Investigating Biomaterial Scaffolds in Peripheral Nerve Regeneration in Gryllodes sigillatus: Effects of Developmental Stages on Hinge Leg Nerve Recovery

AUTHORS

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Background & Literature Review

The PNS is critical for survival in invertebrates, where decentralized nervous systems make nerve injury especially consequential. While C. elegans and Drosophila have advanced our understanding of axon regeneration, both face significant limitations in regenerative capacity and anatomical complexity. Crickets provide a more robust model: their segmentally organized nervous system, accessible hinge leg nerves, and SpikerBox compatibility make them ideal for neurophysiology research even in resource-limited settings.

Hydrogels are polymer networks that retain water and mimic the ECM, making them strong candidates for nerve scaffolding. A hydrogel must promote cellular adhesion, guide axonal growth, and degrade at a rate matching the nerve's recovery timeline. Their properties are largely determined by their crosslinking, the chemical or physical bonds that give the gel its structure and stability. Crosslinkers can be natural or synthetic, and they directly affect cytotoxicity, mechanical strength, and degradation rate. Hydrogels are broadly classified by origin (natural, synthetic, or hybrid) and by functional properties (conductivity and biodegradability)

Research Question and Hypotheses

- How do developmental stages (juvenile vs. adult) affect hinge leg nerve regeneration on G. *sigillatus*?
- How hydrogel scaffold types influence the PNS recovery?

H1: Juvenile G. *sigillatus* will exhibit superior recovery to the adult cohort due to greater regenerative capacity.
H2: Hybrid and conductive hydrogel scaffolds will outperform natural and synthetic scaffolds by integrating superior structural, biochemical and electroactive support.

Hydrogel Composition

Group	Material	Crosslinker	Key property
Natural	Gelatin	Genipin	Biocompat. · blue dye
Synthetic	PEG (~20%, MW 3000–4000)	None	Bioinert control
Hybrid	Alginate + Gelatin	$CaCl_2$	Toughness + adhesion
Conductive	Activated carbon + Gelatin	None	Lowers impedance
Control	Saline	—	Baseline reference

Fig. 1. Proposed hydrogel scaffold groups by composition and function.

Experimental Design/Methods

1. **Grouping & Housing:** 140 G. sigillatus (70 juvenile ~2wk, 70 adult ~4–5wk) across 5 treatment groups of 10 per age cohort: natural, synthetic, hybrid, conductive, and saline control. Housed in ventilated terrariums with vermiculite bedding, cardboard enrichment, and standard nutrition.

2. **Baseline Assessment:** Behavioral data collected via camera: jump height/distance measured against calibrated grid lines, and startle response recorded via pressurized air puffs. Electrophysiological baseline recorded via SpikerBox under brief ice anesthesia: recording electrode (+) inserted into hinge leg, ground electrode (−) at thorax.

3. **Nerve Injury:** Following anesthesia (1–4 min, −20°C), hinge leg nerve is crushed or transected under dissecting microscope using micro-scissors or forceps.

4. **Hydrogel Application:** ~10 μL of assigned scaffold applied to injury site via micropipette immediately post-injury. Control group receives saline.

5. **Post-Treatment Observation:** Crickets returned to divided terrariums. Tinted microscopy performed on hinge leg tissue at designated intervals to monitor scaffold integration and nerve tissue response at the injury site.

6.**Post-Injury Assessment & Data Analysis:** Pre/post behavioral metrics and spike frequency data analyzed in SIGVIEW across age × treatment groups.

Anticipated Outcomes & Data Analysis Plan

Juvenile crickets are expected to show faster and more complete recovery than adults across all measures. Electrophysiologically, a return toward baseline spike frequency and amplitude in SIGVIEW would indicate functional axon recovery. Behaviorally, recovery will be tracked through camera grid-referenced jump distance and height, as well as latency and magnitude of air-puff startle responses, both expected to progressively restore toward pre-injury baselines in more actively regenerating groups. Tinted microscopy will provide histological context, allowing visual assessment of scaffold integration and tissue response at the injury site. Among scaffolds, hybrid and conductive groups are anticipated to show the greatest recovery across all three measures. PEG's bioinert nature makes it a useful reference: recovery above saline but below biologically active scaffolds would isolate mechanical support as a contributing but insufficient factor in nerve repair.

Significance

This study demonstrates that meaningful neuroengineering research can be conducted in a state college setting using ethical, low-cost invertebrate models. Comparative scaffold design allows simultaneous evaluation of mechanical, biochemical, and electrophysiological contributions to nerve recovery, findings relevant to both invertebrate biology and the broader challenge of peripheral nerve repair in vertebrates.

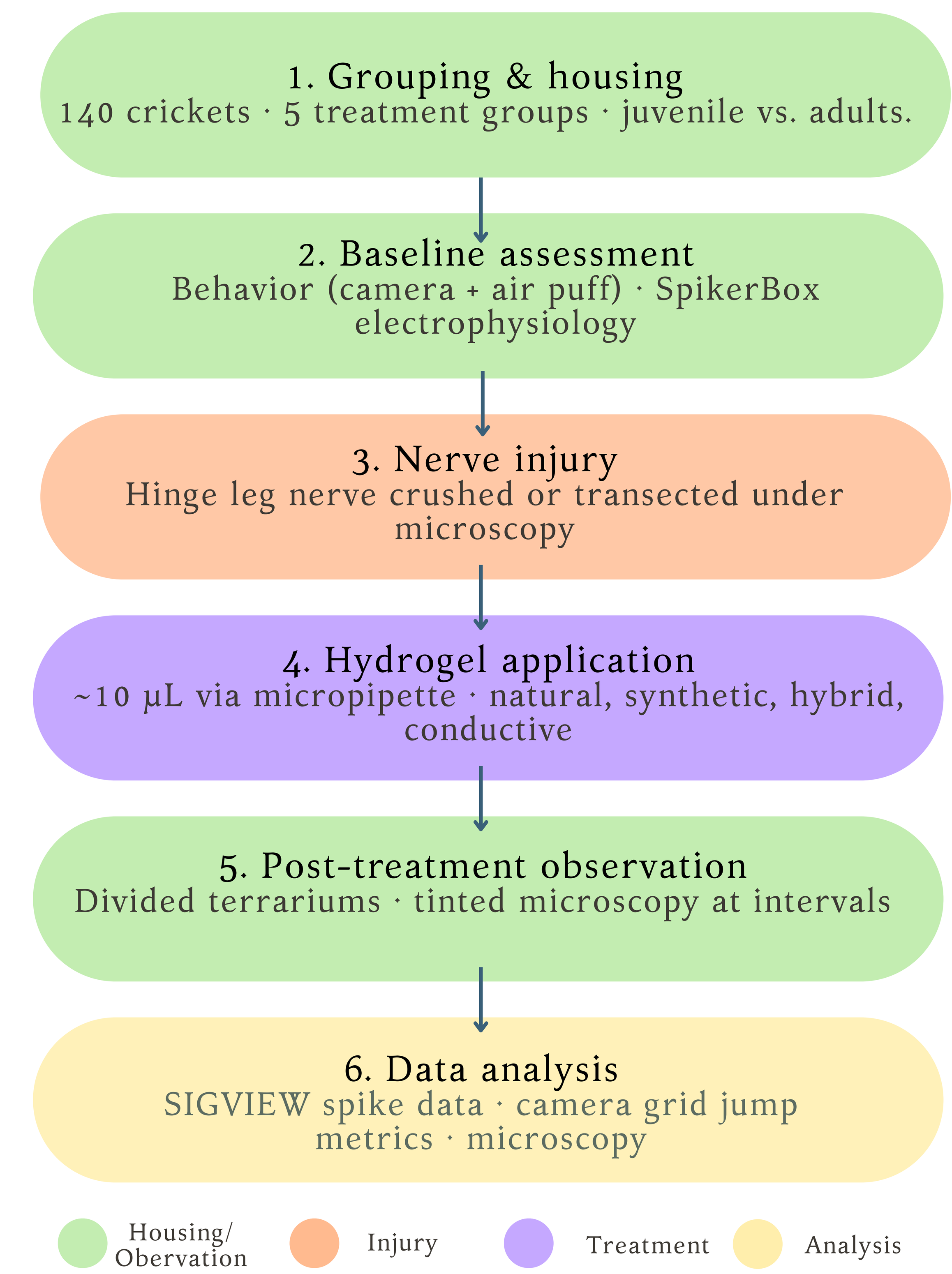

Fig. 2. Experimental procedure flowchart

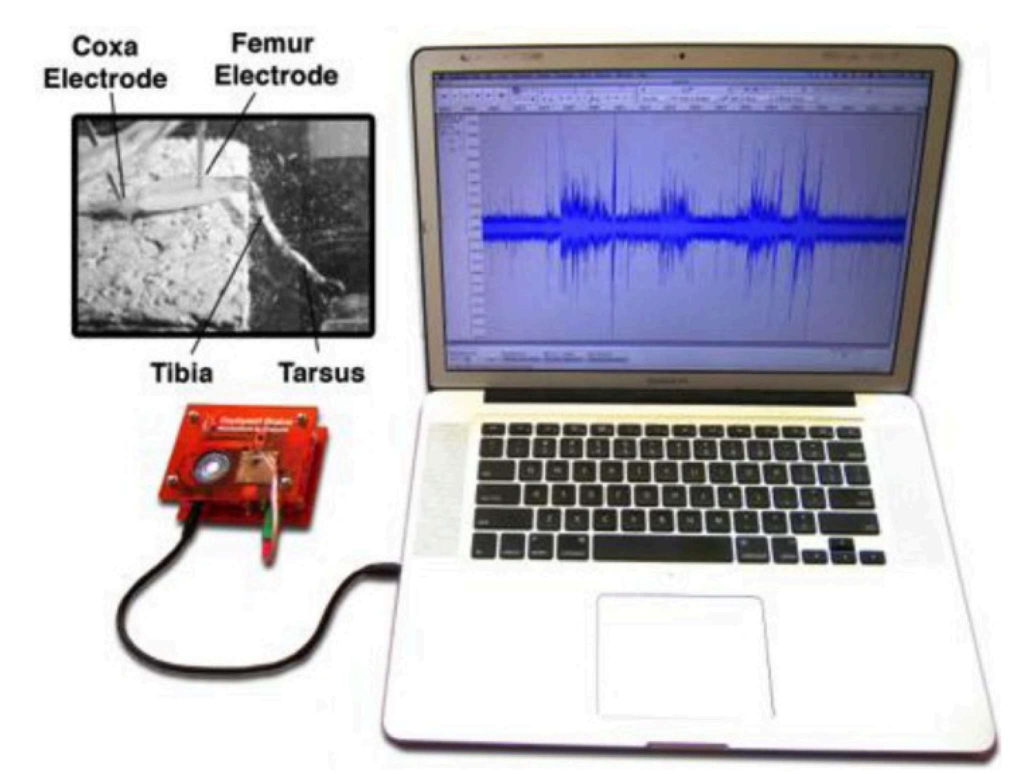

Fig. 3. SpikerBox setup and neuronal spike recording from cricket leg. Adapted from Dagda et al. (2013). J. Undergrad. Neurosci. Educ., 12(1), A66.

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