UNIVERSITÀ DEGLI STUDI
DELL'INSUBRIA

"Integrated Electrophysiology"

Coordinator: Prof. **Elena Bossi**

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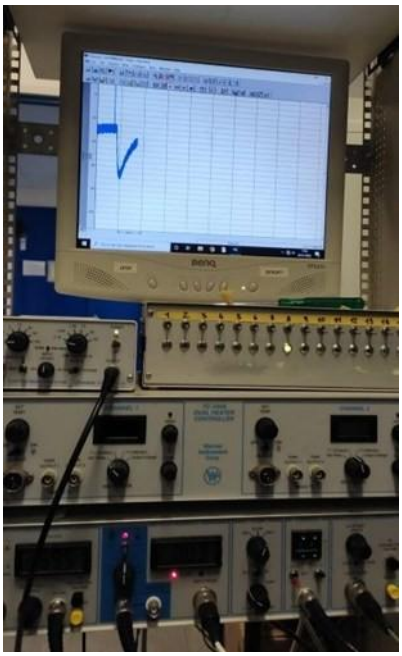
Keywords: SLC (solute carrier), voltage-clamp, patch-clamp, fluorescence imaging, brain slices, field potential recordings, ion channels, receptors, heterologous expression, Xenopus oocytes.



Purpose: The Electrophysiology Facility provides scientific and technological support for the functional study of electrogenic proteins: ion channels, receptors, and transporters (SLCs), as well as neuronal circuits. The facility integrates complementary and scalable electrophysiological platforms, analyzing function from the single protein level up to neuronal network activity, enabling rapid translation of results toward preclinical and translational applications. Pathogenic variants, recombinant proteins, chimeras, and mutants can be analyzed to identify therapeutic and nutritional targets.

Location: Department of Biotechnology and Life Sciences

Organization: The facility brings together laboratories already operating within the University.

It is organized into two highly specialized functional sub-units, each dedicated to specific experimental techniques:

- Varese site: Heterologous expression and Two-Electrode Voltage Clamp
- Busto site: Neuronal circuits in brain slices, neurons, and cell lines – Patch Clamp and Field Recordings. Fluorescence measurements in vitro, in live cultures or fixed preparations.

Connections with the CRIETT Technological Platforms and University Scientific Platforms:

La The facility operates in close connection with the University Scientific Platforms and the CRIETT Technological Platforms, particularly in the areas of health technologies and biomedical research, promoting multidisciplinary approaches and integration with existing infrastructures. In particular, it collaborates with:

- the **Technology Platform for Energy, Health and Environment**, within the scope of the objective: technologies, methods, and approaches for biomedical investigation.
- the **Frailty Platform**, within the scope of the following objectives:
    - frailty in the elderly: new therapeutic approaches.
    - early-life frailty: neurodevelopment, vulnerability, and neuropsychiatric disorders.

Substructure "Functional study of membrane transporters and organelles (SLC)" and of ion channels

Coordinators: Prof.ssa Elena Bossi, Prof.ssa Cristina Roseti

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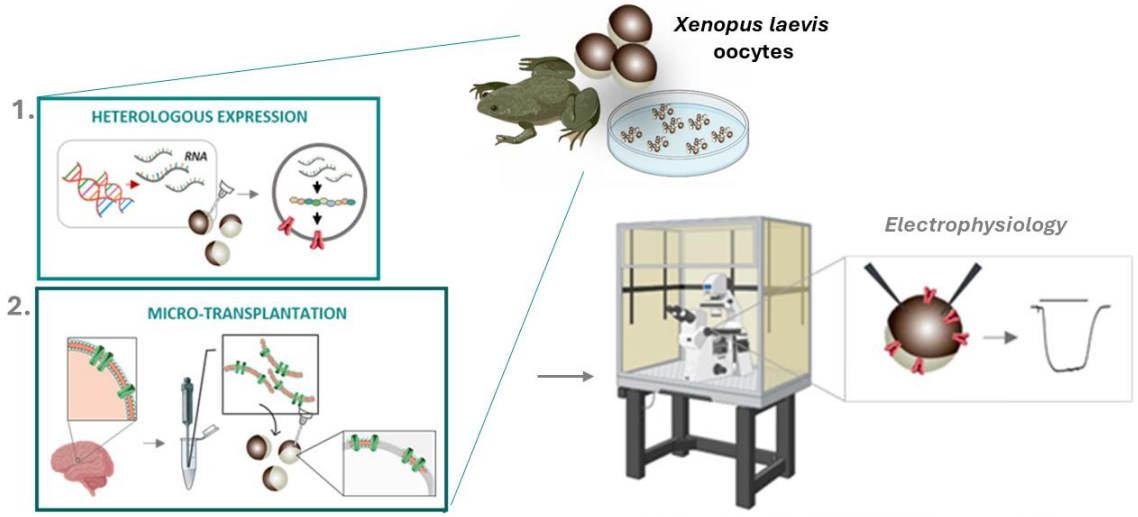
Keywords: heterologous expression, *Xenopus laevis* oocytes, SLC (solute carrier), receptors, ion channels, neurotransmitters



Brief description:

The substructure uses the *Xenopus laevis* oocyte expression system, a well-established model for the functional study of electrogenic proteins characterized by high sensitivity and signal amplification. Both heterologous (over)expression from cDNA is employed for detailed functional and pharmacological analyses of transporters, receptors, and channels, and membrane microtransplantation from murine or human tissues is used to investigate the native activity of proteins present in their tissue of origin and their modulation. This approach enables the testing and screening of potentially therapeutic molecules on channels and receptors, including those derived from human tissues, under controlled conditions, reducing the use of animal models and posing no risk to humans. Recordings are performed using two-electrode voltage clamp, ensuring stable and reproducible measurements, ideal for biophysical studies and pharmacological target validation.

Substructure "Modulation of ion channels and synaptic circuits"

Coordinators: Prof.ssa Lia Forti, Prof. Stefano Giovannardi

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Keywords: ion channels, neurotransmitter receptors, brain slices, patch-clamp, field potential recordings, cell cultures, transfection, immunofluorescence, fluorescent proteins (GFP).

Electrophysiological and fluorescence techniques enable the study of interactions between ion channels, receptors, and their modulators, as well as the assessment of pharmacological effects on these interactions. The application of electrophysiological techniques to thin neuronal tissue slices allows the analysis of neuronal excitability, synaptic transmission, and synaptic plasticity in animal models of neuropathologies, including the evaluation of the effects of specific drugs on synaptic alterations and excitability. Fluorescence imaging techniques also enable subcellular protein localization and the dynamic monitoring of their distribution under physiological and pathological conditions.

The substructure is equipped with a setup for patch-clamp/field recording experiments on slices/cultures, with temperature control and a fast solution exchange system; a vibratome (Leica VT1000S) and a puller (Sutter P-87) for the preparation of microelectrodes.

Links to available instrumentation/materials/technologies

For LAB Busto: equipment EQP-0101, EQP-0102, EQP-0103, EQP-0065 available in IRIS Insubria.

Publications:

<u>Publications on the study of membrane transporters and channels through heterologous expression and membrane transplantation.</u>

1. Bhatt M*, Di Iacovo A*, Romanazzi T*, Roseti C, Cinquetti R, Bossi E. (2022) *The "www" of Xenopus laevis Oocytes: The Why, When, What of Xenopus laevis Oocytes in Membrane Transporters Research*. Membranes (Basel). ;12(10):927. doi: 10.3390/membranes12100927. PMID: 36295686.

2. Di Iacovo A, D'Agostino C, Bhatt M, Romanazzi T, Giovannardi S, Cinquetti R, Roseti C, Bossi E. (2025) The kinase LRRK2 is *required for the physiological function and expression of the glial glutamate transporter EAAT2 (SLC1A2)*. J Neurochem.;169(1): e16265. doi: 10.1111/jnc.16265. PMID: 39655696.

3. Bhatt M, Lazzarin E, Alberto-Silva AS, Domingo G, Zerlotti R, Gradisch R, Bazzone A, Sitte HH, Stockner T, Bossi
4. E. (2024) *Unveiling the crucial role of betaine: modulation of GABA homeostasis via SLC6A1 transporter (GAT1)*. Cell Mol Life Sci. 2024 Jun 17;81(1):269. doi: 10.1007/s00018-024-05309-w.

5. Vacca F, Gomes As, De Gennaro M, Rønnestad I, Bossi E, Verri T. The teleost fish PepT1-type peptide transporters and their relationships with neutral and charged substrates. Front Physiol. 2023 Aug 21;14:1186475. https://doi.org/10.3389/fphys.2023.1186475. eCollection 2023. PMID: 37670771.

6. Iovino L, Giusti V, Pischedda F, Giusto E, Plotegher N, Marte A, Battisti I, Di Iacovo A, Marku A, Piccoli G, Bandopadhyay R, Perego C, Bonifacino T, Bonanno G, Roseti C, Bossi E, Arrigoni G, Bubacco L, Greggio E, Hilfiker S, Civiero L. (2022) *Trafficking of the glutamate transporter is impaired in LRRK2-related Parkinson's disease*. Acta Neuropathol.; 144(1):81-106. doi: 10.1007/s00401-022-02437-0. PMID: 35596783.

7. BURACCO S, PERACINO B, CINQUETTI R, SIGNORETTO E, VOLLERO A, IMPERIALI F, CASTAGNA M, BOSSI E,
8. BOZZARO S. Dictyostelium Nramp1, structurally and functionally close to mammalian DMT1 transporter, mediates phagosomal iron efflux. J Cell Sci. 2015 Sep 1;128(17):3304-16. pii: jcs.173153. https://doi.org/10.1242/jcs.173153 PMID: 26208637.2015.

9. Puhakka N, Cifelli P, Ruffolo G, Gaeta A, Roseti C, Di Iacovo A, Tiilikainen J, Ndode-Ekane XE, Lipponen A, Drexel M, Sperk G, Pitkänen A, Palma E. (2025) *Molecular and functional changes in GABAergic transmission during epileptogenesis in a rat model of post-traumatic epilepsy*. Exp Neurol.;387:115183. doi: 10.1016/j.expneurol.2025.115183. PMID: 39938858.

10. Gaeta A, Lissner L. J, Alfano V, Cifelli P, Morano A, Roseti C, Di Iacovo A, Aronica E, Palma E and Ruffolo G. (2024). *Membranes and Synaptosomes Used to Investigate Synaptic GABAergic Currents in Epileptic Patients*. Membranes 14, no. 3: 64. doi: 10.3390/membranes14030064.

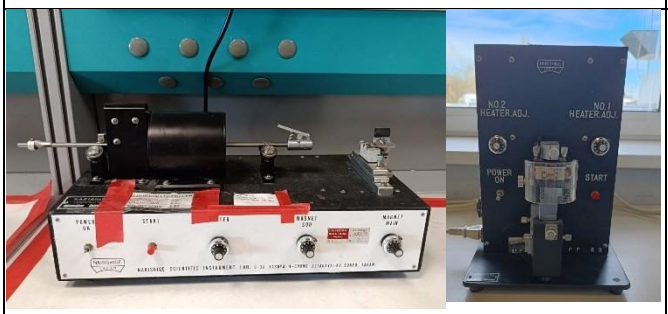
11. <u>Pubblicazioni per lo studio di circuiti neuronali attraverso fettine di tessuto cerebrale e tecniche di immunofluorescenza:</u>

12. Forti L, Ndoj E, Mingardi J, Secchi E, Bonifacino T, Schiavon E, Carini G, La Via L, Russo I, Milanese M, Gennarelli M, Bonanno G, Popoli M, Barbon A, Musazzi L. (2023) *Dopamine-Dependent Ketamine Modulation of Glutamatergic Synaptic Plasticity in the Prelimbic Cortex of Adult Rats Exposed to Acute Stress*. Int J Mol Sci.; 24(10):8718. doi: 10.3390/ijms24108718.

13. Sala N, Paoli C, Bonifacino T, Mingardi J, Schiavon E, La Via L, Milanese M, Tornese P, Datusalia AK, Rosa J, Facchinetti R, Frumento G, Carini G, Salerno Scarzella F, Scuderi C, Forti L, Barbon A, Bonanno G, Popoli M, Musazzi L. (2022) *Acute Ketamine Facilitates Fear Memory Extinction in a Rat Model of PTSD Along With Restoring Glutamatergic Alterations and Dendritic Atrophy in the Prefrontal Cortex*. Front Pharmacol.; 13:759626. doi: 10.3389/fphar.2022.759626.

14. De Lerma Barbaro, Andrea; Balkhi, Sahar; Giovannardi, Stefano; Vianelli, Alberto; Ribatti, Domenico; Lorenzo, Mortara. *The Quest for Antibodies and Other Acquired Immune Receptors: A Historical Perspective*. DOI: 10.1111/iji.12712. 2025 In INTERNATIONAL JOURNAL OF IMMUNOGENETICS vol. 52 (3).

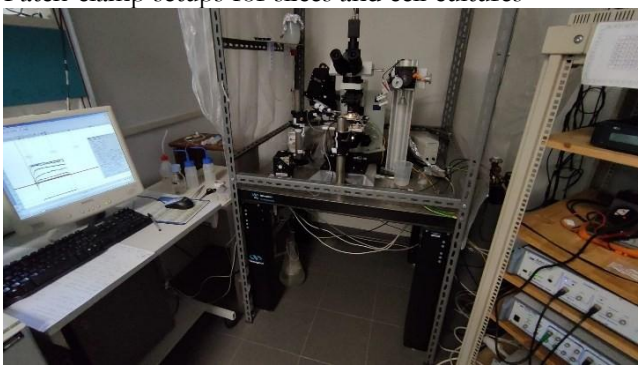
15. Malacarne, Miryam Chiara; Randisi, Federica; Marras, Emanuela; Giovannardi, Stefano; Dognini, Paolo; Mark Simm, Alan; Giuntini, Francesca; Gariboldi, Marzia Bruna; Caruso, Enrico. *EGFR-Targeted Photodynamic Treatment of Triple Negative Breast Cancer Cell Lines Using Porphyrin–Peptide Conjugates: Synthesis and Mechanistic Insigh*t. DOI: 10.3390/molecules30173533. 2025 In MOLECULES vol. 30 (3533).

1) Facility for the study of ion channels and transporters – Varese	
	**Two-Electrode Voltage Clamp (TEVC):** **3 complete electrophysiology setups for electrical recordings on *Xenopus laevis* oocytes.** **Digidata (Molecular Devices) acquisition system and analysis software (pCLAMP – Clampfit).** One setup is equipped with a temperature control system.
	**2 *Xenopus laevis* oocyte microinjection systems.**
	**2 Pullers**

	**System for preparation and maintenance/incubation of *Xenopus* oocytes.**
	**Fully equipped laboratory with instruments and reagents for molecular biology, including preparation of cDNA, expression vectors, and mRNA. Includes tools and materials for membrane preparation from tissues.**

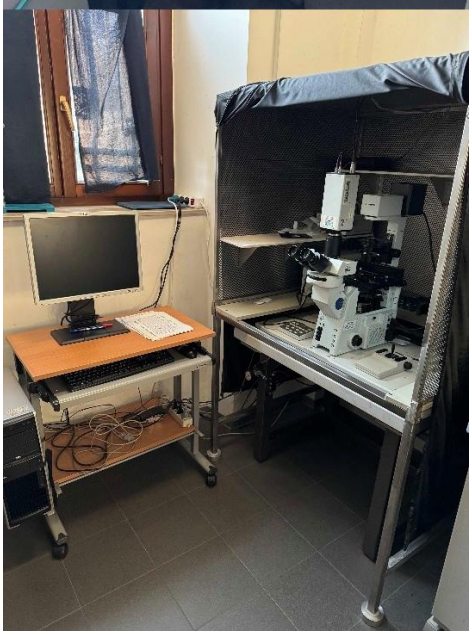
2) Facility for the study of ion channel modulation and synaptic circuits – Busto Arsizio

Patch-clamp setups for slices and cell cultures



Vibratome (slicer)

One dedicated patch-clamp setup for recordings on brain slices.

Puller  	
 	**One dedicated patch-clamp setup for cultured cells**

List of available materials

Expression vector	***Description***	***Resistance***	***Promoter***
pAMV	Vector for overexpression in *Xenopus* oocytes and in vitro transcription; it can be used for the expression of most cDNAs encoding plasma membrane proteins.	AMP	T7
pGeMHJ(m)	Vector for overexpression in *Xenopus* oocytes and in vitro transcription; it can be used for the expression of most cDNAs encoding plasma membrane proteins.	AMP	T7
pGemHJ(mDMT)	Vector for overexpression in *Xenopus* oocytes with targeting sequences for plasma membrane localization of intracellular transporters and in vitro transcription.	AMP	T7
Vectors for expression in neurons and cell lines, with or without GFP, YFP, CFP			
cDNA			
Numerous cDNAs encoding different orthologs of the SLC6A, SLC1A, and SLC15 families, as well as potential accessory proteins, including cDNAs encoding other transporters, ion channels, G proteins, etc.			