FACULTY OF INFORMATION TECHNOLOGY AND ELECTRICAL ENGINEERING

DEGREE PROGRAMME IN BIOMEDICAL ENGINEERING

HYBRID HEAD CAP FOR MOUSE BRAIN STUDIES

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ABSTRACT

In this thesis, I present a hybrid head cap in combination with non-invasive multi-channel Electroencephalogram (EEG) and Near-Infrared Spectroscopy (NIRS) to measure brainwaves on mice's scalps. Laboratory animal research provides insights into multiple potential applications involving humans and other animals. An experimental framework that targets laboratory animals can lead to useful transnational research if it strongly reflects the actual application environment. The non-invasive head cap with three electrodes for EEG and two optodes for NIRS is suggested to measure brainwaves throughout the laboratory mice's entire brain region without surgical procedures. The suggested hybrid head cap aims to ensure stability in vivo monitoring for mouse brain in a non-invasive way, similarly as the monitoring is performed for the human brain. The experimental part of the work to study the quality of the gathered EEG and fNIRS signals, and usability validation of the head cap, however, was not completed in the planned time frame of the thesis work.

Keywords: Head cap, Electroencephalogram, Functional near-infrared spectroscopy, Laboratory mouse, 3D printing.

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FOREWORD

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Arup Pal

ABBREVIATIONS

AVA	Agri-Food and Veterinary Authority
BCI	Brain-Computer-Interfaces
BL	Bregma to Lambda
CA	Cornu Ammonis
CS	Coronal Sutures
CT	Computer Tomography
CARS	Coherent Anti-Stokes Raman Spectroscopy
DG	Dentate Gyrus
ERP	Event-Related Potential
EC	Entorhinal Cortex
EEG	Electroencephalogram
ECoG	Electrocorticogram
fNIRS	Functional Near-Infrared Spectroscopy
fMRI	Functional Magnetic Resonance Imaging
IACUC	Institutional Animal Care and Use Committee
LEA	Lateral Entorhinal Area
MRI	Magnetic Resonance Imaging
MEG	Megnetoencephalogram
MEA	Medial Entorhinal Area
NIRS	Near-Infrared Spectroscopy
NACLAR	National Advisory Committee for Laboratory Animal Research
PET	Position Emission Tomography
PCPSS	Pyramidal Cells Perpendicular to the surface
PTFE	Polytetrafluoroethylene
SPECT	Single Photon Emission Computer Tomography
SNR	Signal to noise ratio
SS	Sagittal Sutures
VEP	Visual Evoked potential

1. INTRODUCTION

Neuroscience has benefited notably from a current gain in genetic engineering. In both neuroscience and medicine, brain development, function, and dysfunction are investigated by multimodal analysis of brain activity. Brain disorder such as epilepsy, dementia, and different intellectual ailments result in growing expenses on health care system with getting elderly population. The most wonderful cure of this disorder would be either prevention or intervention of the sickness before irreversible damage develops (1).

Furthermore, it has been proven that one imaging modality is not sufficient to cover the brain function appropriately. Therefore, a multimodal approach is required (2). To acquire information about brain structure and physiology include electrical, magnetic, structural, and hemodynamic measurements, methods are available. Magnetic resonance imaging (MRI), computerized tomography (CT), positron emission tomography (PET), magnetoencephalography (MEG) and functional MRI every have their benefits with recognize to temporal, spatial and anatomical decision and specificity. Genetically modified mice are essential tools for reading human diseases and their treatment. Unequally, their benefits have been spread for the duration of the neuroscience community (3).

Transgenic mice can be used significantly to our understanding of their underlying cellular and molecular pathways. The use of transgenic mice has untangled more than a few problematic phenomena occurring in the brain, which is the most complicated system in nature (4). However, the small size of the mouse brain (1 cm3 in volume) has been a challenge to apply current neuroimaging technology, such as functional magnetic resonance imaging (fMRI) or PET (5). In the past years, electroencephalography (EEG) and functional near-infrared spectroscopy (fNIRS) instrumentation have been seen significant advances towards miniaturization and mobility, making them well suited for bimodal setups. Being noninvasive, relatively low cost and similar in setup complexity, EEG and fNIRS collectivity allow the optimized combined acquisition of electrical and local metabolic/hemodynamic activity (6). While constricted to near-surface brain regions, they can be applied under several conditions at the bedside. EEG conductively acquires the electric-powered recreation of the brain with an excessive temporal, but the low spatial resolution (7); NIRS optically acquires the metabolic (hemoglobin / oxygen-dependent) activity of the brain measuring physiological brain activity (8). Combined EEG and NIRS allow the investigation of interactions between neuronal electrical activity and regional microcirculation changes. This combination enables new approaches in many domains related to neuroscience and neuro technology, e.g., advanced diagnostic tools for medicine (for instance in Parkinson's and epilepsy research), cognitive science, psychology, Brain-Computer-Interfaces (BCI), Neuro ergonomics and adaptive neuro technology research (9).

1.1. Comparison of different type of neuroimaging techniques

EEG is a common device for monitoring the brain activity of human. By using a mouse model, EEG study can be facilitated under electrophysiological, pharmacological, or molecular manipulation by understanding the neural activity with molecular and cellular mechanisms. It is used effectively as an extensive neuropsychological evaluation instrument for directly measuring the electrical variation on the brain scalp. The change in power spectral density or connectivity energy in particular brain areas can be tracked, and great attempts have been made with neurological, physiological or psychological correlates to these changes by EEG research. Usually, there are two types of electrical activities generated in the brain (10). When the nerve cell of neuron communicates with each other, quick pulses of electrical current is produced called action potential which passes along the neuron fiber, whereas a chemical substance neurotransmitter adjacent by a neuron (11). Another neural electrical activity, which is provided by the connection of two neurons called postsynaptic potential, can be seen in the extracellular region of neuron by putting the electrode in this type of area on the skull brain function can be monitored easily. If we comprise between EEG and other techniques we can realize the benefit of EEG easily, such as MRI which is used for example, for examining the abnormalities of the brain and spinal cord and injuries of joint and other abnormalities also with high spatial resolution without ionizing radiation and contrast (12). Low sensitivity and long scanning time characteristic of MRI makes complication of the study on the moving animal (13).

Another medical imaging techniques such as CT, Ultrasonography, and some radionuclide imaging techniques like single-photon emission computed tomography (SPECT), PET is also accessible in the biomedical field (14). CT is a noninvasive diagnostic technology that use to examine many individual parts of the body included with the brain with an excellent spatial resolution. However, it produces too much ionizing radiation; this influence the possibility of developing cancer later life even some side effect –skin reddening, and hair loss also occur. A similar technique to SPECT, PET offers practical information which is mostly accurate and specific for diagnosing epilepsy, Alzheimer's diseases, Parkinson diseases, and various neurological diseases (15). However, using ionizing radiation need to take extra care for handling radioactivity. Relatively low spatial resolution and high cost must be considered. However, nowadays combining PET/CT or PET/MRI are accessible to diagnosis more accurate and precisely (16). The comparison between the different medical imaging techniques is summarized in Table 1.

Table 1. Comparisons between Medical Imaging Techniques

Imaging Technique	**Image quality Good contrast/ Spatial Resolution**	**System availability**
CT	Hard tissue (bone, tooth, enamel, dentin, cementum) and soft tissue (skin, fat, muscles, nerves, blood vessels, fascia)/0.5 mm	Cost: high Real-time information: no Ionizing radiation effect: yes Heating effect: low

MRI	Hard and soft tissue/0.5 mm	Cost: high Real-time information: no Ionizing radiation effect: no Heating effect: medium
Radiography	Soft tissues and fluid/1 mm	Cost: medium Real-time information: no Ionizing radiation effect: yes Heating effect: low
EEG	Hard and soft tissue/10mm	Cost: medium Real-time information: possible some cases Ionizing radiation effect: no Heating effect: low
Ultrasonography	soft tissue/1mm	Cost: low Real-time information: yes Ionizing radiation effect: no Heating effect: Negligible
Radionuclide	soft tissue/3mm	Cost: high Real-time information: no Ionizing radiation effect: yes Heating effect: medium
Thermography	soft tissue/15nm	Cost: low Real-time information: no Ionizing radiation effect: no Heating effect: high
Terahertz	soft tissue/40nm	Cost: high Real-time information: no Ionizing radiation effect: no Heating effect: high
NIRS	Hard and soft tissue/10 mm	Cost: medium Real-time information: possible some cases Ionizing radiation effect: no Heating effect: medium

In the cerebral cortex when apical dendrite is activated then extracellular current flows from the tip of the apical dendrite into a deep layer where the surface positive and deep negative electrical field is distributed respect to the cortical region (17). These electrical fields are generated from several neurons and recorded by EEG from

the cortical surface or the head surface. The EEG is the record of brain electrical fields, whereas the MEG, is the record of brain magnetic fields. The EEG field is a scalar and relative measure; it is susceptible to dipolar sources' tangential and radial parts. In theory, a radially focused dipolar source does not create a magnetic field outside the aspheric volume conductor; therefore, the MEG is not susceptible to the radial parts of dipolar sources but to the tangential parts. MES's are its excellent spatial resolution in the separation of cortical sources owing to less spatial spread than in the EEG and its selectivity to fissural cortex activity (18). When various kinds of cells being intermixed in the neocortex, then cellular architecture is more complex, at that moment tangentially or radially orientation is not essential discussion, but a maximum portion of large pyramidal neurons are tangentially or radially concerning the head surface under the cortical area (19).

1.1.1. Mouse brain EEG monitoring

With the progress in genetic engineering, the mouse is growing importance in the neuroscience. The transgenic mouse has become significant models for investing the behavioral contribution of genes (20). The development of genetically engineered mouse in latest years has enabled more extensive views on regulating oscillatory dynamics by the neurological, electrophysiological difference between a normal mouse that promotes to understand of brain oscillations and produce prospective objectives for drugs for associated illnesses (21). Lack of spatial resolution making mouse EEG recording even more difficult to evaluate the cortical rhythm of the brain. Taking into account that field oscillations were detected in various cortical construction at frequency bands (22). Effective EEG topological mapping techniques can be applied to the large-scale neural network in a mouse model for solving spatial resolution complication.

Monitoring brain function activity by mouse EEG is essential when investigation neurological functions. Invasive and noninvasive EEG recordings from the mouse brain, whereas electrodes are implanted in internal the cranium for invasive and electrodes are attached to the scalp surface for noninvasive recording. Invasive EEG can be split into two main types of recordings depending on the types of the electrode used: (1) stereo-EEG, acquired on the surface of the brain from electrode implanted in the hippocampus or neocortical region, and (2) electrocorticogram (ECoG), acquired straight on the surface of the brain from electrode implanted below the dura (23). An invasive method, electrodes are implanted are much closer to the brain so that the recording signals is considerably high amplitudes and high spatial resolution than scalp EEG that gives distinctive possibilities for electrophysiological inquiries of brain function. Recently, a growing amount of research has used invasive EEG information to investigate motor-sensory and cognitive systems (24). The characteristic of the detection signal from concealed cortex and recording signal from small pools of neurons without attenuation by scalp and muscle artifact using useful cortical mapping function make this method more effective. In the invasive process, the animal must prepare for surgery, which requires highly trained personal and specific medical equipment, which increase the total cost of such studies. The rate of serious permanent complication is 1% or less experienced hands are infrequent for surgery and implementing electrode in the head because the regions of cortex are narrowed and hard possibilities to find the right place to put intracranial electrodes. Though invasive method, there is less suffer from artifact such as eye blinks invasive

EEG faces some technical challenges with significant clinical risks of infection and other damage to the brain (25). For acquiring brain signals by invasive EEG, stainless steel screws type electrode is used in most research work. As the skulls are not too much strong for holding the screws in young or diseases model mice skull, implement of such electrodes can be problematic. Furthermore, for setting this type of electrode, surgery is needed, and such kind of surgery increases the risk of infection, bleeding, and traumatic injury. Moreover, replacement of multi-functioning electrode, the risk for complications, limited cortical sampling, biased by a signal from the adjacent cortex, dipole angle affects signal are made the invasive method more sophisticated (26).

Many invasive EEG studies in mouse have been concentrated to describe the functional feature of the neuron. On the other hand, non-invasive EEG research has gained more attention for mouse brain study without the complication of surgery during different cognitive activity. EEG is frequently evaluated in human studies using non-invasive electrodes of the scalp (27). But experimental trials using the animal are not common for the non-invasive EEG. Previously, noninvasive mouse EEG was used in few experiments. Noninvasive multichannel mouse EEG scheme would provide a reliable and efficient output that can impose to other animal non-invasive paradigms. Generally, the electrode is placed on the mouse scalp without undergoing any surgical processes in the noninvasive method. Different noninvasive EEG model like as flexible multi-channel mouse EEG electrodes to cover the curvature of the scalp (28), noninvasive EEG for cortical study (29), dry non-invasive multichannel EEG sensor (30), bipolar electrode method by covering the head (31) were developed. Noninvasive EEG method has been tested to overcome the problem caused by screw implantation in the invasive EEG method. By considering the reusability and safety guarantees, noninvasive mouse EEG may be a new novel approach to acquis brain signal without any surgery. But it is not simple to maintain a substantial number of electrodes on the mouse brain during a certain recording period with continuous touch. Moreover, all the electrode must be kept within the equal impedance range, and strength is needed to allow plug-in/out the action. In this thesis, we represent a novel hybrid head cap to gain brain signal from the mouse without any surgery.

1.1.2. Mouse brain NIRS monitoring

NIRS is a noninvasive imaging technique that can provide information on oxygen saturation and hemoglobin concentration in tissues. Infrared light propagates through the tissue, and eventually, part of it is backscattered to the surface and collected by photon detector. The detected attenuated light encodes information about brain activity because of absorption and scattering dominated light tissue interaction (32). Investigating and monitoring the neonatal brain provides us with important clinical information. Besides continuous EEG monitoring, cranial ultrasound, MRI and magnetic resonance spectroscopy, NIRS based regional cerebral oxygen saturation (rScO2) is increasingly used. Neuronal activity of the brain depends on the rate of the blood flow and metabolic modification. NIRS can evaluate cerebral hemodynamics and metabolic modification for recording brain activity (33). NIRS uses infrared light and the wavelength of infrared light is 700 to 900 nm (34). Continuously it is fit for measuring tissue oxygenation utilizing versatile instrumentation and a minimal effort (35).

In contemporary neuroscience, insight is acquired through the growth of transgenic mice models in significant pathophysiological mechanisms underlying Alzheimer's and Parkinson's disease (36). For searching prospective therapies, information of the cerebral oxygenation, hemodynamics, cerebrovascular reactivity, and mitochondrial function is crucial from the validating model is essential. Moreover, the transgenic mouse models would be very important to study cerebral blood flow and mitochondrial function (37). A suitable technique for such measurement does not exist at the moment. Therefore, NIRS technology, a minimally invasive technique, can be used as a potential tool to examine cerebral oxygenation and hemodynamics in the anesthetized mice (38). There is currently no appropriate method for such measurement. De Geofrey et al. has therefore evaluated whether multi-wavelength NIRS technology, a minimally invasive method, can be used as an instrument for examining anesthetized mouse's cerebral oxygenation and hemodynamics (39). Furthermore, based on the known brain penetration and general pharmacokinetic profile, distinct compounds from distinct chemical classes that change in brain metabolism as measured by NIRS could be a helpful brain study. The information collected in mouse treated with exogenous O(2) show an initial connection between NIRS assessment of brain metabolism and electrical alterations engaged in neurophysiological and pathological operations in this significant nucleus of CNS (40). Finally, the possibility of coupling NIRS with another method like as noninvasive EEG for corresponding cerebral firing assessment in separate brain fields are evaluated to study the correlation between neuronal activity, brain metabolism and blood concentration (41). In mouse, NIRS was used in a broad spectrum of studies to explore the impact of various stimuli on brain activity, like as direct-current transcranial stimulation of the cortex barrel, subcutaneous injections of amphetamine, electrical stimulation of the somatosensory cortex (42). In addition, NIRS has been applied to mouse with epilepsy, brain damage, stroke or pain. During seizures, fNIRS can evaluate the distinct activity of the cortical and subcortical region and differentiate between distinct kinds of seizures categorized by the EEG pattern (43). But lacking’s of standardization in data analysis of NIRS must be mentioned. NIRS systems provide readings with a spatial resolution of 2–3 cm from the cortical surface of two hemodynamic signals—HbO2 and HbR, as outlined above. Investigating the interconnectedness between these two signals enables us to draw judgments that are more precise on functional brain activity. If a participant walks or move the body slowly, a well-positioned fNIRS cap will continue to offer a strong signal, but it is really difficult to regulate animal movement (44).

Due to rapid head movements, the optical displacement of the optodes (i.e., both NIRS light source sand detectors) is quite hard, and motion artifacts can appear as quick and shifts from standard values in NIRS signals. Besides, the positioning of the optodes can be time-consuming for hairy areas and less convenience for high-coverage fiber optic measurements combined with the head and Impossibility of collecting structural pictures and anatomical data systemic interfaces variable SNR makes the research complex (45).

2. ANIMAL WELFARE AND RISKS MANAGEMENT

2.1. Ethical consideration of the experimental animal

For growing usage of animals in the scientific research and develop to a new medication and to test the safety of other products, the welfare and ethics consideration has drawn more attention in an experimental animal study (46). International guidelines about ethical concern for the experimental animal are approved by different organization like as National Advisory Committee for Laboratory Animal Research (NAC LAR, Singapore), Institutional Animal Care and Use Committee (IACUC), Agri-Food and Veterinary Authority (AVA) can be counted of the duration of investigation of animal study. In the following are listed some specific areas that covered in ethical considerations (47):

1) All use of animals must be scientifically justified, and pain and distress must be minimized, and the overall purpose of animals must be minimized by reducing the number of animals used, refining experiments and replacing vertebrate animals with either lower or non-animal alternatives.
2) Pain and distress accruing at the time of the examination must be minimized and treated appropriately by recommended anesthesia Regimen.
3) High standard housing, pure feeding environment, comfortable transportation has to establish for the experimental animal.
4) Personal animal or pet animal are forbidden for the experiment. Laboratory animal monitored by the expert veterinarian must oversee the program.
5) Duration of Experiment must be limited to that just enough to achieve the purpose of the experiment.
6) Repeated use of animal in the experiment should be avoided. IACUC approves one animal for one experiment.
7) A committee of at least one scientist, veterinarian, and a non-institutional member must approve all animal use and any exceptions to the above guidelines.

2.2. Selection of experimental animals

By examining the anatomy, physiology, and metabolism of the mouse, scientists can gain many standard genetic features between human and mouse. For alleviating the symptoms of diseases such as cancer, Alzheimer's diseases, or inherited disorder, transgenic laboratory mouse can play a significant role in drug development (48). The selection of animal model researchers always considers some issues like as types of epilepsy being modeled, minimization of suffering and number, limitation of these model, mortality rate variability between the animal even financial cost. Models can be classified by acquired and genetic, focal and generalized, acute and chronic. The model has to focus on the critical features of the corresponding diseases but no need to identical. In this circumstance, the controlling of seizure rate in the testing method of the model must be higher, because the lower rate of the seizure is impractical for current scientific setting and timelines (49).

Consideration:

1) Choice of the breeder: There is some complexity to select perfect animal breeding to research wide cause range of potential criteria and variation of

the generation. It is a critical factor to obtain an animal from commercial breeders. As an example, if we focus on C57BL/6 mice, a high mortality rate is observed after injection with anxiety-like behavior and seizure susceptibility. So appropriate commercial colonies should be used for biomedical research with taking care (50).

2) Choice of age: From neural properties and connectivity of the brain, study it is proved that the age of animals is likely to affect some essential factors like as seizure latency, mortality, and sensitivity to chemo-convulsions, behavioral, physiological, and pharmacological responses to anticonvulsant. Young Mouse: 6-month-old, aged mouse: 18-month-old, aged mouse: 24-month-old (51).
3) Choice of sex: In the animal model, sex hormone may be involved with the different types of epilepsies like, as females are susceptible to epilepsies: juvenile myoclonic epilepsy and childhood absence epilepsy while males in Dravet syndrome with Centro temporal spikes. Gender difference also creates an effect on the signaling pathways and brain function, and the use of antiseptic must be calculated for this issue. Generally, most of the biomedical researchers suggest an adult male model for performing their studies (52).
4) Choice of Strain: For genetic background, generally Mouse plays a crucial role in neuropathological consequences and genetically modified phenotypes and the susceptibility of these strains to seizures. It is vital to control the genetic background for avoiding genetic drift and same characterized colon problem. It is also must be noted that mouse strains can differ in the consequences of seizure activity, seizure susceptibility even effect of drugs (53).

Recommendations:

1) For searching animal mode, it is needed to ensure that types of animal model, which is scientifically relevant, the least severe model for the scientific purpose, and that any model-specific refinement opportunities are identified.
2) Consideration of harmfulness to animals and potential benefits of the research, it is crucial to take account of the lifetime experience of the animals and the whole epilepsy syndrome (not just seizures).
3) When designing, conducting, and reporting studies, must be considered age and sex of animals, variations in the strain, genetic background, source that influence seizure susceptibility and mortality.
4) Considered the appropriate littermate controls with the same genetic background and controlled Genetic background, for example, use age-matched wild-type littermates as controls.
5) Animals of both sexes should be used because sometimes sex type makes the impact of the estrus cycle on seizure susceptibility, and it needs to be considered.

2.3. Animal housing and pretreatment of mouse

Environmental complexity is responsible for functional changes in the nervous system of the laboratory animals, including mice, which make a negative impact on the brain study of the animal (54). For this issue, the animal must be provided

standardized housing conditions by considering the economic and ergonomic requirements where controlled climate, right hygienic conditions, nutritionally well-balanced diet, and other physical needs are available for the animal (55). For minimizing suffering and more safety at the time of experimentation of animal must be needed to follow the guidelines of the local council on animal care. European Communities Council Directive of 24 November 1986 (86/609/EEC) or individual regional or national legislation where radio telemetry may be played a vital role in the replacement, reduction and refinement strategy that is summarized in Table 2.

Table 2. Optimized environment condition to reduce mouse stress (56)

Environment condition	**Description**
Temperature and humidity	Recommended temperature is 65-75°F (~18-23°C), and humidity is 40-60% of concerning temperature.
Light cycle	The lighting system is a critical issue for mouse housing. The light should not enter the room during the dark period. Recommended cycle: 14-hour light/10-hour dark 12-hour light/12-hour dark
Diet	Fat content: 4% to 11%; Recommended by Animal Care and Use Committee (ACUC)
Water	Water supply must be ensured.
Minimize handling/noise/vibration	These can cause stress, so it must be minimized.
Enrichment	Stress can be reduced, and breeding can be improved by-Nestlets (Animal Specialties and Provisions, LLC), NestPaks (WF Fisher and Son), Shepherd Shacks (Shepherd Specialty Papers)

The facility of cages (57):

1) Food and fluids: By using modified food with dietary glucose, which enhances to accelerate weight gain following SE. The source of modification food should have minimized the adverse effect, which helps to weight loss. Recommended food may be fruit juices mixed with sweetened milk or mashed food pellets, which are beneficial for seizure induction.it, should be noted that sometimes-feeding behavior of animals might disrupt.
2) Infection management: During the experimental procedure, the infection must be avoided by using good practices, including the proposed technique though it is challenging to maintain the complex implant procedures. At the time of choosing antibiotics, it should be considered the effect of anti-apoptotic and anti-inflammatory, where the advice of veterinarian may be helpful.
3) Welfare assessment: Regular monitoring of welfare is effective and facilitated by surveying body weight, the color of the skin, mortality rate, aggressive behavior, and social interaction in the undistributed state.

2.4. Mouse anesthesia, analgesia, and care

As animal permits, in vivo monitoring for noninvasive studies as well as research to evaluate diseases with investigations, the effects of new therapies. Mouse anesthesia is challenging for several reasons. Anesthesia and analgesia are often a requirement as experimental procedures to obtain adequate immobilization and to reduce stress or pain but there is high risk of hypothermia and hypoglycemia (58). Besides, anesthetic agents influence physiological parameters, interfering further with the result of experiments. Steps to ensure animal safety and anesthesia efficacy must be taken before, during, and after anesthesia (59). Laboratory mice have specific anatomical and physiological characteristics that influence anesthetic drug effect. Drug metabolism and excretion are speedy. Due to their small body size, reducing the half-life of injectable drugs and making anesthesia duration a more critical factor compared to more abundant species. The primary factors to consider when selecting an anesthetic technique for mice are a strain, age, weight, the model of disease to be investigated, and the type of experimental procedure to be used (60).

2.4.1. Anesthetic monitoring and physical management

Careful monitoring and support of mice body temperature, heart and respiratory rates, mucous membranes, and the degree of CNS depression are imperative during sedation and anesthesia. Animal care staff should position the animal on a heated platform or use a heating lamp to keep the temperature above 95 to 99°F. Core temperature should be measured by an esophageal or rectal probe, pulse-oximetry monitoring tissue oxygenation, and electrocardiogram heart rate and rhythm. Anesthesia is considered adequate when the animal remains quietly, is unresponsive to external stimuli, and has constant heart and breathing. The absence of the palpebral reflex in mice suggests a fair anesthetic depth (61).

2.4.2. Anesthetic regimen

Pre-anesthetic care diminishes the incidence of complexity that may occur during anesthesia by ensuring that the most appropriate technique and regimen are chosen. Tranquilizers and analgesics to reduce apprehension, promote stress-free induction and recovery, reduce doses and side effects of other anesthetic agents, and achieve preventive analgesia generally administer pre-anesthetic drugs (62). Anesthesia in laboratory animals is a condition of unconsciousness, analgesia, muscle relaxation, and the main indication of general anesthesia in imaging procedures is the need for constant immobility, avoiding artifacts of movement. Anesthetic depth in mice can be clinically monitored by observing the loss of the righting and palpebral reflexes, and by evaluating muscle tone, response to painful stimulation, and respiratory rate and depth of anesthesia can be clinically monitored. An ideal mice anesthetic agent should be easy to administer, produce quick and adequate immobilization, safe for both animals and operators, have limited side effects, and be reversible (63). Therefore, it is essential to select the appropriate anesthetic agents and to implement appropriate structures for monitoring anesthetized animal in the course of image acquisition because of this type of agent effect on the physiology of the animal and image data also. Unfortunately, such an anesthetic is not available in the market, and

according to different experimental circumstances, the best drug selection is highly variable. There may be two types of anesthetic regimens: injectable, inhaled, depending on the nature of the administration of the drug (64). Imaging procedures sometimes-involved sometimes painful or invasive procedures such as intracavitary, or intravascular injections, catheterization of the blood vessel, or penetration of the endocavitary probe. In these conditions, it is needed to take enough analgesia protocol by recommended dosage for analgesia that is summarized the Table 3 and Table 4.

Table 3. Recommended dosages for anesthetic agents (65)

Types of anesthesia	**Agent/ Duration of anesthesia/ Dosage/**	**Comments**
Injectable anesthetic agents	Ketamine/xylazine*(20-30 minutes) ketamine 80-100 mg/kg IP xylazine 10-12.5 mg/kg IP	May not produce a surgical plane anesthesia for a major procedure through more reliable than in Mice.
	Ketamine/xylazine/acepromazine (60 - 90 minutes) ketamine 60-100 mg/kg IP xylazine 10-15 mg/kg IP acepromazine 2-5 mg/kg IP	May not produce surgical- plane anesthesia for major procedures, if redosing, use 1/3 dose of ketamine alone may loss surgical anesthesia.
	Ketamine/xylazine cocktail*(20-30 minutes) Ketamine 87.5 mg/kg IP Xylazine 12.5 mg/kg IP	potential health risks
	Ketamine + dexmedetomidine (15-25 minutes) Ketamine 50-75 mg/kg IP dexmedetomidine 0.5-1 mg/kg IP	May not produce surgical-plane anesthesia for major procedures If redosing, use 1/3 dose of ketamine alone may loss surgical anesthesia. Dexmedetomidine may be reversed with Atipamezole.
	Pentobarbital (20-40 minutes) 50 mg/kg IP	Loss of balance or co-ordination, vomiting, constipation maybe happen as the side effect
Inhalational anesthetic agents	Isoflurane 4-5% + 0.8-1 L/min	Survival surgery requires concurrent preemptive analgesia. Must use precision vaporizer.
	Sevoflurane + 0.8-1.0 L/min	Individualized based on the mouse response

Table 4. Recommended dosages for analgesics (66)

Drugs	**Dosage/Duration**
Tramadol (opioid)	10-30 mg/kg IP or 1mL 5% solution in 150 mL of water
Buprenorphine(opioid)	0.05-0.1 mg/kg SC q12h
Ketoprofen (NSAID)	2-5 mg/kg SC q12-24h
Buprenorphine(opioid)	5 mg/kg SC PO q24h
Meloxicam (NSAID)*	1 mg/kg SC, PO 30 min pre-surgery and q24h post-surgery

Recommendations (67):

1) To avoid excessive depression of cardiac and respiratory function must be monitored under anesthesia where following body temperature should be above 97°F and Oxygen saturation and heart rate should be greater than 95% and between 300-800 beats/minute with respiratory rate: ~180/minute.
2) Due to their high body surface area mouse are especially susceptible, so that mouse should not be connected directly with a heating source. Supplemental heat sources like as electric heating pads, circulating water blankets or other commercial product may be used.
3) For ill, aged or debilitated animals, fluid (SQ-warm subcutaneous, IP-intraperitoneal fluid) support can be more helpful. Recovery and Eye protection must be considered.

3. MOUSE BRAIN STUDY

3.1. Brain anatomy of the mouse

In mouse models, human neurophysiology and neuropathology, in particular, can be well studied. The structures the human brain and the mouse brain are the same that shown in the Figure1. Although they are organized differently and in different volume proportions, mouse brain model can be considered as a valid and registered model with the comparison for investigation of different types of brain diseases (68).

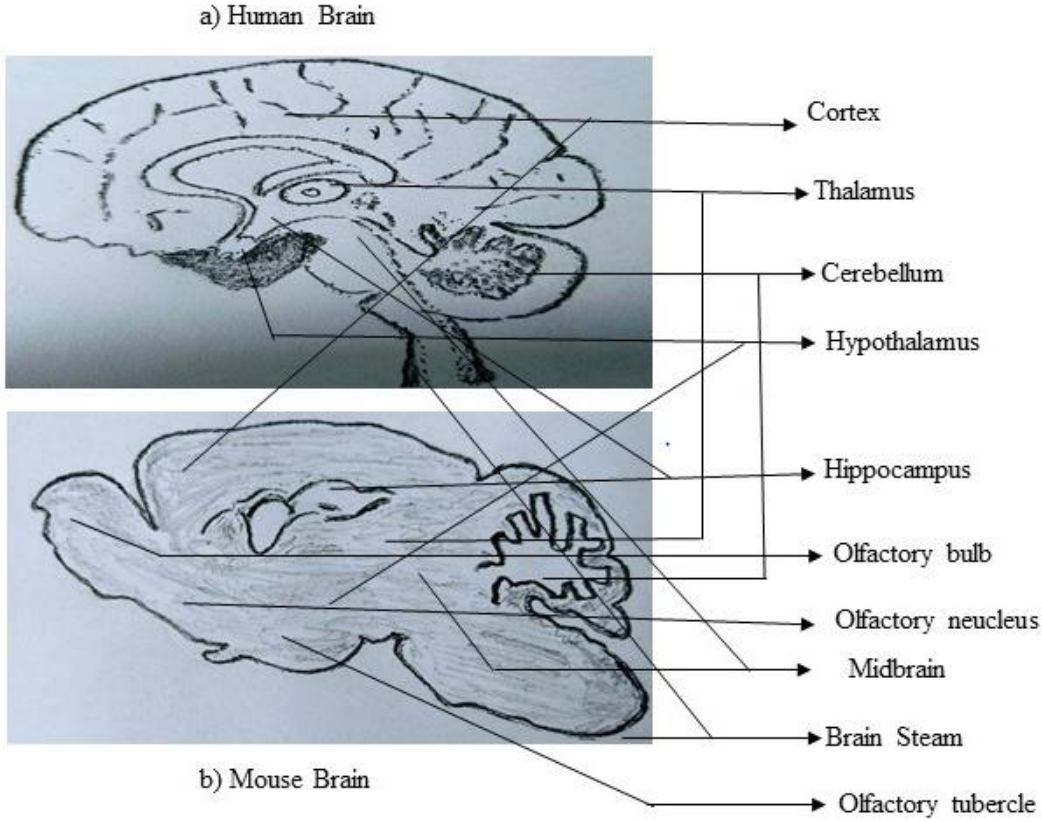

Figure 1. Anatomical comparison between human and the mouse brain (69).

Hippocampus is the key point of the anatomical and functional investigation in mouse brain study, which is the oldest components of the brain. Varieties phase of the limbic system, connected by the other brain areas like as Prefrontal /orbitofrontal cortices, Cingulate cortex, Piriform cortex, perirhinal and postrhinal cortices, striatum, amygdala, Septum, mammillary bodies, thalamus, hypothalamus and defined by the dentate gyrus and Cornu Ammonis (CA) (70). Cornu Ammonis is anatomically and functionally differentiated into distinct subfields named CA1, CA2, CA3, and CA4. In the latest years, the CA3 region has drawn significant attention for its particular role in memory mechanisms, seizure sensitivity, and neurodegeneration (71). In the hippocampus region, recurrent connection between CA3 with CA1 by the pyramidal cell makes the excitation in the axonal terminals to mid apical dendrites where the synaptic operation was acceptable. These types of recurrent connection

between cells in the same area develop a high quality linking with the chain of the neuron (72).

A strong commissural projection is created where almost 30% to 70% of the total proportion of synapses are constructed by a CA3 pyramidal cell might be connected with another CA3 cell and CA1 neurons. In the mouse skull, total axonal length of CA3 pyramidal cell is almost 150-3mm with 30% ramification. Total 30000 to 60000 terminals are targeted to pyramidal cells and interneurons with a similar frequency, but for intra-regional difference stratum oriens are more targeted than stratum radiatum (73). Many recurrent CA3 axon collaterals can make exciting contacts with other essential and inhibitory neurons. This circuit involves encoding spatial representations and episodic memories. Coherent population synchrony, together with gamma, theta, and sharp waves are presumed for firing indifference type of behavioral conditions generated by this circuit (74). The output of entorhinal cortex both directly by the perforant path and not straight from the dentate gyrus through the mossy fibers. The pass way of soft fiber works as a high pass filter that converts the cortical signal to a sparse, especially hippocampal code, which is essential for memory formation. Distinct cell types supply upward thrust to unique axonal fiber pathways in the dentate gyrus, CA3 sub-region (75). This location can also exhibit differential molecular profiles in response to a variety of behavioral paradigms and pharmacological and genetic treatments.

A cognitive map is a sort of mental representation that acquires, codes, stores, recalls, and decodes data about the relative places and characteristics of events in their everyday or metaphorical spatial setting. A specific functional mapping of various cortical areas in the hippocampus has not possible to publish clearly for limitation of the particular optical method (76). A more difficult problem that limits imaging depth is light attenuation caused by absorption and scattering, both of which reduce intensity exponentially with depth. Light attenuation induced by absorption and scattering is a more challenging issue that limits image depth, both of which decrease intensity exponentially with depth. Even overlaying tissue of hippocampus in the mouse makes a significant impact on the investigation period. EEG with NIRS that can be an effective solution for solving this problem (77). Declarative memories (autobiographical/episodic/semantic), spatial memories, memory formation, memory optimization and navigation in sleep are associated in the networking node, which is placed in the medial temporal lobe in the brain region of the mouse skull called entorhinal cortex. The neuron is divided into a various layer in the Entorhinal cortex as the other cortex (78). The first group of Neurons, which are amongst the predominant recipient of incoming axons and represent the necessary supply of entorhinal output to a variety of cortical and subcortical structures, is called the principle Neuron works as the neurotransmitter. The second group of Neuron, i.e., interneuron that supplies intrinsic local connection works as an inhibitory transmitter. LEA and the MEA are a significant region or the entorhinal cortex (79). Where perforant path closes in the outer one-third of the molecular of the dentate gyrus, LEA is originated from there, and MEA is originated where medial perfortant path terminates in the middle one-third of the atomic layer of the DG as shown in Figure 2.

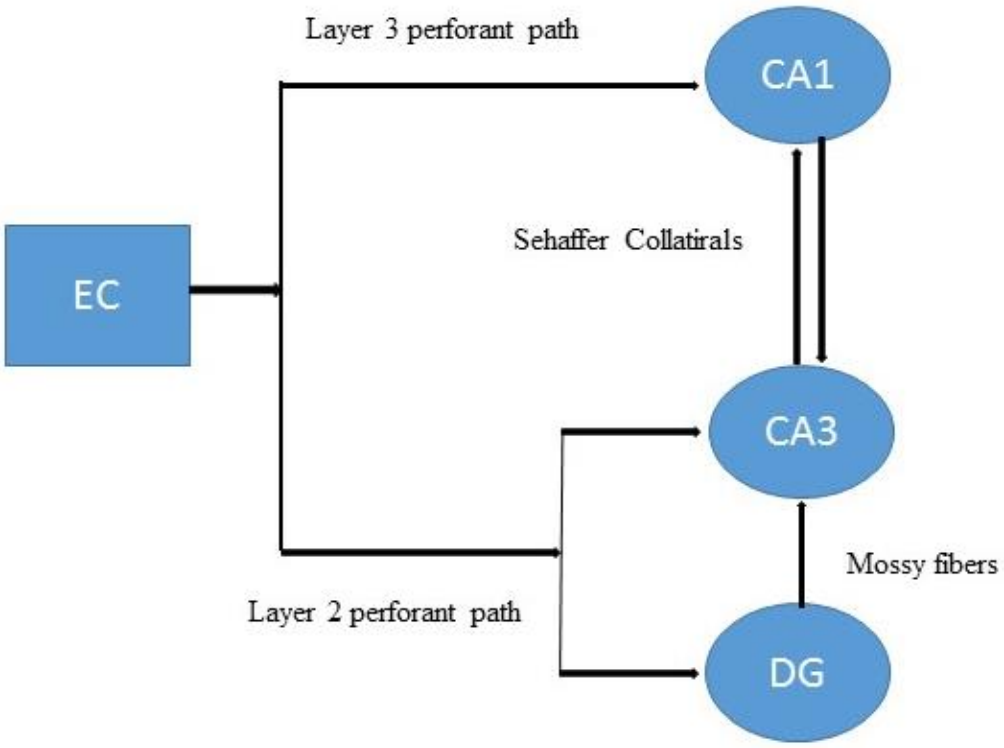

Figure 2. Performant path of EC networking with the mossy fiber synapse; one of the most potent synapses in the brain (80).

3.1.1. EEG and NIRS sensor positioning in the mouse head

Head cap must be placed in a suitable position on the mouse skull to collect satisfied data. There are many landmarks in the mouse skull, where bregma and lambda are the preferable landmarks for reference point. As another technique using the degree of the ear bar works as the reference point (81). But a perfect reference point may be varied animal to animal for their gradation difference of external auditory meatus. However, our experience has taught us that for in-depth monitoring of the brain activity bregma is the most preamble reference point where monitoring success rate increases at least 15% to 20% more (82). Fontanelles are the membrane filled spaces at the meeting point of sutures. Anterior fontanelle called bregma and posterior fontanelle called lambda. The joint position of CS and SS called bregma and intersection of sagittal and lambdoidal sutures called lambda shown in figure 3. The striatum, substantia nigra, hippocampus, dorsal raphe, locus coeruleus, hypothalamus, motor cortex, are situated in the BL (Bregma to Lambda) region (83). Generate the motor cortex controls neural impulses, Sensory guidance of movement, spatial voluntary movement, even though the hypothalamus controls specific metabolic processes, activities of the automatic nervous system. Therefore, most powerful activities are associate in these parts and the distance these part from the BL point is not so far (84).

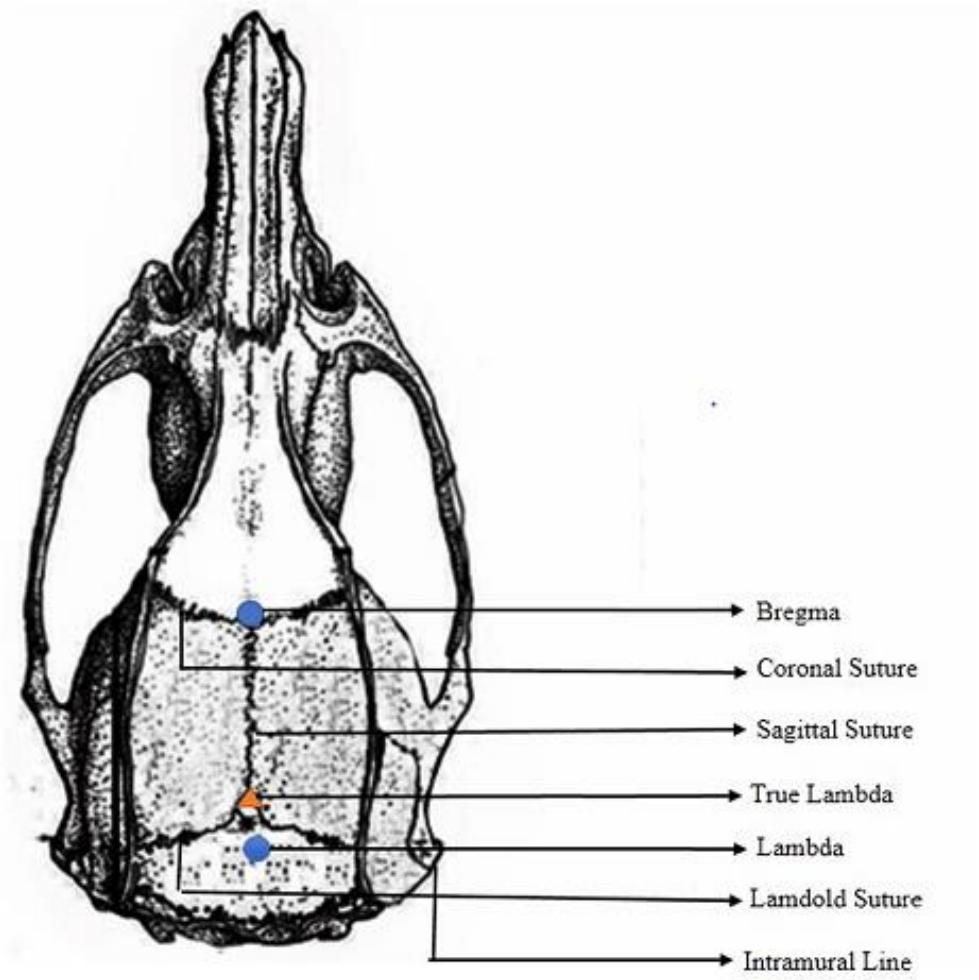

Figure 3. A. Skull surface sutures the most common reference points used are bregma and lambda (85).

NIRS offers a low-cost approach for non-invasive or invasive measurement of modifications in cortical and subcortical activity of the brain. In this process fiber optic wire installs on the suitable region of the mouse brain and it can transmit or capture photons in the target region (86). A significant characteristic of the cortical area or cortical patterning is the establishment of accurate intra-neocortical (INCs) links that serve as the main cortical function network. It is thought that neocortical gene expression will drive the original region and targeting of INCs during preliminary developmental phases before eye-opening (87). After the original patterning, subsequent sensory experience is hypothesized to refine the growth of the visual cortex after eye-opening. The fiber optic cables installed in the target region could be used to monitor and manipulate neuronal activity (88). There was no reported expression of profound brain opsins in the cerebellum. In general, in the caudal brain of teleosts, the number of opsin-expressing neurons in each group is low. The tiny groups of opsin photoreceptors in the caudal brain region may constitute sub-functional participation of photoreceptor activity (89). This strategy would allow correlations and causal relations between regional activity and

physiological or behavioral changes in the animal to be examined by NIRS shown in Figure 4.

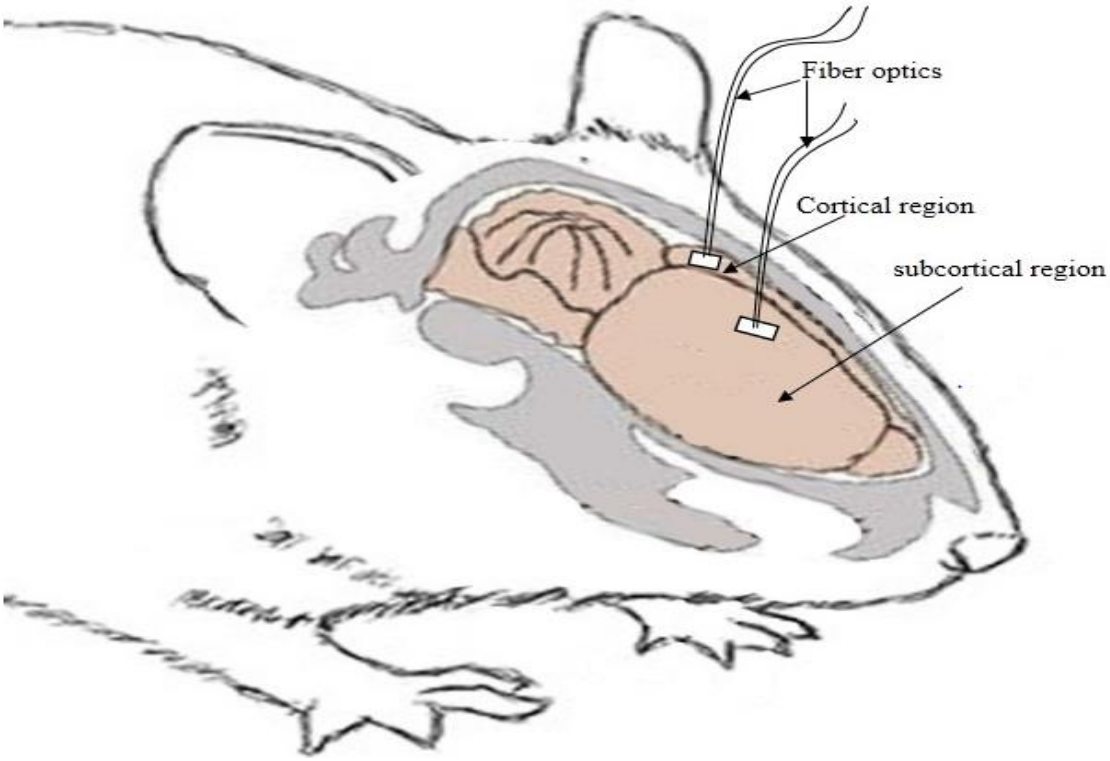

Figure 4. Cortical and subcortical area the most targeting region of the mouse head for installing fiber-optic cable (90).

3.2. Implementation of the hybrid head cap for mouse

Implementation of electrodes in the correct position and hold the electrode on the skull without surgery is challenging for investigation of EEG from the mouse. While electrode head caps make it easier to attach electrodes to the scalp, they may also feel restrictive to participants as they cover the entire scalp and generally have belts fastened across the chin or chest to hold. By ensuring precise positioning of electrodes, maintaining sufficient contact with the scalp, adhering to their proper positions, head cap eradicate mechanical problems with EEG recordings (91). An electrode cap test was performed to assess its technical aspects. The results show that the cap is a significant improvement compared to existing methods in electrode application. Positioning, reproducibility, and recording quality have been highly satisfactory. To reduce artifacts created by operational mistakes such as insufficient electrode cavity fitting and hair disruption removing the electrodes from just the scalp, technicians fitting electrode caps need to have prior experience with the cap placement procedure. The adduction force of the electrode and the ability of the electrode to pass through the hair are the remaining significant effects on the number of operating electrodes of the cap (92). The correct amount of adduction force is applied by the cap's the electrodes make small pressure on the scalp, which improves contact surface, reduces contact impedance, and limits relative movements and

artifacts. Contact pressure interrelationships, optimum contact pressure, substratum hardness, the interfacial impedance will lead to a further rise in the reliability of the dry cap scheme to guarantee mouse comfort. Thus, the usual unpleasant and time-consuming scrubbing of the slap after the paste is applied can be omitted, so no skin preparation is required. After the buttons are filled with paste, the capable electrodes are logged into the cap individually. The method is quick, accurate, and enables to high-density EEG measurement with a minimum of preparation time and subject discomfort (93).

3.3. Technical aspects of the head cap

The head cap is constructed by the double-layered textile fabric with plastic where electrode or optode holder are implanted. The high impedance of the electrode and efficient electrode set up of the cap ensure the reliable and robust experiment where skin preparation is not recommended. By maintaining precise placement of electrodes, adherence to their correct positions, and contact with the scalp, cap assistance eradicates mechanical problems with EEG/NIRS measurements. For faster application, neuroimaging researchers have focused on the theses types of the hybrid cap nowadays. Furthermore, EEG recordings were of comparable quality acquired with individual electrode and caps. In order to reduce artifacts created by operational mistake like as splitting the electrode from the scalp for insufficient of electrodes cavities or disruption of hair caps can provide an ultra-protection.

However, the permeable study demonstrates some technical disadvantage of using cap. Usually, hybrid caps facilitate the attachment of electrodes/optode to the scalp and need to be kept with straps fastened across the neck of the mouse that makes the impact on normal behavior. The light-weighted head cap is suitable for mouse brain study. Selection of electrode and determination of functional brain mapping is the significant technical aspect of the development of the head cap. Materials of the electrode and location of the electrode have to consider at the time of designing the electrode. The electrode should be contained some special characters like hyper-thickness, compact size, high stability, efficiency. The more prominent surface region of the electrode is essential to guarantee proper skin adhesion but low contact resistance. Because of the stable material features, particularly in conjunction with motion, it is hard to obtain stable attachment with the skin. For this specific reason, the non-polarizing electrode can be most preferable. Ag/AgCl ring-type electrode with conductive gel is suitable for full band EEG measurement than disc or cup type electrode. Miniaturized flat and soft features of the Ag/AgCl ring electrode make it more convenient for the user. Quality of skin, ordinary nutrition, averaged head size, and a range of hair lengths are essential for cap testing. For the conventional cap, conductive EEG gel is applied at every electrodeposition by syringe, but the gel is not necessary for the dry cap. An additional or ground electrode is positioned between the recording electrodes in both caps and it provides a secure ground communication between amplifier and user.

Distinct types of signals are recorded by a head cap like as resting and active state (the eye is closed), visually evoked potential (VEP), eye blink artifact under the recommended sampling rate performed by mentionable amplifier and software for data acquisition. In order to the excellent performance of the head cap with minimizing influences attention on the test, the mouse should be placed at room temperature and average air humidity. Performance of the head cap depends on the

number and types of the electrode and production time of the head cap is faster than direct electrode implantation system on the scalp or another invasive system. Before starting the test, the experiment of the supervisor must be aware of setting up the head cap exactly with the mouse head to record a high quality signal.

Specialty:

1) Comfortable Fit and Quick Application: Thin electrode connected with a lightweight rubber holder and Lycra type flexible fabric is the main element for relaxed recordings. The high indomitable and stretchy material is attached gently on the mouse skull for recording the optimal signal. Cap is significantly faster and more comfortable compared to another conventional method due to the unique design of the electrodes (Ag/Ag-Cl material).
2) Optimal solution for high-quality signal: High-quality EEG signal is obtained by Ag/Ag-Cl electrode with minimal noise promoted by the most excellent materials of the latest technology and well -defined production process where shielding technique contribute actively for recording data by connecting any EEG system.
3) Fastening Options: the main part of the cap is attached with the rubber type fabric for matching any head size of the mouse, which allows a free size cap, and this fastening option permits the free movement of the mouse duration recording data.
4) The lifetime of Cap: Without any interruption how, the long-time cap can be used depends on many factors like as maintenance of electrode sensors, design of fabrication, cleaning process and number of cycles for recording data can be considered.
5) Maintenance of electrode: After every time using head cap need to take more care of electrode cause oxidation layer on the electrode damage, the electrode and signal quality is going to bad. To remove oxidation layer of a cotton swab or wooden stick can be used just after fix out from the scalp but any chemical or electrolyte method must not apply because these methods are responsible for degrading the function of the electrode, which reduces the efficiency of head cap.
6) Contra-indication: Any abnormal state or the event of defibrillation, the cap should be disconnected from the head as soon as possible. Cap does not permit to use in Magnetic Resonance Imaging (MRI) scanner or any other high-intensity electromagnetic field. Cap should not be used in any skin injuries like as burns, blisters, wounds from an operation, or any transmissible diseases.
7) Disinfecting Cap: Some disinfectant solution like as Cavicide, Korsolex Extra, or Cidex OPA may be used for an advanced solution. However, keep in mind that the use of extra disinfectant may reduce the lifetime of cap and alcohol must be avoided thought there no mentionable effect on the plastic part of the cap.
8) Side effects: As this proposed model cap is generally used in the non-invasive process, usually there is no mentionable side effect hare. However, need to caution at the time of fixing cap, keep in mind that not too much tight for avoiding a headache. After putting the head, the cap needs to check the normal breathing of the animal is okay or not.

4. FABRICATION OF THE EEG-NIRS HEAD CAP

The size of the mouse head is very small; thus, the placement of the electrodes and optodes is difficult to realize in this small area of the mouse skull. Even though the selection of a suitable electrode is one of the big challenges. Micro size electrode is not available in the market. Sometimes the selection of suitable electrode for the mouse from different types of the electrode is hard that makes our study more challenges.

Our proposed non-invasive combined EEG-NIRS head cap is placed on the mouse scalp without undergoing any surgical processes can solve this problem. The elements, composition, and use of the suggested mouse EEG electrode are described in detail in this chapter. We illustrate the characteristics and functionality operability of the basic components that configure the structural characteristics of the electrode. Subsequently, a presentation is provided on how to use the suggested sensor in animal studies. The suggested sensor comprises of a total of three electrodes and a substratum panel to fix the electrodes as shown in Figure 5. This high conductivity and bio-compatibility plunger contracts with the skin of the mouse's scalp to obtain EEG signals. Gold (Au) plated bronze is selected as the sample material to improve tensile strength conductivity for high-quality EEG signals and to avoid harmful effects on the skin-electrode interface owing to its anti-toxic and non-corrosive properties. The diameter of the probe head is 1.3 mm so that it can contain a ring electrode and cover all suitable area. Each tetra-spiked probe head shape not only improves vulnerability through an expanded contact region but also disseminates the pain reduction force when the probes are positioned firmly on the underside of the scalp (94).To keep the suitable amount of electrode-skin contact impedance, a sufficiently big spring force is desirable. The high carbon steel spring is positioned inside each plunger and force of the spring proportionally increases with the spring distance (2 mm) which feedback force is 20g. The pre-loaded spring allows a certain quality of strength before the contact between electrode and scalp. The innerspring associated with the plunger is perfectly fixed, pulling back after being positioned on the scalp (95). The barrel retains the plunger and promotes it. Since it deserves to be sufficiently reliable to bear the stress during most of the electrode-skin interaction, a barrel of nickel-plated beryllium-copper (1% Be–Cu) was used. Moreover, with its elevated electrical conductivity, the barrel enables the signal obtained by the plunger to pass through it. Finally, the ring-type electrode is installed smoothly inside the plunger-spring-barrel embedded in the probe head. Each electrode is connected to the wire for EEG signal acquisition with a touch-proof connector. The probe-fixing substratum has a total of four holes, and electrodes attach with that hole according to the 10/20 international system (96). For separating the obtained signals from the different electrode, the non-conducting plastic substrate acts as an insulator was used. Each electrode operates at the specified place as a single channel. The positionable electrode-a1, a2, and b are placed on the substratum cross ponding by the 10/20 international system where a=f3, b=f4, c=cz, an electrode positioned on the P4 works as a ground electrode which is shown in figure 5(c). The distance one electrode to another electrode is: a-b=2.4mm, b-c=2mm, c-a=2mm (97).

From the brain anatomy study of the mouse, a most important region of the brain like as striatum, substantia nigra, hippocampus, dorsal raphe, locus coeruleus, hypothalamus, motor cortex, is situated in the standard position from the BL (Bregma to Lambda) reference point. Generate neural impulses, Sensory guidance of

movement, spatial voluntary movement are controlled by the motor cortex, even though certain metabolic processes, activities of the automatic nervous system are controlled by the hypothalamus. So, most powerful activities are associate in these parts and the distance these part from the BL point is not so far (9.0 mm). Two detector fibers (D1, D2) were linked and positioned between the bregma and lambda points at the midpoint (CZ-FZ) to accumulate diffuse photons, enhancing the signal-to-noise ratio (SNR).

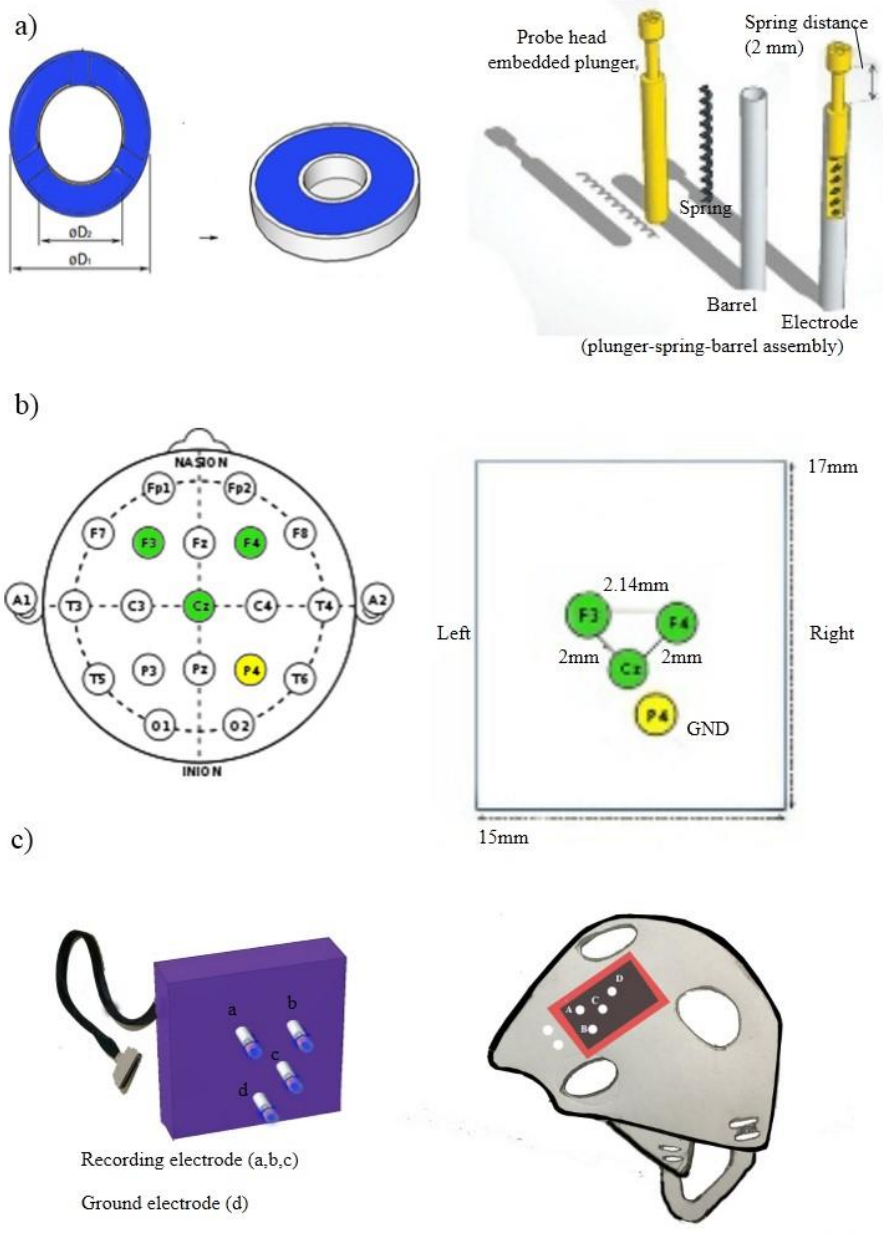

Figure 5. Mouse electroencephalography sensor description: (a) Front view and side view of the ring-type electrode, (b) probe head embedded plunger, spring, and barrel, (b) a schematic design of the proposed mouse EEG sensor (c) electrode placement on the sensor according to 10/20 international system. (d) Side and frontal view of the manufactured sensor with the highlighted of electrode: ground electrode (GND), one reference electrode, and recording electrodes.

4.1. Considerations in the head cap design

My aim as a designer is to develop the hybrid head-cap for both the investigator and the subject to enhance the experience of an EEG and NIRS measurement. The difficulties facing us in this development were to build an ergonomic fit for all topics, the researcher's flexibility to measure any region of the brain, to manage the weight of our fiber, specially designed for optode/electrode holder (classic, pinch, NIRS/EEG, ring electrode holder, etc.). From a technical view, a question may come which part of the brain is more important to study. The answer might be interested in every brain region. That is why I think the cap should cover the whole scalp. It seems apparent, but I have seen a lot of placement of EEG electrode network connection that forgets to include the prefrontal cortex, for example. The frontal outline of the cap finishes slightly above the eyebrows, as shown in figure 6(a) while the back of the head-cap covers the entire visual cortex.

Moreover, each head shape is distinctive. There are also so many comparable features, however, that I use as a designer. For example, the overall shape is created by the relationship between the size of the head circumference, the distance eye to eye and the ear to the ear. An equivalent quantity of stress is generated by the sweeping curve on the neck, the flat region on the side of the head, or the tiny bump (inion) on the back of the head. During a measurement, we used the space below D-ring type chin to secure the head-cap and restrict artifacts of motion. Our design in Figures 6 shows that we used the features to shape the head-cap and create a proper fit for each while the cap covers all areas of concern in the brain. To produce a satisfactory cap neoprene fabric is selected from multiple structures that have proven to be the most appropriate material after numerous iterations of prototypes. To prevent electrolyte solution diffusion high effectiveness of the silicone frame is adjusted in the graphical linear part for the brain research, and our developed EEG sensor is surrounded by it.

Optode holder for optic fiber is fixed with the cap in front of the EEG sensor. We used D-ring to connect the chain strap to the head cap, which ensures the free size of the cap and modular/exchangeable comfort soft sleeve over the chain-strap is used for relaxation.

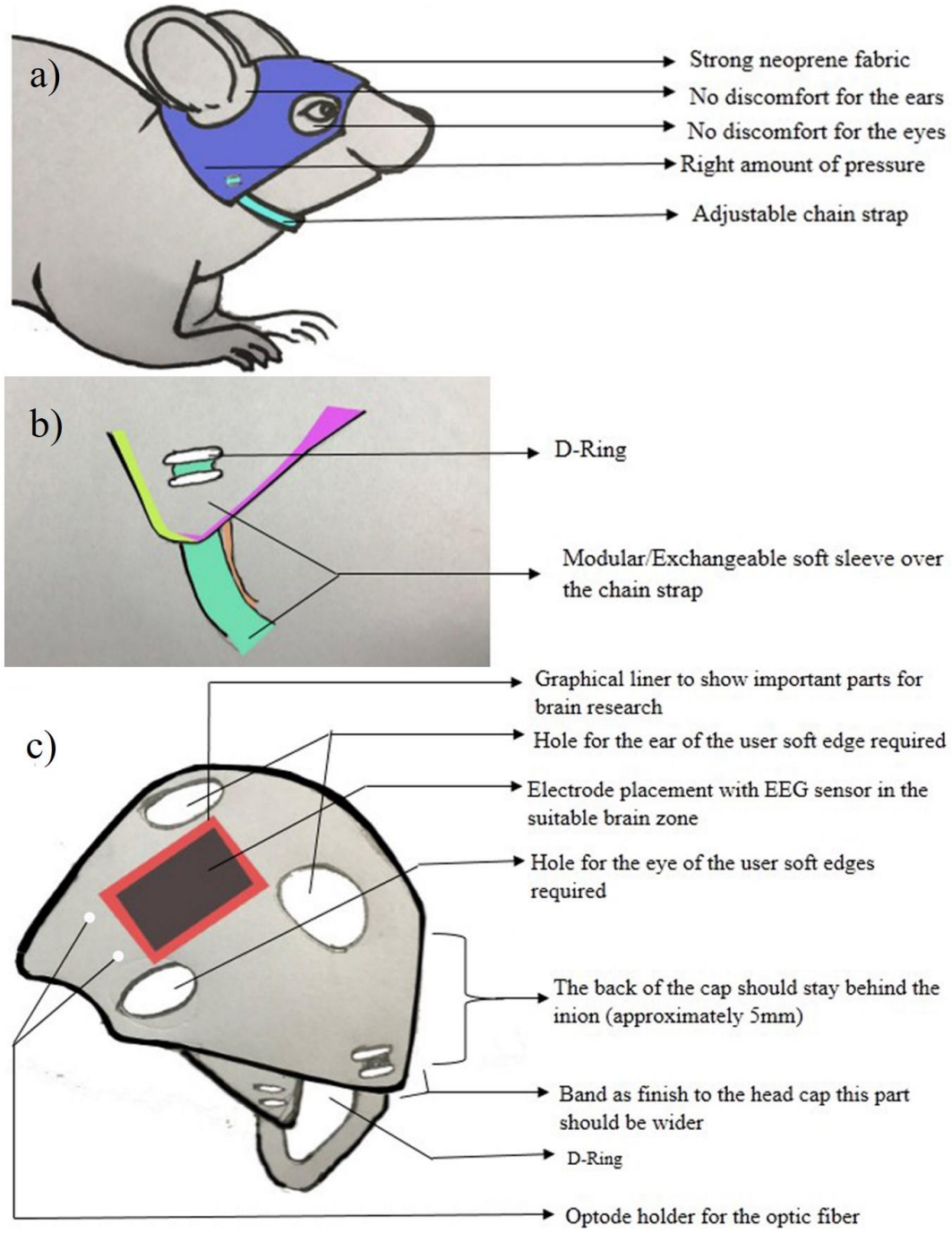

Figure 6. Implementation of the hybrid head cap. (a) Head-cap design features, (b) Graphical line for the suitable position of the placement of EEG and NIRS (c) Exchangeable Soft sleeve over the chain strap connected with D-ring (d) commercially designed hybrid head cap using EEG electrode and NIRS optode corresponding brain structures.

The multiple electrodes are fixed on the flat insulated plate. As the material of the insulated plate, we have used the non-conducting material polytetrafluoroethylene-Teflon (Teflon™/PTFE) that serves as an insulator so that the substrate plate (insulated plate) separates the acquired signals from the electrode. PTFE has a very excellent chemical strength, is a material that repels other materials, is easy to

fabricate, and has the smallest known metal and metal friction coefficient. Fluorine resins have a unique position among plastics because of their elevated and low temperatures, climate, chemicals, solvent strength, and high melting point. We have used Epilog laser fusion M2 40 Laser for cutting the PTFE plate. In addition, we made the hole (diameter-2mm) on the PTFE plate according to our design that works as the substrate plate shown in Figure: 7(a). We used ribbon cable which one side is shouldering with the electrode another is connected to the connector that can make a proper connection with our 3-channel EEG amplifier that shown in the Figure: 7(b).

The holder has a minimized distance from the transmitter to receiver, leading in a + /-10 mm inter-optode range. These short-separation channels measure the extra-cerebral signal that subtracts the normal channels ' hemodynamic reaction signal. A sample of short channel optode holder was developed in our laboratory. All holder parts are produced of inert materials that provide high resistance to most chemicals. Teflon is well known for its resistance to chemicals. Butyl rubber has excellent resistance to many harsh chemicals, including hydrazine, methanol, and fluoride from hydrogen, acetone, and nitric acid. The sample holder for the thin film sensor material consists of the following parts: two-part Teflon housing (substrate) and an O-ring for butyl rubber. The Teflon two-part house has been intended as follows. The housing was grooved for feedthrough feed and thin-film positioning. The sample port was then designed with an O-ring of butyl rubber, giving a sample region of roughly 7.5 cm2. Using LOCTITE- super glue, the two-part Teflon housing is connected. The thin film sample is positioned in the sample holder in the present embodiment, the wire leads are positioned in the wire groove, and the two-part housing is attached using the super glue LOCTITE. The holder of the sample is then connected to a sample chamber with a receptacle that matches the holder's sample port. Any analytic of concern may fill the sample chamber. The chamber atmosphere can be altered at any moment, allowing a broad range of environmental circumstances to be sampled (multiple combinations of temperature/humidity/ analytic).

The suitable sized panel of fabric was sectionized from neoprene fabric sheet by computerized laser beam fabric cutter for the fabrication of head cap in the Fablab. This cutter can be cut higher than the manual cutter that is controlled by the computer system. We have loaded the cutter disc and positioned the laser on the fabric in the right position in the computer.

Placement of EEG electrode and NIRS optode holder on the fabric is the most essential part of the head cap. Holes are pre-punched by Grommet Eyelet Setter Plier, Hole Punch Tool with 100 Silver Metal Eyelets Grommets on the fabric for placement of EEG electrode and NIRS optode holder. Then our fabricated EEG sensor and optode holder are fixed with fabric in the exact point. D- Ring is connected with the chain strap fixed with the backside of the head cap that makes our head capsize free. It is comprised of a hybrid head cap (housing the optical and EEG components), cabling, and a control unit.

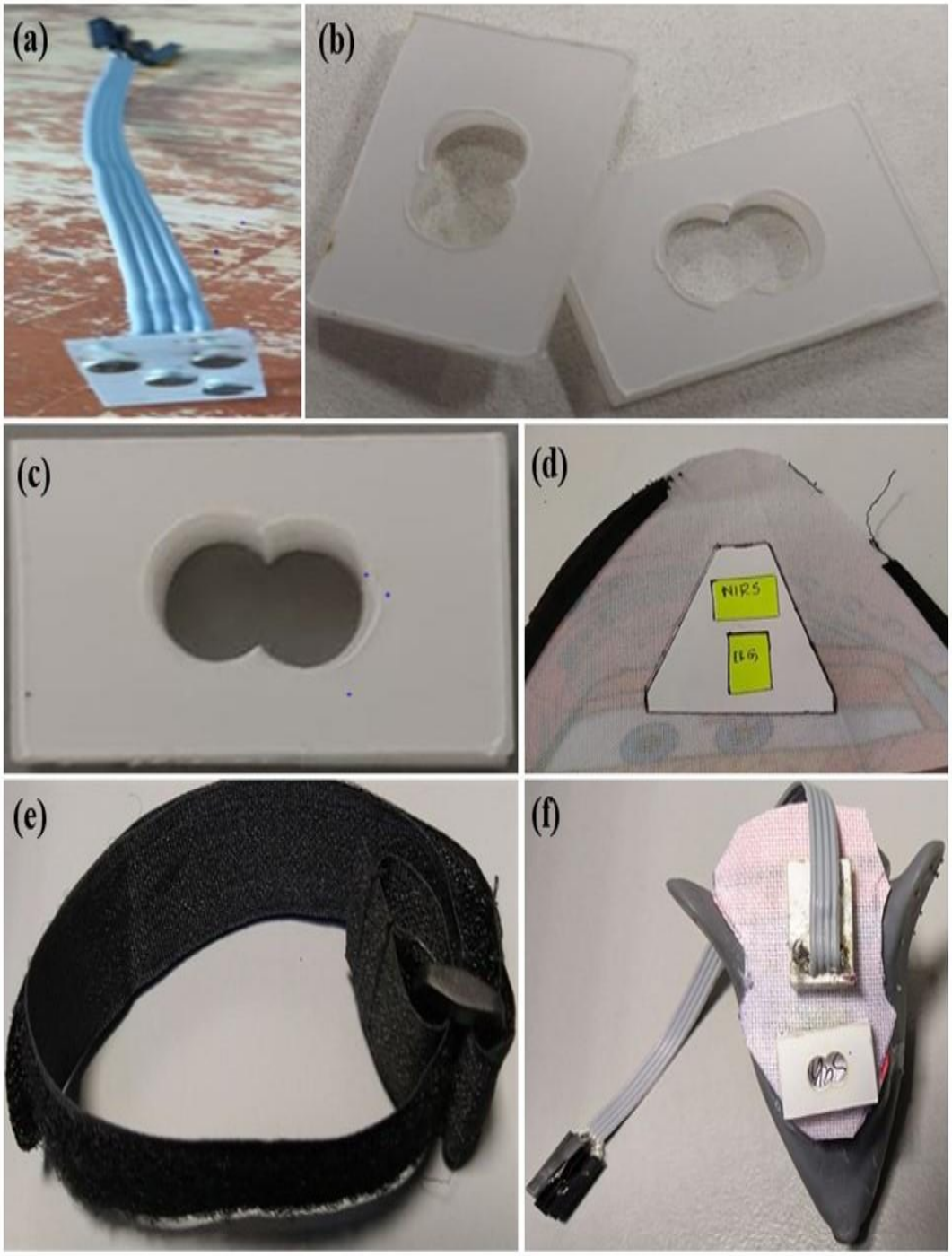

Figure 7. 3d printed EEG/NIRS headset system, (a) 3d printed multi-channel EEG sensors, (c) two thin Teflon film cutting by laser cutter, (d) optode holder: two thin-film were intended with an O-ring of butyl rubber attached by super glue, (e) neoprene fabric sheet sectionized by laser beam fabric cutter, (f) soft chain strap with D-ring (g) portable hybrid cap.

5. DISCUSSION

In this thesis, it was provided the first version of the design and fabrication of EEG/NIRS mouse head cap with multi-channel, ring-type, and non-invasive characteristics.

The thickness, elasticity, and impedance level of skin layer characteristics affect noninvasive EEG technique. The epidermal layer of the mouse skin is comparatively dense where dermis contains many hair follicles at ~4–5 mm−2. At the moment of our inquiry, we observed that to improve EEG and electrode technology, the majority of study apps still require low concentrations of skin impedance: optimum signal quality makes detection of artifacts simpler, improves in data reliability, and smaller groups can be retained to achieve important distinctions. Due to the size of the brain, arranging various sensor on the mouse brain is very difficult. Building fiber holders and applying dental cement to the fixation sensors were critical considerations for effective of various detectors on the mouse brain. Light mixed into one fiber and aligning fibers generally take most of the preparation moment and using the fiber holder with manipulation feature decreased the installation time dramatically. The dental cement also fixed the tips of the fiber and preserved a coherent skull interface. At the time of our investigation, we have felt the shortage of appropriate sized electrode, and Mouse EEG sensor is not available in the market. We have selected a ring-type electrode that covers the more area and developed an EEG sensor according to our personalized designed to collect adequate information.

Non-toxic gold is used in our investigation to produce the sensor's electrode characteristics. Gold is an outstanding heat and electric conductor, and it is not affected by air, water, alkalis and any substances other than aqua regia (a mixture of hydrochloric acid and nitric acid). We have chosen a ring-type electrode considering some distinctive feature as being simple to install around current facilities, obtaining low hardware component, achieving low resistance and accessibility of hardware element. If we attempt to concentrate on stimulation via concentrated ring electrodes verse via conventional electrodes, it can be observed that TcES via concentrated ring electrodes did not cause noticeable contractions in rats, whereas TES caused powerful tonic contractions via conventional disk electrodes. One potential explanation is that the pattern of stimulation of Ring electrodes is much more concentrated, while stimulation via disk electrodes activates significant tissue quantities. The proposed electrode feature is not possible to acquire the EEG signals without conductive gel.

The scalp of the mouse brain is small. Placement of electrode and NIRS optode in this small region is complicated, and distance from one electrode to another electrode is not so far. Sometimes recording signals from different electrodes are interfaced and create artifacts that makes an impact of the final result. In our proposed head cap electrode and optode are holding with fabric and fabric works as an insulator, and there no possibilities to interface the signal. Our head cap covers the whole head gently that reduce the motion artifact.

5.1. Areas of future research

The next step of the EEG technology is to measure brain activity across the whole lifecycle of the animal which may promote initial diagnoses of neurological disorder. (98). As hardware costs reduce, the use of multiple sensors in original research will become more affordable, to both deepen knowledge, but also to cross-validate experiments (99). As an example, we can consider a new type EEG sensor that has wearable physiological signal amplifier system for multi-channel of high-fidelity wireless EEG data with 3D accelerometer and trigger channel where water electrode will be used, and they are not sensitive for movement, blood flow and light artifacts (100). The use of more open-access big data projects and faster analytical capacities will, therefore, probably be seen by EEG studies. With a solid and rigorous basis, the large-scale multi-sensor study will provide the next wave of findings in psychology and behavioral science (101). The multichannel NIRS system is developed in the recent year for monitoring the brain diseases and improving the accuracy of the image with potential applicability for future clinical application (102). Recently, various study groups have been able to miniaturize NIRS imaging and create a wireless sensor. Such miniaturized NIRS systems will make a significant contribution not only to neuroscience research but also to tissue oxygenation monitoring, which was the original objective of NIRS development (103). Thus, NIRS will demonstrate excellent promise to provide a unique direction for functional mapping research that could not be achieved by other neuroimaging methods.

6. CONCLUSION

A lot of study expenditure on neuroscience and released results correspond to studies in mice. These findings are usually submitted across species as universally representative of brain function. Often, these studies were based on analyzing changes in electrical neuronal activity. More lately, methods were created and implemented based on measuring hemodynamic or metabolic modifications. EEG and NIRS are complementary, as they enable the concurrent study of the neuronal and hemodynamic elements of brain activation with elevated temporal resolution. However, the mouse's tiny size brain presents a serious restriction in EEG/NIRS spatial data. In this thesis work was designed a ring-type microelectrode to overcome this restriction and created a compact head cap with manufacturing techniques. The EEG head cap includes 4 electrodes, weighs 100 mg. Our demonstrated 3D printed hybrid head cap aims to capture signal in both the neuronal and hemodynamic dynamics by monitoring functional mapping of the mouse brain.

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8. APPENDICES

Appendix 1. Different types of software for analyzing data

Software	**Performance**
Matlab® software (MathWorks, Inc., MA, USA) with Fourier transform (FT)	Analyzing the EEG recorded data to measure the system
BCI2000 software (Schalk lab, Albany, NY, USA) and g.USBamp (g.tec, Schiedlberg, Austria)	Measuring the impedance (1 M-ohm for all mice) to check the adherence between electrodes and scalp.
USBamp (g.tec, Schiedlberg, Austria)	Measuring the impedance (1 M-ohm for all mice) to check the adherence between electrodes and scalp.
Mitsar-EEG 202-24 (MITSAR, Sankt-Peterburg, Russia) amplifier, EEGStudio EEG acquisition software (MITSAR, Sankt-Peterburg, Russia), and Photo Stimulator (MITSAR, Sankt-Peterburg, Russia)	VEP Recording