	Biology
University of California, Berkeley, CA	Ph.D.	06/1988	Molecular Biology
Vanderbilt University, Nashville, TN	Postdoctoral	06/1992	Development
Columbia University, New York, NY	Postdoctoral	07/1996	Dev. Neurobiology

A. Personal Statement

The research in my lab focuses on two areas of research (a) characterization of spinal networks that control movement, and (b) novel gene therapy approaches using RNA splicing. Our research on the spinal circuits for locomotion can be further divided into characterization of the genetic pathways that control neuronal diversification, connectivity, and circuit function; and studies to understand diseases that affect the spinal cord including amyotrophic lateral sclerosis (ALS) and peripheral neuropathies. In the mid-90s it was unclear how neuronal subtypes acquired their unique identities and the extent to which they were even genetically distinct. One of the most significant discoveries to emerge from my lab is the first demonstration that combinatorial genetic codes based on transcription factors are involved in neuronal subtype specification. We demonstrated that a small number of regulartory factors (LIM-HD genes) could drive emense neuronal diversity. The observation that neuronal subtypes can be identified based on their genetic signature serves as a foundational principle neuroscientists use in which genetic markers of neuronal subtypes are leveraged to investigate neuronal function, and provide some of the logic for stem cell biologists using transcription factors to induce neuronal differentiation from fibroblasts (iN). Following the characterization of LIM-HD transcription codes published in a series of papers, we began to investigate how motor neuron connectivity is established by studying axon guidance systems. We showed that motor neurons are guided to their muscle targets using “push-pull” mechanisms and “synergy detectors” involving receptor-ligand systems that are compartmentalized within different membrane domains within the same cell to prevent cis-interactions but allow trans-signaling. We pioneered the use of forward genetic screens in mice to uncovered new axon guidance factors such as presenilin, and uncovered genetic pathways that allow motor axons to clear endothelial cells (forming nasanct blood vessels) from their pathway.

Single cell sequencing and bioinformatics have become major tools used in my laboratory and in one application we investigated spinal neuron diversity by asking how cell populations are related to one another rather than trying to identify differences (a logic similar to that used by taxonomists in comparing species). This uncovered a surprising distinction in the spinal cord – a genetic signature for neurons with short range local connections distinct from neurons with long range connections that transcends the classical diffisions of neuronal subtypes such as their cardinal identity or neurotransmitter phenotype. This finding is a major research direction we have applied for funding to pursue. In addition to investigating how spinal circuits function we have investigated molecular pathways that are relevant to diseases and have found a critical miRNA within motor neurons that may be a central target of ALS pathways, and we have studied how Schwann cell plasticity is regulated during diseases that affect peripheral nerves. Aside from the neuroscience projects in my lab the other major research area we now study is based a fundamental discovery we made that RNA-transplicing can be enginnered to function efficiently in mammalian cells. This discovery has opened new possibilities for using gene

therapy to treat genetic diseases caused by very large genes (i.e. Stargardt and DMD), and we are trying to exploit this observation to make a genetic system for detecting specific transcripts within cells.

The lab is equally comprised of men and women from different URM groups including South Amerian, African, Central-Asian and Asian descent. During the last five years the lab has had 5 graduate students receive PhD degrees and 6 post doctoral fellows start research positions as Assistant Professors (2) or senior scientists in Biotech (4). The lab currently has 4 graduate students, 1 post doc, 2 senior staff scientists, 3 technical support members, and 1 undergraduate.

B. Positions, Scientific Appointments, and Honors

Positions

2005-present	Professor, The Salk Institute for Biological Studies, Gene Expression Laboratories; and Adjunct Professor, University of California, San Diego
2001-05	Associate Professor, The Salk Institute for Biological Studies, Gene Expression Laboratories; and Adjunct Associate Professor, University of California, San Diego
1996-01	Assistant Professor, The Salk Institute for Biological Studies, Gene Expression Laboratories; and Adjunct Assistant Professor, University of California, San Diego
1992-96	Research Associate (Post doc), Columbia University, Center for Neurobiology and Behavior and Department of Biochemistry and Biophysics, Mentor: Thomas Jessell
1988-92	Research Associate (Post doc), Vanderbilt University, Molecular Physiology and Biophysics, Mentor: William Taylor

Honors and Awards

2022	Fellow, American Association for the Advancement of Science (AAAS)
2012-present	Benjamin H. Lewis Chair in Neuroscience
2008-19	Howard Hughes Medical Institute Investigator
2007-14	Javits Neuroscience Investigator Award
1999-01	PEW Scholar Award
1998-00	Alfred P. Sloan Research Fellow Award
1998-01	March of Dimes Basil O'Connor Scholar Award
1997-00	Whitehall Foundation Scholar Award
1997-00	McKnight Scholar Award in Neurobiology

Professional Activities

2018	External Reviewer: Max Delbruck Centrum
2015-2023	*Science* Board of Review Editors
2013	Issue Editor: *Current Opinions in Neurobiology*, Development
2012	Reviewing editor: eLIFE
2011-present	Scientific Advisory Committee: Cure SMA
2009	Editor and Chief, *Molecular and Cellular Neuroscience*
2008	Scientific Advisory Committee: Halifax Brain Repair Center, Canada
2006-2008	Chair of Gordon Research Conference: Developmental Neurobiology
2004-2009	Scientific Advisory Committee: March of Dimes Basil O'Connor Scholar Awards 2004-
2004	Organizer of Christopher Reeves Paralysis Foundation Grant Holder Meeting
2003	Issue Editor: *Current Opinions In Neurobiology*, Development
2002	Associate Editor: *The Journal of Neuroscience*
2001-2010	Scientific Advisory Committee: Christopher and Dana Reeve Foundation

Federal Government Public Advisory Committees

2020	Clinical Biomarker ALS Panel Reviewer: DOD
2017	Intramural Scientific Reviewer: NINDS
2011-2017	Scientific Review Panel Study Section Member: NINDS
2012	Panel Organizer: Neurons and Cancer: NCI
2005-2010	Scientific Advisory Committee, Intramural Board: NICHD
1998-2004	Adhoc Reviewer, NINDS

C. Contributions to Science

1. Transcription codes and neuronal identity. As a post doctoral fellow with Tom Jessell and early independent investigator at the Salk I was one of the first to demonstrate that transcription factors could be used in combinatorial arrays to define neuronal subtypes. Our early studies focused on spinal motor neurons where we established that LIM homeodomain factors regulate motor neuron subtype development and connectivity. More recent studies have characterized the genetic diversity of spinal interneuron subtypes using methods such as next generation sequencing (NGS) and viral tracing.

- Tsuchida, T., Ensini, M., Morton, S.B., Baldassare, M., Edlund, T., Jessell, T.M., and Pfaff, S.L. (1994).Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes. **Cell** 79: 957-970.
- Sharma, K., Sheng, H., Lettieri, K., Li, H., Karavanov, A., Potter, S., Westphal, H., and Pfaff, S.L. (1998). LIM homeodomain factors Lhx3 and Lhx4 assign subtype identities for motor neurons. **Cell** 95: 817-828.
- Thaler, J.P., Lee, S-K., Jurata, L.W., Gill, G.N., and Pfaff, S.L. (2002). LIM Factor Lhx3 contributes to the specification of motor neuron and interneuron identity through cell-type-specific protein-protein interactions. **Cell** *110*, 237-249.
- Hayashi, M., Hinckley, C.A., Driscoll, S.P., Moore, N.J., Levine, A.J., Hilde, K.L., Sharma, K., Pfaff, S.L. (2018). Graded arrays of spinal and supraspinal V2a interneuron subtypes underlie forelimb and hindlimb motor control. **Neuron** 97 (4), 869-884. Doi:10.1016/j.neuron.2018.01.023. PMID: 29398364.

2. Molecular studies of neuromuscular disease. My lab has used genetic approaches to better understand the molecular pathways that affect motor neuron survival. We investigated the non-cell autonomous interactions of astroctyes and motor neurons using next generation sequencing, we identified miR-218 as a higly enriched motor neuron microRNA and characterized its function using mouse knockouts, and we defined a novel non-cell autonomous function for tRNA synthetase mutations associated with motor degeneration.

- Amin, N.D., Bai, G., Klug, J.R., Bonanomi, D., Pankratz, M.T., Gifford, W.D., Hinckley, C.A., Sternfeld, M.J., Driscoll, S.P., Dominguez, B., Lee, K.F., Jin, X., Pfaff, S.L. (2015) Loss of motoneuron-specific microRNA-218 causes systemic neuromuscular failure. **Science** 18;350 (6267), 1525-1529. PMCID: PMC4913787.
- He, W., Bai, G., Zhou, H., Wei, N., White, N.M., Lauer, J., Liu, H., Shi, Y., Dumitru, C.D., Lettieri, K., Shubayev, V., Jordanova, A., Guergueltcheva, V., Griffin, P., Burgess, R.W., Pfaff, S.L., and Yang, X-L. (2015) Peripheral neuropathy is linked to the neomorphic binding activity of tRNA synthetase. **Nature** 526 (7575), 710-714. PMCID: PMC4754353. *Corresponding Authors.
- Amin ND, Senturk G, Costaguta G, Driscoll S, O'Leary B, Bonanomi D, Pfaff SL. (2021). A hidden threshold in motor neuron gene networks for survival revealed by modulation of miR-218 dose. **Neuron** 109 (20): 3252-3267. PMID: 34450025**,** PMCID: PMC8542606
- Daboussi, L., Costaguta, G., Gullo, M., Jasinski, N., Pessino, V., O'Leary, B., Lettieri, K., Driscoll, S., Pfaff, S.L. (2023). Mitf is a Schwann Cell Sensor of axonal integrity that drives nerve repair. **Cell Reports** 42, 113282.

3. Spinal circuit mechanisms. My laboratory has used genetic, optogenetic, viral and electrophysiological tools to characterize spinal motor circuitry. We identified basic principles that underlie the function of the spinal CPG and characterized a premotor neuron population that integrates descending and sensory motor commands.

- Levine, A.J., Hinckley, C.A., Hilde, K.L., Driscoll, S.P., Poon, T.H., Montgomery, J.M., Pfaff, S.L. (2014) Identification of a cellular node for motor control pathways. **Nature Neurosci.** 17(4), 586-593. PMID: 24609464. PMCID: PMC4569558.
- Hinckley, C.A., Alaynick, W.A., Gallarda, B.W., Hayashi, M., Hilde, K.L., Driscoll, S.P., Dekker, J.D., Tucker, H.O., Sharpee, T.O., Pfaff, S.L. (2015) Spinal locomotor circuits develop using hierarchical rules based on motorneuron position and identity. **Neuron** 87(5), 1008-1021. PMCID: PMC4592696.
- Hilde, K.L., Levine, A.J., Hinckley, C.A., Hayashi, M., Montgomery, J.M., Gullo, M., Driscoll, S.P., Grosschedl, R., Kohwi, Y., Kohwi-Shigematsu, T., Pfaff, S.L. (2016). Satb2 is required for the development of a spinal exteroceptive microcircuit that modulates limb position. **Neuron** 91, 763-776. PMCID: PMC5011456.
- Sternfeld, M.J., Hinckley, C.A., Moore, N.J., Pankratz, M.T., Hilde, K.L., Driscoll, S.P., Hayashi, M., Amin,

N.D., Bonanomi, D., Gifford, W.D., Sharma, K., Goulding, M., Pfaff, S.L. (2017). Speed and segmentation control mechanisms characterized in rhythmically-active circuits created from spinal neurons produced from genetically-tagged embryonic stem cells. **Elife**, FEB 14;6. Doi:10.7554/eLife.21540. PMCID: PMC5308898.

- Osseward PJ II, Amin ND, Moore JD, Temple BA, Barriga BK, Bachmann LC, Beltran F, Gullo M, Clark RC, Driscoll SP, Pfaff SL, Hayashi M. (2021). Conserved genetic signatures parcellate cardinal spinal neuron classes into local and projection subsets. **Science** 372 (6540): 385-393. PMID: 33888637

4. <u>Axon guidance mechanisms.</u> My group has investigated the signaling mechanism that control motor neuron axon guidance and revealed fundamental mechanisms that control the timing and specificity of signaling mediated by ligand-receptor systems. We uncovered examples of signaling coincidence detection, hierarchical interactions, sequential timing-based controls, and the role of membrane compartmentalization in receptor activation.

- Marquardt, T., Shirasaki, R., Ghosh, S., Carter, N., Andrews, S.E., Hunter, T., and Pfaff, S.L. (2005). Co-expressed EphA receptors and ephrin-A ligands mediate opposing actions on growth cone navigation from distinct membrane subdomains. **Cell** 121: 127-39.
- Bai, G., Chivatakarn, O., Bonanomi, D., Lettieri, K., Franco, L., Xia, C., Stein, E., Ma, L., Lewcock, J.W., Pfaff, S.L. (2011). Presenilin-dependent receptor processing is required for axon guidance. **Cell** 144(1):106-118. PMCID: PMC3034090.
- Bonanomi, D., Chivatakarn, O., Bai, G., Abdesselem, H., Lettieri, K., Marquardt, T., Pierchala, B.A., Pfaff, S.L. (2012). Ret is a multifunctional co-receptor that integrates diffusible- and contact-axon guidance signals. **Cell** 148: 568-582. PMCID: PMC3286831.
- Bonanomi, D, Valenza, F, Chivatakarn, O, Sternfeld, MJ, Driscoll, SP, Aslanian, A, Lettieri, K, Gullo, M, Badaloni, A, Lewcock, JW, Hunter, T, Pfaff, SL. (2019). p190RhoGAP Filters Competing Signals to Resolve Axon Guidance Conflicts. **Neuron** 102: 602-620. Doi:10.1016/j.neuron.2019.02.034. PMID:30902550.
- Martins, L.F., Brambilla, I., Motta, A., De Pretis, S., Bhat, G., Badaloni, A., Malpighi, C., Amin, N.D., Imai, F., Almeida, R.D., Yoshida, Y., Pfaff, S.L., Bonanomi, D., (2022). Motor neurons use push-pull signals to direct vascular remodeling critical for their connectivity. **Neuron** 110(24):4090-4107. PMID: 36240771

D. Publications:

Wu Y, Temple BA, Sevilla N, Zhang J, Zhu H, Zolotavin P, Jin Y, Duarte D, Sanders E, Azim E, Nimmerjahn A, *Pfaff SL, *Luan L, *Xie C. Ultraflexible electrodes for recording neural activity in the mouse spinal cord during motor behavior. Cell Rep. 2024 May 28;43(5):114199. doi: 10.1016/j.celrep.2024.114199. Epub 2024 May 9. PMID: 38728138; PMCID: PMC11233142. (*co-corresponding author)

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Globig AM, Zhao S, Roginsky J, Maltez VI, Guiza J, Avina-Ochoa N, Heeg M, Araujo Hoffmann F, Chaudhary O, Wang J, Senturk G, Chen D, O'Connor C, Pfaff S, Germain RN, Schalper KA, Emu B, Kaech SM. The β_1-adrenergic receptor links sympathetic nerves to T cell exhaustion. Nature. 2023 Oct;622(7982):383-392. doi: 10.1038/s41586-023-06568-6. Epub 2023 Sep 20. PMID: 37731001; PMCID: PMC10871066

Hayashi M, Gullo M, Senturk G, Di Costanzo S, Nagasaki SC, Kageyama R, Imayoshi I, Goulding M, Pfaff SL, Gatto G. A spinal synergy of excitatory and inhibitory neurons coordinates ipsilateral body movements. bioRxiv [Preprint]. 2023 Mar 21:2023.03.21.533603. doi: 10.1101/2023.03.21.533603. PMID: 36993220; PMCID: PMC10055247.

Kobayashi Y, Shigyo M, Platoshyn O, Marsala S, Kato T Jr, Takamura N, Yoshida K, Kishino A, Bravo-Hernandez M, Juhas S, Juhasova J, Studenovska H, Proks V, Driscoll SP, Glenn TD, Pfaff SL, Ciacci JD, Marsala M. (2023) Expandable Sendai-Virus-Reprogrammed Human iPSC-Neuronal Precursors: *In Vivo* Post-Grafting Safety Characterization in Rats and Adult Pig. Cell Transplant: 32:9636897221107009. doi: 10.1177/09636897221107009. PMID: 37088987; PMCID: PMC10134149.

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N, Beato M. (2022). Spinal premotor interneurons controlling antagonistic muscles are spatially intermingled. Elife. Dec 13;11:e81976. doi: 10.7554/eLife.81976. PMID: 36512397; PMCID: PMC9844990.

Martins, L.F., Brambilla, I., Motta, A., De Pretis, S., Bhat, G., Badaloni, A., Malpighi, C., Amin, N.D., Imai, F., Almeida, R.D., Yoshida, Y., *Pfaff, S.L., *Bonanomi, D., (2022). Motor neurons use push-pull signals to direct vascular remodeling critical for their connectivity. Neuron 110(24):4090-4107. PMID: 36240771 (*co-corresponding author)

Tadokoro T, Bravo-Hernandez M, Agashkov K, Kobayashi Y, Platoshyn O, Navarro M, Marsala S, Miyanohara A, Yoshizumi T, Shigyo M, Krotov V, Juhas S, Juhasova J, Nguyen D, Kupcova Skalnikova H, Motlik J, Studenovska H, Proks V, Reddy R, Driscoll SP, Glenn TD, Kemthong T, Malaivijitnond S, Tomori Z, Vanicky I, Kakinohana M, Pfaff SL, Ciacci J, Belan P, Marsala M. (2022) Precision spinal gene delivery-induced functional switch in nociceptive neurons reverses neuropathic pain. Mol Ther. 30(8):2722-2745. doi: 10.1016/j.ymthe.2022.04.023. Epub 2022 May 5. PMID: 35524407; PMCID: PMC9372322.

*Amin, N.D., Senturk, G., Hayashi, M., Driscoll, S.P., Pfaff S.L. (2022). Detecting microRNA-mediated gene regulatory effects in murine neuronal subpopulations. STAR Protocols 3 (1): 101130. PMID: 35146446, PMCID: PMC8801384 (*corresponding author)

*Amin ND, Senturk G, Costaguta G, Driscoll S, O'Leary B, Bonanomi D, *Pfaff SL. (2021). A hidden threshold in motor neuron gene networks for survival revealed by modulation of miR-218 dose. Neuron 109 (20): 3252-3267. PMID: 34450025**,** PMCID: PMC8542606 (*co-corresponding author)

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deficits in Charcot-Marie-Tooth neuropathy. Nat. Commun. Mar 8;9(1): 1007. Doi: 10.1038s41467-018-03461-z. PMCID: PMC5843656.

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Hilde, K.L., Levine, A.J., Hinckley, C.A., Hayashi, M., Montgomery, J.M., Gullo, M., Driscoll, S.P., Grosschedl, R., Kohwi, Y., Kohwi-Shigematsu, T., Pfaff, S.L. (2016). Satb2 is required for the development of a spinal exteroceptive microcircuit that modulates limb position. Neuron 91, 763-776. PMCID: PMC5011456.

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Geeven, G., Zhu, Y., Kim, B.J., Bartholdy, B.A., Yang, S.M., Macfarlan, T.S., Gifford, W.D., Pfaff, S.L., Verstegen, M.J., Pinto, H., Vermunt, M.W., Creyghton, M.P., Wijchers, P.J., Stamatoyannopoulos, J.A., Skoultchi, AI., and de Laat, W. (2015). Local compartment changes and regulatory landscape alterations in histone H1-depleted cells. Genome Biol.16, 289-304. PMCID: PMC4699363.

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