CARSS Integration Blueprint

Rev. 1 - June 2024

The goal of CARSS is to develop and verify a platform for clinical neuromodulation of the autonomic nervous system. CARSS is organized into five NESTs, each handling a different part of the overall project. The goal of this Blueprint is to provide a high-level description of how components developed by each NEST will be integrated into a completed platform and tested to verify compatibility, as well as to describe collaborations conducted between NESTs which were necessary to form the goals of the project. The collaboration between NESTs will include both co-development and testing of integrated components and systems.

The following describes the goals of each NEST:

- NEST 1, located at Med-Ally LLC: develop the mechanical enclosure for the implantable pulse generator (IPG)
- NEST 2, located at Medipace Inc.: Develop the electronic hardware, firmware, and software, including the PCBA for the IPG and charger, firmware for the IPG, and control software to run on a tablet or computer
- NEST 3, located at Med-Ally LLC: develop a set of leads for stimulation and recording based on existing commercial leads. Leads include a bipolar cuff for vagus nerve stimulation (VNS), linear array for sacral nerve stimulation (SNS), a sensor for electrocardiogram signals (ECG), a sensor for electromyogram signals (EMG), and an accelerometer motion sensor lead (AMS)
- NEST 4, located at USC under the direction of Ellis Meng: develop a thin-film cuff electrode for stimulating sub-mm diameter nerves, as well as a placement tool
- NEST 5, located at USC under the direction of Maral Mousavi and Hangbo Zhao: develop advanced sensors to enable closed-loop biosensing, including sensors for acetylcholine concentrations, catecholamine concentrations, pH, strain, and temperature

Close collaboration between NESTs will be necessary at the development stage to ensure that components are integrated into a complete system. Co-development projects are described below, along with project leadership and group responsibilities

NESTs	Project Description	Collaboration Plan	Leadership
1 & 3	Ensure that all NEST 3 leads are compatible with connectors on the IPG developed by NEST 1	Both NESTs are headquartered at Med-Ally LLC and will co-develop devices	Jayme Coates, Med-Ally LLC
1 & 2	Ensure that the PCBA is compatible with the IPG case	CAD of both PCBAs and enclosures will be shared and evaluated for fit before manufacturing to ensure compatibility	Jayme Coates, Med-Ally LLC
3, 4, & 5	Develop robust electrical and mechanical connection between NEST 3 lead bodies and thin-film sensors from NEST 4 and 5	Interconnection methods will be developed and prototyped at USC and transferred to Med-Ally for manufacturing	Ellis Meng, USC
3 & 5	Develop robust electrical and mechanical connection between NEST 3 lead bodies and thick-film flexible strain sensors	Attachment methods will be developed and prototyped at USC and transferred to Med-Ally for manufacturing	Hangbo Zhao, USC
2 & 5	Develop electronics and protocols for communication between IPG electronics and sensors with acceptable signal-noise ratio and without compromising safety	Medipace will develop a wired communication protocol and front-end hardware to be placed both on the PCBA and at the sensor site. USC will test to ensure electronic hardware compatibility with sensors. Med-Ally will encapsulate	Victor Pikov, Medipace Inc.

		sensor attachments and peripheral electronic hardware.	

To verify that components for all NESTs are integrated a rigorous benchtop testing protocol will be conducted. For NEST 1-3 devices this will follow existing standards for implantable neurostimulation devices. For NEST 4 and 5 devices, developmental benchtop testing will be conducted using protocols developed internally for the novel sensing methods.

Benchtop testing plan

NESTs	Tests	Responsibility
1 & 2	ISO11607-1:2006/ASTM F1980-07(2011) - Accelerated ageing for 60 months ISO14708-1:17.1/EN60601-1:11.1.2.2 – Self-heating and heat detection testing ISO14708-3: 23.2 (2017)/EN60068-2-47/EN60068-2-64 – Vibration testing ISO14708-3: 23.7 (2017)/EN60068-2-27 – Shock testing ISO14708-1: 10.1,23.2,23.7 – Drop testing Record BLE packet error rate and RSSI at 1m and 3m when implanted in 3cm phantom	Jayme Coates, Med-Ally LLC
1 & 3	ISO14708-2: 23.6 – Lead connector pull test at 10N ISO14708-2: 16.2 – Lead DC charge <= 0.75uA/mm2 ISO14708-2 6.2.3 – Lead connector contact impedance <1kOhm	Jayme Coates, Med-Ally LLC
1 & 4	No testing necessary	
1 & 5	No testing necessary	
2 & 3	No testing necessary	
2 & 4	No testing necessary	
2 & 5	I2C signal integrity testing across lead body	Victor Pikov, Medipace Inc.
3 & 4	Accelerated lifetime testing Contact resistance <1kOhm Stimulation reliability testing Pull testing	Ellis Meng, USC
3 & 5	Accelerated lifetime testing Contact resistance <1kOhm Stimulation reliability testing Pull testing	Ellis Meng, USC (thin-film) and Hangbo Zhao, USC (thick film)
4 & 5	No testing necessary	

Finally, animal testing will be used as a preliminary validation test of the integrated system. Two different animal testing campaigns will be conducted, one to validate the NEST 1, 2, and 3 components which are at a higher technological readiness level and a second to validate the NEST 4 and 5 devices.

Test 1: validating safety of NEST 1, 2, and 3 devices for 90 days in large animals

This study will validate the combined NEST 1, 2, and 3 system in 6 pigs for 99 days. It will assess: 1) functionality of VNS and SNS by measuring the gastric and duodenal motility and 2) safety of the implanted IPG and leads. The gastric and duodenal motility study will use chronically implanted AMS and EMG leads. IPG and lead safety will be assessed as: 1) after the sacrifice, amount of tissue injury around the IPG, VNS/SNS stimulation leads, and ECG/EMG/AMS sensing leads in 50-μm tissue sections with H&E staining to be performed at USC pathology lab; *2)* after the sacrifice, amount of VNS/SNS-induced axonal pathology in the vagal and sacral nerves assessed in semi-thin 1-μm sections stained with Toluidine blue performed at USC Core Center of Excellence in Nano Imaging, and subjected to morphometric analysis with automated counting tool AxoNet; and 3) amount of mechanical lead damage, during implantation via weekly impedance testing and after the sacrifice via examination of explanted leads.

Test 2: evaluating the safety and performance of NEST 4 and 5 devices in rodents

We will initially evaluate all components of NESTs 4 and 5 acutely in up to 30 rats and then chronically in up to 40 rats. We will evaluate the capability of the NEST 4 cuff to be deployed on a small nerve with no damage and successfully activate the nerve fibers, and the capability of the strain, temperature, pH, acetylcholine (ACh) and catecholamine sensors to sense in an *in vivo* environment with pre-defined accuracies compared to a control sensor. In addition, we will evaluate their feasibility to stimulate and sense chronically through weekly stimulation, sensing, and impedance measurements. Following the chronic study, we will euthanize the animal, and the amount of foreign body response and its effect on the device functionality will be assessed histologically. We will section the target tissue for sensing on a cryostat at 30 µm and use H&E staining to observe changes in the tissue and perform microscopic examination of explanted leads. Similar to the pig study, we will also assess the amount of nerve induced axonal pathology in 1-µm nerve sections stained with Toluidine blue and subject to morphometric analysis with automated counting tool AxoNet. The IPG PCBA from NEST 2 with a user interface will be used to perform stimulation and sensing. The IPG PCBA and NEST 4 and 5 components will be tested on a benchtop together ahead of surgery for the purpose of training the operator to use all hardware and software components and to debug any possible issues. The IPG board will also be used during chronic studies with a head stage and a swivel for tethered recording, stimulation, and impedance measurements once a week.