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Planning Report for MSc Project

Closed-Loop Auditory Stimulation System for mPFC-Amygdala Modulation in Free-Moving Marmosets

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1. Project Specification

This Master's project is part of a study which is a collaboration between the University of Newcastle and Imperial College London. Whilst most of this Master's project work is focused on the technical developments of the hardware supporting the study, it is important to outline the context around the development of the system, which is done in the following background.

Background

Closed-loop auditory stimulation has long been proven as a mechanism to modulate brain activity non-invasively (1,2). Some frequency patterns introduced into the auditory cortex through sound can travel and affect other existing brain activity on a wide spatial scale both for potentiation and suppression depending on their phase (3,4).

This modulatory effect is dependent on neurological networks linking the auditory cortex with the target region. In alignment with this, neurological pathways exist between the auditory cortex, the medial prefrontal cortex (mPFC) and the amygdala (5). The amygdala activity is mediated by the mPFC; it plays an essential role in emotional processing and is involved in many mental health disorders (5–7). This leads us to believe there may be great potential for psychiatric treatments adjusting amygdala activity by leveraging non-invasive auditory stimulation.

This study aims to prove the hypothesis that closed-loop auditory stimulation with sounds aligned to the oscillations in the mPFC-amygdala circuit can increase or decrease their activity and therefore regulate cognitive control. In the pursuit of this objective, marmoset monkeys were selected because of their brain topology, since it makes electrophysiological readings easier to perform than for other primates (8), and due to their similarities to the human brain in vocalisation and social abilities (9).

Hardware System Specification

Within this broader study, the project's aim is to develop the electro-mechanical device which will enable the testing of our hypothesis. The device performing electrophysiological recordings from the free-moving marmosets will be a battery powered, head-mounted data logging, and data streaming device. The device will need to transmit brain signals wirelessly to a computer which will generate the sounds used as auditory stimuli.

The neural data will be recorded from invasive electrodes implanted into the marmoset's cortex and amygdala regions. These electrodes will be routed into a connector mounted on a "crown" which will remain permanently attached on the monkey's skull and will act as a "docking station" for the active electronics.

The active electronics and battery will be housed in a removable module which will attach to the head crown. Within this active package a sequence of components will amplify, filter, digitise, store, and stream neural signals to generate the sounds. Moreover, the electronics will need to be powered through a removable or easily rechargeable battery ensuring a substantial autonomy. Lastly, the active electronics will need to store the data on an easy-to-offload storage platform.

The housing for the electronics will need to be carefully designed to provide the necessary shielding of internal components whilst being as non-intrusive as possible to the primates. Additionally, its geometry may need to be individualised depending on the specimen's skull curvature. Lastly, the removable electronics will need to be very easy to access and quickly replace, both for the monkey's and neuroscientist's comfort.

Technical Quantitative Specifications

The full quantitative specifications were determined with the Newcastle University team and are still subject to change. They are outlined in *Table 1* below:

Table 1 – Project Specifications

Code	Name	Value	Description
E1	**Autonomy**	2 hrs - ideally 24 hrs	If restricted, the maximum allowed recording time is 2 hrs. However, the marmosets should be able to be continuously monitored for as long as possible to minimise interventions and swapping systems.
E2	**Channel Count**	16 to 32	For the sole aims of the study, 4 channels would be enough, however recovering as much data as possible is desirable.
E3	**Sampling Rate**	100 Hz/channel – ideally capable of 10 kHz/channel	Local field potentials recordings should be sufficient for the study. However, in the initial weeks, action potentials might be recordable, for which a kHz-range sampling rate is necessary.
M1	**Dimensions**	15mm x 15mm	The device must be as small as possible, but the chamber size of the crown is limited to these dimensions.
M2	**Weight**	< 12 g	The device must not weigh more than 3% of the monkey's weight.
P1	**Wireless Range**	≥ 50m	In line with standard Bluetooth Low Energy, the operating range of the device should be comfortably in the dozens of meters.
P2	**Latency**	< 10 ms	The signals are highly time sensitive and should reach the receiver in under 10 milliseconds.
D1	**Plug-&-Play**	Yes	The headstage should made to be easily and quickly removed and replaced.
D2	**Remote Operation**	Yes	Ideally, the device should be able to switch modes without requiring the immobilisation of the animal.

2. Ethical Analysis

Due to the use of large live animals, a strong ethical basis must be laid out to support this work. Moreover, the collaborative nature of the project assigns different ethical responsibilities to both parties of the project.

The marmoset monkeys are sourced from the University of Newcastle. The surgeries they will undergo, their captivity and handling will also be managed by the University of Newcastle. The procedures will involve behavioural training, electrophysiology, non-invasive imaging (MRI, CTscan), and fluid or food control procedures if necessary. These ensure the animals are motivated in experimental procedures and remain safe and healthy at a high state of animal welfare. The role of this project and Imperial's ethical responsibility in this work, is the technical development of a device aiming to minimise invasiveness and maximise welfare throughout the device's entire usage life cycle.

The marmoset monkeys will need to wear the headstage for multiple hours at a time. The level of disturbance this will cause the monkeys is primarily determined by the 3D volume of the device and its weight. Therefore, our primary ethical consideration is the implementation of such a low-impact device through careful engineering, balancing mechanical objectives with the attainment of the study's data acquisition goals.

In a secondary manner, the animals will need to be "head fixed" at regular intervals for data offloading, device recharging, maintenance, and simply for removal during non-study hours. This restrained period can take up to a few minutes due to some fine connectors needing precise alignment. Minimising this duration as much as possible is one of the objectives of the project. Another secondary objective is the full teleoperation of the device, as rebooting, mode switching, and other checks would otherwise necessitate the immobilisation of the primates. In fact, ensuring the immobilisation duration is not only short but sparse is an important aim.

It must be highlighted that all the ethical considerations mentioned above also find themselves in alignment with the production of more reliable results. Previous studies have shown that motility constraints modify marmoset behaviour (10), and therefore reducing impact on animal behaviour from our device is a considerable performance objective. In a similar way, the secondary objectives of head-fixing reduction are great improvements to the scientists' user experience and may indirectly improve the reliability of results by reducing any potential negative impact on the animals.

Given the non-invasive potential of the intervention (auditory stimuli), and the potential applicability of the amygdala modulation mechanism to a wide range of mental disorders, this research holds great promise for the development of better, accessible psychiatric treatments. Moreover, the additional data collected from the device beyond closed-loop auditory stimulation hours is set to provide insight into neurological mechanisms related to autism and potentially sleep. These factors offer a positive risk-benefit-ratio, and with ethical goals aligned, promote direct applications in human healthcare.

As per Appendix G of the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, regarding severity classification, such research needs to be prospectively classified as severe. This recognises the sensitive nature of research with nonhuman primates and the possibility, however, remote of any animal suffering severely. To minimise this risk, the Home

Office approved PPL (PPL PP8119034: Kikuchi as Project License Holder), which this marmoset study will be under, is structured such that the humane endpoints are in place to prevent any animal suffering severely and to reduce cumulative impact to a bare minimum, by for example providing sufficient spacing between moderate severity events.

3. Literature Review

The following is a short review of existing literature on the growing use of the marmoset as a neuroscience model, and the development of neural loggers for electrophysiology in animals.

The Marmoset Model

Marmosets are growing as a valuable neuroscience model for studying circuits related to social behaviour and disorders. They are a small primate featuring complex social signalling and cooperative strategies more akin to humans than for other animal models (11). In close relation to the present research, marmosets have recently been shown to process part of their vocalisations in the amygdala (12). This paper also showed the likely encoding of vocalisations' emotional features through the auditory thalamus to amygdala circuit. These results are promising, they lay a solid neuroscientific foundation for the present research and make a strong case for using this primate as a model.

Moreover, techniques have been developed to monitor single-unit activity in freely moving marmosets as early as 2008 (13). This is in part thanks to the marmoset's smooth brain, allowing for facilitated access to target recording regions compared with other primates (14). Overall, these characteristics make the marmoset a very valuable asset in the context of this research, both thanks to its similarities with human cognition and accessibility of physiological recordings.

Developing Minimally Invasive Neural Loggers

Neural loggers are invasively implanted devices which record brain activity. Due to the interference of electrophysiology equipment with standard animal behaviour, and the need for animal models in neuroscience, much of the recent research in electrophysiology loggers has been directed towards developing lightweight, small, and efficient devices (15).

As the system will be performing closed-loop auditory stimulation (CLAS), performing the neural recording-to-sound emission steps directly on the headstage could be explored. Studies have demonstrated such systems with non-invasive EEG devices in humans (16). However, for humans, the dimensions of a CLAS device far exceed what would be possible within the 12g weight budget of the marmoset headstage. Therefore, the philosophy of the system's design is to keep only the essential components on the headstage, and to outsource as much of the processing and sound generation to an external computer.

The most limiting constraint for the neural recording of freely moving animals is the device's wired vs wireless functionality. Whilst wired devices are still advantageous in high bandwidth, high processing applications (17,18), the vast majority of studies now leverage wireless methods. Within the wireless "branch", transmitting digital rather than analog brain signals is also a preferred option. In fact, the transmission of analog signals is subject to noise, necessitates RF/EMI shielded chambers and does not benefit from error correction methods which can be implemented through digital transmission protocols (9,10). These findings strongly support the development of a device emitting digital wireless signals.

Neural signals require pre-processing to be usable and stored. This is due to their very low amplitude in the microvolts (µV) range, and various frequency bands of interest. Whilst local field potentials (LFPs) are found in the 1-300Hz range, action potentials (APs) are in the 300Hz-10kHz band (19). This segmentation and the wide dynamic range of the frequency bands call for specialised analog front-ends which can perform the appropriate amplification, filtering, and digital conversion (20). In the literature, performant, off-the-shelf, low-power devices such as the Intan RHD2000 chips are widely used (20–22).

Another factor in line with the goal of reducing complexity on the headstage is the maximisation of the autonomy. As the battery is often a bulky and heavy component with a predetermined geometry, some studies opt for modular designs, whereby various autonomy levels can be enabled (23). Also, snap-fit, easily replaced rechargeable units have been successfully implemented in a recent marmoset recording study with an average autonomy of 4 hours (22). Lastly, in ultra-low power systems which leverage custom solutions or advanced signal processing methods, autonomy can be measured in days for mice (24) and rhesus macaques (20).

Head Mounting

Raw volume or weight are not the only mechanical factors in designing neural recording headstages, in fact, the geometry of the device is crucial to maximise animal welfare and nullify disturbances. Only a few studies factor this in, as most traditional head-mounted systems feature crude cylinders or cuboids to house the electronics (23,25).

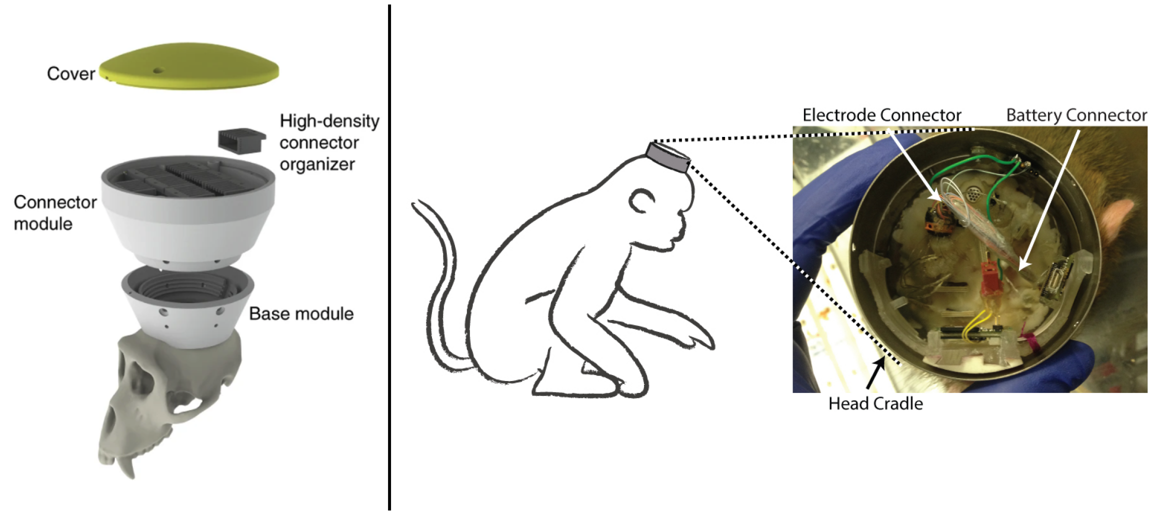

Figure 1: Rhesus monkey large scale recording headstage (left) (23), rhesus monkey compact standalone neural recording platform (right) (25)

Two marmoset papers seem to factor in the geometry of the monkey's skull to design their headstage. The first features a permanently attached "bent" cuboid, which follows the curvature of the skull and is bonded through a layer of dental cement (21). The second provides a modular system composed of a permanent screwed-in titanium pedestal, which is topped with modular components such as a helmet, a multiplexer, and the transmitter-battery package (22). It is also important to note the conformal shape of the headstage which would intuitively appear as organic and familiar.

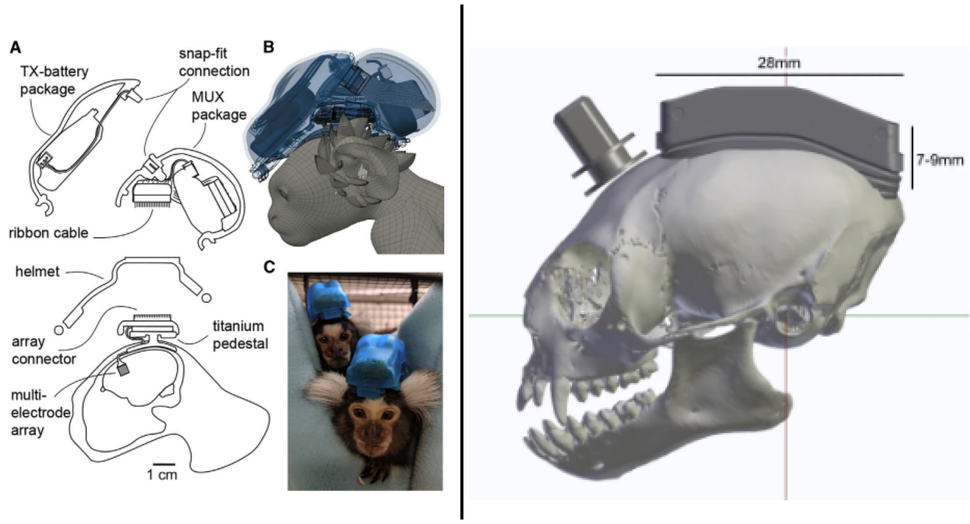

Figure 2: Marmoset modular headstage (left) (22), Marmoset curved cuboid permanent headstage (right) (21)

4. Implementation Plan

The following plan was determined based on the initial research performed and known workload for the rest of the master's degree. It follows four phases (*Figure 3*), with each phase containing a specific set of work blocks, with independent aims.

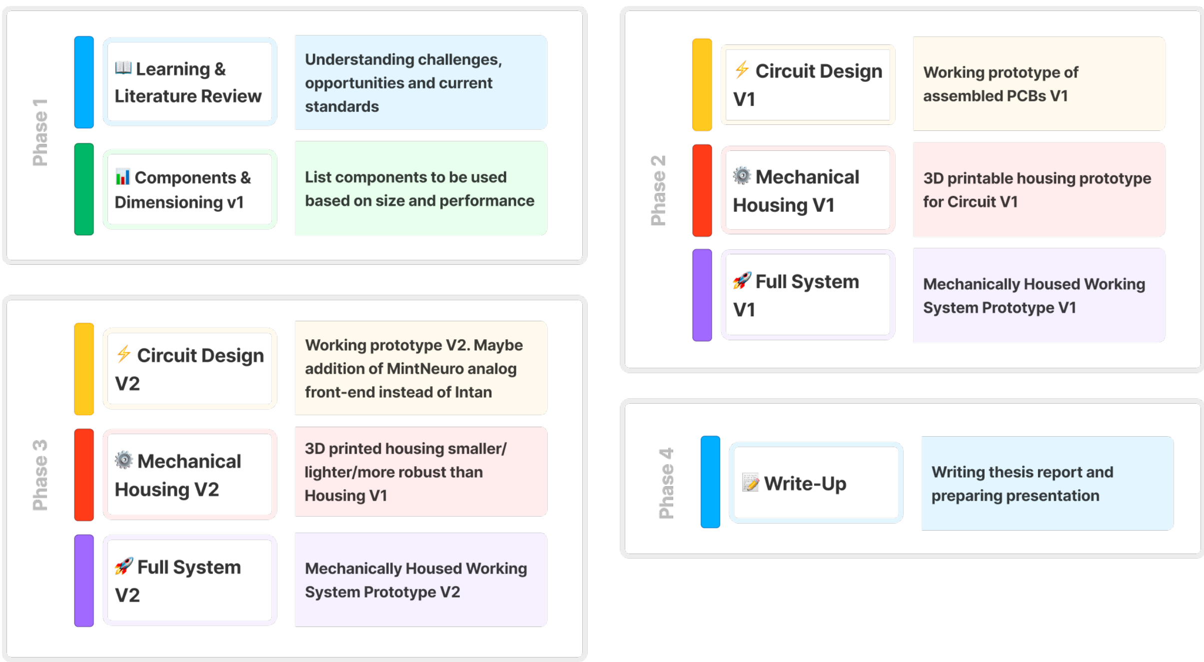

Figure 3: High level implementation plan work blocks

The week-by-week Gantt chart is below (*Figure 4*) for illustrative purposes. A full-scale readable version can be found here and at the end of this report.

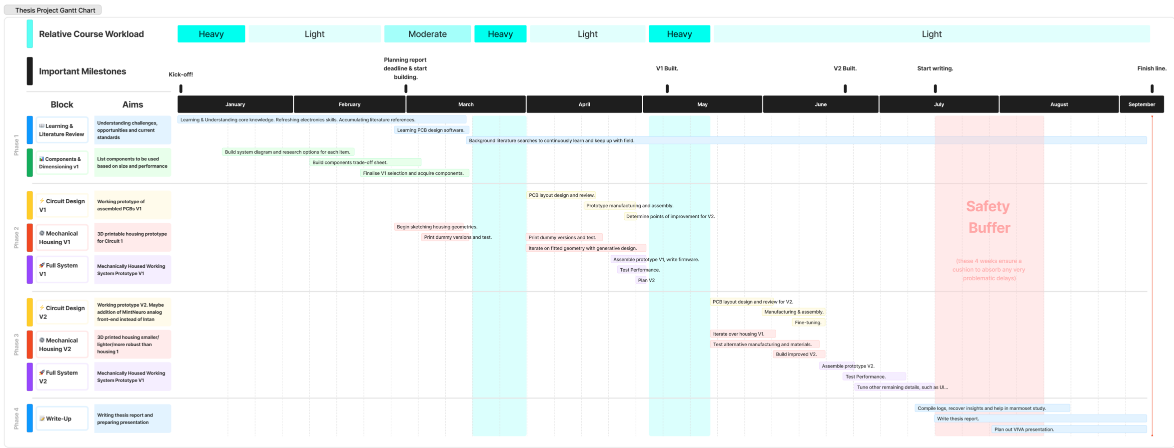

Figure 4: Detailed implementation plan Gantt chart

5. Risk Register

To manage the risks associated with the development of this project, we must identify challenges which could hinder progress and the attainment of objectives. The following risk register (*Table 2*) outlines potential uncertainties, categorised by project phase (P1-4), with their likelihood, impact, and mitigation strategies.

Table 2 – Project Risk Register

Code	Risk	Likelihood	Impact	Mitigation Strategies
P1A	Inadequate understanding of current standards and technologies.	**Medium**	**High**	- Engage in continuous learning. - Establish a routine of literature documentation in the field. - Refresh basic knowledge. - Routinely check assumptions with expert advisors.
P1B	Delays due to the learning curve associated with PCB design software.	**Low**	**Medium**	- Pre-allocate time for software training. - Identify learning curve very early on, before jumping into training. - Seek mentorship and help.
P2A	Component unavailability or delays in acquiring components.	**Medium**	**High**	- Develop list of suppliers and identify availability risk per component. - Incorporate backups with minimal compromises in performance or development.
P2B	Inadequate testing leading to design	**Low**	**High**	- Implement a thorough and standardised testing protocol for each testing milestone.

	flaws not being identified.			- Enforce feedback loops to address issues early on.
P2C	Underestimation of electronics prototype development time.	**High**	**Low**	- Add conservative time estimations and buffers for unforeseen delays. - Consult with experts in a tight feedback loop to dissolve bottlenecks immediately.
P2D	Underestimation of mechanical prototype development time.	**Medium**	**Low**	- Add conservative time estimations and buffers for unforeseen delays. - Use rapid prototyping to accelerate iterations. - Consult with experts in a tight feedback loop to dissolve bottlenecks immediately.
P3A	System integration issues lead to functional shortcomings.	**Medium**	**High**	- Develop system components in parallel to ensure seamless integration early. - Schedule integration reviews to identify and fix issues early.
P3B	Mechanical/ Electronic objectives are not met by V2.	**Low**	**High**	- Ensure improvement objectives from V1 towards V2 are clear and realistic. - Involve Newcastle team in a tight feedback loop to manage objectives if the original set becomes unattainable. - Take a modular approach allowing the scaling of the solution to different performance levels. - Introduce a solid time buffer to iron out potential flaws and develop a "V2.5" if needed.
P4A	Inadequate documentation or logs hinder report write-up.	**Low**	**High**	- Maintain diligent record-keeping practices throughout the project. - Regularly review and update logs to ensure they are comprehensive and up to date.
P4B	General delays in writing and VIVA preparation.	**Medium**	**Medium**	- Develop a clear writing and presentation schedule early in the project timeline. - Allocate time for reviews and rehearsals to refine the content and delivery.
G1	Unidentified risks exist.	**High**	**Medium**	- Introduce sanity checks regularly. - Ensure plans are updated at regular intervals and are validated by expert advisors.

6. Evaluation

The developed system will be evaluated and validated against its specification objectives at regular intervals using a set of tests. These tests are straight forward:

To validate the device's main objective of performing electrophysiology recordings, it will be attached to a test bench simulating neural signals, these will be recorded with the system's electronics. The measurements will then be checked against the known emitted signals to validate the ability of the system to accurately record and store electrophysiological data. These measurements will also allow us to quantify the performance of the system and provide insight into future iteration steps.

For the validation of the autonomy (E1), the system will be connected to an electrophysiology simulation rig and a range of power draw measurements will be taken with varying device settings. These power consumption values will be extrapolated to the in-vivo sustained usage and will allow us to validate the battery autonomy of the device.

The dimensions and weight of the device (M1-2) should be predictable and controlled upfront. However, given the small dimensions of the system, production methods may introduce significant errors to the predicted targets. Therefore, the device will be measured and weighed at significant progress milestones.

The wireless communication performance (P1-2) will be tested with dummy data sent out from the device to a receiver PC. This setup will test for maximum latency and wireless range.

Lastly, regular checks with the Newcastle University primate and neuroscience team will validate the system's adherence to usability objectives such as remote operation and easy device swapping (D1-2).

7. Preliminary Results

From the beginning of the project until today, Phase1 has been underway and will finish in the next few weeks. The initial learning objectives and literature review have been performed. Challenges, opportunities, and trade-offs have been laid out. Candidate components have also been explored and initial selections have been made. Lastly, some mechanical engineering ideation has been performed.

8. References

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9. Gantt Chart