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PROJECT REPORT

"Feasibility of DREADDs in Non Human Primates"

by

Leafy Behera
SURGE Roll no: 2142500
Department of Biological Sciences
Indian Institute of Science Education and Research, Kolkata

under guidance of:

Dr.Arjun Ramakrishnan
Assistant Professor
Department of Biological Sciences and Bioengineering
Indian Institute of Technology,Kanpur

CERTIFICATE

This is to certify that the project entitled "**Feasibility of DREADDs in Non Human Primates**"submitted by **Leafy Behera**(2142500) as a part of Summer Undergraduate Research and Graduate Excellence 2021 offered by Indian Institute of Technology, Kanpur,is a bonafied record of the work done by her under my guidance and supervision at the Indian Institute of Technology ,Kanpur from 14th June to 31st August,2021.

Dr.Arjun Ramakrishnan
Assistant Professor
Department of Biological Sciences and Bioengineering
Indian Institute of Technology,Kanpur

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Abstract

Current literature in neuroscience is heavily influenced by correlational studies, where we often compare our observational data against association based models.However in systems with complex dynamics like human brain, it is very hard to understand the interactions using correlational measures.Such situations where things get complicated,causational studies are needed to be done that involves direct perturbations of neural correlates which are known to be involved in generating the corresponding behaviour.In the current scenario where mental health has become a primary issue in the population, where one in seven people from India have suffered from mental illnesses ranging from depression,anxiety to severe conditions like schizophrenia, it's really important to shift to methods that involves targeted manipulation of the system to better understand the problem in hand.
Taking into account the current situation,improvements have been made on the technological front that involves selective modulation of circuits using chemo-genetic techniques like designer receptors exclusively activated by designer drugs(DREADDs), thereby allowing remote control of neuronal functions by drug administration.Literature aiding DREADDs only focuses on small laboratory animals like mice and rodents, whereas its effect on complex animal models like non-human primates (NHP) are either minimal or non-existent. To support the scientific community using/planning to use DREADDs as a tool on NHP models, we intend to come up with a centralised database listing all attempts of DREADDs on primates. The database is focused on use of chemo-genetics on non-human primates,hence all results related to application of chemo-genetics in primates are included. This collection is going to be a valuable resource showcasing the expression of receptors on the cell surface as well as the efficiency at using different drugs to activate those designer receptors followed by behavioral tests to gauge the outcome of the perturbations.

Contents

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List of Abbreviations

DREADDs - Designer Receptors Exclusively Activated by Designer Drugs
NHP - Non Human Primates
tACS - Transcranial Alternating Current Stimulation
tDCS - Transcranial Direct Current Stimulation
fMRI - Functional Magnetic Resonance Imaging
PET - Positron Emission Tomography
WHO - World Health Organisation
MDD - Major Depressive Disorder
tACS - Transcranial Alternate Current Stimulation
tDCS - Transcranial Direct Current Stimulation
hmDREADDs - Human muscarine DREADD
KORD - Human k-opiod receptor DREADD
SalB - Salvinorin B
CNO - Clozapine-N- oxide

Chapter 1

1 Introduction

1.1 Mental Health Crisis

Increasing globalisation and automation in today's fast paced world has tremendously transformed our experience with the reality.Technological advancements and increase in digital information surge has improved our lives optimising mundane activities, albeit it has a devastating effect on the health of the population. Still when it comes to physical health people are very concious and aware, whereas mental health is given the least importance.According to the World Health Organisation(WHO), 7.5 % Indian population suffers from some form of mental health condition. Mental health constitutes of one-sixth of the health related disorders while 15 % of the global mental health, neurological and substance abuse burden is born by India alone.

But what exactly causes mental illness? And who are most likely to get it? Although the exact answers are unknown, increasing research in the field has managed to decipher various factors in combination of biology and environment which could be a potential reason for the upsurge in cases. With the emergence of neuroscience as a booming field of research,various new technologies and strategies have been developed to address this global issue.Development of cutting edge technologies and state-of-the art strategies can help us in understanding the underlying molecular factors behind mental health disorders as well as where and how to focus research and treatment.Collaborations between various domains of biology with significant contributions on multiple levels could notably help in understanding brain-development which in turn could give us a deeper insight into mental disorders.

1.2 Current problems with neuroscience

Neuroscience at present is heavily influenced by correlational studies (popularly used in psychology), which involves comparision of two observable values to predict the outcome of one based on other. In correlational studies,one can formulate a relationship between two variables but we can never correctly state that changing one variable will always change the other, i.e we cannot confine them to a cause-and-effect relationship.To address these problems we need to change our strategies and implement causational methods to better understand the inner working of complex systems like human brain.

To subdue the issues with traditional methods, neuroscientists have been using perturbation techniques for targeted manipulation of regions of interest. Brain lesions as a result of injury or due to some disease could give deeper insights about the actual functions of the area. To take this method forward, technological advances in the field has revolutionised the whole process of dealing with psychological and psychiatric diseases. The introduction of new chemogenetic (DREADDs) and optogenetic techniques in the recent years, has helped to evince the circuitry underlying different behaviours in animals models. The power of these technologies lies in the fact that they allow us to manipulate specific neurons to determine causal relationships between the brain and behaviour. Applications in understanding the neural circuitry underlying psychological issues could be the next big step in the field, and techniques like chemogenetics could offer unique advantages of probing long term effects of manipulating neural circuits.

Chapter 2

2 Animal models in neuroscience

In biology animal models are widely used to understand human diseases. Evolutionary lineage and easy manipulations are the primary reasons why they are frequently selected for experimental disease research. Mouse models are one of the most common systems used to study behaviour and diseases, which has revolutionised the whole medical field with the development of state-of-the art treatments for most baffling diseases found in the population.
When it comes to brain disorders, most mouse models fail in humans due to the large gap between the complexity of the brains. To develop treatments that could make an impact on human brain disorders, we need a different model which at least shares a homology with human brain and it's architecture. NHP models solve the problem of most experimental neuroscientist, giving them a brain template to manipulate, making assumptions about human brain. Non human primates are closest to humans sharing a common central nervous system and neural circuitry, which gives them an edge over other other animal models. This similarity helps us to work on treatments or test behaviour that could have work out for humans in curing and treating all sorts of diseases. NHPs are going to be an integral part of neuroscience research for decades to come, but they come with a cost. Closer we get to humans in the evolutionary lineage, greater the ethical concern. It is the responsibility of the scientific community to choose the right model to answer their questions, because some questions can be answered using simple existing model systems. Such thoughtful experimentations could avoid unnecessary sacrifices of life in the name of research.

Chapter 3

3 Tools of the trade

3.1 Imaging Techniques

3.1.1 fMRI

Fucntional magnetic resonance imaging (fMRI) is one of the most powerful technologies used to examine living brain and its activity by detecting changes associated with blood flow. Primarily fMRI uses BOLD(blood oxygen level dependent) signals to identify brain regions functioning during a particular task. The non invasive nature of this method increases it's preference in neuro-scientific studies. The concept is built up on the earlier MRI scanning technologies and oxygen rich blood. The firing of neurons in response to a stimulus is followed by an increase in the metabolic rate of the neurons, oxygenated blood displaces de-oxygenated blood in few seconds. The magnetic properties of blood haemoglobin molecules generate an MR signal which is mapped to visualise which neurons were active at a time.

3.1.2 PET Scan

Positron Emission Tomography (PET) is another imaging technique that used radiotracers to visualise defective cells in a particular region of the body. This technique can be used as an indirect measure of the changes in the oxygen and glucose levels in the brain. Radioactive markers like *oxygen-15* and *18F-FDG* are used to detect the changes in the level of oxygen and glucose in certain brain disorders like Alzheimer's.

3.2 Neural Stimulation

3.2.1 Deep Brain Stimulation

Invasive neural stimulation techniques involves electrode implantation at specific brain regions. These implants generate electrical impulses to adjust the chemical imbalance in the brain, controlling the abnormality. These implant generated stimulation techniques are generally used to treat motor disorders the likes of which is observed in Parkinson's disease. This method involves surgical procedure to treat the brain abnormality, which makes it one of the last options to be considered when all medications fail.

3.2.2 Transcranial Magnetic Stimulation

Transcranial Magnetic Stimulation (TMS) is one of the most popular magnetic therapies used to treat conditions like major depressive disorders(MDDs). This non-invasive therapy uses changing magnetic field to induce electric currents in specific areas of the brain. Repetitive transcranial magnetic stimulations (rTMS) has shown therapeutic benefits in mental disorders and has the potential to treat treatment resistant diseases.

3.3 Transcranial Alternate(direct) Stimulation (tACS/tDCS)

Transcranial alternate/direct current stimulation (tACS/tDCS) has recently become a popular choice to treat neuropsychiatric disorders like MDD. This non invasive technique interacts with various brain rhythms, synchronising or desynchronising the cortical oscillations. Recent applications of this technique has been very successful in improvement of anxiety and mood related disorders. However future large scale clinical trials are highly recommended to understand the full potential of this method.

3.3.1 Technologies for neuron selective stimulation

3.3.2 Optogenetics

Optogentics is a new kid in the block! This invasive biological technique involves using light to control neurons those have been genetically modified to express light sensitive ion channels. This technology has made a powerful impact in brain research, allowing the researchers to control the activities of specific neurons in living tissue. Such genetic perturbations controlling neuronal activity could be used in causational studies to better understand the neural architecture.

3.3.3 DREADDs

Just like optogenetics, chemogenetics a.k.a DREADDS is also a new perturbation technique which has recently become a popular approach in the field. This causational studies mediated by DREADDs succeeds DBS and TMS to understand the relationship between brain activity and behaviour. Both these techniques show similarity in approach to modify neural activity with spatial precision. Unlike optogenetics, chemogenetics is a non-invasive technique, accomplished by administering ligands to activate designed receptors without which they are inert in general.

Chapter 4

4 DREADDs

4.1 What are DREADDs?

Neurological disorders like depression, anxiety and mood alterations are the most prevalent issues suffered by the population currently. To address these issues we need to conduct studies that could provide remote control of neural circuits with high spatial precision. Technologies like DREADDs that use cellular signalling to control neuronal circuits has gained wide utility in the past decade.
DREADDs are designer G-protein coupled receptors expressed on the cell surface that can be activated only by inert ligands which in general don't have any off side effects. They provide broad control on full circuits and tissue wide systems. To use the method to its full potential we need to understand the system's component parts and their applications. Identifying the type of intervention, i.e activation, suppression or bidirectional control is crucial for the selection of the appropriate DREADD. Currently used DREADDs are modified human muscarine acetylcholine receptors (hmDREADDs) that either inhibit or excite neuronal firing in the presence of the inert ligand CNO. hmDREADDs have been extensively used for unideirectional control of brain activity, but to achieve bidirectional control, researchers have developed a new type of DREADD called KORD (human k-opioid receptor DREADD). KORD provides bidirectional control over neuronal circuits and is activated in presence of inert SalB. While considering DREADD systems for therapeutic relevant studies it is important to select proper transactivators that can exhibit strong signalling activity during the protein-ligand docking. this would result inactivation of various downstream signalling pathways aiding proper activation.

4.2 Advantages of chemogenetics

Chemogenetics has gained it's popularity in the recent decade, with the wide spread use of designer receptors to remotely control neuronal circuits. It is a huge technological improvement compared to previous lesion and implantation based studies which were irreversible and costly respectively. Chemogenetics allows modulations of neural activity through neuro-transmitter receptors those are designed to bind with specific inert ligands. This technique provides high spatial accuracy by injecting genetically modified receptors to specific regions of the brain surgically, lacking the complications generated during implant surgery. The emergence of optogenetics was a huge success in the last decade, it has revolutionised the study of brain giving researches an opportunity to manipulate the neurons in awake moving animals. Although the instant effects of the technique provides a good temporal resolutions, but the therapeutic effects are not well established which can be made possible using DREADDs. DREADDs activation heavily relies on availability of the inert ligands and systemic circulation for their transfer. Compared to tradition DBS and optogenetics which involves invasion into the skull, chemogenetics could have an upper hand due to it's remote nature. The use of G-protein coupled receptors, which is one of the most commonly found receptors in the human body may prove to be a tool for therapeutic studies. Like any new technique, DREADDs approach also has a few limitations which are important to highlight for better future implementations. Since the transmission relies on systemic circulation, there are very high chances of off target effects which could be related to unwanted retrograde viral spread or designer drug chosen for the therapy. Even though, applications of DREADDs on higher complex animals is very few, studies are still being conducted to address these limitations and embrace this new technique which hold immense potential to used for therapeutic purpose.

4.3 Development of NHP database

4.3.1 Introduction

Chemogenetics is a relatively new technique which has gained its popularity in the recent decade. This technique provides high spatial resolutions using inert drugs and designer receptors, aiding precise control of neuronal circuits. This method has potential to come very useful in understanding brain disorders and development of therapeutic treatments. However currently there is no use of chemogenetic techniques for human therapies or clinical trials, but to get an approval for extending the studies to human subjects, necessary experiments are needed to be done on higher animals like non human primates to assess its feasibility. Therefore, this review is focused on addressing the research question underlying above mentioned concerns and assist future experiments involving application of DREADDs on NHP.

4.3.2 Methods

The application of DREADDs has boomed over the recent years with numerous publications in both rodents and monkey models (Figure 1) with most studies focusing on memory, reward learning and fear. A search through PubMed resulted in a total of 57 results. While 16 of these were review articles focusing on application of DREADDs in neuroscience using different models, 8 articles focused on different ligands and their applicability in non human primates. Only 14 articles were filtered out to be added to the database. Other remaining articles were rejected based on their abstract and title. The primary aim of this exercise was to filter out articles which had information about robust experiments conducted on NHP model.

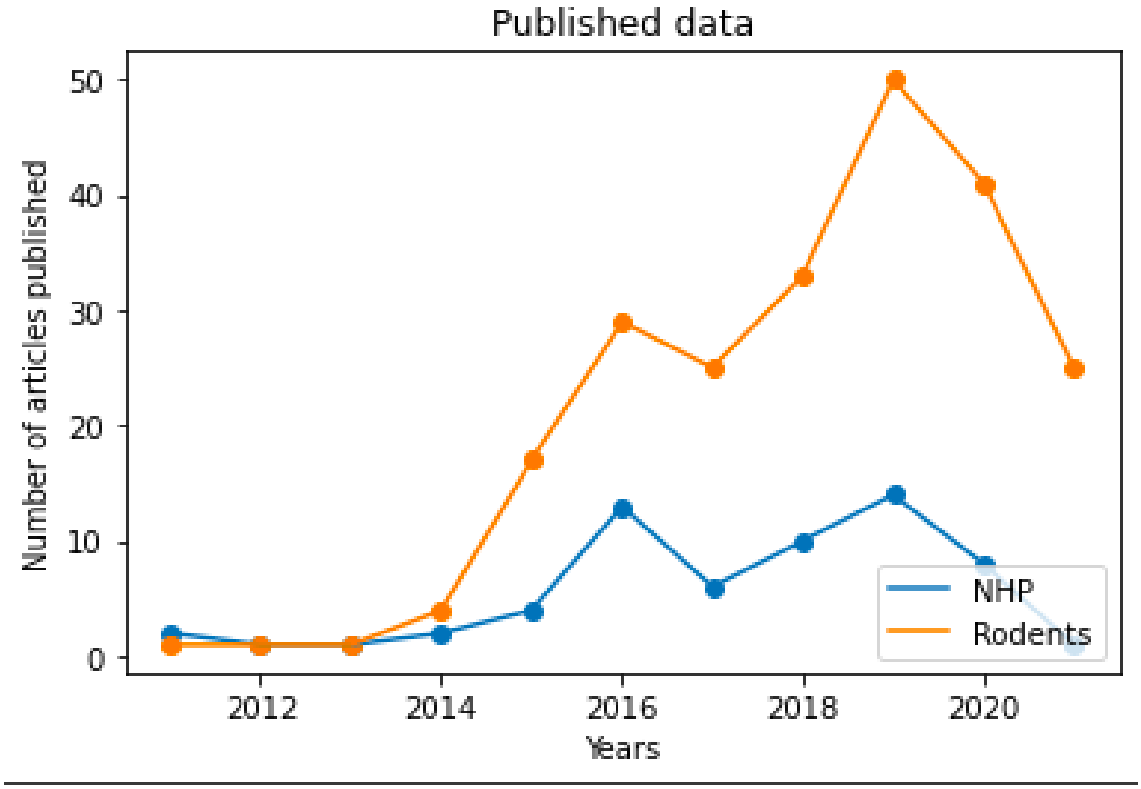

Figure 1: Number of published paper

4.3.3 Results

Statistics: We entered information obtained from all 14 published articles (Table 1). As of august 2021, 335 viral vector injections were included in the database. Each entry in the database was a single injection of viral vector. Multiple injections could have been given to a single animal, and each injection was considered as a separate entry.Injections given at multiple depths using the same needle penetration were considered part of a single entry unless they targeted multiple regions through the same track.

Table 1

Viral Vector	N	Promoter	N	Ligand	N	Reporter	N
Lenti	127	hSyn	276	CNO	128	CFP	125
AAV2	48	CMV	38	CNO/	114	mCherry	101
AAV1	38	CAg	6	DCZ/PBS	45	GFP	49
AAV5	34	CaMKII	2	DCZ	24	mKO	12
AAV8	19			DCZ&CNO/	7	AcGFP	12
AAV7	18			Not Used	15	mCitrine	8
AAV6	18					EYFP	3
rAAV2	13					HA	1
AAV	5					Fluroscent	1
AAV9	4					Not used	22
FuG	2						
AAV4	2						
AAV3	2						
AAV10	1						
AAV12	1						
AAV11	1						

Figure 2: Table 1: Compressed contents of the database

Current database includes experiments done on 4 different NHP species (Figure 3). Most of the experiments were performed on rhesus macaques (88.8%), rest were conducted on japanese macaques (7.79%), marmoset (2.1%) and cynomolgus (1.2%).

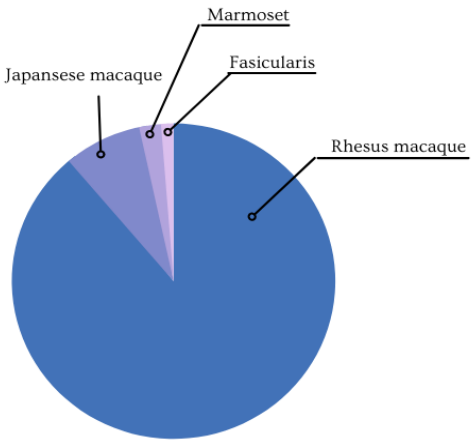

Figure 3: Proportion of species used across all experiments

Lentivirus was mostly used as a viral vector for the delivery of the gene which accounted for (38.1%)(Figure 4). Adeno-associated virus (AAV) were the next popular viral vectors of which the AAV2 serotype was most commonly used (14.4%) followed by AAV1(11.4%).

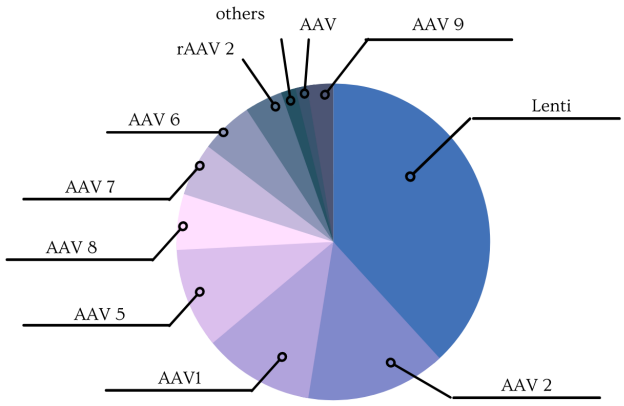

Figure 4: Proportion of viral vector used across all experiments

Commonly used promoters was human synapsin neuron-specific promoter (Figure 5) (hSyn;85.7%), followed by cytomegalovirus (CMV;11.8%), chicken beta-actin promoter (CAG;1.9%) and Calmodulin-dependent protein Kinase II (CaMKII;0.6%).

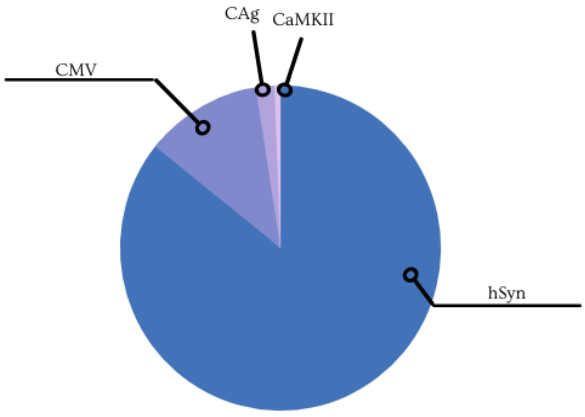

Figure 5: Proportion of promoter used across all experiments

To test the efficacy and spread of the viral vector spread studies used a reporter gene. To visualise the targeted regions most studies used is cyan fluorescent protein (Figure 6) CFP (36.4%) followed by mCherry (29.4%) and GFP (14.2%).

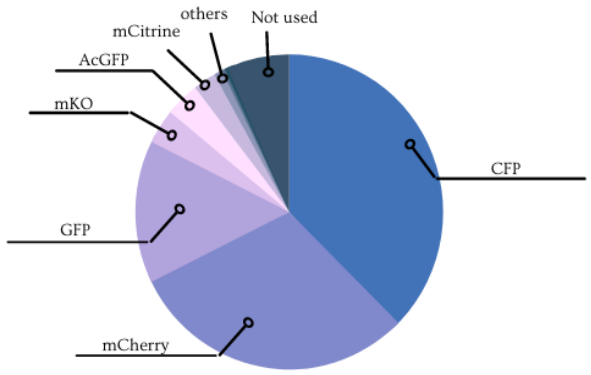

Figure 6: Proportion of reporter used across all experiments

The most widely used ligand was clozapine-N-oxide (Figure 7) (39%). Some experiments also used DCZ(7.2%) and a combination of DCZ/PBS(13.7%).

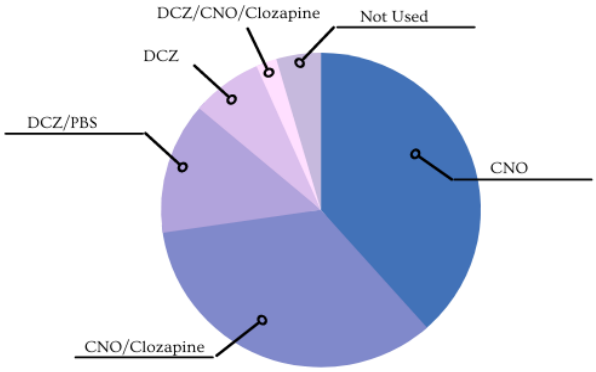

Figure 7: Proportion of ligands used across all experiments

Viral delivery method: To deliver viral vector into the brain different surgical techniques were conducted across different experiments (Figure 8). 40% of the studies used carniotomy, while other methods like burr hole, chamber,iMRI each were used in 20% of the studies.

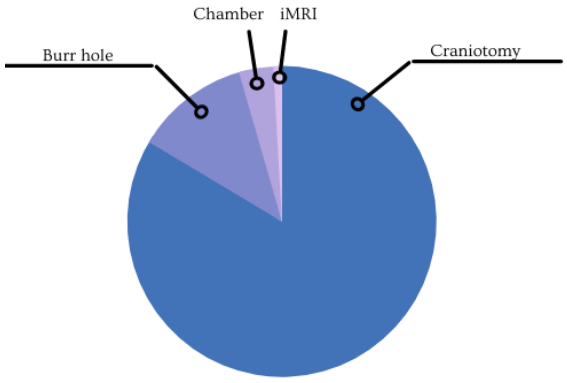

Figure 8: Proportion of each surgical method used to deliver the virus

Injections of the viral solution was made at various infusion speeds(Figure 9). The infusion speeds were governed by the size of the transduced area. However slower injection rates are known to reduce the tissue damage. Most of the studies conducted has injection rate within the rage of 1-2.5 μl followed by 2.5-5 μl.

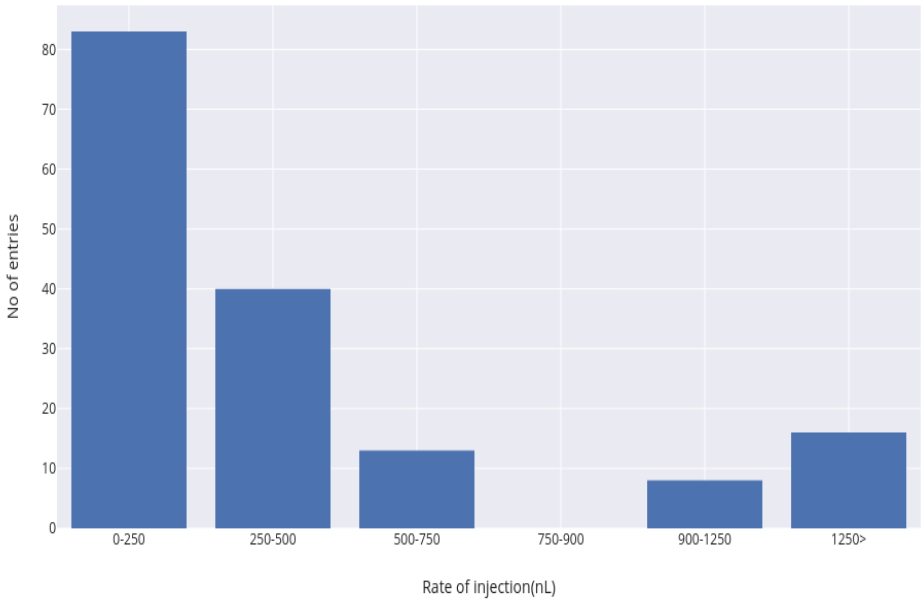

Figure 9: Rate of injection of the viral solutions across all experiments

Statistics of the volume of viral solution that is infused is also a very important because they affect the number of cells that will encounter the viral particles. This measure can affect the density and volume of the transduced tissue.

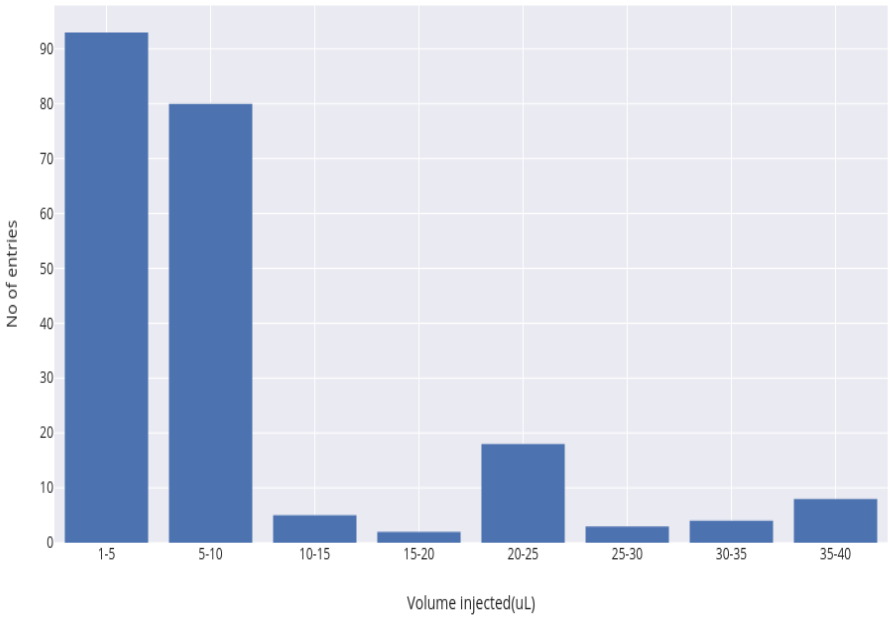

Figure 10: Volume of injection of the viral solution across all experiments

4.3.4 Discussion

The current initiative aims to collect all studies done on non human primates using DREADDs and make it accessible for researchers to consult and contribute for future studies. The DREADDs NHP database is a work in progress which is going to be an open resource and will continue to accept contributions to enrich the contents.As of now, the database contains information only from published articles on the topic, however we intent to expand it by also including unpublished articles at the later stages of its making.

Identifier	Lab	User (s)	Animal	Species	Viral Construct	Viral Vector	Promoter	Ligands	Fluorescent Protein
1				Rhesus	AAV5-hSyn-HA-hM4Di- IRES-mCitrine	AAV5	hSyn	CNO/clozapine	mCitrine
2				Rhesus	AAV5-hSyn-HA-hM4Di- IRES-mCitrine	AAV5	hSyn	CNO/clozapine	mCitrine
3				Rhesus	AAV5-hSyn-HA-hM4Di- IRES-mCitrine	AAV5	hSyn	CNO/clozapine	mCitrine
4				Rhesus	AAV5-hSyn-HA-hM4Di- IRES-mCitrine	AAV5	hSyn	CNO/clozapine	mCitrine
5				Rhesus	AAV5-hSyn-HA-hM4Di- IRES-mCitrine	AAV5	hSyn	CNO/clozapine	mCitrine
6				Rhesus	AAV5-hSyn-HA-hM4Di- IRES-mCitrine	AAV5	hSyn	CNO/clozapine	mCitrine
7				Rhesus	AAV5-hSyn-HA-hM4Di- IRES-mCitrine	AAV5	hSyn	CNO/clozapine	mCitrine
8				Rhesus	AAV5-hSyn-HA-hM4Di- IRES-mCitrine	AAV5	hSyn	CNO/clozapine	mCitrine
9				Rhesus	AAV5-hSyn-hM4Di- mCherry	AAV5	hSyn	CNO/clozapine	mCherry
10				Rhesus	AAV5-hSyn-hM4Di- mCherry	AAV6	hSyn	CNO/clozapine	mCherry
11				Rhesus	AAV5-hSyn-hM4Di- mCherry	AAV7	hSyn	CNO/clozapine	mCherry
12				Rhesus	AAV5-hSyn-hM4Di- mCherry	AAV8	hSyn	CNO/clozapine	mCherry
13				Rhesus	AAV5-hSyn-hM4Di- mCherry	AAV9	hSyn	CNO/clozapine	mCherry
14				Rhesus	AAV5-hSyn-hM4Di- mCherry	AAV10	hSyn	CNO/clozapine	mCherry
15				Rhesus	AAV5-hSyn-hM4Di- mCherry	AAV11	hSyn	CNO/clozapine	mCherry
16				Rhesus	AAV5-hSyn-hM4Di- mCherry	AAV12	hSyn	CNO/clozapine	mCherry
17				Rhesus	AAV5-hSyn-hM4Di- mCherry	AAV5	hSyn	CNO/clozapine	mCherry
18				Rhesus	AAV5-hSyn-hM4Di- mCherry	AAV5	hSyn	CNO/clozapine	mCherry
19				Rhesus	AAV8-hSyn-hM4Di- mCherry	AAV8	hSyn	CNO/clozapine	mCherry
20				Rhesus	AAV5-hSyn-HA-hMDi	AAV5	hSyn	CNO/clozapine	Not Used
21				Rhesus	AAV5-hSyn-HA-hMDi	AAV5	hSyn	CNO/clozapine	Not Used
22				Rhesus	hSyn-hMDq-mCherry		hSyn	CNO/clozapine	mCherry
23			Marmo 1	Marmoset	AAV1-hSyn1-hM3Dq-IRES-AcGFP	AAV1	hSyn	DCZ & CNO/cloza	GFP
24			Marmo 1	Marmoset	AAV2-CMV-mKO	AAV2	CMV	DCZ & CNO/cloza	mKO
25			Marmo 2	Marmoset	AAV1-hSyn1-hM3Dq-IRES-AcGFP	AAV1	hSyn	DCZ & CNO/cloza	GFP
26			Marmo 2	Marmoset	AAV2-CMV-mKO	AAV2	CMV	DCZ & CNO/cloza	mKO
27			Marmo 3	Marmoset	AAV2-CMV-hM3Dq	AAV2	CMV	DCZ & CNO/cloza	Not Used
28			Marmo 3	Marmoset	AAV2-CMV- AcGFP	AAV2	CMV	DCZ & CNO/cloza	GFP
29			Marmo3	Marmoset	AAV2- CMV-mKO	AAV2	CMV	DCZ & CNO/cloza	mKO
30			Monkey F	Rhesus	AAV2-hSyn-hM4D(Gi)-mCherry	AAV2	hSyn	CNO/clozapine	mCherry
31			Monkey F	Rhesus	AAV2-hSyn-hM4D(Gi)-mCherry	AAV3	hSyn	CNO/clozapine	mCherry
32			Monkey F	Rhesus	AAV2-hSyn-hM4D(Gi)-mCherry	AAV4	hSyn	CNO/clozapine	mCherry
33			Monkey F	Rhesus	AAV2-hSyn-hM4D(Gi)-mCherry	AAV5	hSyn	CNO/clozapine	mCherry
34			Monkey E	Rhesus	AAV2-hSyn-hM4D(Gi)-mCherry	AAV2	hSyn	CNO/clozapine	mCherry
35			Monkey E	Rhesus	AAV2-hSyn-hM4D(Gi)-mCherry	AAV3	hSyn	CNO/clozapine	mCherry
36			Monkey E	Rhesus	AAV2-hSyn-hM4D(Gi)-mCherry	AAV4	hSyn	CNO/clozapine	mCherry

Figure 11: NHP DREADDs Database (snapshot)

Chapter 5

5 Future Directions

NHP DREADDs database is being created with an aim of providing an open source resource to assist future experiments to be conducted on non human primates using DREADDs. Currently the database only contains information from published articles adding up to a total of 336 entries. As the next step we intend to invite all labs focusing on the application of DREADDs on non human primates to contribute to the database by sharing their published and unpublished data. Addition of unpublished data will enrich out database and help future experiments tremendously, which otherwise would stay unknown forever. Finally after compiling all studies related to the topic at hand we will go though the same statistical analysis to make the final conclusions about the current application of DREADDs on non human primates. This database is going to be an open resource and will be accessible for everyone. It will also encourage investigators to share their data on a broad range of parameters which could be added to the database in the future.

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