From Cells to Circuits: Ethical Challenges and Opportunities in 3Rs-Aligned Modeling of Sleep Disorders

Laura Clara Grandi[1,2], Francesca Gullo[1], Andrea Becchetti[1]
E-mail: laura.grandi@unimib.it
[1] Dipartimento di Biotecnologie e Bioscienze, Università di Milano Bicocca, Milano, Italia
[2] Dipartimento di medicina sperimentale e clinica, Università degli studi di Firenze, Firenze, Italia

Keywords: Narcolepsy, Epilepsy, Sleep disorders, 3Rs, Animal models, Neuronal cultures, Brain slices, Translational neuroscience

Abstract:

Sleep disorders with a genetic basis, such as narcolepsy or certain forms of epilepsy, affect millions worldwide and have a profound impact on quality of life and healthcare systems. These conditions are classically studied using genetically modified animal models that have revealed key neurobiological mechanisms and supported preclinical testing of candidate therapies. However, interspecies differences and ethical concerns challenge their translational validity. To address these issues within the 3Rs framework (Replacement, Reduction, Refinement), alternative approaches are urgently needed. Existing alternatives, such as human-derived cultures, brain organoids or computational models, struggle to capture the systemic intricacy of sleep-regulating circuits.
Our research focuses on genetically defined mouse models of narcolepsy (with conditional deletion of orexin receptors 1 or 2) and sleep-related hypermotor epilepsy (carrying mutations in neuronal nicotinic ACh receptors). To study these models efficiently and ethically, we adopt a tiered strategy that progresses from isolated neurons to structured brain tissue, each level reflecting increasing network complexity: 1) dissociated postnatal neurons are used to study cell-autonomous pharmacological responses; 2) primary neuronal cultures allow investigation of synaptogenesis and early network dynamics with reduced animal use; and 3) acute brain slices preserve local circuits for electrophysiological recordings (e.g., patch-clamp and multi-electrode arrays).
This tiered “complexity ladder” design enables selection of the minimal neural architecture necessary for each research question, balancing ethical imperatives with scientific rigor. Functional data from in vitro/ex vivo assays support the calibration of pharmacological interventions, streamlining subsequent in vivo testing and reducing animal use.
Adopting this strategy enables the calibration of pharmacological treatments on defined neuronal networks, streamlining subsequent in vivo pharmacokinetic studies. Further Reduction is achieved by using slice preparations that preserve long-range connectivity and approximate the complexity of intact brain circuits.