

**BEHAVIOURAL INFLUENCES ON THE STABILITY OF POINTS WITH
OPTOGENETIC STIMULATION OF MOTOR CORTICES**

BEHROO MIRZA AGHA

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BEHROO MIRZA AGHA

Date of Defence: August 15, 2023

Dr. Ian Q. Whishaw	Professor	Ph.D.
Dr. Majid H. Mohajerani	Associate Professor	Ph.D.
Thesis Co-supervisors		

Dr. Bryan E. Kolb	Professor	Ph.D.
Thesis Examination Committee		

Dr. Jon Doan	Associate Professor	Ph.D.
Internal External Examiner		
Department of Kinesiology		
Faculty of Arts and Science		

Dr. Rochelle M. Hines	Associate Professor	Ph.D.
External Examiner		
University of Nevada		
Las Vegas, USA		

Dr. Robbin Gibb	Professor	Ph.D.
Chair, Thesis Examination Committee		

DEDICATION

For my family and all the Bahá'í students in Iran who are deprived of higher education because of their beliefs.

ABSTRACT

This thesis investigates the stability of points on the motor cortex of the mouse produced using optogenetic stimulation. Mice were implanted with fiber optic probes in four areas of the motor cortex and one in a non-motor area for control. They received stimulation of various frequency and duration as they engaged in six different voluntary and automatic behaviour. Control stimulation was also administered via a separate optic probe located on the side of the apparatus. The general findings are that the outcome of the stimulation depends on the ongoing behaviour of the mouse at the time of stimulation. Thus, stimulation effects are unstable, and behaviour is a contributing factor.

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LIST OF ABBREVIATIONS

AC	Alternating current
ALM	Anterior lateral motor
AMM	Anterior medial motor
CFA	Caudal forelimb area
Chr2	Channelrhodopsin-2
CT	Corticothalamic
DC	Direct current
ET	Extratelencephalic
FDR	Frontal discrete rhythmic
FEF	Frontal eye field
ICMS	Intracortical microstimulation
IT	Intratelencephalic
M1	Primary motor cortex
MOP-ul	Primary motor cortex upper limb area
PB	Peanut butter
PLM	Posterior lateral motor
PMM	Posterior medial motor
PPC	Posterior parietal cortex
pTOS	Prolonged transcranial optogenetic stimulation
RFA	Rostral forelimb area
SP	String-pulling task
Thy1	Thymus cell antigen 1
V	Visual cortex
YFP	Yellow fluorescent protein

CHAPTER 1: INTRODUCTION

The story of cerebral cortex begins with Rene Descartes theory of the brain function. Descartes thought that the cerebral cortex is a protective tissue for the underlying subcortical areas. This covering protected the pineal body which Descartes proposed to be the seat of the mind (Descartes, 1972). Following Descartes reasoning, subsequent investigators did come to accept that the cerebral cortex is an important part of the brain but unlike other areas of the brain they thought that it was electrically unexcitable.

Fritsch and Hitzig in 1870 refuted the doctrine of unexcitability, the position that although many parts of the brain produce behaviour when electrically stimulated, stimulation of the neocortex resulted in no evoked behaviour. Their paper provided three important insights into the function of the cerebral cortex; that it is a functional part of the brain, it is electrically excitable, and it has a motor function. Fritsch and Hitzig had observed that passing a constant galvanic current, or direct current (DC), from a battery to the posterior part of the human head resulted in eye movements.

This prompted Fritsch and Hitzig to adopt a different view from the dominant view of their time. They do note, however, that the eye movement could have been the result of stimulating the corpora quadrigemina (the colliculi, subcortical structures, which were known to have a visual function). They then conducted a preliminary experiment on a rabbit, which encouraged them to continue addressing the question of whether the elicited movements are due to excitability of the cerebrum or due to transfer of current to the colliculi. They also stimulated the cerebral cortex of an awake and anesthetized dog and reported a combination of twitches and movements of the opposite side of the body: twitching of neck muscles, adduction of foreleg, extension of foreleg,

movement of hindleg, and facial twitching (Figure 1). Thus, Fritsch and Hitzig confirmed that stimulation of the cortex could result in behaviour and in doing so discovered the motor cortex.

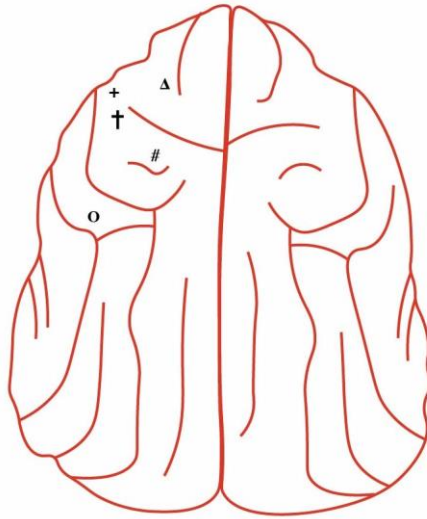


Figure 1. Map of excitable areas of dog cerebral cortex generated by galvanic electrical stimulation. Δ center for twitching of neck muscle, + center for extension of foreleg, † center for adduction of foreleg, # center for movement of hindleg, and O center for facial twitching. Adapted from Fritsch and Hitzig (2009).

Soon after Fritsch and Hitzig published their results, David Ferrier (1874) initiated a series of experiments on various animals including dogs, cats, guineapigs, rabbits, jackals, monkeys, and others. Unlike Fritsch and Hitzig, Ferrier used Faradic stimulation, alternating current (AC) electrical stimulation, in Chloroform anesthetized animals. He described a motor map on the monkey cortex in which he used numbers to describe unique centers from which he elicited movements (Figure 2). Movements of grasping arms and legs and raising the hand to the mouth and closing a fist are described to be evoked along the medial part of the cortex, whereas the movement of facial muscles including lips and nose are described to occur more laterally. He then damaged the motor centers in monkeys and observed the loss of voluntary power over the

movement elicited by electrical stimulation at that location. He also described the boundary of the motor and sensory region by drawing the line that connects Sylvian fissure with parieto-occipital fissure, the anterior portion of which being the motor region and the posterior portion the sensory region. His work confirmed and extended the findings of Fritsch and Hitzig in different animal species. Nevertheless, his studies used quite long durations of stimulation and less controlled pulses of electrical current.

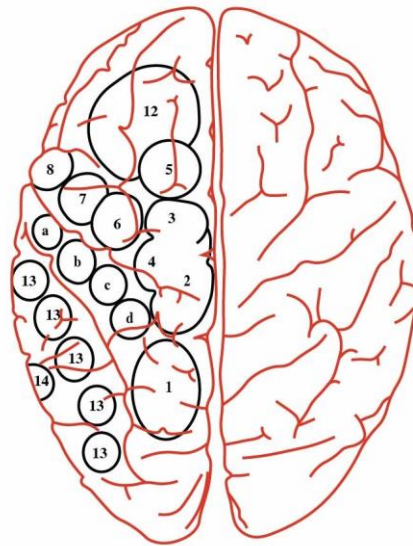


Figure 2. Map of motor and sensory cortex of monkey using Faradic electrical stimulation. Adapted from Ferrier (1886). Note that numbers and circles designated each part as a center of motor action.

The experiments Ferrier and others must have appeared relatively crude because the subsequent history of cortical stimulation featured improved methods in which location, stimulation and electrode type were varied. In following studies, experimenters refined their stimulation paradigm by using finer electrodes with smaller stimulation points, more stimulation sites, and briefer stimulation duration. Sherrington and Grunbaum (1901) conducted experiments

that extended stimulation studies to anthropoid apes including a chimpanzee, orangutan, and gorilla using AC stimulation. Unlike the result of their predecessor's experimenters, they describe the boundary of the motor cortex differently. They reported that in the post central gyrus the application of even the strongest current failed to evoke movement whereas application of the weakest current to the precentral gyrus induced a movement. They refuted a general belief that in higher primates only the most intense current excited the cortex. Their finding that only brief and low levels of current could evoke a motor movement in anthropoids including apes became the experimental standard for defining the motor cortex.

Some years later, Leyton and Sherrington (1917) further defined the function of motor cortex by stimulation experiments of cortex in apes. They used short duration alternating electrical pulses with lowest current to avoid its spread to the rest of the brain tissue. They used anesthetized chimpanzees, orangutans, and gorillas (Figure 3) because those non-human primates are phylogenetically close to humans. With their stimulation, they observed sequences of movement occurring which they defined as first, second, third and fourth movements, although their intention was to identify the primary movements elicited from each point. An example of such a sequence is stimulation of point #187 in chimpanzee. The stimulation resulted first in flexion of all fingers except the thumb, followed by flexion of wrist, followed by supination of the wrist, followed by flexion of elbow. They reported more than 400 first movements mapped on the motor cortex followed by second, third and even fourth movements, summarizing them in a table that occupies seven pages of their original publication. Although the localization of responses followed a well-fixed topographical scheme, they emphasized the functional instability of points. When revisiting a point after the adjacent points were stimulated, they could obtain a different movement from that point. They proposed that the motor cortex was the brain region that combined many partial and

fractional movements and postures into a precise spatial and temporal manner to produce patterns and sequences of motor actions. Presumably, instable points were part of the behavioural repertoire.

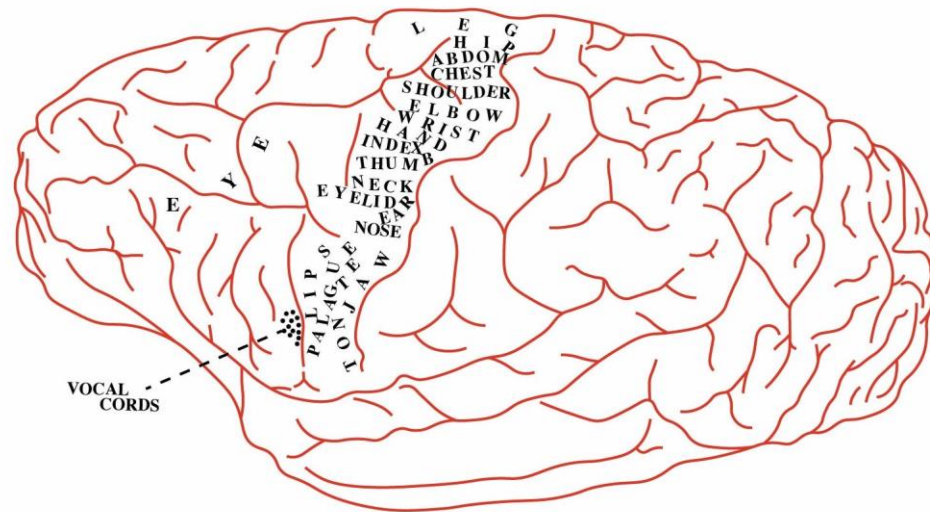


Figure 3. Map of the motor cortex in gorilla using Faradic electrical stimulation. Adapted from Leyton and Sherrington (1917). Note that map consists of body parts.

Lashley (1923) conducted a systematic experiment to test the temporal variation of the outcome of the excitable points on the motor cortex. He performed a craniotomy and created a chronic window over the precentral gyrus of a rhesus monkey. He used bipolar stimulation in which two electrodes were placed on the brain that were 1mm apart every 2.5mm for less than 1sec. He adjusted the current intensity threshold based on a single point at the beginning of every test and it increased until responses appeared. He mapped the motor cortex of the monkey on two consecutive days, fifteen days after the second mapping, and twelve days after the third mapping. He used the same order of points he stimulated in every mapping day and recorded the behavioural outcomes of 57 points in a three-page table. Lashley reported that the movements elicited in each

day were quite constant, however, the stimulation of the same points in different days usually produced different movements.

Penfield and Boldrey (1937) extended electrical stimulation experiments to humans. They summarized results obtained from the electrical stimulation of the cortex of 163 patients made over a period of nine years. These patients were undergoing elective surgery for epilepsy, in which part of their brain from which seizures began was to be removed. To minimize the damage from removal of cortical tissue, functional mapping of the brain was a therapeutic justification. Patients were awake during the stimulation, so they were able to report on what they felt at the time of stimulation. Low voltage and short duration stimulation was used so that no seizure was induced. Penfield and Boldrey summarized their experimental findings as a homunculus (Figure 4).

The homunculus is humanoid creature with exaggerated body parts; for example, enormous hands and mouth. It is based on the order and comparative size of those body parts appeared around the central sulcus. Thus, in the original paper the homunculus is an upside-down creature with its head and tongue detached underneath the upside-down body. The size of the body parts of the homunculus corresponds to the amount of brain area dedicated to function of those body parts. In addition to mapping the motor cortex, they mapped the sensory cortex on the postcentral gyrus. They did so by stimulating body parts and recording the response of the cortex to the stimulation. The sensory homunculus is also upside down with exaggerated mouth and hands similar to the motor homunculus. This exaggerated size of the same body parts shows the sensitivity and dexterity in these body parts and is due to the large portion of the brain dedicated to the hands and mouth.

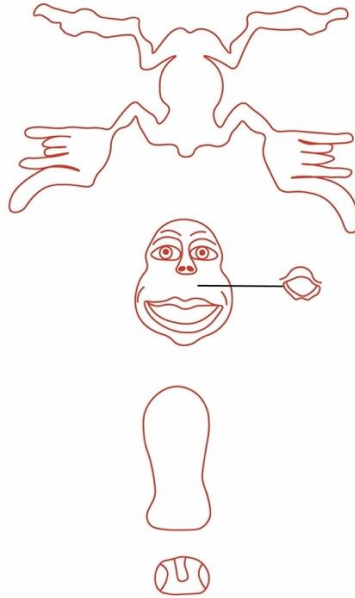


Figure 4. Sensory and motor homunculus. The order and comparative size of the body parts depicted as they appear on the motor cortex from above down. Adapted from Penfield and Boldrey (1937).

Penfield and Welch (1949) elaborated on the instability of response to cortical stimulation in human. They categorized the obtained response into four categories: facilitation, extinction, reversal, and deviation of response. Facilitation occurred when the response of cortical stimulation was enhanced due to previous stimulation of the same (primary facilitation) or a different (secondary facilitation) cortical point. For example, restimulation of point#2 after less than 10sec that previously resulted in no response led to tingling in the left thumb. Extinction occurred when previous stimulation of the same point resulted in a decreased response. For example, restimulation of a certain point on the cortex that elicited flexion of the hand during mapping, resulted in no response three times out of four trials when the interval between stimulations was 4sec. Reversal occurred when stimulation of a cortical point that usually gave rise to extension of a body part resulted in flexion rather than extension when the same or another point was stimulated previously. Deviation of response occurred when stimulation of the same point elicited the same response in

different body parts due to previous stimulation. For example, stimulation of a point that previously led to a sensation in thumb produced a lip sensation.

Subsequent experimenters adopted Penfield and Boldrey's approach by describing homunculi maps on the motor cortex in other animal species. They also used ever finer microelectrodes and briefer stimulation duration to define body parts in ever smaller regions of the brain. Eventually the experimental procedures were reduced to the point that muscle twitches were monitored with EMG signals. The idea generated by this approach was that individual muscle twitches were controlled by the cortex and could be combined to produce movements of body parts.

The idea that points on the brain corresponding to individual muscles was altered by Michael Graziano and his coworkers (M. S. Graziano, Taylor, & Moore, 2002). They produced the surprising result that rather than representing muscles, the motor cortex represents actions. Graziano used electrical microstimulation of monkey brain with half a second duration pulses, a duration much longer than the conventional durations and considered whatever actions that were elicited as his data.

The inspiration behind using longer stimulation came from the work of his colleague, Tirin Moore who studied the frontal eye field (FEF) area of motor cortex. Moore was using long duration stimulation to produce movement of the eyes. The elicited eye movements resembled movements that a monkey would make when looking around the room. Graziano (2009) describes in his book "The intelligent movement machine" how Tirin one day rushed to call for him to see an astonishing phenomenon. Upon stimulation, a monkey sitting on a chair in the middle of a room would extend his arm forward and shape his finger as if reaching for something. Clearly the implant had missed the FEF and was instead placed in primary motor cortex.

Graziano and Moore together with graduate student Charlotte Taylor decided to study the motor cortex of monkey using long duration stimulation. They studied one cortical site a day by applying long duration stimulation and observing the outcomes. From one site they could elicit integrated movements of shoulder, arm, and hand (Figure 5). From another site they observed the monkey extended a hand to a fixed position in space regardless of hand's starting point as if reaching in a goal-direct manner. From still another site the monkey closed its fingers, brought a hand to the mouth, and then opened its mouth as if feeding. To rule out that the monkey is intentionally fooling them, they stimulated an anesthetized monkey. They saw the same behaviour as before: fingers closed, a hand brought upward towards the mouth, and the mouth opening. They proposed that they were driving movements regardless of monkey's behavioural context.

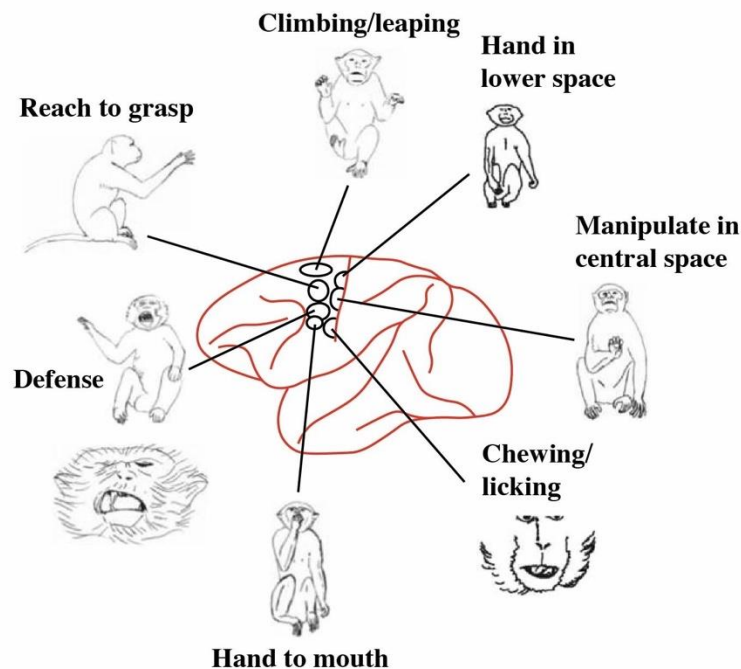


Figure 5. Action zones of the motor cortex of macaque monkey generated by long duration electrical stimulation. Images show the final posture at the end of stimulation-evoked movement. Adapted from Graziano et al. (2002). Note that rather being organized as many muscles, the motor cortex is organized as a few movements.

As Graziano has noted, it should not have been surprising that movements could have been obtained by stimulation of the motor cortex. Fritsch and Hitzig, Sherrington, and Ferrier had all described movements, as opposed to muscle twitches. However, the importance of the earlier observations was not recognized because investigators had not recognized the concept that behaviour is organized as movement units. Graziano solidified his view by going on to document the movements that monkeys actually make as part of their natural activity. These results confirmed his view that behaviour can be described as actions and the motor cortex represents the movements that a monkey actually makes as part of its daily activity.

Graziano's results are also relevant to the question of stability of points. His work recognized that the movement elicited by stimulation was directed to an end point; for example, a hand going to the mouth. The initial location of the hand was not relevant to the response, because notwithstanding its position, it would always find the mouth. Consequently, were only the initial movement of the hand considered, *a la* Sherrington, it may flex from one location and extend from another on its passage to the mouth, if the mouth were the target. The stimulation effect could appear unstable from the perspective of the initial movement but would appear stable were its end point considered.

Subsequently, a number of experimenters have adopted Graziano's approach in finding action maps on the motor cortex in other simians. Jon Kaas and Omar Gharbawie studied galago, squirrel monkey, owl monkey, and macaque monkeys (Gharbawie, Stepniewska, & Kaas, 2011; Gharbawie, Stepniewska, Qi, & Kaas, 2011; Stepniewska, Fang, & Kaas, 2005). Using the same stimulation paradigm as Graziano, they stimulated the posterior parietal cortex (PPC) of anesthetized animals with Ketamine/Xylazine anesthesia. They reported action maps with zones for various behaviours in the parietal cortex. They further investigated the neuronal connectivity

between the action maps in motor and parietal cortex by injecting retrograde tracers in the behavioural zones. They found matching action zones on both maps are connected. PPC receives a dense projection from motor (M1) and densest input to motor cortex is from PPC zones. These results point to the existence of parietal-frontal networks with body representations of sensory input in parietal cortex connected to body representation of motor output in motor cortex, which together represented movements.

Rat and mouse studies

To address many questions related to the function of the motor cortex, many studies have been conducted in rodents, with mapping experiments in mice and rats the focus of motor cortex research. The feasibility of rodent models allows the use of different techniques in mapping the motor cortex. Hall and Lindholm (1974) were the first scientists to map the sensory and motor cortex of the rat in detail. Their initial interest was the representation of the eye muscle on the cortex. Since previous studies had not specifically address their interest, they included an investigation of the entire topography of the rat cortex. They used two sinusoidal stimulation paradigms and created two maps. First, they used 25ms bursts of 1KHz sinusoidal stimulation every 2sec with current at 200 μ A. This resulted in a smaller map in the anterior cortex (Figure 6). Then, they used 250ms bursts at 300Hz sinusoidal every half a minute current at 200 μ A which resulted in a bigger map occupying anterolateral cortex. Electrodes used for stimulation were made up of tungsten or stainless-steel wire with a tip diameter of 10 μ . They reported that both maps of somatosensory and motor cortex of the rat agreed with earlier studies on primates. Nevertheless, they found that the hindlimb and forelimb areas were highly overlapped. This must mean that they do not have an independent movement representation. It is noteworthy, however, when their two

stimulation approaches are contrasted, it can be concluded that points of stimulation were unstable as somewhat different results could be obtained depending upon the parameters of stimulation.

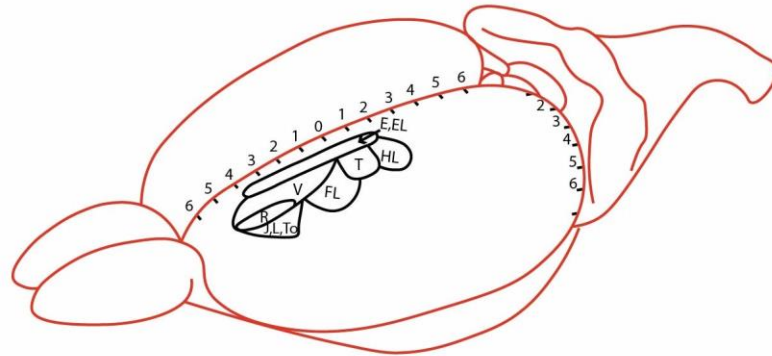


Figure 6. Motor map of rat brain. E, eye; EL, eyelids; V, vibrissae; FL, forelimb; HL, hindlimb; T, trunk; R, rhinarium; J, jaw; L, lip; To, tongue. Adapted from Hall and Lindholm (1974).

Tennant et al (2011) used intracortical microstimulation (ICMS) in wildtype C57 mouse. They overlay a grid across the cortex and applied stimulation at designated points, with adjustments around the blood vessels in anesthetized mice receiving Ketamine/xylazine anesthesia. They delivered a 40ms pulse duration at 350Hz for 200 μ s, at a maximum current of 60 μ A via a glass microelectrode of 25 μ m in tip diameter. They documented the slightest movement of body parts. Their map shows that a large part of the motor cortex of the mouse dedicated to the forelimbs (Figure 7). They emphasize two areas of motor cortex, the rostral forelimb area (RFA) and the caudal forelimb area (CFA). CFA comprises a large proportion of the map and included movements of digits, wrist, and elbow. RFA covering a smaller portion of the map anterior to the CFA included movement of wrist and elbow.

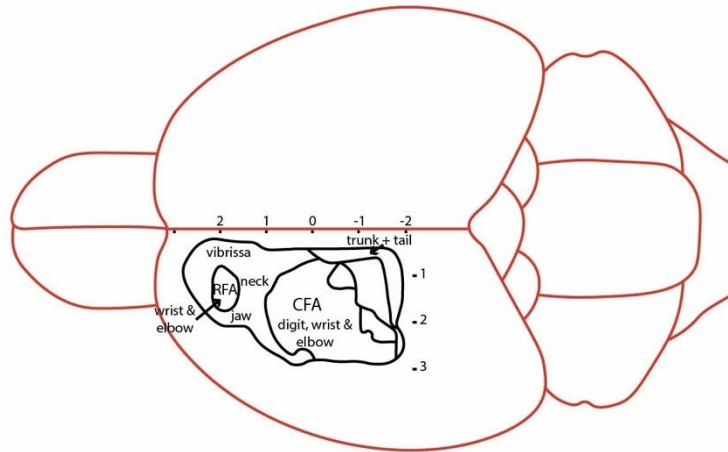


Figure 7. Motor map of mouse brain. Intracortical microstimulation (ICMS) technique under anesthesia is used. Adapted from Tennant et al. (2011).

Brown and Teskey (2014) were the first investigators to apply Graziano-type long duration stimulation to the motor cortex of the rat. They suggested that the rat motor cortex is organized in terms of movements, as is the monkey motor cortex. Moreover, they suggested that the CFA mediates arm movements and the RFA mediates hand movements. They examined the function of CFA and RFA in awake rats performing single-pellet skilled reaching task. In this task, the rat is trained to pick up a piece of food with its hand and bring it to mouth for consumption (Whishaw & Pellis, 1990). They implanted cryoloops over the CFA and RFA which could be filled with cooled methanol. While engaged in this task, the CFA and RFA were inactivated using a reversible cooling deactivation technique. The results showed that both CFA and RFA cooled rats had lower number of attempts and lower average success scores. Moreover, movement component analysis revealed that RFA cooled rats had a significant impairment in grasping the pellets. Thus, both stimulation and inactivation results confirmed that there is a distinction between reaching and grasping with respect to motor cortex function. They also mapped the brain of those rats using short and long duration intracortical microstimulation (ICMS), and they obtained different maps

based on which duration stimulation was used. Moreover, mapping the motor cortex of the rats that were trained in skilled reaching task as compared to naïve rats revealed that the points became dual responsive producing forelimb and non-forelimb movements. Thus, the stimulation of the motor cortex was unstable because different effects could be obtained as a result of changing experimental condition.

Optogenetics stimulation

Optogenetics uses light to manipulate activity of neurons in living tissues in a rapid and reversible manner (Fenno, Yizhar, & Deisseroth, 2011; Zhang, Boyden, Nagel, Deisseroth, & Bamberg, 2005). Light-sensitive protein channels that exist naturally in algae are expressed in the neurons via engineered viral vectors or transgenic methods. These protein channels, upon shining light on them, specifically allow the passage of ions that result in excitation or inhibition of the cell depending upon the channel. For example, channelrhodopsins are cation channels that allow the passage of $H^+/Na^+/K^+/Ca^{2+}$ upon illumination with blue light and result in depolarization of neurons. Transgenic mice have channelrhodopsin tagged with a fluorescent reporter protein inserted into the nucleus of the fertilized mouse egg. This mouse and its offspring will have this gene in its genome and will express it wherever it is engineered to be expressed. For example, line 18 of the transgenic Chr2-YFP mouse is generated to express channelrhodopsin-2 (Chr2) fused to yellow fluorescent protein (YFP) under the control of the mouse thymus cell antigen 1 (Thy1) promoter (Figure 8). This transgenic mouse shows a high level of Chr2-YFP expression in layer 5 cortical neurons, neurons in the hippocampus CA1 and CA3, cerebellum, thalamus, midbrain, brainstem, and olfactory bulb (Arenkiel et al., 2007; Wang et al., 2007).

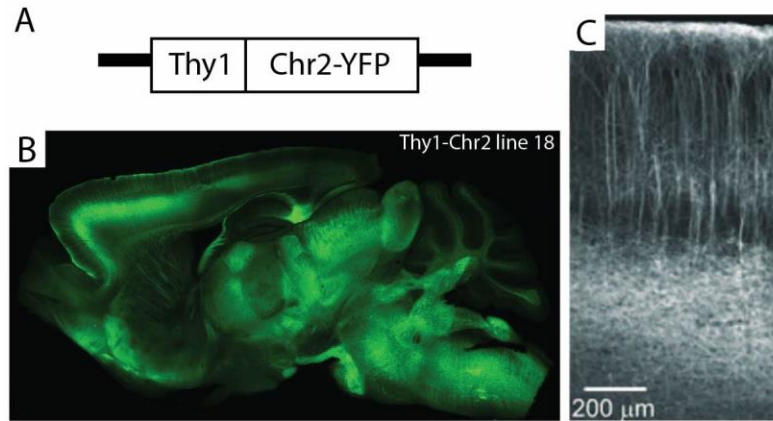


Figure 8. Transgenic expression of Chr2-YFP from Thy1 promoter in mouse brain. A) Thy1-Chr2 gene. B) Sagittal brain section from a transgenic mouse line 18. C) High levels of Chr2-YFP expression in layer 5 neurons and their apical dendrites. Adapted from Wang et al. (2007).

Hira et al (2015) used awake head-fixed mice and optogenetic stimulation to map the frontal cortex. They described two sets of fundamental movements: discrete and rhythmic. Discrete movements are those movements that have a start and an end. For example, reaching, grasping, or tongue protrusions are discrete movements. Rhythmical movements are those movements that are repetitive and oscillatory such as locomotion. A combination of discrete and rhythmic movements can be combined to produce more complex movements. Two areas on the cortex produced rhythmical and discrete hand movements. Stimulation of anterolateral motor area (ALM) produced a movement that resembled a hand-to-mouth movement, and caudal forelimb area (CFA) stimulation produced a movement resembled a locomotion-like movement. Thus, they referred to ALM as discrete hand module and to CFA as rhythmical hand module. A border between these two modules is specified and called frontal discrete-rhythmic (FDR) border (Figure 9). It is a straight line drawn from anteromedial to posterolateral on the cortex. Each module has intracortical synaptic connections within its zone but not with the other zone and each has an output that independently projects to the spinal cord.

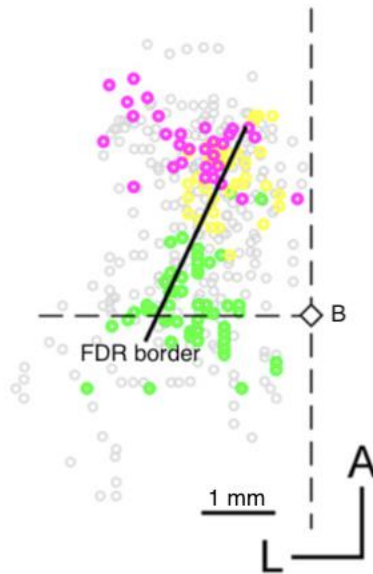


Figure 9. Map of ethologically relevant movements on mouse motor cortex. Prolonged transcranial optogenetic stimulation (pTOS) in awake head-fixed mice is used. Magenta, hand to mouth movement; green, locomotion-like movement; yellow, defensive-like movement; B, bregma; FDR border, frontal discrete-rhythmic border. Adapted from Hira et al. (2015).

Origin of instability of points

One possible expectation of brain stimulation studies is that stimulating a brain location should result in much the same response every time the stimulation is administered. This idea can be referred to as the theory of the stability of points. The idea is that there is a pathway from motor cortex to the spinal cord and from there to muscles that when activated produces a contraction.

Instability of points refers to lack of stability of stimulation sites on the brain either because some stimulation events are ineffective, the same stimulation given at different times results in different behaviour, or because the same stimulation produces inconsistent results. Sherrington and his colleagues (1912; 1917), Lashley (1923), and Penfield and Boldrey (1937) specifically mention in their studies that points on the brain are unstable (Table 1).

Nevertheless, different studies define instability of points in different ways. Based on the experiments done by Leyton and Sherrington (1917), revisiting point A after stimulation of that

location or stimulation of an adjacent point produced a different response. For example, restimulation of point A on the brain that first resulted in flexion of elbow, after stimulation of adjacent point, resulted in rotation of shoulder. They further reported that stimulation of the same points on the opposite hemisphere did not produce the same behaviour all the time. Moreover, the points in different animals of the same and different species exhibit variations as well. Furthermore, in Lashley's experiments (1923), restimulation of a point after days or weeks produced different movements. For example, in one case, restimulation of point X on four different days produced four different movements, adduction to the upper arm, rotation of the same segment, shrugging of the shoulder, and turning of the eyes. Penfield and Boldrey (1937) suggest that the instability of cortical points could also occur as physical displacement of the functions on the cortex due to lesions. For example, point Z that produced motor response Z could move from posterior edge of the precentral gyrus to the anterior edge.

In this thesis, instability of points refers to any different behavioural outcome of a point when repeatedly stimulated. The objective of this thesis is to examine the influence on the outcome of cortical stimulation in relation to behaviour. It is obvious that if stimulation presented to an anesthetized animal vs. an awake animal, behaviour would be different and stimulation effects might be different. It is also obvious that if stimulation is presented to a head-fixed animal vs. a freely moving animal, behaviour is different and stimulation effects might be different. These different conditions could result in instability of points as is described in Table 1. What is less certain is the outcome of stimulation in a freely moving animal engaged in different species-typical behaviours, say sitting still vs. walking. The question is, would stimulation produce a different outcome depending upon ongoing behaviour?

Table 1. Summary of the studies that mapped the motor cortex.

Author	Year	Title	Journal	Subject	Stimulation paradigm	Findings	Example	Stable
Fritsch and Hitzig	1870	Electrical Excitability of cerebrum	Epilepsy and Behavior (2009)	Dog	-Electrical -Faradic stimulation -Direct current (DC) -Low current causing a sensation on the tongue -Duration of few seconds	-Identified centers of muscle movements -Stimulation of the same point did not produce the same twitch in all cases.	“We must add that in not all cases was it possible to put the neck muscles in motion from the first-named site.” “a circumscribed site from which the contraction could be induced in isolation could not be determined with certainty.”	No
Ferrier	1874	On the Localisation of the Functions of the Brain	The British Medical Journal	Guineapigs, rabbits, cats, dogs, monkeys	-Electrical -Alternating current (AC) -Variable intensity electrical energy -Duration of few seconds	-Identified centers of voluntary motion -Identified boundary of motor and sensory region	“Integrity of the centres depends on the maintenance of the equilibrium of the body.”	No
Brown and Sherrington	1912	On the Instability of a Cortical Point	Proceedings of the Royal Society of London	Chimpanzee, baboon, small monkeys	-Electrical -Alternative current (AC) -Unipolar method -Duration of few seconds	-First to point out instability of points	“The functional instability of some cortical points seems to be greater than that of others.”	No
Lashley	1923	Temporal Variation in the Function of the Gyrus Precentralis in Primates	American Journal of Physiology-Legacy Content	Rhesus monkey	-Electrical -Bipolar method -current intensity below limen -Duration of less than 1sec	-Stimulation of same point in different days resulted in different movements.	“Within the arm area stimulation of the same point in different tests resulted in widely different movements and at different times the same movement was obtained from widely separated and shifting areas.”	No

Penfield and Boldrey	1937	Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation	Brain	Human	-Electrical -Alternative current (AC) -Thyratron 30 and 24 V -Bipolar electrode -Duration not mentioned	-Physical displacement of the functions occur due to lesions.	"In Case 110 we have shown that a motor response may be moved from the posterior lip of the precentral gyrus to the anterior lip and a sensory response from the anterior lip of the posterior gyrus across that gyrus to its posterior lip by the activating effect of rhythmic repeated advancing stimulation."	No
Penfield and Welch	1949	Instability of response to stimulation of the sensorimotor cortex of man	Journal of Physiology	Human	[Presumably same as above]	-Instability of response to stimulation of points in the sensorimotor cortex of man has been demonstrated.	"The present observations indicate that instability of response is a property of the human central nervous system as well, and that it applies to sensory as well as motor responses."	No
Graziano, Taylor, and Moore	2002	Complex movements evoked by microstimulation of precentral cortex	Neuron	Macaque monkey	-Electrical -Current 25-150 μ A -Frequency 200Hz mostly, 50-400Hz -Duration of 500ms mostly, 100ms, 1000ms	-Movements elicited was directed to an endpoint.	"Different directions of arm movement could be elicited depending on the starting position of the hand." with stimulation of the same point.	No
Tennant, et al.	2011	The Organization of the Forelimb Representation of the C57BL/6 Mouse Motor Cortex as Defined by	Cerebral Cortex	Mouse	-Intracortical microstimulation (ICMS) -Glass microelectrode with a platinum wire -350Hz -Max intensity of 60 μ A	-They lowered the stimulation threshold to detect the first detectable body movement. -They did not revisit points	"In animals in which no distinct RFA could be resolved, vibrissa and neck movements could be found in the area of the	No

		Intracortical Microstimulation and Cytoarchitecture			-Duration of 40ms	and could not repeat mapping in different days. -Points are unstable between animals.	expected RFA.”	
Brown and Teskey	2014	Motor Cortex Is Functionally Organized as a Set of Spatially Distinct Representations for Complex Movements	Journal of Neuroscience	Rat	-Intracortical microstimulation (ICMS) -Glass-coated platinum/iridium microelectrode -333Hz -Intensity of 100 μ A -Short duration of 39ms -Long duration of 500ms	-Short or long duration ICMS produced different maps -After reaching training, points were dual responsive, making forelimb and non-forelimb movements	“We document a specialization and segregation of complex movement representations eliciting grasping movement in the RFA and reaching movement (forelimb elevate, advance, and retract) in the CFA in the rat under LD-ICMS, indicating a functional dissociation between the two motor areas that is not observed under SD-ICMS.”	No
Hira, et al	2015	Distinct Functional Modules for Discrete and Rhythmic Forelimb Movements in the Mouse Motor Cortex	Journal of Neuroscience	Mouse	-Prolonged transcranial optogenetic stimulation (pTOS) -Power of 1.2-7.2mW -50Hz -Duration of 500ms	-Identified rhythmic and discrete movements. -Elicited rhythmic and discrete movements have different trajectories in every trial.	“During pTOS of the ALM, the right forepaw moved to a narrow space in every trial. By contrast, during pTOS of the CFA, the right forepaw moved circularly for >1 cycle and its end point varied.”	No
Guo, et al.	2015	Cortex commands the performance of skilled movement	eLife	Mouse	-Optogenetic -Power of 1-15mW -40Hz -Duration of 0.1-4sec	-Cortical stimulation does not inhibit the movement in all trials.	“Prehension progression blocked by optogenetic inhibition of contralateral sensorimotor cortex during Grab.”	No

							“Prehension progression impeded but not prevented by optogenetic inhibition of contralateral sensorimotor cortex during Grab.”	
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One source of instability of points is the complexity of the motor cortex. Original descriptions of the motor cortex outputs emphasize the corticospinal tract, a pathway from the cortex to the spinal cord. Most of this pathway runs from layer 5 of the motor cortex to the spinal cord with collateral synapses in a number of brain stem structures. Munoz-Castaneda et al. (2021) provided a new cellular anatomy of the mouse upper limb motor cortex. They identified thirty-eight types of neurons based on their laminar position, projection class and target (Figure 10). All previous electrical stimulation studies that have been done are nonspecific with respect to the neurons targeted and so cannot address this complexity. Therefore, if behaviour is to be considered in relation to the stability of points, there need be some control of the complexity of motor cortex anatomy. This can be achieved by optogenetic methods.

The optogenetic technique used in the present thesis enables specific targeting of one group of neurons, the layer 5 pyramidal neurons, extratelancephalic pathway, (See figure 10, the green neurons in ET) and so is very specific as is described in the previous section “optogenetic stimulation”. No doubt future studies will address the function of the many other output neurons of the motor cortex. Nevertheless, it is clear that minor differences in stimulation parameters could differentially affect the output of the motor cortex, thus resulting in different movements from apparently the same stimulation. This difficulty can be overcome where only one population of motor neurons activated by the stimulation.

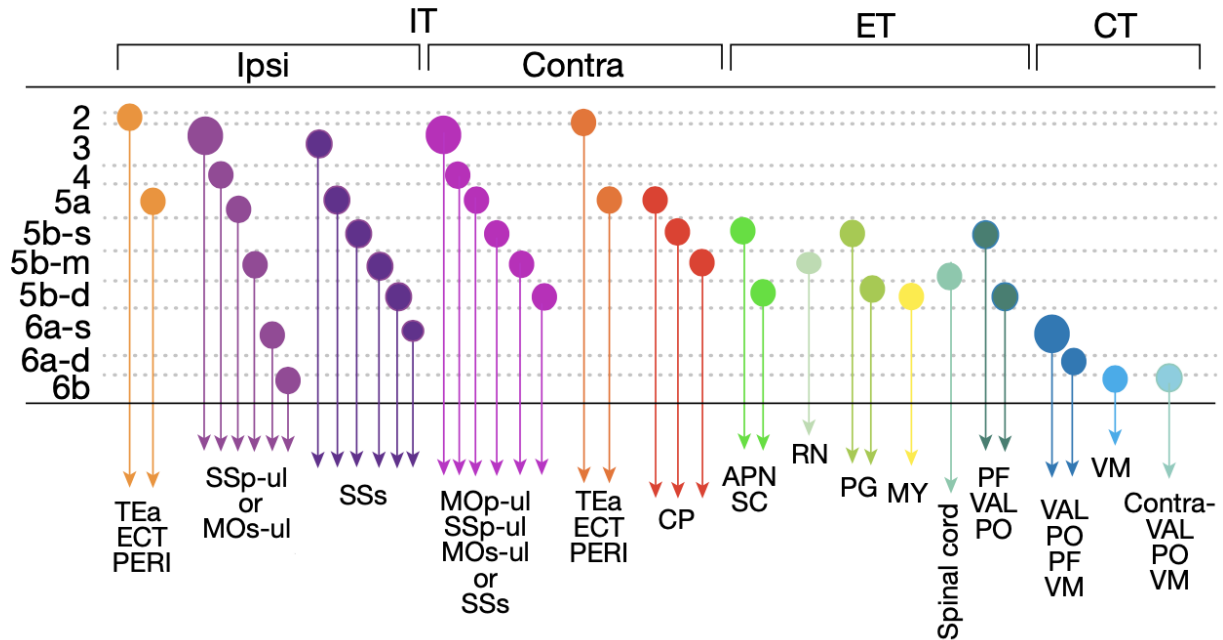


Figure 10. Classification of cortical neuron types in primary motor cortex, upper limb area (MOP-ul). IT, intratelencephalic; ET, extratelencephalic (also known as pyramidal tract); CT, corticothalamic. Adapted from Munoz-Castaneda et al. (2021).

Experimental Design

It is established in the results of preceding experimental descriptions that sometimes motor cortex stimulation produces a response and sometimes it does not produce the same response. One possible cause of this instability could be the ongoing behaviour of an animal. The hypothesis that underlies the present study is that there may be behavioural conditions that are conducive to evoked responses produced by motor cortex and other conditions that are not so conducive to similar evoked responses. Accordingly, to optimize the investigation of this idea, freely moving mice received cortical stimulation while engaged in different behaviours that are a part of their behavioural repertoire.

In nearly all studies that have been conducted on the function of motor cortex, experimental subjects were either anesthetized or awake and head fixed. Thus, studies were limited by methods

in which the subject was not free to behave. I removed this bias out by employing a methodology which examines freely moving mice in different behavioural conditions. The behaviour of an animal can vary moment to moment, of course, and that allows for the testing of stimulation over the full behavioural repertoire of the animal.

In this study, I used freely moving mice that are engaged in various types of tasks in a box, and the behaviours that were selected include learned behaviours, voluntary or Type 1 behaviours, and automatic or Type 2 behaviours (Vanderwolf & Robinson, 1981; Whishaw & Vanderwolf, 1973). Voluntary behaviours are defined as movements that take one from point A to point B and can be associated with any motivational state. For example, walking towards the food source, or standing up to examine the space above for objects. Automatic behaviours are defined as movements that are not generated by training and are limited to a single motivational state. Examples of automatic movements are eating, grooming, and licking. I optically stimulated the mice while they were engaged in the voluntary movements of walking, rearing, and the automatic movements of grooming, eating, and licking peanut butter (Figure 11A). The idea is that the motor cortex is more likely to be occupied in producing voluntary movement than automatic movements and so likely to be differentially sensitive to stimulation depending upon which type of movement is occurring.

I also used a learned behaviour. The learned behaviour was string-pulling, a task that the animals are trained to do fairly quickly. It enables assessment of skilled bimanual hand movements, posture, and learning. In this task, the mouse stands on its hind feet and pulls on a string with hand-over-hand movements to retrieve a piece of food attached to the end of the string. While pulling, the mouse makes alternating reach, grasp, pull, push, and release movements, and at all times the nose follows the string closely. The mouse learns this task fairly quickly because it

falls within its natural behavioural repertoire. String-pulling behaviour is similar to pulling on tall grass to retrieve the seed on top or pulling on nesting materials (Blackwell, Banovetz, Qandeel, Whishaw, & Wallace, 2018).

Five stimulation areas on the cortex are chosen for stimulation: anterior lateral motor area (ALM), anterior medial motor area (AMM), posterior lateral motor area (PLM), posterior medial motor area (PMM), and primary visual cortex (V) (Figure 11B). The ALM region is regarded as a hand to mouth area (Hira et al., 2015). Having the mouse engaged in tasks that involve hand or mouth or both hand and mouth movements, such as string-pulling, licking, and eating provides a more comprehensive look at the function of the ALM region. ALM and PMM areas are known to be involved in discrete vs. rhythmical movements respectively (Hira et al., 2015). Therefore, stimulating those areas during rhythmical behaviour of walking and discrete behaviour of rearing encouraged a closer investigation of the function of those regions. Medial and lateral cortical areas are considered posture and hand areas respectively (Hall & Lindholm, 1974). Therefore, investigating the function of AMM and PLM areas specially in string-pulling task where posture and hands are important examining features is deemed befitting. Stimulation of a non-motor area such as primary visual area served as a control region for other motor areas. The aim was to have six fiber optics for each of the mentioned brain regions. However, due to unexpected mouse mortality less than six mice are in two of the groups.

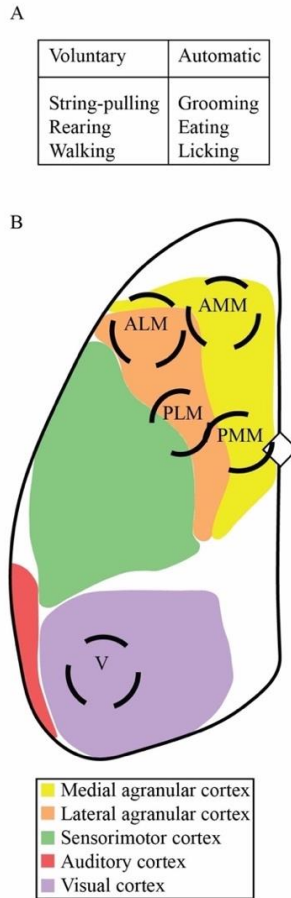


Figure 11. Behavioural tasks and fiber optic implant areas. A. Voluntary and automatic behaviours that the mice were engaged in when they received either stimulation or control stimulation. B. Top view of the left hemisphere of the mouse brain with dashed circles depicting the fiber optic implant sites. White diamond depicts bregma. ALM, anterior lateral motor; AMM, anterior medial motor; PLM, posterior lateral motor; PMM, posterior medial motor; V, visual cortex. Note that the fiber optic implant surgery was actually done on the right hemisphere.

CHAPTER 2: MATERIALS AND METHOD

Animals

Sixteen (9 males, 7 females) Thy1Chr2 mice (B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J; Jax stock number 007612) (Arenkiel et al., 2007) weighing 20-30g and 2-5months of age, raised at the Canadian Center for Behavioural Neuroscience Vivarium at the University of Lethbridge, were used. The animals were housed in pairs under a 12:12 h light/dark cycle with light starting at 7:30am and temperature set at 22°C. After fiber optic implantation, the mice were housed singly. Testing and training were performed during the light phase of the cycle at the same time each day. The animals received water ad libitum. Procedures were approved by the University of Lethbridge Animal Care Committee.

Surgical procedures

To implant the fiber optic, animals were anesthetized with isoflurane (1–2%), and stereotaxic surgery was conducted using aseptic methods. The mice were placed in a stereotactic frame (Kopf Instruments) on a 37–38°C heating pad. The animal's eyes were covered with a thick layer of lubricating ointment (Refresh, Allergan Inc.). Using scissors, a flap of skin about 1cm² in area was retracted from the skull and the gelatinous periosteum was removed with small scissors. The skull was cleaned and dried with sterile cotton swab. Prior to the implantation, the fiber optic ferrule and electrode pins were disinfected with 70% isopropyl alcohol and allowed to air dry. Coordinates for fiber optic placement were: ALM (AP= 2.4mm, ML= 2.0mm), PLM (AP= 0.2mm, ML=1.5mm), AMM (AP=2.0mm, ML= 1.25mm), PMM (AP=0.3mm, ML=1.2mm). The skull was covered with a thin layer of Metabond (C&B Metabond) and a layer of dental cement.

Optogenetic stimulation

Fiber optic procedure. The fiber optics were prepared in house following guidance from the ThorLabs Manual (FN96A). Briefly, fiber optic cores (Part # 460HP, Thor Labs) were cut to 12 mm lengths, inserted into ceramic ferrules (Part # CFLC230-10, Thor Labs) and cemented in place with epoxy (Part # F120, Thor Labs). After allowing the epoxy to set for 24 hours, the “bare” end was cut to 1.0 mm in length with a diamond cutter (Part # S90R, Thor Labs). This “bare” end was eventually implanted into the brain. The other end of the fiber optic core was trimmed and polished until flush with the ceramic ferrule and showed an appearance of “polished glass” under a dissecting microscope at 10x power. Before implantation, the ferrules were tested for light transmittance with an optical power meter (Part # PM100D, Thor Labs) by attaching the polished end to a 473 nm laser (Shanghai Dreams Lasers Technology, SDL-473-100T.) All fiber optic implanted in the cortex had an average measured output of ~1-2mW.

Fiber optic stimulation. Mice were stimulated with four different frequencies of 10Hz (25ms on and 75ms off), 20Hz (25ms on and 25ms off), 30Hz (20ms on and 13.3ms off), and 40Hz (20ms on and 5ms off) with durations of 0.5sec, 0.7sec, 1sec, 1.5sec, and 2sec except for string-pulling and eating in which the duration used was 0.7sec (Table 2). The 2 sec length of stimulation is the duration defined as “long” by Graziano et al. (2002).

Table 2. Frequency and Duration of stimulation and control stimulation used for behaviours of walking, rearing, grooming, and licking peanut butter. For behaviours of string-pulling and eating all frequencies but only 0.7sec duration was used. Freq, frequency; Dur, duration.

Freq Dur	10Hz	20Hz	30Hz	40Hz
0.5sec				
0.7sec				
1sec				
1.5sec				
2sec				

Control stimulation. A laser cable was placed at one side of all the behaviour boxes (Figure 12), and it was turned on semi-randomly while the mice were engaged in each behaviour. The frequency and duration of control stimulation was the same as real stimulation as described above (Table 2). The control stimulation provided the same sound and light as was produced by the intracranial stimulation, and its relation to ongoing behaviour was semirandom while still occurring during the designated behavioural conditions. The control stimulation served to assess whether the animals were responding to the light or sound produced by the stimulation as opposed to its effects on motor cortex.

Video recording

All behaviours were filmed from a frontal view with a Panasonic HDC-SDT750 camera at 60 frames per second at an exposure rate of 1ms. Illumination for filming was obtained by using a

two-arm cold light source (Nikon Inc.), with the arms positioned to illuminate the behaviour boxes from a frontolateral location on each side. Videos were captured with an SD card, stored on a Hard drive for offline analysis, and replayed frame-by-frame on a computer using QuickTime player (Version 10.5 (1126.4.1), (<https://support.apple.com/downloads/quicktime>), Apple Inc) for scoring.

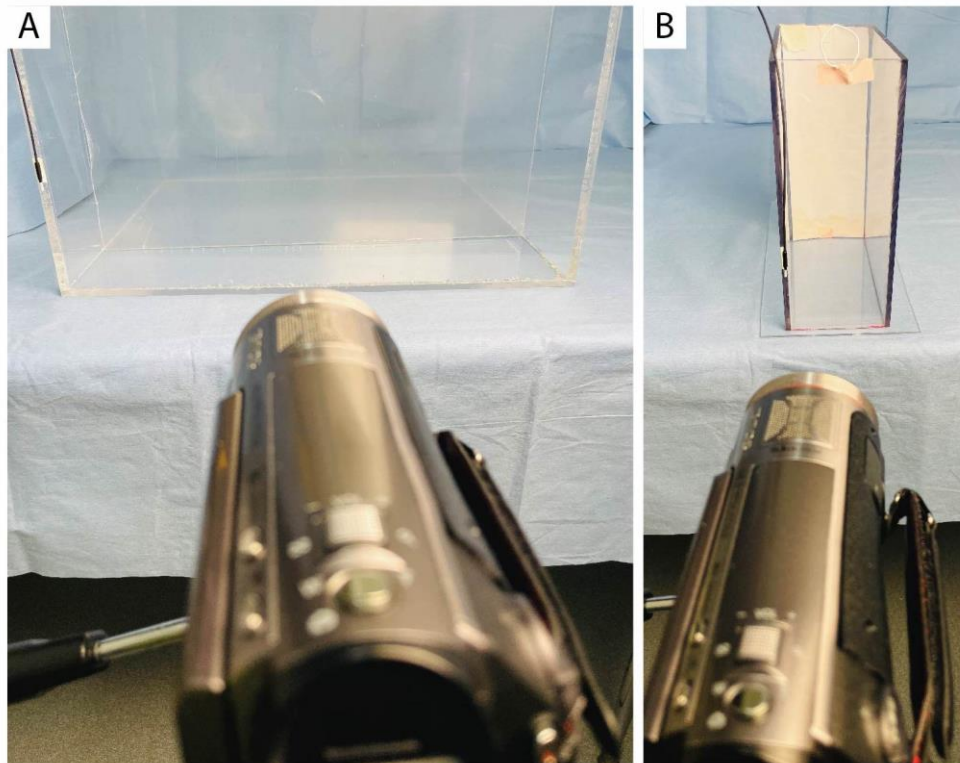


Figure 12. Photographs of the video recording and behaviour box setup. A) Behaviour box used for behaviour of walking. B) Behaviour box used for behaviours of string-pulling, eating, and licking peanut butter. A white hook is added to the center front top of the box for the string to go through it so that it stays in the centre of the box. *Note that in either box the mice would groom or rear where stimulation or control stimulation was applied at random. Also, for both boxes a laser cable is placed on the right side for control stimulation trials.

Behavioural tests

String-pulling

Pretraining to string-pulling. After fiber optic implant surgery and prior to food restriction, all mice were given Honey Nut Cheerios (referred to as cheerios henceforth; <https://www.cheerios.com/products/honey-nut-cheerios>) attached to pieces of string in their home cage to familiarize them with the food (Blackwell, Banovetz, et al., 2018). Then mice were food restricted to maintain 90% of their body weight and were fed once daily. After food restriction on the first day of training, the mice were placed individually in a transparent transfer tub (46cm x 26cm x 26cm) with a wired mesh top. Six strings of ~10-15cm baited with half a cheerio were lowered on each side for the tub (Blackwell, Banovetz, et al., 2018; Inayat et al., 2020). The mouse could pull the string to get the cheerio. Mice were removed from the tub once all the strings were pulled, and cheerios consumed or fifteen minutes had elapsed.

String-pulling test. The following day mice were placed in the string-pulling apparatus, a transparent rectangular box (20cm x 8cm x 20cm). The apparatus was positioned on a table 50cm above the floor in a small testing room (Figure 12B). Red strings made of 100% cotton with 0.1 cm diameter were used. The camera was positioned perpendicular to the wall of the apparatus. From day two onward, 60cm string was used baited with 1/3 of a cheerio. The string was hung over the edge and in the middle such that the portion of string on the inside was at mouse's nose level and not touching the floor. Mice were given five strings to pull daily for four days while laser cable was attached to the implant. Filming started on day five onward. Each mouse was given 4-6 trials during which either stimulation or control stimulation occurred. Mice were not taken out of apparatus in between trials and filming was never paused in between trials, i.e., mice spent the

entirety of filming session in the apparatus. The apparatus was cleaned with 70% isopropyl alcohol for the next mouse. Same string was used for all the mice.

Eating

When mice were consuming the retrieved cheerios after string-pulling, if they were sitting facing the front wall, stimulation or control stimulation was given semi-randomly for the experimental condition of eating. Since video recording was not paused in between string-pulling trials as mentioned above, the same recording track contained eating data.

Walking

After fiber optic implant surgery, mice were placed individually in a transparent rectangular box (44 cm x 10cm x 44 cm) for habituation for 5 min (Figure 12A). The next two days, they were placed in the box with the laser cable attached to the implant to habituate them for 5 minutes. Filming started on day four. The camera was placed perpendicular to the length of the box. Stimulation and control stimulation was given semi-randomly when the mice were walking. Filming duration was approximately 5 minutes for each mouse.

Rearing

Occasionally when the mice were rearing in the middle or at the walls except the back wall of the behaviour box, stimulation or control stimulation was given semi-randomly. Rearing data was contained in the same video track for behavioural box recording.

Licking peanut butter

After optic fiber implantation, mice were given peanut butter (KRAFT Smooth Peanut Butter, <https://www.kraftcanada.ca/brands/kraft-peanut-butter/product/kraft-smooth-peanut-butter-00068100084276?categoryid=20180001>) in their home cage to avoid neophobic response. The same string-pulling apparatus was used for peanut butter licking task (Figure 12B). Approximately at the height of the mice when rearing, peanut butter was applied to the front wall. The camera was positioned perpendicular to the wall of the apparatus. Stimulation and control stimulation were applied semi-randomly while the mouse was licking peanut butter.

Grooming

When the mice groomed in either the behaviour box or in the string-pulling apparatus (Figure 12), after eating cheerio or after licking peanut butter, stimulation or control stimulation was given semi-randomly only during the syntactic chain, highly stereotyped behavioural sequences shown by rodents (Berridge, 1990; Berridge & Fentress, 1986). Therefore, grooming in this thesis only refers to the syntactic chain portion of grooming.

Behavioural scoring

All videos were assessed frame-by-frame and a description of the behaviour before during and after stimulation and control stimulation was recorded on the excel sheet.

Behavioural effect. Any behavioural change, or occurrence of a forced movement than that of the normal ongoing behaviour during the stimulation or control stimulation was noted as an evoked behaviour. For example, if the mouse was rearing and then stimulated, and the mouse then dropped to the floor, it was noted that the stimulation affected rearing. If the mouse was walking

and during the stimulation the mouse stopped walking, it was noted that the stimulation affected walking.

Percentage of behavioural change for stimulation. Percentage of behavioural change for stimulation was calculated by counting the number of trials that the behaviour was affected during stimulation and dividing it by total number of stimulation trials multiplied by a hundred (Percentage = number of stimulation trials which behaviour was affected/total number of stimulation trials x 100).

Percentage of behavioural change for control stimulation. Percentage of behavioural change for control stimulation was calculated similarly by counting the number of trials that the behaviour was affected during control stimulation and dividing it by total number of control stimulation trials multiplied by a hundred (Percentage behavioural change for control stimulation = number of control stimulation trials which behaviour was affected/total number of control stimulation trials x 100).

Posture. Posture of the mice for all stimulation and control stimulation trials was scored as affected or not affected. For example, if the mouse was string-pulling and during the stimulation the mouse's posture changed, then it was scored that the stimulation affected the posture. A percentage of change for posture due to stimulation was calculated by counting the number of trials which posture was affected divided by the total number of stimulation trials multiplied by a hundred (Percentage change for posture for stimulation trials = number of stimulation trials which posture was affected / total number of stimulation trials x 100). A percentage of change for posture due to control stimulation was similarly calculated by counting the number of trials which posture was affected divided by the total number of control stimulation trials multiplied by a hundred

(Percentage change for posture for control stimulation trials = number of control stimulation trials which posture was affected / total number of control stimulation trials x 100).

Function. Function of the ongoing behaviour of the mice for all stimulation and control stimulation trials was scored as affected or not affected. For example, if the mouse was grooming and during the stimulation the mouse stopped grooming, then it was scored that the stimulation affected that behaviour. A percentage of change of function due to stimulation was calculated by counting the number of trials which function was affected divided by the total number of stimulation trials multiplied by a hundred (Percentage change for function for stimulation trials = number of stimulation trials which function was affected / total number of stimulation trials x 100). A percentage of change for function due to control stimulation was similarly calculated by counting the number of trials which function was affected divided by the total number of control stimulation trials multiplied by a hundred (Percentage change for function for control stimulation trials = number of control stimulation trials which function was affected / total number of control stimulation trials x 100).

Forced movement. Occurrence of a forced movement in the mice for all stimulation and control stimulation trials was scored as occurred or not occurred. For example, if the mouse was licking peanut butter and during stimulation extension of digits happened, although licking itself was not affected, then it was noted that the stimulation produced a forced movement. A percentage of occurrence of forced movement due to stimulation was calculated by counting the number of trials which forced movement occurred divided by the total number of stimulation trials multiplied by a hundred (Percentage change for forced movement for stimulation trials = number of stimulation trials which forced movement was affected / total number of stimulation trials x 100). A percentage of occurrence of forced movement due to control stimulation was similarly

calculated by counting the number of trials which forced movement occurred divided by the total number of control stimulation trials multiplied by a hundred (Percentage change for forced movement for control stimulation trials = number of control stimulation trials which forced movement was affected / total number of control stimulation trials x 100).

Stability score: Each fiber optic was scored as 0 or 1, where 0 means no effect and 1 means effective, based on whether the optogenetic stimulation through that fiber optic had a behavioural effect in the animal. This scoring was done across all six behaviours. The score of all fiber optics for each behaviour was summed up and divided by the highest potential score multiplied by a hundred (Percent stability score for each behaviour = sum of all fiber optic scores/ 25 x 100).

Statistical Analyses

All behavioural percentages were subjected to repeated measure ANOVA using SPSS (v.26.0.0.0) and follow-up post-hoc comparison and pairwise analyses were subjected to Bonferroni corrections. A p-value<0.05 was considered significant. Partial eta squared and observed power is reported along with p-value for all repeated measure ANOVA.

CHAPTER 3: RESULTS

The following result section includes statistical analysis for the effects of optogenetic and control stimulation on behaviour and in different cortical regions. Moreover, the effect of frequency and duration of optogenetic stimulation is included. Figure 13 is a summary of all the implants depicted with a circle and whether the optogenetic stimulation had no effect on any of the Type 1 or Type 2 behaviours tested, affected some of Type 1 or Type 2 behaviours tested, or affected all Type 1 or Type 2 behaviours tested, the circles are shown empty, half colored, or full colored respectively.

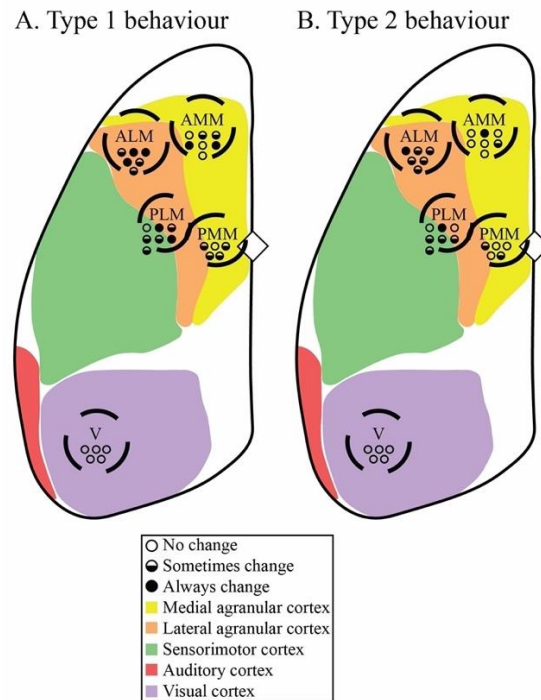


Figure 13. Summary of implant sites, number of implants, and the effect of optogenetic stimulation on Type 1 and Type 2 behaviours depicted on the top view of the left hemisphere of the mouse brain. Each circle depicts one implant. ALM, anterior lateral motor; AMM anterior medial motor; PLM, posterior lateral motor; PMM, posterior medial motor; V, visual cortex. A) Type 1 behaviours include walking, rearing, and string-pulling. B) Type 2 behaviours include grooming, eating, and licking peanut butter. *Note that the actual implants were done on the right hemisphere in all mice.

The average behavioural effect of optogenetic stimulation

All behavioural data for optogenetic stimulation (n = 5,056) and control stimulation (n = 3,318) were considered together to evaluate the relative effects of optogenetic stimulation in sensorimotor cortex. As shown in Figure 14A, the optogenetic stimulation applied to the neocortex had a greater effect in affecting behaviour than did the control stimulation. This conclusion was confirmed by the main effect of a repeated measure ANOVA comparing cortical to control stimulation, Stimulation (F (1,21) = 62.742, $p < 0.001$, $\eta_p^2 = 0.749$, observed power = 1.000).

Figure 14B shows the effects of optogenetic stimulation of the cortex vs. control stimulation which was also examined as a function of the cortical region stimulated. Stimulation of four brain regions of the motor cortex (ALM, AMM, PLM, and PMM) produced very similar percentages of behaviours affected in each region. This result was confirmed by a repeated measure ANOVA in which there was no region effect, Stimulation by Brain region (F (3,21) = 1.394, $p > 0.05$), $\eta_p^2 = 0.166$, observed power = 0.316).

Figure 14C shows the effects of optogenetic stimulation as a function of ongoing behaviour of the mice. In general, the more automatic behaviours of licking and chewing were less affected by stimulation and the learned behaviour of string-pulling was most affected by optogenetic stimulation. These conclusions were confirmed by a repeated measure ANOVA in which the effects of optogenetic stimulation of each the six behaviours were compared, Stimulation by Behaviour (F (5,105) = 24.507, $p < 0.001$, $\eta_p^2 = 0.539$, observed power = 1.000). Post-hoc pairwise comparisons of the behaviours revealed a significant difference between Type 1 and Type 2 behaviours where Type 2 behaviours were significantly less affected (Figure 14C). Grooming and eating compared to walking, rearing and string-pulling ($p < 0.01$, $p < 0.001$, $p < 0.001$) were

significantly less affected. Licking peanut butter compared to rearing and string-pulling ($p < 0.001$, $p < 0.001$) was also significantly less affected.

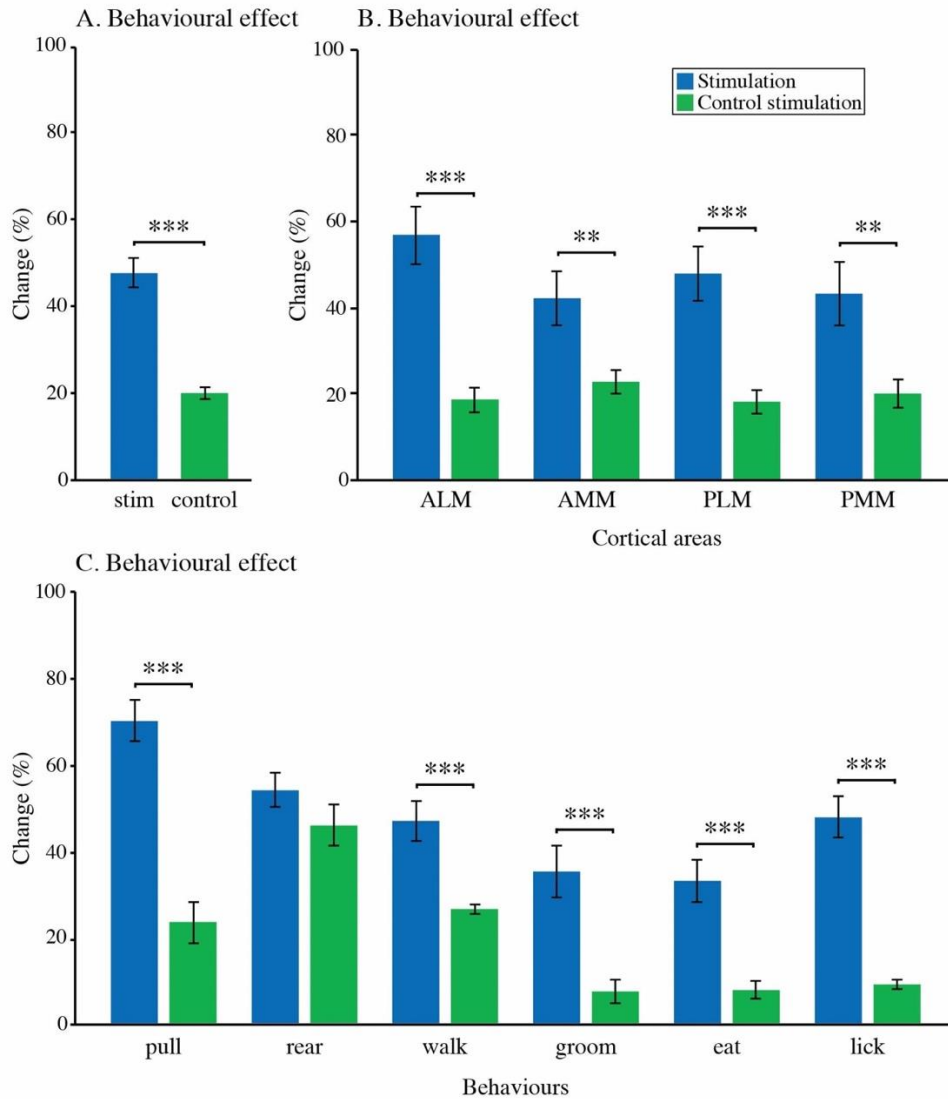


Figure 14. The behavioural effects (mean \pm s.e.) of optogenetic stimulation or a control stimulation application. A) The total behavioural effect of optogenetic or control stimulation in all cortical areas, B) The behavioural effect of optogenetic stimulation as a function of cortical region, and C) The behavioural effect of optogenetic stimulation as a function of ongoing behaviour. Stim (optogenetic stimulation), control (control stimulation), ALM (anterolateral motor area), AMM (anteromedial motor area), PLM (posterolateral motor area), PMM (posteromedial motor area). (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Note that the behavioural effect refers to any behavioural change than that of the normal ongoing behaviour.

In summary, the overall optogenetic stimulation of sensorimotor cortex affected the behaviour of mice. Stimulation of different regions had similar effects. All behaviours except for rearing were affected by optogenetic stimulation, and string-pulling task was most affected by the stimulation. Thus, the effect of the stimulation was not all or nothing but depended upon ongoing behaviour.

Behavioural effects of optogenetic stimulation as a function of behaviour

In order to assess the relative effects of optogenetic stimulation, a repeated measure ANOVA compared the effects of stimulation on each behaviour in each brain region, Stimulation by Brain region by Behaviour ($F(15, 105) = 2.340, p < 0.05, \eta_p^2 = 0.251, \text{observed power} = 0.917$). In order to compare the optogenetic effects on individual behaviours on each brain region follow-up Bonferroni test were performed. Figure 15 shows main optogenetic effects on each behaviour on each region and summarizes the result of the follow-up test. In summary, optogenetic stimulation of mice affected licking peanut butter and string-pulling in all four brain regions. In addition, rearing was the only behaviour that was not significantly affected in any region. The effects of optogenetic stimulation on the remaining behaviours depended on brain region that was stimulated.

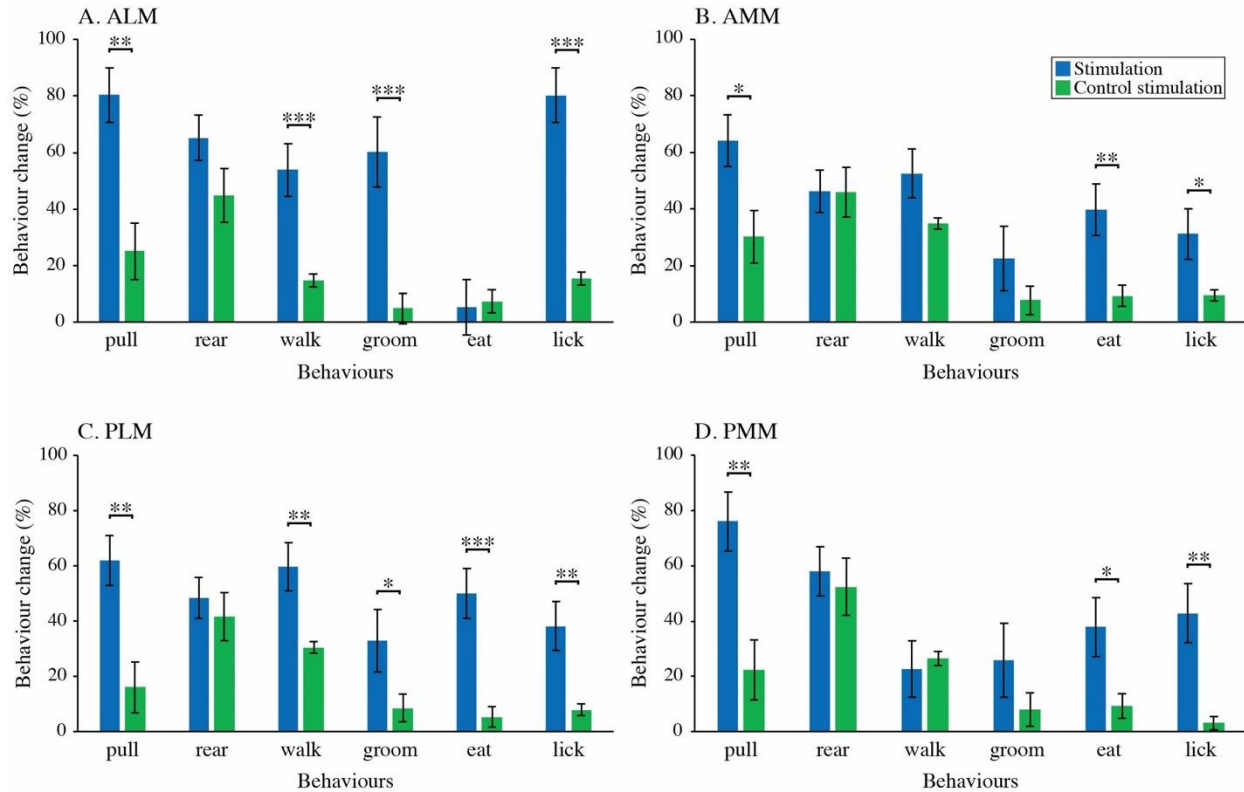


Figure 15. The behavioural change (mean \pm s.e.) of optogenetic stimulation or control stimulation applied during ongoing behaviour. A) anterolateral motor (ALM) area. B) anteromedial motor (AMM) area. C) posterolateral motor (PLM) area. D) posteromedial motor (PMM) area. Pull is string-pulling task. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Note that the behavioural change refers to any behavioural change from the normal ongoing behaviour.

Behavioural effects of optogenetic stimulation of the primary visual cortex

In order to determine whether visual cortex stimulation affected behaviour, the responses from 5 fiber optics implanted in 5 mice were compared to the effects of control stimulation. In total, the behavioural data of 904 stimulation to the primary visual cortex was compared to 677 control stimulation to evaluate the relative effects of optogenetic stimulation in the visual cortex. As shown in Figure 16A, the optogenetic stimulation applied to the primary visual cortex did not affect behaviour. This result was confirmed by the main effect of a repeated measure ANOVA

comparing primary visual to control stimulation, Stimulation ($F(1,4) = 0.086$, $p > 0.05$, $\eta_p^2 = 0.021$, observed power = 0.056).

In order to determine whether optogenetic stimulation of the primary visual cortex has any effect on the behaviour of mice in different behaviours, stimulation and control stimulation was applied during various behaviours. In total, 5 mice were stimulated during behaviours of grooming, eating, licking peanut butter, walking, rearing, and string-pulling. Figure 16B shows the effects of optogenetic stimulation as a function of ongoing behaviour of the mice. Optogenetic stimulation of primary visual cortex did not affect any of the six behaviours mentioned above. This conclusion was confirmed by a repeated measure ANOVA in which the effect of optogenetic stimulation of each of the six behaviours was compared with the control stimulation, Stimulation by Behaviour ($F(5,20) = 1.750$, $p > 0.05$, $\eta_p^2 = 0.304$, observed power = 0.253).

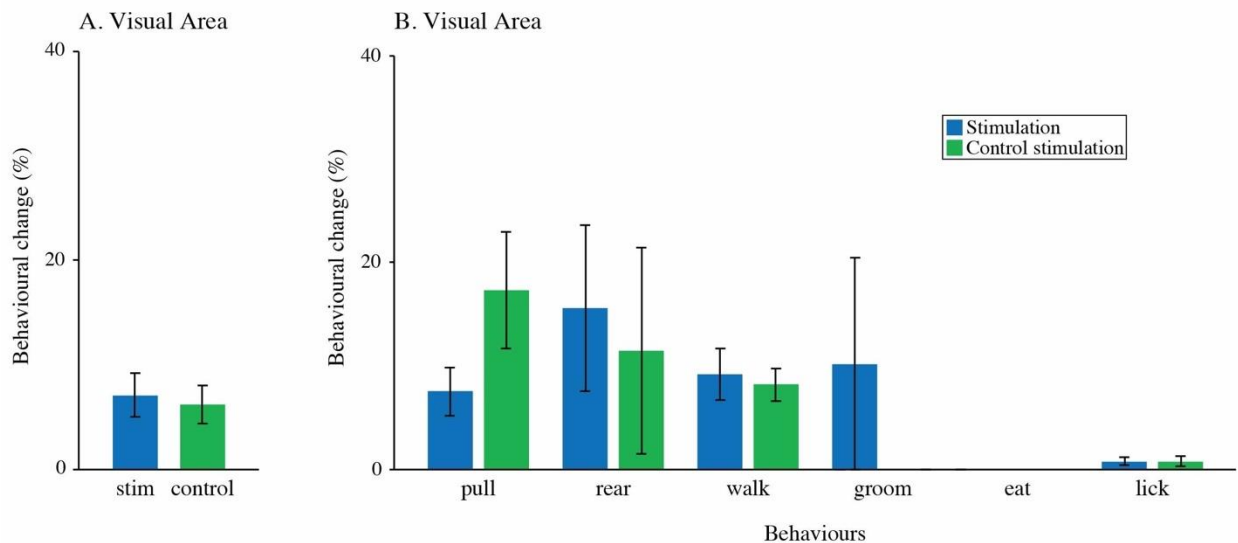


Figure 16. The behavioural change (mean \pm s.e.) of optogenetic stimulation of the primary Visual cortex or a control stimulation application. A) The total behavioural effect of optogenetic or control stimulation in all behaviours. B) The behavioural effect of optogenetic stimulation as a function of ongoing behaviour. Stim, optogenetic stimulation; pull, string-pulling task. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Note that the behavioural change refers to any behavioural change than that of the normal ongoing behaviour.

In order to determine whether stimulation of the visual cortex had an effect on behaviour compared to optogenetic stimulation of sensorimotor regions, the responses from 5 fiber optics implanted in 5 mice were compared to the overall effects of optogenetic stimulation in sensorimotor cortex in 25 fiber optic implants in sensorimotor cortex. As shown in Figure 17, the behavioural effects of optogenetic stimulation in sensorimotor regions of ALM, AMM, PLM, and PMM are greater than those for Visual Cortex. This result was confirmed by a repeated measure ANOVA on behavioural effects of Visual Cortex vs. Sensorimotor Cortex stimulation, Stimulation by Brain region ($F(4, 25) = 4.083, p < 0.05, \eta_p^2 = 0.395, \text{observed power} = 0.853$). Post-hoc pairwise comparison tests with Bonferroni correction revealed that optogenetic stimulation of all sensorimotor areas of ALM, AMM, PLM, and PMM compared to the Visual area have a greater effect in behaviour ($p < 0.001, p < 0.001, p < 0.001, p < 0.001$).

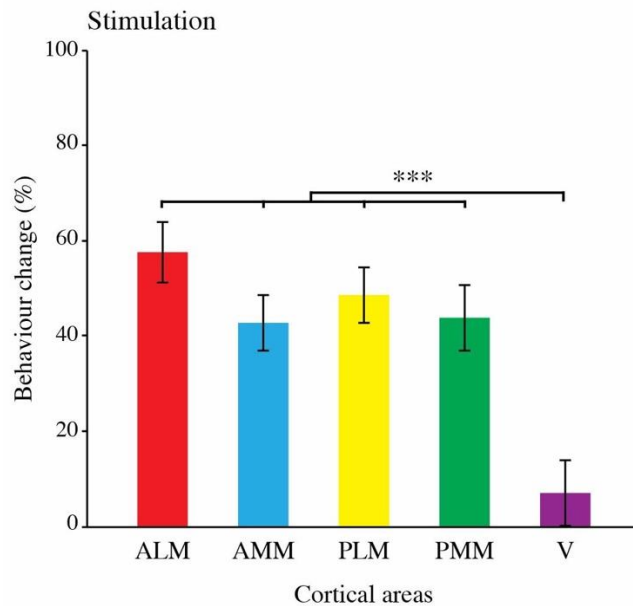


Figure 17. The behavioural change (mean \pm s.e.) of optogenetic stimulation of different cortical regions. ALM, anterolateral motor area; AMM, anteromedial motor area; PLM, posterolateral motor area; PMM, posteromedial motor area; V, primary visual area. (* < 0.05 , ** $p < 0.01$, *** $p < 0.001$). Note that the behavioural change refers to any behavioural change than that of the normal ongoing behaviour.

In summary, overall optogenetic stimulation of Visual cortex did not affect the behaviour of the mice in any of the six behavioural tasks as compared to stimulation of sensorimotor cortex.

Behavioural effects of frequency of optogenetic stimulation

In order to evaluate the effect of different frequency pulses of optogenetic stimulation on behaviour of mice, all frequency data for optogenetic stimulation ($n = 5,056$) were pulled together regardless of duration of stimulation. As shown in Figure 18, frequencies of 10Hz, 20Hz, 30Hz and 40Hz were similar in affecting the behaviour of mice. This result was confirmed by a repeated measure ANOVA in which the effect all four mentioned frequencies was compared and found not to be different, Frequency ($F(3,63) = 3.341, p > 0.05, \eta_p^2 = 0.137, \text{observed power} = 0.564$).

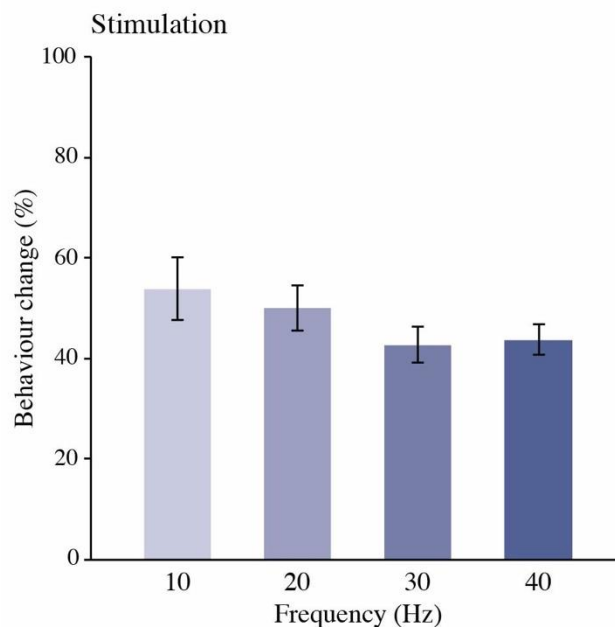


Figure 18. The behavioural change (mean \pm s.e.) of optogenetic stimulation applied with different frequency pulses of 10Hz, 20Hz, 30Hz, and 40Hz. Note that the effect of different frequencies of optogenetic stimulation on the behavioural change of the mice is the same. Note that the behavioural change refers to any behavioural change than that of the normal ongoing behaviour.

In summary, the different frequency of the optogenetic stimulation did not have a significant effect on affecting the behaviour of the mice.

Behavioural effect of various durations of optogenetic stimulation

In order to evaluate the effect of different duration of optogenetic stimulation on the behaviour of the mice, all frequency data for optogenetic stimulation in four different behaviours (n = 4,211) were pulled together. Stimulation durations of 0.5, 0.7, 1, 1.5, and 2 seconds were compared for behaviours of grooming, licking peanut butter, walking, and rearing. As shown in Figure 19, all five durations had similar effects in affecting the behaviour of the mice. This result was confirmed with a repeated measure ANOVA in which the effect of five different duration of stimulation were found to be equivalent, Duration ($F(4,84) = 2.749, p > 0.05, \eta_p^2 = 0.116, \text{observed power} = 0.671$).

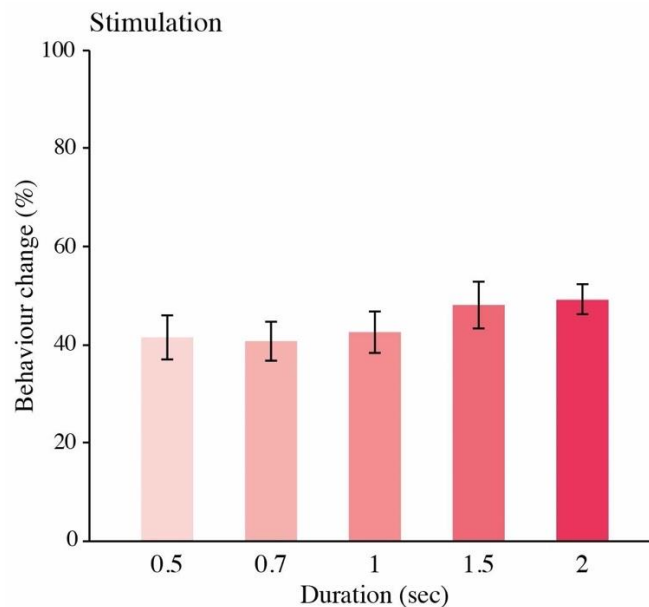


Figure 19. The behavioural change (mean \pm s.e.) of optogenetic stimulation of different durations of stimulation at 0.5, 0.7, 1, 1.5, and 2 seconds. Note that the behavioural change refers to any behavioural change than that of the normal ongoing behaviour.

In summary, the duration of the optogenetic stimulation did not have a significant effect in affecting the behaviour of the mice.

Effect of optogenetic stimulation on posture, function, and forced movement

In order to evaluate the effect of optogenetic stimulation on body posture, all stimulation trials (n = 5,056) and control stimulation trials (n = 3,318) were scored to indicate whether the posture of mice changed when applying the stimulation during various behaviours. A repeated measure ANOVA on the percentage of change of posture revealed a main effect of Behaviour (F(5,105)=153.501, $p < 0.001$, $\eta_p^2 = 0.880$, observed power=1.000), Stimulation by Behaviour (F(5,105)=4.607, $p < 0.05$, $\eta_p^2 = 0.180$, observed power=0.787), but no main effect of Stimulation (F(1,21)=3.161, $p > 0.05$, $\eta_p^2 = 0.131$, observed power=0.396), Brain Region (F(3,21)=1.834, $p > 0.05$, $\eta_p^2 = 0.208$, observed power=0.407), Stimulation by Brain Region (F(3,21)=0.604, $p > 0.05$, $\eta_p^2 = 0.079$, observed power=0.154), Behaviour by Brain Region (F(15,105)=0.489, $p > 0.05$, $\eta_p^2 = 0.853$, observed power=0.201), or Stimulation by Behaviour by Brain Region (F(15,105)=0.390, $p > 0.05$, $\eta_p^2 = 0.053$, observed power=0.156) (Figure 20A).

In order to evaluate whether optogenetic stimulation affects the function of a behaviour, all stimulation trials (n = 5,056) and control stimulation trials (n = 3,318) were scored to indicate the function that was affected by applying the stimulation in various behaviours. A repeated measure ANOVA on the percentage of change of function revealed a main effect of Stimulation (F(1,21)=12.807, $p < 0.01$, $\eta_p^2 = 0.379$, observed power=0.927), Behaviour (F(5,105)=39.765, $p < 0.001$, $\eta_p^2 = 0.654$, observed power=1.000), Stimulation by Behaviour (F(5,105)=7.856, $p < 0.001$, $\eta_p^2 = 0.272$, observed power=0.994), but no main effect of Brain Region (F(3,21)=0.291, $p > 0.05$, $\eta_p^2 = 0.040$, observed power=0.097), Stimulation by Brain Region (F(3,21)=1.672, $p > 0.05$,

$\eta_p^2=0.193$, observed power=0.373), Behaviour by Brain Region ($F(15,105)=1.549$, $p>0.05$, $\eta_p^2=0.181$, observed power=0.762), or Stimulation by Behaviour by Brain Region ($F(15,105)=1.204$, $p>0.05$, $\eta_p^2=0.147$, observed power=0.598) (Figure 20B).

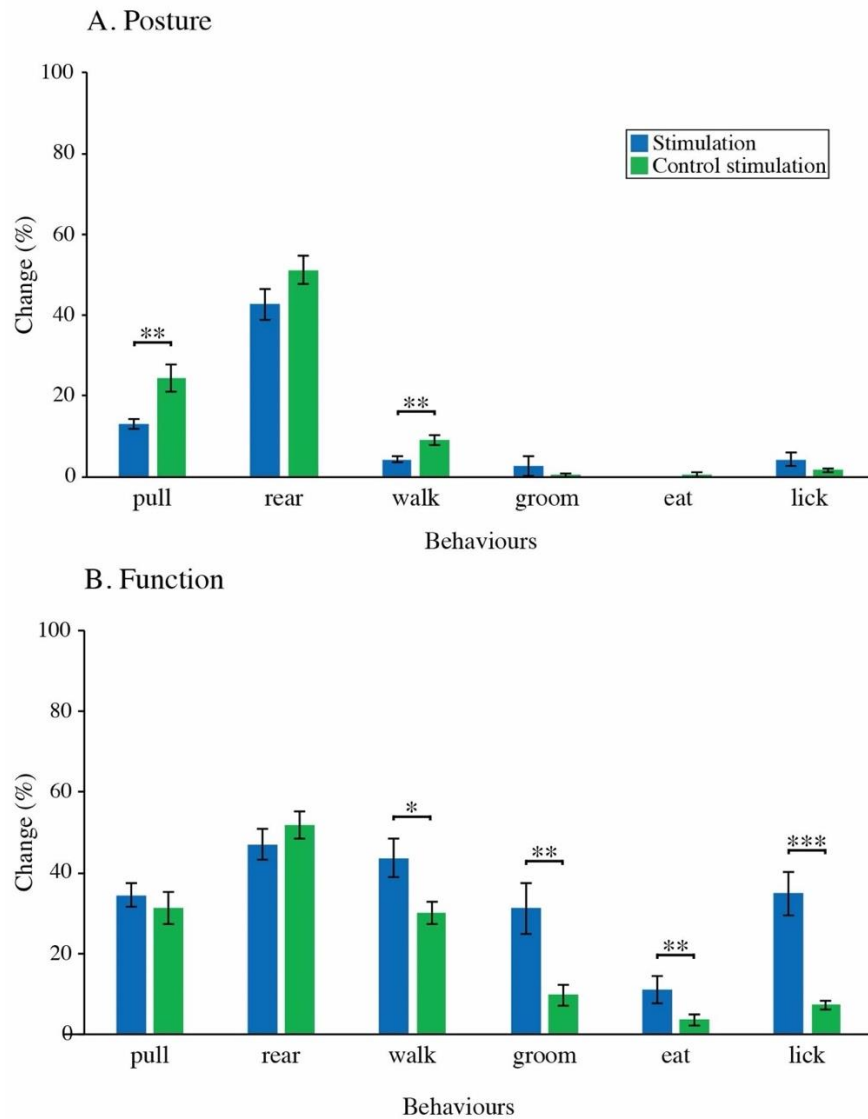


Figure 20. The postural and functional change (mean \pm s.e.) of mice during optogenetic stimulation and control stimulation in different behaviours of groom, eating, licking, walking, rearing, and pulling (string-pulling). A. Postural change of mice from all sensorimotor stimulation and control stimulation as a function of ongoing behaviour. B. Functional change of mice from all sensorimotor stimulation and control stimulation as a function of ongoing behaviour. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Note that the postural and functional change refers to any change in the posture and function of the normal ongoing behaviour.

In order to evaluate whether optogenetic stimulation induced any forced movement, all stimulation trials (n = 5,056) and control stimulation trials (n = 3,318) were scored to indicate whether a forced movement occurred by applying the stimulation in various behaviours. A repeated measure ANOVA on the percentage of forced movement revealed a main effect of Stimulation ($F(1,21)=13.149$, $p<0.01$, $\eta_p^2=0.385$, observed power=0.933), Behaviour ($F(5,105)=7.114$, $p<0.001$, $\eta_p^2=0.253$, observed power=0.974), Brain Region ($F(3,21)=4.548$, $p<0.05$, $\eta_p^2=0.394$, observed power=0.816), Stimulation by Brain Region ($F(3,21)=3.948$, $p<0.05$, $\eta_p^2=0.361$, observed power=0.753), Behaviour by Brain Region ($F(15,105)=2.621$, $p<0.05$, $\eta_p^2=0.272$, observed power=0.910), Stimulation by Behaviour ($F(5,105)=5.325$, $p<0.001$, $\eta_p^2=0.202$, observed power=0.918), and Stimulation by Behaviour by Brain Region ($F(15,105)=2.329$, $p<0.05$, $\eta_p^2=0.250$, observed power=0.873) (Figure 21).

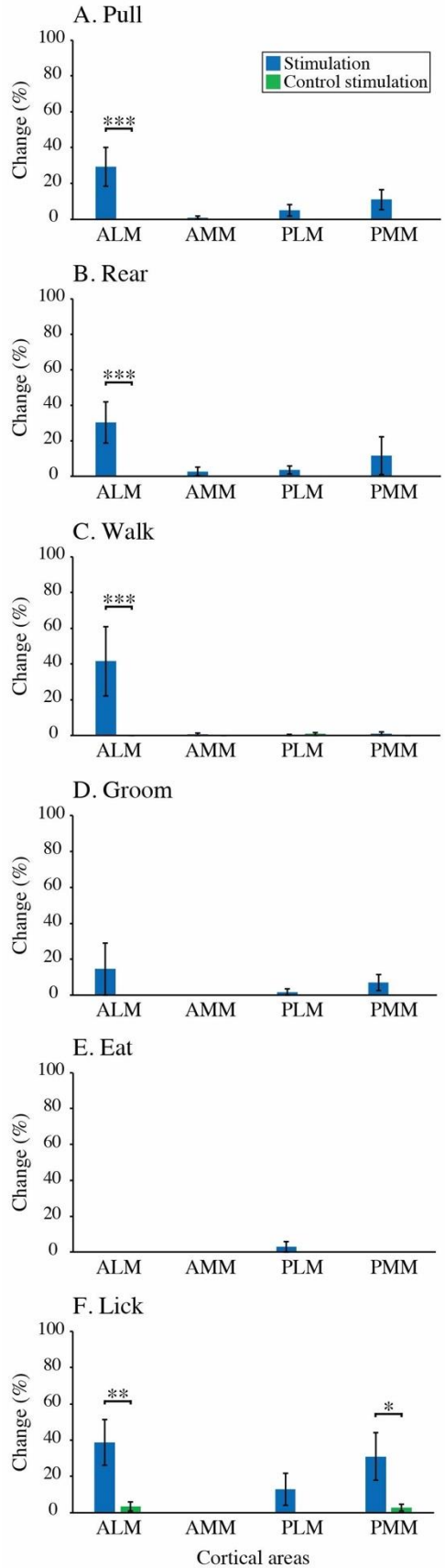


Figure 21. The forced movement occurred (mean \pm s.e.) with optogenetic stimulation or control stimulation at different sensorimotor brain regions during ongoing behaviour. A. String-pulling behaviour. B. Rearing behaviour. C. Walking behaviour. D. Grooming behaviour. E. Eating behaviour. F. Licking behaviour. ALM, anterolateral motor area; AMM, anteromedial motor area; PLM, posterolateral motor area; PMM, posteromedial motor area. Note that the stimulation of the ALM region produces forced movements in four out of six behaviours and stimulation of AMM or PLM regions does not produce forced movements in any of the behaviours. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

In summary, optogenetic stimulation affected the posture of mice during string-pulling and walking behaviours. Optogenetic stimulation interrupted the function of behaviours of walking, grooming, eating, and licking peanut butter. Forced movement occurred during optogenetic stimulation of the ALM region when mice were engaged in string-pulling, rearing, walking, and licking peanut butter and of the PMM region during licking peanut butter.

Overall summary of the results

In summary, various frequency and duration of the optogenetic stimulation did not significantly affect the behaviour of the mice. Optogenetic stimulation of different sensorimotor regions had similar effects in affecting the behaviours. However, optogenetic stimulation during different behaviours had different effects. Stability scores indicate that voluntary behaviours of string-pulling and rearing are more resistant than automatic behaviours of grooming and eating (Figure 22).

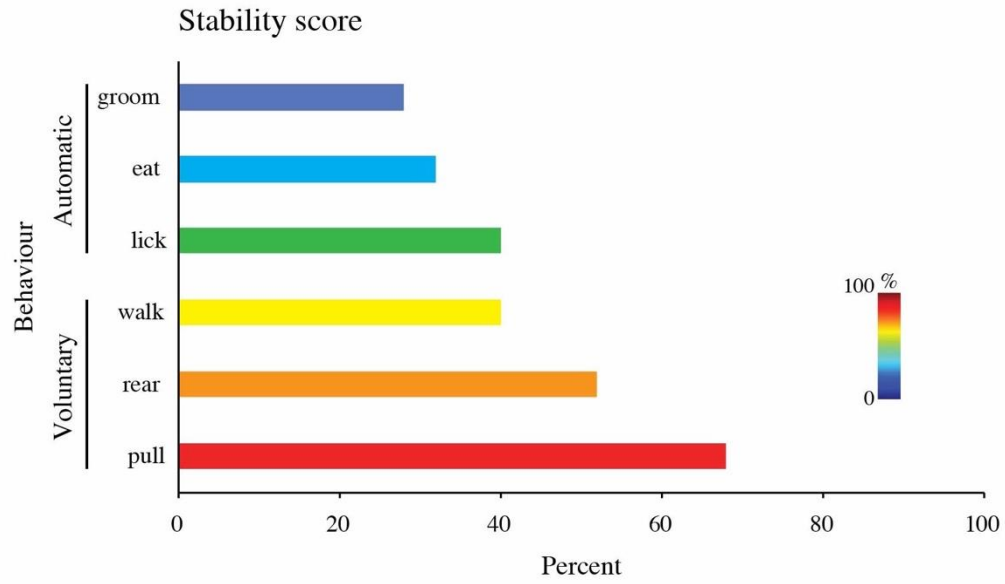


Figure 22. Stability score of different behaviours. Pull, string-pulling. Note that automatic behaviours of grooming and eating have the lowest scores.

CHAPTER 4. ANTERIOR LATERAL MOTOR (ALM) AREA

String-pulling

String-pulling is a skilled behaviour in which a mouse stands on two feet and uses hand-over-hand movements to pull down a string to get to the food reward at the end of the string (Blackwell, Banovetz, et al., 2018). This behaviour consists of two acts: a reach in which the mouse advances the hands to grasp the string, and a withdraw in which the mouse pulls on the string. During these two acts, four arm movements and four hand movements occur. The arm movements include 1) Lift, in which the lower arm is brought upward at the level of chest during which fingers are closed and flexed. 2) Advance, in which the upper arm extends, and the elbow opens to extend the lower arm at the level of mouth during which the fingers extend and fully open. 3) Pull, in which the upper arm is lowered to the level of chest and the elbow closes while holding the string. 4) Push, in which the elbow opens, and the arm extends to the level of abdomen still holding the string. The hand movements include 1) Release, in which the fingers fully extend at the completion of the push. 2) Collect, in which the fingers are lightly closed and flexed at the level of chest. 3) Overgrasp, in which the fingers are fully open and extended while advancing towards the string at the level of mouth. 4) Grasp, in which the fingers close around the string at the beginning of pulling. Overall, the mouse makes alternate hand movements of reach and withdraw to advance towards the end of the string (Blackwell, Banovetz, et al., 2018).

Video 1 is an example of the effect of stimulation of the ALM region while string-pulling. In this video, as the left hand releases the string and is in the collect posture in which fingers are lightly closed and flexed with the hand at the level of the chest, as the stimulation begins. During the stimulation, the left hand continues advancing towards the string, but misses a grasp. It again attempts to grasp the string and bringing it towards the mouth, but misses the string and only closes







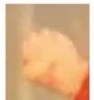





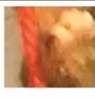











digits and bringing a closed empty hand towards the mouth. During all of this, the mouth keeps opening and closing. The right hand holds onto the string without releasing it and the mouse maintains its posture throughout the stimulation.

Overall, string-pulling is affected. The mouse remains standing, the right hand stays closed and does not release the string, the left hand whether holding the string or not approaches the mouth, and the mouth makes open and close movements. The relationship between the hand, string and the mouth resembles eating behaviour as if the mouse is putting the string into its mouth. However, the hand does not actually put the string in the mouth neither does the mouse bite the string.

Table 3 summarizes the state of the posture, right and left hands, and the mouth in 6 mice that received optogenetic stimulation of the ALM region while engaged in the string-pulling task. Here is a description of the individual mice since not all mice show the exact same behaviour. Mouse #1F: As illustrated in the first row of Table 3, this mouse remains standing up during the stimulation. The right hand remains grasping the string. Left hand grasping the string and bringing it towards the mouth. Mouth does not open and close. Mouse #5F: As illustrated in the second row of Table 3, this mouse remains standing up during the stimulation. Right hand releases the string, but the digits are closed and flexed. Left hand is brought towards the mouth. Mouth makes open and close movement. Mouse #6F: As illustrated in the third row of Table 3, this mouse remains standing up during the stimulation. Right hand remains grasping the string. Left hand grasping the string and bringing it towards the mouth. Mouth makes open and close movement. Mouse #7M: As illustrated in the fourth row of Table 3, this mouse remains standing up during the stimulation. Right hand remains grasping the string. Left hand is brought towards the mouth. Mouth makes open and close movement. Mouse #10M: As illustrated in the fifth row of Table 3,

this mouse remains standing up during the stimulation. Right hand remains grasping the string. Left hand is grasping the string. Mouth does not open and close. Mouse #11F: As illustrated in the last row of Table 3, this mouse does not remain standing up, but quits string-pulling by lowering its posture during the stimulation. Right hand releases the string, but the digits are closed and flexed. Left hand releases the string and is open. Mouth does not open and close.

Table 3. The state of mouth, left hand, right hand, and posture during stimulation of the anterolateral motor (ALM) region in string-pulling task for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#1F	no movement 	closed and approaching mouth 	closed, does not release string 	remain standing up 
#5F	opening and closing 	closed and approaching mouth 	closed, release string 	remain standing up 
#6F	opening and contacting string 	closed and approaching mouth 	closed, does not release string 	remain standing up 
#7M	opening and closing 	closed and approaching mouth 	closed, does not release string 	remain standing up 
#10M	no movement 	closed and holding string 	closed, does not release string 	remain standing up 
#11F	no movement 	open and release string 	closed, release string 	going down 

In summary, the string-pulling is interrupted by stimulation of the ALM region. The mouse remains standing, the right hand remains closed and does not release the string, the left hand





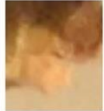











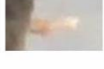





whether holding the string or not approaches the mouth, and the mouth makes open and close movements. The relationship between the hand, string and the mouth resembles eating behaviour as if the mouse is putting the string in the mouth. However, the hand does not actually put the string in the mouth, neither does the mouse bite the string.

Rearing

Rearing is a voluntary behaviour in which the mouse raises hands from the floor, puts its weight on the feet and extends head upward (Whishaw et al., 2010). As the mouse pushes off from the floor and digits release and lose contact from the floor surface, they close. As the hands are lifted, the digits are closed and flexed, a position named collection. If the mouse makes an unsupported rear, in which the hands make no contact with the wall, the mouse stands on two feet with hands remaining collected for the duration of the rear. If the mouse makes a supported rearing in which the hands have contact against the walls, then as the hands approach the wall, the digits extend and open to contact the wall. This contact can be seen as digit tips rest against the wall, or hand palm fully rests against the wall. When the mouse goes back to its initial quadruped position, first the digits/hands release the wall and acquire the collect position, then they lower and as the hands approach the floor, then the digits extend and hands open to be placed on the floor (Whishaw et al., 2010). Video 2 demonstrates the effect of stimulation of the ALM region while the mouse makes a supported rear. In this video, both hands are resting against the wall when the stimulation begins. The mouse remains standing, right hand adjusts its position by withdrawing contact from the wall and going into a collected position, then reaching forward to make a wall contact. Head moves slightly to the left side. Then the left hand withdraws contact from the wall and positions closer to the mouth during which the mouth keeps opening and closing.

Table 4 summarizes the state of the posture, right and left hands, and the mouth in 6 mice that received optogenetic stimulation of the ALM region while rearing. Here is a description of the individual mice since not all mice show the same behaviour. Mouse #1F: As illustrated in the first row of Table 4, this mouse remains standing up during the stimulation. Right hand is closed and flexed. Left hand is also closed and flexed. Mouth does not make any movements. Mouse #5F: As illustrated in the second row of Table 4, this mouse remains standing up during the stimulation but lowers its back so that the mouth goes towards the hands. Right hand is closed and flexed at the mouth. Left hand is also closed and flexed at the mouth. Mouth makes open and close movements. Mouse #6F: As illustrated in the third row of Table 4, this mouse quits standing up during the stimulation. Right hand is semi-closed and semi-flexed. Left hand is also semi-closed and semi-flexed. Mouth does not open and close. Mouse #7M: As illustrated in the fourth row of Table 4, this mouse remains standing up during the stimulation. Right hand is open at the front wall. Left hand makes grasping movements, opening and closing. Mouth makes open and close movement and follows the left hand. Mouse #10M: As illustrated in the fifth row of Table 4, this mouse quits standing up during the stimulation. Right hand is semi-closed and semi-flexed. Left hand extends forward before reaching the floor. Mouth does not open and close. Mouse #11F: As illustrated in the last row of Table 4, this mouse quits standing up during the stimulation. Right hand is open at the front wall. Left hand is also open at the front wall. Mouth does not open and close.

Table 4. The state of mouth, left hand, right hand, and posture during stimulation of the anterolateral motor (ALM) region in rearing for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#1F	no movement 	closed and flexed 	closed and flexed 	remains standing up 
#5F	opening and closing, approaching hands 	closed and flexed, close to mouth 	closed and flexed, close to mouth 	remains standing up 
#6F	no movement 	semi-closed, semi-flexed 	semi-closed, semi-flexed 	goes down 
#7M	opening and closing, approaching left hand 	grasping movements 	open at the wall 	remains standing up 
#10M	no movement 	extends forward 	semi-closed, semi-flexed 	goes down 
#11F	no movement 	open at the wall 	open at the wall 	goes down 

In summary half the mice in this group remain standing and the other half quit rearing when stimulation occurs. All mice show a collect hand posture when they rear. Two of the standing mice show a hand to mouth/mouth to hand behaviour and mouth movement, in which the mouse opens and closes with no tongue protrusion. The mice that quit rearing exhibit a normal hand collection and placement of hand on the floor.



Walking

Walking is a voluntary behaviour in which the mouse changes in limb position and changes the configuration of its hands and digits as it moves (Whishaw et al., 2010). A limb cycle consists of: 1) Lift, as a mouse moves forward, the hand releases contact with the surface, and the fingers remain extended at first but begin to close. 2) Collect, as the limb is flexed and carried in the transport phase of the step, the fingers are lightly closed and flexed into a posture termed collect. 3) Advance, as the limb is advanced to make contact with the surface, the fingers extend. 4) Place, to make contact with the surface, the hand pronates so that the 5th finger first contacts the surface followed in sequence by fingers 4, 3, and 2 in an arpeggio movement. 5) Stance, during the stance phase of the step, the fingers are extended and open and the surface of the fingers and palm contact the surface to support the body's weight (Whishaw et al., 2010). Video 3 gives an example of stimulation of the ALM region while a mouse is walking. In this video, the left hand is placed on the floor when the stimulation begins. The mouse stops walking, head is lowered towards the floor and moves towards the left side to the location of the left hand, and the mouth makes open and close movements.

Table 5 summarizes the state of the posture, right and left hands, and the mouth in 6 mice who received optogenetic stimulation of the ALM region while walking. Here is a description of the individual mice since not all mice show exactly the same behaviour. Mouse #1F: As illustrated in the first row of Table 5, this mouse keeps its posture and continues walking during the stimulation. Right and left hands take normal steps. Mouth does not make any movements. Mouse #5F: As illustrated in the second row of Table 5, this mouse keeps its posture, but stops walking during the stimulation. Right hand is not visible since the view captures the left side of the mouse. Left hand is semi-closed and close to the mouth. Mouth movements is not visible since the left

hand is blocking the view of the mouth. Mouse #6F: As illustrated in the third row of Table 5, this mouse keeps its posture, initially stops walking but then takes steps during the stimulation. Right hand is on the floor. Left hand is semi-closed and semi-flexed. Mouth makes open and close movement and tongue protrudes at one point. Mouse #7M: As illustrated in the fourth row of Table 5, this mouse keeps its posture, but stops walking during the stimulation. Right hand is on the floor. Left hand is closed and flexed and moves to the left side. Mouth makes open and close movement and follows the left hand. Mouse #10M: As illustrated in the fifth row of Table 5, this mouse keeps its posture and continues walking during the stimulation. Right and left hands take normal steps. Mouth does not make any movements. Mouse #11F: As illustrated in the last row of Table 5, this mouse keeps its posture and continues walking during the stimulation. Right and left hands take normal steps. Mouth does not make any movements.

Table 5. The state of mouth, left hand, right hand, and posture during stimulation of the anterolateral motor (ALM) region in walking for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#1F	no movement 	semi-closed, semi-flexed, stepping 	on the floor 	remains the same, keeps walking 
#5F	not visible	semi-closed, close to mouth 	not visible	remains the same, stops walking 
#6F	opening and closing, tongue protrusion 	semi-closed, semi-flexed 	on the floor 	remains the same, takes steps 
#7M	opening and closing, following left hand 	closed, flexed, moving to the left 	on the floor 	remains the same, stops walking 
#10M	no movement 	semi-closed, semi-flexed, stepping 	on the floor 	remains the same, keeps walking 
#11F	no movement 	semi-closed, semi-flexed, stepping 	on the floor 	remains the same, keeps walking 

In summary, half the mice in this group stop walking and the other half keep walking during the stimulation. All mice exhibit collected fingers while stepping. In two mice the mouth moves towards the left hand and makes open and close movements.

Grooming








Grooming is an automatic behaviour in which the mouse starts by licking its hands and uses the wet hands to clean the nose and face by making elliptical brushing movements (Berridge, 1990). Then the mouse proceeds to clean the torso by licking the belly and the back, anogenital

area, and feet and tail. In this study, only syntactic chain of grooming and specifically phases 1-3 was looked. These phases are described by Berridge and Fentress (1986) as followed: Phase 1 consists of a set of 5-9 rapid strokes bilaterally forming small elliptical trajectories over the nose; phase 2 consists of a single unilateral (or bilateral asymmetrical) stroke or short series of strokes that ascend to the dorsal border of the mystacial vibrissae; and Phase 3 consists of a series of repeated, large-amplitude strokes often over the ear that typically are bilateral and symmetrical. Video 4 is an example video demonstrating stimulation of the ALM region while the mouse is grooming. In this video, the hands are licked, and as the mouse raises hands to wash the face/nose the stimulation begins. Both hands stay closed and flexed in a fist at the mouth level, and the mouth moves towards the left hand makes open and close movements. Once the stimulation ends, both hands are licked, and face washing starts.

Table 6 summarizes the state of the posture, right and left hands, and the mouth in 6 mice who received optogenetic stimulation of the ALM region while grooming. Here is a description of the individual mice since not all mice show the exact same behaviour. Mouse #1F: As illustrated in the first row of Table 6, this mouse keeps its posture but stops grooming during the stimulation. Right and left hands are closed and at the mouth and not moving. Mouth is not visible in the video to assess whether it makes any movements. Mouse #5F: As illustrated in the second row of Table 6, this mouse keeps its posture, but stops grooming during the stimulation. Right and left hands are closed and at the mouth and not moving. Mouth makes open and close movements, but no tongue protrusion is seen. Mouse #6F: As illustrated in the third row of Table 6, this mouse keeps its posture and continues grooming during stimulation. Right and left hands continue face washing. Mouth keeps opening and closing and tongue protrusion is seen to wet the hands. Mouse #7M: As illustrated in the fourth row of Table 6, this mouse keeps its posture, but stops grooming during

the stimulation. Right and left hands are closed and at the mouth and not moving. Mouth makes open and close movement, but no tongue protrusion is seen. Mouse #10M: As illustrated in the fifth row of Table 6, this mouse keeps its posture and continues grooming during the stimulation. Right and left hands are closed and at the mouth without moving. Mouth does not make any movements. Mouse #11F: As illustrated in the last row of Table 6, this mouse keeps its posture and continues grooming during the stimulation. Right and left hands continue face washing. Mouth keeps opening and closing and tongue protrusion is seen to wet the hands.

Table 6. The state of mouth, left hand, right hand, and posture during stimulation of the anterolateral motor (ALM) region in grooming for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#1F	not visible 	closed, at mouth 	closed, at mouth 	remains the same, on haunches 
#5F	opening and closing, no tongue protrusion 	closed, at mouth 	closed, at mouth 	remains the same, on haunches 
#6F	normal grooming 	normal grooming 	normal grooming 	remains the same, on haunches 
#7M	opening and closing, no tongue protrusion 	closed, at mouth 	closed, close to left hand 	remains the same, on haunches 
#10M	no movement 	closed, close to right hand 	closed, close to left hand 	remains the same, on haunches 
#11F	normal grooming 	normal grooming 	normal grooming 	remains the same, on haunches 

In summary, grooming is interrupted in two thirds of the mice in which the hands freeze at the mouth while being closed during the stimulation.

Eating

Eating is an automatic behaviour in which the mouse displays a distinctive pattern of movements in consuming the food, the rodent common eating pattern (Whishaw, Dringenberg, & Pellis, 1992). The pattern is: 1) Sniff, the mouse first sniffs the food to identify it. 2) Mouth grasp, the mouse then opens its mouth and grasps the food between its incisors. 3) Sit, with food held in the mouth, the mouse shifts its weight backward so that it is sitting on its haunches. 4) Grasp, as the mouse takes a sitting position, it lifts first one hand then the other hand to position the hands on either side of its mouth, in opposition to the food. Using an elbow-in movement, it reaches to grasp the food with one or both hands. 5) Handle, the mouse uses the fingertips and palm of both hands to grasp, support and manipulate the food using many asymmetrical movements in relation to its mouth.

Table 7 summarizes the state of the posture, right and left hands, and the mouth in 5 mice who received optogenetic stimulation of the ALM region while eating. All mice in this group show normal eating behaviour that was unaffected by the stimulation. They keep their posture during the stimulation. Right and left hands hold and manipulate the food as done in normal eating. Mouth opens when biting the food and closes during chewing.

Table 7. The state of mouth, left hand, right hand, and posture during stimulation of the anterolateral motor (ALM) region in eating for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#1F	not available	not available	not available	not available
#5F	normal eating 	normal eating 	normal eating 	remains the same, on haunches 
#6F	normal eating 	normal eating 	normal eating 	remains the same, on haunches 
#7M	normal eating 	normal eating 	normal eating 	remains the same, on haunches 
#10M	normal eating 	normal eating 	normal eating 	remains the same, on haunches 
#11F	normal eating 	normal eating 	normal eating 	remains the same, on haunches 

In summary, eating is not interrupted during the stimulation in any mice.

Licking













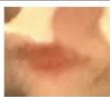



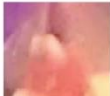



Licking is an automatic behaviour in which the mouse protrudes tongue to consume in this case peanut butter that is on the wall. The way this task is set up is that the mouse approaches the wall that has a dab of peanut butter at about 10cm above the floor. In order to lick the peanut better, the mouse has to lift hands from the floor and rear against the wall. The hands are either unsupported or supported on the wall. Video 5 is an example video demonstrating stimulation of

the ALM region while the mouse is licking peanut butter. In this video, the mouse stood up and licking peanut butter on the wall. Its right and left hands are in contact with the wall. When the stimulation begins, the mouse maintains posture and continues licking, however head and left hand synchronously move to its left side. Once the stimulation ends, the mouse lowers its body and hands let go off the wall as if going towards the floor, but halfway to the floor the mouse stands up and licks peanut butter on the wall with hands supported on the wall.

Table 8 summarizes the state of the posture, right and left hands, and the mouth in 5 mice who received optogenetic stimulation of the ALM region while licking peanut butter (PB). Here is a description of the individual mice since not all mice show the exact same behaviour. Mouse #5F: As illustrated in the second row of Table 8, this mouse's posture tilts to the left side and away from the peanut butter during the stimulation. Left and right hand and digits are opened and extended. Mouth makes opening and closing movements, but no tongue protrusion and no licking occurs. Mouse #6F: As illustrated in the third row of Table 8, this mouse's posture tilts to the right side and away from the peanut butter during the stimulation. Left hand remains closed and semi-flexed. Right hand remains on the floor. Mouth makes opening and closing movements, but no tongue protrusion and no licking occurs. Mouse #7M: As illustrated in the fourth row of Table 8, this mouse's posture tilts to the left side and away from the peanut butter during the stimulation. Left hand remains on the floor. Right hand and digits remain open and semi-flexed. Mouth makes opening and closing movements, but no tongue protrusion and no licking occurs. Mouse #10M: As illustrated in the fifth row of Table 8, this mouse's posture remains the same. Left hand remains on the floor. Right hand and digits remain close and semi-flexed. Mouth makes opening and closing movements, but no tongue protrusion and no licking occurs. Mouse #11F: As illustrated

in the sixth row of Table 8, this mouse's posture remains the same. Left and right hands and digits are opened and extended. Mouth keeps its function and continues licking.

Table 8. The state of mouth, left hand, right hand, and posture during stimulation of the anterolateral motor (ALM) region in licking peanut butter for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#1F	not available	not available	not available	not available
#5F	opening and closing, no licking 	digits open and extended 	digits open and extended 	tilted left, away from PB 
#6F	opening and closing, no licking 	closed, semi-flexed 	on the floor 	tilted right, away from PB 
#7M	opening and closing, no licking 	on the floor 	open and semi-flexed 	tilted left, away from PB 
#10M	opening and closing, no licking 	on the floor 	closed, semi-flexed 	remains the same, on haunches 
#11F	remains the same, licking 	digits open and extended 	digits open and extended 	remains the same 

In summary, licking peanut butter is interrupted in two thirds of the mice in which tongue protrusion to lick the peanut butter ceased during stimulation. The mouth keeps opening and closing in a way that resembles eating. Posture in half the mice is disturbed, in that the mice show tilting of the posture away from the peanut butter. Forced movement of opening and extension of digits is seen in one third of the mice.

Overall summary of ALM optogenetic stimulation

In summary, stimulation of the ALM region affects three voluntary behaviours of string-pulling, walking, and rearing and two automatic behaviours of grooming and licking. No effect in the automatic behaviour of eating was observed. In the behaviours affected, the posture of the mice is not changed during the optogenetic stimulation, but the function of the hands and mouth is affected. There is forced movement of hand to mouth or mouth to hand. The relationship between the hands and the mouth resembles eating behaviour because the hand is withdrawn towards the mouth and the mouth makes opening and closing movements.

CHAPTER 5. ANTERIOR MEDIAL MOTOR (AMM) AREA

Stimulation of the anteromedial motor area produced effects on all behaviours, but these were different from those obtained from anterolateral motor cortex. First there were no elicited movements, yet the stimulation did interfere with ongoing behaviour. Second, there were large differences in the effects of stimulation depending on ongoing behaviour in individual animals. Third, the most consistent effect was on string-pulling in which most animals quit pulling and adopt a quadrupedal posture at the stimulation onset. For this reason, the following describes the general effects of stimulation on individual animals for each behaviour.








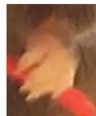







String-pulling

Video 6 is an example video demonstrating stimulation of the AMM region while string-pulling. In this video, the left hand is advancing towards the string when the stimulation begins. The mouse makes an overgrasp but does not grasp the string. The mouse gradually lowers body while the left elbow is extended. The right hand remains grasping the string during the stimulation. The stimulation ends when the mouse has lowered half its body, yet the mouse keeps lowering body and before reaching the floor, the left hand opens and is placed on the floor. Right hand is not visible so it is unclear at what point it lets go of the string.

Table 9 summarizes the state of the posture, right and left hands, and the mouth in 7 mice who received optogenetic stimulation of the AMM region while engaged in the string-pulling task. Here is a description of the individual mice since not all mice show the exact same behaviour. Mouse #13F: As illustrated in the first row of Table 9, this mouse remains standing up during the stimulation. Right and left hands keep string-pulling. Mouth remains closed and no movement is seen. Mouse #14F: As illustrated in the second row of Table 9, this mouse lowers body during the

stimulation. Right hand keeps grasping the string. Left hand is unable to grasp the string. Mouth remains closed and no movement is observed. Mouse #15F: As illustrated in the third row of Table 9, this mouse lowers body during the stimulation. Right and left hands keep grasping the string. Mouth remains closed and no movement is observed. Mouse #16M: As illustrated in the fourth row of Table 9, this mouse lowers body during the stimulation. Right and left hands keep grasping the string. Mouth remains closed and no movement is observed. Mouse #17M: As illustrated in the fifth row of Table 9, this mouse remains standing up during the stimulation. Right and left hands keep string-pulling. Mouth remains closed and no movement is seen. Mouse #18M: As illustrated in the sixth row of Table 9, this mouse lowers body during the stimulation. Right and left hands keep grasping the string. Mouth remains closed and no movement is observed. Mouse #19M: As illustrated in the seventh row of Table 9, this mouse remains standing up during the stimulation. Right and left hands keep string-pulling. Mouth remains closed and no movement is seen.

Table 9. The state of mouth, left hand, right hand, and posture during stimulation of the anteromedial motor (AMM) region in string-pulling task for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#13F	remains closed, no movements 	keeps string pulling 	keeps string pulling 	remains standing 
#14F	remains closed, no movement 	unable to grasp 	holds string 	lowers body 
#15F	remains closed, no movement 	holds string 	holds string 	lowers body 
#16M	remains closed, no movement 	holds string 	holds string 	lowers body 
#17M	remains closed, no movement 	keeps string pulling 	keeps string pulling 	remains standing 
#18M	remains closed, no movement 	holds string 	holds string 	lowers body 
#19M	remains closed, no movement 	keeps string pulling 	keeps string pulling 	remains standing 

In summary, string-pulling is disturbed in four of seven mice, in which the mice lower their body and quit string-pulling. Lowering body is not abrupt and no forced movement is inflicted upon the hands. The mouth remains closed and no mouth movement or tongue protrusion is seen in any of the mice.

Rearing

Table 10 summarizes the state of the posture, right and left hands, and the mouth in 7 mice who received optogenetic stimulation of the AMM region while engaged in rearing. All mice in this group except one show normal rearing behaviour. They keep their posture during the stimulation. The right and left hands remain on the wall. The mouth remains closed and no mouth movement or tongue protrusion is seen in any of the mice. Mouse #16M: As illustrated in the fourth row of Table 10, this mouse lowers body during the stimulation. Right and left hands are semi-closed and semi-flexed. Mouth remains closed and no movement is observed.

Table 10. The state of mouth, left hand, right hand, and posture during stimulation of the anteromedial motor (AMM) region in rearing for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#13F	remains closed, no movements 	on the wall 	on the wall 	remains standing 
#14F	remains closed, no movement 	on the wall 	on the wall 	remains standing 
#15F	remains closed, no movement 	on the wall 	on the wall 	remains standing 
#16M	remains closed, no movement 	semi-closed, semi-flexed 	semi-closed, semi-flexed 	lowers body 
#17M	remains closed, no movement 	on the wall 	on the wall 	remains standing 
#18M	remains closed, no movement 	on the wall 	closed, semi-flexed 	remains standing 
#19M	remains closed, no movement 	on the wall 	on the wall 	remains standing 

In summary, rearing is not disturbed during the stimulation in six of seven of the mice.

Walking

Table 11 summarizes the posture, right and left hands, and the mouth in 7 mice who received optogenetic stimulation of the AMM region while engaged in walking. All mice in this group except three show normal walking behaviour. They keep their posture and continue walking

during the stimulation. Their right and left hands take normal steps. Their mouth remains closed and no mouth movement or tongue protrusion is seen. Mouse #14F: As illustrated in the second row of Table 11, this mouse keeps its posture, but stops walking during the stimulation. Right and left hands are on the floor. The mouth does not make any movements. Mouse #16M: As illustrated in the fourth row of Table 11, this mouse keeps its posture, but stops walking during the stimulation. Right and left hands are on the floor. Mouth does not make any movements. Mouse #18M: As illustrated in the sixth row of Table 11, this mouse keeps its posture, but stops walking during the stimulation. Right and left hands are on the floor. Mouth does not make any movements.

Table 11. The state of mouth, left hand, right hand, and posture during stimulation of the anteromedial motor (AMM) region in walking for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#13F	remains closed, no movements 	on the floor 	lifting from the floor 	remains the same, keeps walking 
#14F	remains closed, no movement 	on the floor 	on the floor 	remains the same, stops walking 
#15F	remains closed, no movement 	on the floor 	lifting from the floor 	remains the same, keeps walking 
#16M	remains closed, no movement 	on the floor 	on the floor 	remains the same, stops walking 
#17M	remains closed, no movement 	on the floor 	lifting from the floor 	remains the same, keeps walking 
#18M	remains closed, no movement 	on the floor 	on the floor 	remains the same, stops walking 
#19M	remains closed, no movement 	semi-closed, advancing 	on the floor 	remains the same, keeps walking 





























In summary, walking is arrested in three mice and not disturbed during the stimulation in four mice.

Grooming

Table 12 summarizes the posture, right and left hands, and the mouth in 7 mice who received optogenetic stimulation of the AMM region while engaged in grooming. All mice in this

group except one show normal grooming behaviour and were unaffected by optogenetic stimulation. They maintained their posture and continued grooming during the stimulation. Their right and left hands continue face washing. Their mouth continued opening and closing and tongue protrusion is seen as they lick their hands. Mouse #14F: As illustrated in the second row of Table 12, this mouse keeps its posture but stops grooming during the stimulation. Right and left hands are closed at the mouth without moving. Mouth is closed and does not make any movement.

Table 12. The state of mouth, left hand, right hand, and posture during stimulation of the anteromedial motor (AMM) region in grooming for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#13F	movement, tongue protrusion 	closed, normal grooming 	closed, normal grooming 	remains the same 
#14F	no movement, no tongue protrusion 	closed, at mouth 	closed, at mouth 	remains the same 
#15F	movement, tongue protrusion 	closed, normal grooming 	closed, normal grooming 	remains the same 
#16M	movement, tongue protrusion 	closed, normal grooming 	closed, normal grooming 	remains the same 
#17M	movement, tongue protrusion 	closed, normal grooming 	closed, normal grooming 	remains the same 
#18M	movement, tongue protrusion 	closed, normal grooming 	closed, normal grooming 	remains the same 
#19M	movement, tongue protrusion 	closed, normal grooming 	closed, normal grooming 	remains the same 

In summary, grooming is not disturbed during the stimulation in six out of seven mice.

Eating

Table 13 summarizes the state of the posture, right and left hands, and the mouth in 7 mice who received optogenetic stimulation of the AMM region while engaged in eating. All mice in this group except two show normal eating behaviour. Mouse #14F: As illustrated in the second row of Table 13, this mouse keeps its posture. Right and left hands are holding the food at the

mouth. Mouth is closed and does not make any movement. Mouse #19M: As illustrated in the last row of Table 13, this mouse keeps its posture. Right and left hands are holding the food close to the mouth. Mouth is closed and makes movements.

Table 13. The state of mouth, left hand, right hand, and posture during stimulation of the anteromedial motor (AMM) region in eating for the individual mice in this group.














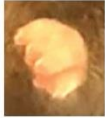




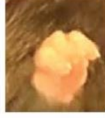


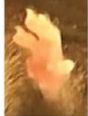






	Mouth	Left hand	Right hand	Posture
#13F	movement 	closed, normal eating 	closed, normal eating 	remains the same, on haunches 
#14F	closed, no movement 	closed, at mouth 	closed, at mouth 	remains the same, on haunches 
#15F	movement 	closed, normal eating 	closed, normal eating 	remains the same, on haunches 
#16M	movemnet 	closed, normal eating 	closed, normal eating 	remains the same, on haunches 
#17M	movement 	closed, normal eating 	closed, normal eating 	remains the same, on haunches 
#18M	closed, movement 	closed, at mouth 	closed, at mouth 	remains the same, on haunches 
#19M	movement 	closed, normal eating 	closed, normal eating 	remains the same, on haunches 

In summary, eating is not disturbed during the stimulation in five out of seven mice.

Licking

Table 14 summarizes the state of the posture, right and left hands, and the mouth in 7 mice who received optogenetic stimulation of the AMM region while engaged in licking. All mice in this group except one continued to show normal licking behaviour. Mouse #14F: As illustrated in the second row of Table 14, this mouse lowers its body during the stimulation. Right and left hands are semi-closed and semi-flexed before placing on the floor. Mouth keeps opening and closing but no tongue protrusion is seen.

Table 14. The state of mouth, left hand, right hand, and posture during stimulation of the anteromedial motor (AMM) region in licking peanut butter for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#13F	remains the same, licking 	open, at the wall 	semi-closed, semi-flexed 	remains the same 
#14F	movement 	semi-closed 	semi-closed 	lowers body 
#15F	remains the same, licking 	open, at the wall 	open 	remains the same 
#16M	remains the same, licking 	semi-closed, semi-flexed 	open, at the wall 	remains the same 
#17M	remains the same, licking 	semi-closed, semi-flexed 	semi-closed, semi-flexed 	remains the same 
#18M	remains the same, licking 	open, at the wall 	open, at the wall 	remains the same 
#19M	remains the same, licking 	open, at the wall 	open, at the wall 	remains the same 

In summary, licking peanut butter is not disturbed during the stimulation in six out of seven mice.

Overall summary of AMM optogenetic stimulation

In summary, stimulation of the AMM region affected the voluntary behaviour of string-pulling. No effect in automatic behaviours of grooming, eating, and licking peanut butter were

observed. It is interesting that in string-pulling the animals immediately stopped pulling and dropped to the floor. During rearing it might be expected that a similar effect would occur as both in string-pulling and in rearing when both standing on the hind legs. During rearing, however, only one mouse quit rearing and dropped to the floor and the remaining six mice remained rearing during the stimulation. Across all the behaviours, one mouse is consistently showing affected behaviour during stimulation.

CHAPTER 6. POSTERIOR LATERAL MOTOR (PLM) AREA

Stimulation of the posterior lateral motor area produced effects on some of the behaviours that were different from those obtained from stimulation of anterolateral and anteromedial motor cortex. First there was an elicited movement of digit extension during licking behaviour that did not affect licking per se, yet the stimulation interfered with other ongoing behaviours. Second, there were large differences in the effects of stimulation depending on ongoing behaviour in individual animals. Third, the most consistent effect was on eating behaviour in which six out of seven mice stopped chewing the food. For this reason, the following describes the general effects of stimulation on individual animals in each behaviour.














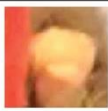














String-pulling

Video 7 is an example video demonstrating stimulation of the PLM region while string-pulling. In this video, left hand is advanced towards the string and makes an overgrasp before grasping the string when the stimulation begins. Left elbow and digits are extended and although the mouse attempts to grasp the string, it is not successful. The mouse lowers body a little and the right hand remains grasping the string during the stimulation. Once the stimulation ends, right hand lets go of the string and grasps it at the mouth level, makes a push and pull while rising up the body to the approximate initial height. Then the left hand grasps the string at the mouth level and continues with normal string-pulling.

Table 15 summarizes the state of the posture, right and left hands, and the mouth in 7 mice who received optogenetic stimulation of the PLM region while engaged in the string-pulling task. Here is a description of the individual mice since not all mice show the exact same behaviour. Mouse #13F: As illustrated in the first row of Table 15, this mouse remains standing up during the

stimulation. Right and left hands keep string-pulling. Mouth remains closed and no movement is seen. Mouse #14F: As illustrated in the second row of Table 15, this mouse lowers body during the stimulation. Right and left hands let go of the string. Mouth remains closed and no movement is observed. Mouse #15F: As illustrated in the third row of Table 15, this mouse remains standing during the stimulation. Right and left hands keep string-pulling. Mouth remains closed and no movement is seen. Mouse #16M: As illustrated in the fourth row of Table 15, this mouse lowers body during the stimulation. Right and left hands keep holding the string. Mouth remains closed and no movement is observed. Mouse #17M: As illustrated in the fifth row of Table 15, this mouse remains standing up during the stimulation. Right and left hands keep string-pulling. Mouth remains closed and no movement is seen. Mouse #18M: As illustrated in the sixth row of Table 15, this mouse lowers body during the stimulation. Right and left hands let go of the string. Mouth remains closed and no movement is observed. Mouse #19M: As illustrated in the last row of Table 15, this mouse lowers body during the stimulation. Right and left hands let go of the string. Mouth remains closed and no movement is seen.

Table 15. The state of mouth, left hand, right hand, and posture during stimulation of the posterolateral motor (PLM) region in string-pulling task for the individual mice in this group.




	Mouth	Left hand	Right hand	Posture
#13F	remains closed, no movements 	keeps string pulling 	keeps string pulling 	remains standing 
#14F	remains closed, no movement 	lets go of string 	lets go of string 	lowers body 
#15F	remains closed, no movement 	keeps string pulling 	keeps string pulling 	remains standing 
#16M	remains closed, no movement 	holds string 	holds string 	lowers body 
#17M	remains closed, no movement 	keeps string pulling 	keeps string pulling 	remains standing 
#18M	remains closed, no movement 	lets go of string 	lets go of string 	lowers body 
#19M	remains closed, no movement 	lets go of string 	lets go of string 	lowers body 

In summary, string-pulling is disturbed in four of seven of the mice. These mice lower their body and hands let go of the string. Lowering body is not abrupt and no forced movement is inflicted upon the hands. Mouth remains closed and no mouth movement or tongue protrusion is seen in any of the mice.

Rearing

Table 16 summarizes the posture, right and left hands, and the mouth in 7 mice who received optogenetic stimulation of the PLM region while engaged in rearing. All mice in this group except three show normal rearing behaviour. They keep their posture during the stimulation. Right and left hands remain on the wall. Mouth remains closed and no mouth movement or tongue protrusion is seen in any of the mice. Mouse #14F: As illustrated in the second row of Table 16, this mouse lowers body during the stimulation. Right and left hands are semi-closed. Mouth remains closed and no movement is observed. Mouse #17M: As illustrated in the fifth row of Table 16, this mouse lowers body during the stimulation. Right and left hands are semi-closed and semi-flexed. Mouth remains closed and no movement is observed. Mouse #18M: As illustrated in the sixth row of Table 16, this mouse lowers body during the stimulation. Right and left hands are semi-closed and semi-flexed. Mouth remains closed and no movement is observed.

Table 16. The state of mouth, left hand, right hand, and posture during stimulation of the posterolateral motor (PLM) region in rearing for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#13F	remains closed, no movements 	on the wall 	semi-closed, semi-flexed 	remains standing 
#14F	remains closed, no movement 	semi-closed 	semi-closed 	lowers body 
#15F	remains closed, no movement 	on the wall 	on the wall 	remains standing 
#16M	remains closed, no movement 	on the wall 	on the wall 	remains standing 
#17M	remains closed, no movement 	semi-closed, semi-flexed 	semi-closed, semi-flexed 	lowers body 
#18M	remains closed, no movement 	semi-closed, semi-flexed 	semi-closed, semi-flexed 	lowers body 
#19M	remains closed, no movement 	digits extended 	digits extended 	remains standing 

In summary, rearing is not disturbed during the stimulation in four of seven of the mice.




Walking

Video 8 is an example video demonstrating stimulation of the PLM region while walking. In this video, the mouse's left hand is collected from the floor when the stimulation starts. The mouse places the left hand on the floor, right hand stays on the floor, so both hands are on the floor

and the mouse stops walking. Just before the stimulation ends the mouse takes a step with the right hand and resumes walking.

Table 17 summarizes the state of the posture, right and left hands, and the mouth in 7 mice who received optogenetic stimulation of the PLM region while engaged in walking. Here is a description of the individual mice since not all mice show the exact same behaviour. Mouse #14F: As illustrated in the second row of Table 17, this mouse keeps its posture and continues walking during the stimulation. Right and left hands take normal steps. Mouth remains closed and does not make any movements. Mouse #15F: As illustrated in the third row of Table 17, this mouse keeps its posture, but stops walking during the stimulation. Right hand is on the floor. Left hand stays lifted from the floor and is semi-closed and semi-flexed. Mouth does not make any movements. Mouse #16M: As illustrated in the fourth row of Table 17, this mouse keeps its posture, but stops walking during the stimulation. Right and left hands are on the floor. Mouth does not make any movements. Mouse #17M: As illustrated in the fifth row of Table 17, this mouse keeps its posture and continues walking during the stimulation. Right and left hands take normal steps. Mouth remains closed and does not make any movements. Mouse #18M: As illustrated in the sixth row of Table 17, this mouse keeps its posture, but stops walking during the stimulation. Right and left hands are on the floor. Mouth does not make any movements. Mouse #19M: As illustrated in the last row of Table 17, this mouse keeps its posture, but stops walking during the stimulation. Right and left hands are on the floor. Mouth does not make any movements.

Table 17. The state of mouth, left hand, right hand, and posture during stimulation of the posterolateral motor (PLM) region in walking for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#13F	not available	not available	not available	not available
#14F	remains closed, no movement 	semi-closed, semi-flexed, stepping 	on the floor 	remains the same, keeps walking 
#15F	remains closed, no movement 	semi-closed, semi-flexed, pause in lift 	on the floor 	remains the same, stops walking 
#16M	remains closed, no movement 	on the floor 	on the floor 	remains the same, stops walking 
#17M	remains closed, no movement 	semi-closed, semi-flexed 	semi-closed, semi-flexed, stepping 	remains the same, keeps walking 
#18M	remains closed, no movement 	on the floor 	on the floor 	remains the same, stops walking 
#19M	remains closed, no movement 	on the floor 	on the floor 	remains the same, stops walking 

In summary, walking is disturbed during the stimulation in four out of six mice in which the mice stop walking during the stimulation. Both hands remain on the floor and mouth makes no movement.

Grooming

Table 18 summarizes the state of the posture, right and left hands, and the mouth in 7 mice who received optogenetic stimulation of the PLM region while engaged in grooming. All mice in this group except one show normal grooming behaviour. They keep their posture and continue grooming during the stimulation. Right and left hands continue face washing. Mouth keeps opening and closing and tongue protrusion is seen to wet the hands. Mouse #14F: As illustrated in the second row of Table 18, this mouse keeps its posture but stops grooming during the stimulation. Right and left hands are closed at the mouth without moving. Mouth is closed and does not make any movement.

Table 18. The state of mouth, left hand, right hand, and posture during stimulation of the posterolateral motor (PLM) region in grooming for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#13F	normal grooming 	normal grooming 	normal grooming 	remains the same, on haunches 
#14F	closed, no tongue protrusion 	closed, at mouth 	closed, at mouth 	remains the same, on haunches 
#15F	normal grooming 	normal grooming 	normal grooming 	remains the same, on haunches 
#16M	normal grooming 	normal grooming 	normal grooming 	remains the same, on haunches 
#17M	normal grooming 	normal grooming 	normal grooming 	remains the same, on haunches 
#18M	normal grooming 	normal grooming 	normal grooming 	remains the same, on haunches 
#19M	normal grooming 	normal grooming 	normal grooming 	remains the same, on haunches 

In summary, grooming is not disturbed during the stimulation in six out of seven mice.

Eating

Table 19 summarizes the state of the posture, right and left hands, and the mouth in 7 mice who received optogenetic stimulation of the PLM region while engaged in eating. All mice in this group except two show disturbed eating/chewing behaviour. Mouse #14F: As illustrated in the

second row of Table 19, this mouse keeps its posture. Right and left hands are holding the food at the mouth. Mouth is closed and does not make any movement. Mouse #16M: As illustrated in the fourth row of Table 19, this mouse keeps its posture. Right and left hands are holding the food close to the mouth. Mouth is closed and makes movements. Mouse #17M: As illustrated in the fifth row of Table 19, this mouse keeps its posture. Right and left hands are holding the food at the mouth. Mouth is closed and does not make any movement. Mouse #18M: As illustrated in the sixth row of Table 19, this mouse keeps its posture. Right and left hands are holding the food at the mouth. Mouth is closed and does not make any movement. Mouse #19M: As illustrated in the last row of Table 19, this mouse keeps its posture. Right and left hands are holding the food at the mouth. Mouth is closed and does not make any movement.

Table 19. The state of mouth, left hand, right hand, and posture during stimulation of the posterolateral motor (PLM) region in eating for the individual mice in this group.











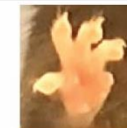















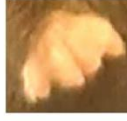

	Mouth	Left hand	Right hand	Posture
#13F	normal eating 	closed, normal eating 	closed, normal eating 	remains the same, on haunches 
#14F	no movement 	closed, at mouth 	closed, at mouth 	remains the same, on haunches 
#15F	normal eating 	closed, normal eating 	closed, normal eating 	remains the same, on haunches 
#16M	no movement 	closed, at mouth 	closed, at mouth 	remains the same, on haunches 
#17M	no movement 	closed, at mouth 	closed, at mouth 	remains the same, on haunches 
#18M	no movement 	closed, at mouth 	closed, at mouth 	remains the same, on haunches 
#19M	no movement 	closed, at mouth 	closed, at mouth 	remains the same, on haunches 

In summary, eating is disturbed during the stimulation in five out of seven mice. Mice keep their posture and hold the food in their hands close to the mouth, however, mouth movement is absent as if the mouse stops chewing the food during the stimulation.

Licking

Table 20 summarizes the state of the posture, right and left hands, and the mouth in 7 mice who received optogenetic stimulation of the PLM region while engaged in licking. All mice in this group except one show normal licking behaviour. Mouse #14F: As illustrated in the second row of Table 20, this mouse lowers its body during the stimulation. Right and left hands show extension of digits. Mouth keeps opening and closing but no tongue protrusion is seen.

Table 20. The state of mouth, left hand, right hand, and posture during stimulation of the posterolateral motor (PLM) region in licking peanut butter for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#13F	remains the same, licking 	semi-closed, semi-flexed 	open, at the wall 	remains the same 
#14F	open and close, no tongue protrusion 	digits extended 	digits extended 	lowers body 
#15F	remains the same, licking 	semi-closed, semi-flexed 	open, at the wall 	remains the same 
#16M	remains the same, licking 	semi-closed, semi-flexed 	open, at the wall 	remains the same 
#17M	remains the same, licking 	open, at the wall 	open 	remains the same 
#18M	remains the same, licking 	digits extended 	digits extended 	remains the same 
#19M	remains the same, licking 	digits extended 	digits extended 	remains the same 

In summary, licking peanut butter is not disturbed during the stimulation in six out of seven mice. Three mice show extension of digits during the stimulation while licking peanut butter.

Overall summary of PLM optogenetic stimulation

In summary, stimulation of the PLM region affected the voluntary behaviours of string-pulling and walking and automatic behaviour of eating. It is interesting that in string-pulling the animals lowered their body and partially dropped to the floor. In fact, most of the mice who lowered their bodies, rose and continued string-pulling right after the stimulation ended. During rearing it might be expected that a similar effect would occur as both in string-pulling and in rearing when both standing on the hind legs. During rearing, however, only one mouse quit rearing and dropped to the floor and the remaining six mice remained rearing during the stimulation.

CHAPTER 7. POSTERIOR MEDIAL MOTOR (PMM) AREA

Stimulation of the posterior medial motor area produced effects on some of the behaviours that were different from those obtained from anterolateral, anteromedial, and posterior lateral motor cortex. First there was an elicited movement of elbow and digit extension during string-pulling and rearing. Second, digit extension occurred in two mice during licking peanut butter, which did not disturb licking per se. Third, the most consistent effect was on string-pulling behaviour in which four out of five mice abruptly adopt a quadrupedal posture at the stimulation onset which is different than stimulation of anterior medial motor area. For this reason, the following describes the general effects of stimulation on individual animals in each behaviour.





















String-pulling

Video 9 is an example video demonstrating stimulation of the PMM region while string-pulling. In this video, left hand is about to grasp the string at mouth level when stimulation begins. The mouse abruptly lowers body and hands let go of string with elbow and digits extended. The hands are placed on floor and the mouse takes a step forward when the stimulation ends.

Table 21 summarizes the state of the posture, right and left hands, and the mouth in 5 mice who received optogenetic stimulation of the PMM region while engaged in the string-pulling task. Here is a description of the individual mice since not all mice show the exact same behaviour. Mouse #5F: As illustrated in the first row of Table 21, this mouse lowers body during the stimulation. Right and left elbows and digits are extended before reaching the floor. Mouth remains closed and no movement is seen. Mouse #8M: As illustrated in the second row of Table 21, this mouse keeps standing up during the stimulation. Right and left hands keep string-pulling. Mouth remains closed and no movement is observed. Mouse #9M: As illustrated in the third row of Table

21, this mouse remains standing during the stimulation. Right and left hands are closed and at the mouth. Mouth bites the string. Mouse #10M: As illustrated in the fourth row of Table 21, this mouse lowers its body during the stimulation. Right and left elbows and digits are extended before reaching the floor. Mouth bites on the string. Mouse #11F: As illustrated in the last row of Table 21, this mouse lowers body during the stimulation. Right and left elbows and digits are extended before reaching the floor. Mouth remains closed and no movement is seen.

Table 21. The state of mouth, left hand, right hand, and posture during stimulation of the posteromedial motor (PMM) region in string-pulling for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#5F	no movement 	digits extended 	digits extended 	lowers body 
#8M	no movement 	string pulling 	string pulling 	remains standing up 
#9M	biting string 	closed, at mouth 	closed, at mouth 	remains standing up 
#10M	biting string 	digits extended 	digits extended 	lowers body 
#11F	no movement 	digits extended 	digits extended 	lowers body 





















In summary, string-pulling is disturbed in three fifth of the mice in which the mice lower their body abruptly with their elbows and digits extended before reaching the floor.

Rearing

Video 10 is an example video demonstrating stimulation of the PMM region while the mouse makes an unsupported rearing. In this video, both hands are semi-closed and semi-flexed when the stimulation begins. As soon as the stimulation starts, both elbows and digits are extended, and mouse takes a quadrupedal posture. Mouth is closed and no mouth movement is seen.

Table 22 summarizes the state of the posture, right and left hands, and the mouth in 5 mice who received optogenetic stimulation of the PMM region while engaged in rearing. Here is a description of the individual mice since not all mice show the exact same behaviour. Mouse #5F: As illustrated in the first row of Table 22, this mouse lowers its posture during stimulation. Right and left elbows and digits are extended. Mouth is closed and makes no movement. Mouse #8M: As illustrated in the second row of Table 22, this mouse keeps its posture during the stimulation. Right and left hands are at the wall. Mouth remains closed and no movement is observed. Mouse #9M: As illustrated in the third row of Table 22, this mouse keeps its posture during the stimulation. Right and left hands are at the wall. Mouth remains closed and no movement is observed. Mouse #10M: As illustrated in the fourth row of Table 22, this mouse lowers body during the stimulation. Right and left digits are extended. Mouth remains closed and no movement is observed. Mouse #11F: As illustrated in the last row of Table 22, this mouse lowers body during the stimulation. Right and left elbows and digits are extended. Mouth remains closed and no movement is observed.

Table 22. The state of mouth, left hand, right hand, and posture during stimulation of the posteromedial motor (PMM) region in rearing for the individual mice in this group.





















	Mouth	Left hand	Right hand	Posture
#5F	no movement 	digits extended 	digits extended 	lowers body 
#8M	no movement 	on wall 	on wall 	remains standing up 
#9M	no movement 	on wall 	on wall 	reamins standing up 
#10M	no movement 	digits extended 	digits extended 	lowers body 
#11F	no movement 	digits extended 	digits extended 	lowers body 

In summary, rearing is disturbed during the stimulation in three fifth of the mice. They lower their body abruptly and extension of digits are seen before they reach the floor.

Walking

Table 23 summarizes the state of the posture, right and left hands, and the mouth in 5 mice who received optogenetic stimulation of the PMM region while engaged in walking. All mice in this group show normal walking behaviour during the optogenetic stimulation. As illustrated in Table 23 all mice keep their posture, right and left hands make normal steps and mouth remains close and does not make any movement.

Table 23. The state of mouth, left hand, right hand, and posture during stimulation of the posteromedial motor (PMM) region in walking for the individual mice in this group.





















	Mouth	Left hand	Right hand	Posture
#5F	no movement 	semi-closed, lift 	on the floor 	remains the same, keeps walking 
#8M	no movement 	semi-closed, lift 	on the floor 	remains the same, keeps walking 
#9M	no movement 	on the floor 	semi-closed, advancing 	remains the same, keeps walking 
#10M	no movement 	semi-closed, advancing 	on the floor 	remain the same, keeps walking 
#11F	no movement 	semi-closed, advancing 	on the floor 	remains the same, keeps walking 

In summary, walking is not disturbed during the stimulation in any of the mice.

Grooming

Table 24 summarizes the state of the posture, right and left hands, and the mouth in 5 mice who received optogenetic stimulation of the PMM region while engaged in grooming. All mice in this group except one show normal grooming behaviour. They keep their posture and continue grooming during the stimulation. Right and left hands continue face washing. Mouth keeps opening and closing and tongue protrusion is seen to wet the hands. Mouse #5F: As illustrated in the first row of Table 24, this mouse lowers its posture during the stimulation. Right and left hands are semi-closed and semi-flexed. Mouth is closed and does not make any movement.

Table 24. The state of mouth, left hand, right hand, and posture during stimulation of the posteromedial motor (PMM) region in grooming for the individual mice in this group.




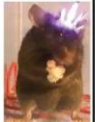




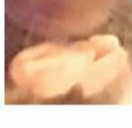
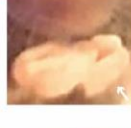
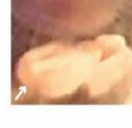

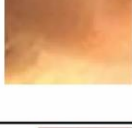





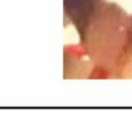

	Mouth	Left hand	Right hand	Posture
#5F	no movement 	semi-closed, semi-flexed 	semi-closed, semi-flexed 	lowers body, stops grooming 
#8M	movement, tongue protrusion 	closed, normal grooming 	closed, normal grooming 	remains the same 
#9M	movement, tongue protrusion 	closed, normal grooming 	closed, normal grooming 	remains the same 
#10M	movement, tongue protrusion 	closed, normal grooming 	closed, normal grooming 	remain the same 
#11F	movement, tongue protrusion 	closed, normal grooming 	closed, normal grooming 	remains the same 

In summary, grooming is not disturbed during the stimulation in four out of five mice.

Eating

Table 25 summarizes the state of the posture, right and left hands, and the mouth in 5 mice who received optogenetic stimulation of the PMM region while engaged in eating. All mice in this group except one show no eating behaviour. Mouse #11F: As illustrated in the last row of Table 25, this mouse lowers its posture. Right and left hands are closed and holding the food. Mouth is closed and does not make any movement.

Table 25. The state of mouth, left hand, right hand, and posture during stimulation of the posteromedial motor (PMM) region in eating for the individual mice in this group.





















	Mouth	Left hand	Right hand	Posture
#5F	normal eating 	closed, normal eating 	closed, normal eating 	remains the same, keeps eating 
#8M	normal eating 	closed, normal eating 	closed, normal eating 	remains the same, keeps eating 
#9M	normal eating 	closed, normal eating 	closed, normal eating 	remains the same, keeps eating 
#10M	normal eating 	closed, normal eating 	closed, normal eating 	remains the same, keeps eating 
#11F	no movement 	closed 	closed 	lowers body, stops eating 

In summary, eating is not disturbed during the stimulation in four out of five mice.

Licking

Table 26 summarizes the state of the posture, right and left hands, and the mouth in 5 mice who received optogenetic stimulation of the PMM region while engaged in licking. All mice in this group except one show normal licking behaviour. Mouse #5F: As illustrated in the first row of Table 26, this mouse lowers its body during the stimulation. Right and left digits are extended. Mouth is closed and does not make any movement. Mouse #11F: As illustrated in the last row of Table 26, this mouse keeps its posture during the stimulation. Right and left digits are extended. Mouth keeps licking peanut butter.

Table 26. The state of mouth, left hand, right hand, and posture during stimulation of the posteromedial motor (PMM) region in licking peanut butter for the individual mice in this group.

	Mouth	Left hand	Right hand	Posture
#5F	no movement 	digits extended 	digits extended 	lowers body 
#8M	remains the same, keeps licking 	semi-closed, semi-flexed 	semi-closed, semi-flexed 	remains the same 
#9M	remains the same, keeps licking 	semi-closed, semi-flexed 	on wall 	remains the same 
#10M	remains the same, keeps licking 	semi-closed, semi-flexed 	on wall 	remain the same 
#11F	remains the same, keeps licking 	digits extended 	digits extended 	remains the same 

In summary, licking peanut butter is not disturbed during the stimulation in four out of five mice. Two mice show extension of digits during stimulation while licking peanut butter.

Overall summary of PMM optogenetic stimulation

In summary, stimulation of the PMM region affected the voluntary behaviours of string-pulling and rearing. In both string-pulling and rearing the animals abruptly lowered their body and dropped to the floor with elbow and digits extended. It is interesting that digit extension also occurred in two of the mice during licking peanut butter, yet the mice kept their posture and continued licking without dropping on the floor.

CHAPTER 8: DISCUSSION

Stimulation of the motor areas of the neocortex has a long history in which elicited movements induced by electrical stimulation have been defined as either muscle twitches or more organized movements. If stimulation at a point in the motor cortex elicits a muscle twitch, that was taken as evidence that the functional unit of the motor cortex was muscle twitches as component movement of more complex motor activity. If stimulation at a point in the motor cortex resulted in an organized movement, that was taken as evidence that the functional unit of the motor cortex was movement. Early summaries of motor cortex involved making a map in which there were numerous labels on the cortex for all of the muscles in the body. More recent maps centered around movements represented by maps containing fewer labels for movements. The conditions under which these studies have been performed involved anesthetized preparations or unanesthetized preparations. Obviously anesthetized conditions would be less conducive to producing organized movements.

In all of this work, there has been uncertainty about the robustness of the movements that are elicited by a repeated bout of stimulation. This uncertainty has been discussed under the label of “stability of points”. If stimulation reliably resulted in the same movement every time it administered, then the point is defined as being stable. Stimulation that results in movement on one occasion and not on other, resulted in the point being defined as unstable. Causes of stability/instability could be related to the changing conditions under which stimulation is administered, i.e., depths of anesthesia, posture of the subject, etc.

The purpose of this thesis is to examine the effect of behaviour on stimulation. Awake and freely moving mice engaged in various voluntary and automatic behaviours as stimulation was delivered. It was expected that if the stimulation always resulted in the same effect, irrespective of

ongoing behaviour, that stimulation point could be defined as stable. If on the other hand, the stimulation resulted in different movements or a movement at one time and not in another, during different behaviours, the point would be defined as unstable. Thus, it was expected that if points were stable, the function of a cortical point is to represent a single motor consequence. On the other hand, if the points were unstable, that might mean the representation of the cortex is one that is behaviourally dependent. For example, if an animal is behaviourally unengaged, the cortex is open for producing a movement. If an animal is engaged in a movement, the cortex is closed to elicited movements.

Design of the experiment

In this thesis, optogenetic stimulation as opposed to traditionally used electrical stimulation was used. Optogenetic stimulation uses light pulses of a certain wavelength depending on the sensitivity of the light-channels genetically inserted into the brain. Light-sensitive channels are in fact light-sensitive ion channels. These open upon illumination of a certain wavelength of light so that ions can flow and change membrane polarity, resulting in activation or inactivation of the cell. In the present thesis, 473nm light, which is the blue light, is used because the light-sensitive channels in the transgenic mice used in the study are sensitive to that specific wavelength of light. Electrical stimulation activates all types of neurons and glial cells in the area stimulated, whereas optogenetic stimulation activates or deactivates only the cells that express the light-sensitive ion channels. Thus, one can selectively manipulate selected neural pathways of the brain. Moreover, prolonged electrical stimulation can burn the brain tissue. Using optogenetic stimulation allows for a longer and repeated stimulation without damaging the brain tissue.

The mouse used in this study is a transgenic mouse that expresses the light-sensitive K^+/Na^+ channels in layer 5 cortical neurons. Thus, applying optogenetic stimulation only activates the neurons in layer 5 of the neocortex. A drawback in this approach is that layer 5 cortical neurons largely project to the brainstem and spinal cord. Other neurons in other cortical layers project to the contralateral hemisphere, the basal ganglia, and the thalamus amongst other targets. Therefore, the selectivity of optogenetic stimulation relative to electrical stimulation has a drawback that not exactly the same circuit gets activated. Future experiments could address the function of the various pathways of the cortex using different driver lines (ion channels in other neurons) selective for different pathways. For the present thesis, a single driver line is used for addressing the main question of the study of whether points are stable in relation to behaviour.

Generation of electrical stimulation pulses require setting the generator at a specific frequency and duration. It is possible to start stimulating at low frequency or duration and gradually increase the parameters until a behavioural effect occurs. This parameter of stimulation is thus the effective current level. Generation of light pulses is not that simple, and it requires taking extra steps because the laser light generated is constant at a constant power. Thus, to change the effects of stimulation it is only possible to increase the duration. In the present experiments, in order to get the on and off phase of a stimulation pulse, a shutter was used to pulse the constant light beam. Opening and closing of the shutter at a certain rate would generate a square pulse of light with on and off phases of set duration, thus generating light pulses of different frequencies. The total duration of the light pulse was controlled by setting how long the shutter would open and close and then remain closed thereafter. The optogenetic stimulation used in the experiments were applied at four different frequencies of 10Hz, 20Hz, 30Hz, and 40Hz in each behavioural task. Moreover, for each of those frequencies five different duration of stimulation was used. Those

durations include 0.5sec, 0.7sec, 1sec, 1.5 sec, and 2sec. It was expected that if using stimulation of different frequency and duration resulted in the same behavioural outcome, then the points were stable. If on the other hand, using stimulation of different frequencies and duration resulted in different behavioural outcomes, the points would be unstable.

Despite these frequency and duration manipulations in the present experiments, it was found variations in frequency and duration of stimulation did not modulate behaviour. One light pulse was very much like another pulse in either inducing a behavioural effect or being ineffective. Consequently, the main effects of the study were obtained by pooling frequency and duration to produce a single score for each stimulation site and for each behaviour.

A caveat in using a shutter is the sound it produces when opening and closing the circuit. The sound can startle the mice or act as a cue for the mice. In any case, the generated sound may interfere with the normal behaviour. In order to overcome this problem, the mice were habituated to the sound when they were habituated to the laser cable and the experimental boxes. Moreover, a control stimulation was applied along with each stimulation session where a different laser cable located at the side of the experimental apparatus was turned on to produce the same stimulation frequency and duration, i.e., the same noise and light as a real stimulation trial. Thus, the control trials were administered in the same way as were the stimulation trials during each behaviour. Then the control trial results were compared with the stimulation trial results. As it turned out it was found that the control trials never reliably induced a behavioural effect whereas the stimulation trials at certain points were effective in producing behavioural effects. Therefore, in the experiments the main results are those obtained from the stimulation trials.

Selection of behaviours

Previous studies on the motor cortex were performed on either anesthetized or awake head-fixed animals whose hands were suspended from the head-fixing platform. This way examining the stability/instability of points was limited to either an unconscious body or one bounded artificial posture when the animal was awake and head-fixed. In this thesis, freely moving mice that were engaged in a variety of naturally occurring voluntary and automatic behaviours were subjected to application of optogenetic stimulation.

A voluntary behaviour is one that takes an animal from one place to another and also one that can be associated with a number of motivational states; e.g., an animal can walk to water, climb to water, or run to water (Vanderwolf & Robinson, 1981). Automatic behaviours are ordinarily performed when an animal remains in the same location and they are associated with a single motivational state; e.g., an animal laps with its tongue in order to drink. Voluntary behaviours included string-pulling, rearing, and walking. Automatic behaviours included grooming, eating, and licking. Each of these behaviours require acquiring a certain posture, adopting a defined hand and digit shape, and performing a specific movement sequence. String-pulling, in addition to being a voluntary behaviour, is one that the animal would not ordinarily perform and requires learning. The string-pulling task specifically allowed for examination of several parameters including digits, hand and arm use, and body posture.

Not only was stimulation of freely moving mice during various behaviours a novel approach in studying the motor cortex, but it also enabled study of stability/instability of the points across a number of species-typical behaviours. It was expected that if stimulation of points in all the different behaviours resulted in the same behavioural outcome, then the points were stable. If on the other hand, stimulation of points in one behaviour resulted in a different behavioural

outcome than the other behaviours, the points would be unstable. In all groups and across all behaviours, the stimulation effect on each mouse was not zero or a hundred percent. For this reason, a threshold percentage of more than fifty percent was set to indicate that the stimulation disturbed the behaviour. Also, if the majority of the mice in a group showed a disturbance in the behaviour, then the behaviour was interpreted as disturbed.

Amongst behaviours, string-pulling was the behaviour that showed the most interruption with light stimulation and rearing was the only behaviour that seemed least affected by the optogenetic stimulation. Both behaviours are voluntary and require the mouse to lift head and stand on two feet, yet string-pulling demands skillful hand use and coordinating hand use with somatosensory tracking of the string by the nose. In rearing, the hands and the nose are not similarly active.

Selection of brain areas

There is a long history in which the cortex has been stimulated with the objective of identifying those parts of the cortex that produce movement and could so define as motor cortex. If all the rodent studies in which the brain has been stimulated (A. R. Brown & Teskey, 2014; Hall & Lindholm, 1974; Hira et al., 2015; Tennant et al., 2011) in search of motor cortex were combined then almost the entire front region of the cortex would be defined as motor cortex.

In light of these findings, the frontal cortex was divided into four areas. The anterior lateral motor (ALM) area is shown to be involved in licking and hand to mouth movements which Hira (2015) characterizes as discrete or aperiodic movements with a straight trajectory. Posterior medial motor (PMM) area is shown to generate reaching or locomotion-like movements which Hira (2015) characterize it as rhythmical or periodic movements with circular trajectories. Hall and

Lindholm (1974) characterize the motor cortex more generally based on the body movements. In their characterization, the medial areas are concerned with the posture and the lateral areas are concerned with hand movements. In the Brown and Teskey (2014) study, anterior motor cortex subserves hand movements and posterior motor cortex subserves arm movements.

In this thesis the brain areas for stimulation were selected so that they almost cover the four regions of the motor cortex (if the motor cortex is identified as a rectangle in one hemisphere on top of the frontal cortex). Also, the points were named based on their corresponding spatial coordinates; e.g., ALM region is named for anterior lateral quadrant. The reasons for the areal names are consistency and to remove confusion of location that have been previously described to produce a particular function.

Behaviours and disruptions

The outcome of stimulation in this thesis could be generally divided into the categories of endpoint behaviour, freezing behaviour, or no change in the ongoing behaviour. The endpoint behaviour was mostly seen when the posture and function of the mouse was affected.

Depending on the brain region stimulated and the ongoing task at the time of the stimulation, the endpoint behaviour manifested with unique elicited movements. For example, stimulation of the posteromedial motor (PMM) area during rearing resulted in abrupt dropping to the floor with elbow and digit extension. Thus, the endpoint behaviour is attaining a quadrupedal posture. The freezing behaviour was seen when the mouse retains its posture, but the function is interrupted during the stimulation. In another words, the mouse pauses during the stimulation and stays motionless without a change in posture. Once the stimulation ends, the mouse resumes the behaviour. For example, stimulation of posterolateral motor (PLM) area during eating resulted in

a pause in eating in which the mouse remained on its haunches with hands holding the food at the mouth, yet the mouth stopped chewing and made no movement. Once the stimulation ended, chewing and biting resumed. No change in the ongoing behaviour was seen when the posture and function remained unchanged, and no elicited movement occurred. In another words, the mouse kept doing what it was doing and the stimulation did not affect the behaviour.

A number of reasons could explain the no change in behaviour outcome. First, it could be the case that activation of the stimulated point is not important to a particular behaviour and that the brain ignores its activation, leaving the behaviour unaffected. Second, it could be the case that the laser power used for activating the points is not powerful enough to drive an effect in the behaviour. Given that at least one behaviour in each group was affected during the stimulation, this scenario is unlikely. Third, it could be the case that the ongoing behaviour at the time of stimulation is already an endpoint behaviour and so no change in the posture and function is required, so it looks as if the stimulation did not interrupt or elicit a behaviour. For example, stimulation of the anterolateral motor (ALM) area in string-pulling, rearing, and walking resulted in a hand to mouth/mouth to hand behaviour and during eating the stimulation had or seemed to have no effect on eating because the hand was already at the mouth. It could be that the endpoint behaviour of hand to mouth/mouth to hand is already met in eating so no change in eating is observed.

Regions that are most sensitive

Previous mapping studies of the mouse motor cortex revealed two distinct forelimb areas (Tennant et al., 2011) that occupy a large region of the frontal cortex of the mouse. These two areas are the caudal and rostral forelimb areas that produce walking or limb movement or hand

movements respectively. It was expected that the stimulation of these two areas would show the biggest effects in all six behaviours. However, the ALM area was the most sensitive region. Stimulation of the ALM area resulted an effect on posture or function, and induction of an elicited movement in four out of six behaviours. One interpretation of the function of the ALM region is that it is the mouse homologue of the primate ventral premotor cortex (An et al., 2022). This region of the cortex integrates the movements of the hands and the mouth. Perhaps the reason that the ALM region is sensitive with respect to the design of the present experiments is that many of the behaviours investigated here involved hand or mouth movements (e.g., string-pulling, eating, licking, walking).

String-pulling as a learned behaviour is special

Numerous motor tasks have been developed for rodents in order to study the hand and arm use. Single-pellet reaching task (Farr & Whishaw, 2002; Whishaw, 1996; Whishaw et al., 1992), tray reaching task (Whishaw, O'Connor, & Dunnett, 1986), string-pulling task (Blackwell, Banovetz, et al., 2018; Blackwell, Widick, Cheatwood, Whishaw, & Wallace, 2018), and staircase task (Baird, Meldrum, & Dunnett, 2001; Montoya, Campbellhope, Pemberton, & Dunnett, 1991) are a few of such tasks. The string-pulling task deserves especial mention because mice learn to pull down the string spontaneously and the behaviour can be maintained with minimal training and reinforcement. Mice perform frequent pulls and pushes on the string which allows a significant number of reaches to be obtained for analysis quickly. The mice use a hand-over-hand movement to pull the string and this allows study of both hands simultaneously. Because the mouse stands up for string-pulling, one can study the posture in addition to the arm and hand movements. Having mice to perform this learned behaviour which presumably requires different neural circuits and

more brain power than for example eating or walking, proved to be befitting for studying the effects of optogenetic stimulation. It turned out based on the obtained results, it was the most sensitive behaviour to optogenetic stimulation. One could study the movements plus how the behaviour itself would get affected because there are several points the brain considers when performing string-pulling. Presence of a food item at the end of the string, standing up on two feet, tracking the string with perioral receptors, using coordinated hand movements in pulling the string, expecting food to drop down at the end of pulling are features that the mouse should consider when performing string-pulling. As such, there are more possibilities of interfering with any of those features with optogenetic stimulation.

Interpretations

In all the mapping studies of the motor cortex, motor cortex was defined when stimulation of points resulted in a muscle twitches, a small limb movement, an organized behaviour, or an endpoint behaviour. A muscle twitch is muscle contraction that could be identified by the naked eye or EMG electrodes. A small limb movement also could be identified by the naked eye or EMG electrodes when part of a limb would move at the joint, like extension of digits. An organized behaviour could be observed by naked eye when a combination of joint movements occurred, for example, abduction of arm and extension of digits during reaching behaviour. Thus, based on the methodology and condition of the experimental subjects, different maps were produced and points on the maps were unstable. Those maps were simply a way of summarizing the findings that one could see at a glance and perhaps did not capture the function of the motor cortex. The action map, however, is very different from the previous maps because previous maps were derived based on body parts and the action map was derived based on the spatial locations in relation to body parts.

An endpoint behaviour is identified as a behaviour that despite a varied starting position would always result in the same endpoint position. In other words, the movement to get to the endpoint would vary depending on the starting position but reaching the endpoint posture is the goal of behaviour. For example, bringing the hand to the mouth regardless of the initial position of the hand whether it is on the side of the body, holding an object, or on the head. The endpoint hypothesis has difficulty in accounting for instability. Thus, the present thesis suggests that behavioural conditions could affect the outcome of stimulating a point in motor cortex. In testing this hypothesis mice received optogenetic stimulation when engaged in learned, voluntary, and automatic behaviours, and depending on the ongoing behaviour of the mice at the time of stimulation, different behavioural effects manifested. Therefore, the results of this thesis suggest that behaviour is a variable in instability of points.

Caveats

A number of caveats can be raised with regards to the selection of areas, selection of behaviours, and number of samples in this thesis. There are multiple possible scenarios one could employ to study the motor cortex. One could select one area of the motor cortex and extensively study it in many animals. One could select the few known areas such as the caudal forelimb and the rostral forelimb areas and concentrate on studying those areas. One could have studied every point on the motor cortex much like the mapping studies performed in the anesthetized animals. The areas chosen for stimulation in this thesis almost cover the four corners of the motor cortex if you regard motor cortex as a rectangle in one hemisphere on top of the frontal cortex. These four areas were distant enough that different stimulation outcomes would have been expected from

each of them. Consequently, the present thesis can be considered to have obtained a representative sample of possible outcomes of optogenetic stimulation.

With respect to the choice of behaviours used in this thesis, one could have used only learned behaviours, or voluntary behaviours, or automatic ongoing behaviours. Yet, in this thesis a combination of voluntary, learned voluntary and automatic behaviours were used. This served as a screen for identifying the potential contribution of a brain region, but also the optimal behavioural assay for evaluating the effect of brain stimulation. The string-pulling task proved to be optimal for studying the motor cortex and perhaps future work could more fully examine the behaviour and possibly compare the results from this behaviour to other learned tasks.

With respect to the number of samples, one could have increased the number of stimulation trials that all animals would systematically receive. However, given the large number of stimulation parameters used in the present experiments, including four different frequencies and five different durations of stimulation for each frequency, it would have been exhausting to increase the number of stimulation trials. Besides, some behaviours, including grooming and rearing were not instructive. Thus, future work could consider not only how stimulation affects sensitive movements such as string-pulling, but also why it does not affect other movements such as grooming.

Conclusion

The purpose of this thesis was to examine whether the repeated stimulation of a point in sensorimotor cortex of the mouse would result in the same outcome with each stimulus incident. This idea was investigated by stimulating a number of sites in each area of the sensorimotor cortex during each of six behaviours. The idea that stimulation of motor cortex may at times result in an

effect and in another times result in a different effect or no effect at all has come to be known as a problem of stability of points. In previous research findings that points are unstable have not been central to the investigation but have been noted in all those studies. Thus, this thesis is the first to dedicate itself to the examination of stability of points. The strategy of varying the behavioural state of the animals during stimulation was to catch the essential features of many previous studies in which animals have been anesthetized, awake but head-fixed, or freely moving or not always engaging in any specific behaviour. The findings of this thesis are that the behavioural outcome of the stimulation largely depended upon the ongoing behaviour of the animals at the time of stimulation, thus, the points on the motor cortex are unstable, and behaviour is a contributing factor to instability. A learned behaviour in which the mice pulled a string to obtain food reward was the behaviour most affected by stimulation. The string-pulling behaviour was most disrupted by stimulation at most cortical sites and these disruptions were relatively stable. Nevertheless, when the same animals received the same stimulation during other behaviours the effects of stimulation were often different or not discernable. Voluntary behaviours and especially the learned behaviour of string-pulling were affected more by optogenetic stimulation of the motor cortex than automatic behaviours. One explanation of the instability of points that can be taken from the present research is that when the cortex is in altered states, stimulation is similarly altered. In the present study it was also found that when behaviours were divided into voluntary as opposed to automatic, the voluntary behaviours were more affected than were the automatic behaviours. Presumably learned behaviours demand distinct brain networks to mediate them and so any interference would interrupt them, whereas automatic behaviours mediated by networks that involve subcortical structures would not get interrupted as easily. During the period in which the present study was conducted, emerging research has revealed that the anatomy of the motor cortex of the mouse is

much more complex than was previously suspected. This complexity is marked by as many as thirty-eight different projection pathways from motor cortex to other brain regions. Perhaps it is not only the complexity of the behaviour but also the complexity of the anatomy that makes the outcome of cortical stimulation studies uncertain with respect to the stability of points.

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APPENDIX 1: SUMMARY OF ALM ANIMALS

Mouse #1F

- ALM
- histology to be done
- summary in following behaviours: walking, string-pulling, grooming, and rearing
- Total # observations = 68 stim + 0 control stim = 68

Walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3		1	3	1
# control stim					

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		2	5	2
# control stim					

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		2	2	2
# control stim					

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2		1		
# control stim					



When walking stimulation is on this behaviour is seen 21/28: walking.
No data for control stimulation.

String-pulling

40 Hz	0.7 sec
# stim	3
# control stim	



When stimulation is on this behaviour is seen 2/3: both hands hold on to the string.
No data for control stimulation.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					1
# control stim					

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3				
# control stim					

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3				
# control stim					

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1				
# control stim					



When stimulation is on this behaviour is seen 8/8: grooming continues however it seems hands are locked at mouth and there is no face washing.

No data for control stimulation.

Rearing or standing up on hindfeet

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2		3	1	1
# control stim					

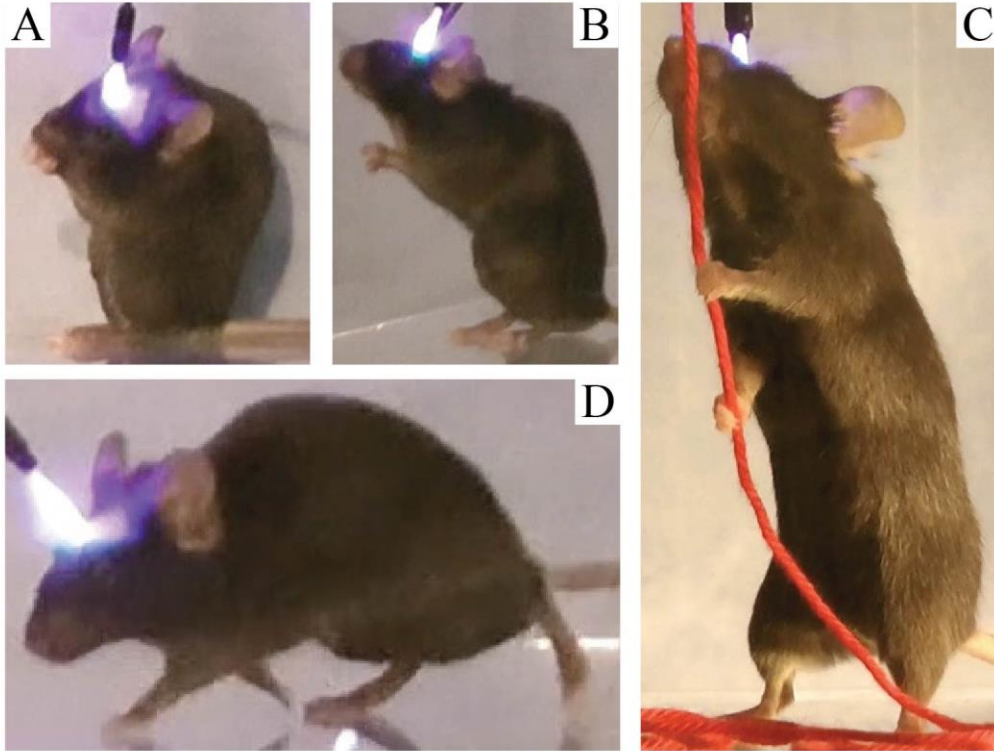
20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0			1	1
# control stim					

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2		2	1	3
# control stim					

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		3	2	6
# control stim					



When stimulation is on rearing seems not to be affected 17/29.
No data for control stimulation.



Common behaviours that occur during optogenetic stimulation of ALM area in mouse #1F A) grooming, B) rearing, C) string-pulling, and D) walking. (Note that the stimulation affected grooming and string-pulling).

Mouse #5F

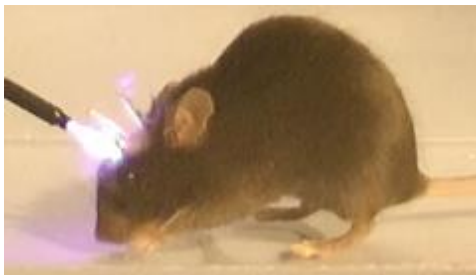
- ALM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- total # observations = 197 stim + 141 control stim = 338

Walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	4	5	4	4
# control stim	2	0	4	2	3

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	7	7	6	4	3
# control stim	6	5	4	6	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	5	6	6	3
# control stim	5	2	3	2	3



When walking stimulation is on this behaviour is seen 69/71: stopping, left hand to mouth.
When control stimulation is on, the mouse keeps walking 40/49.

String-pulling

10 Hz	0.7 sec
# stim	3
# control stim	1

20 Hz	0.7 sec
# stim	6
# control stim	2

30 Hz	0.7 sec
# stim	6
# control stim	2

40 Hz	0.7 sec
# stim	5
# control stim	3



When stimulation is on this behaviour is seen 20/20: pausing string-pulling, both hands grasping string at mouth level, mouth to hands (no sitting down).

When control stimulation is on, the mouse keeps pulling the string 8/8.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	4	4	3	1
# control stim	3	2	5	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	5	6	4
# control stim	4	4	3	6	3

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	5	5	4
# control stim	2	3	2	3	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	4	5	5	5
# control stim	2	2	3	4	2



When stimulation is on these behaviour are seen 80/85: mouth away from peanut butter, mouth movements, head towards right or left, left hand raised waving at the wall (12/85), both hands to the mouth(9/85) (no sitting down).



At higher frequencies (30 and 40 Hz) this behaviour is seen 12/85: mouth away from peanut butter, left hand at raised waving at the wall (no sitting down).



At higher frequencies (30 and 40 Hz) this behaviour is seen 9/85: mouth away from peanut butter, both hands to mouth (no sitting down).

When control stimulation is on, the mouse keeps licking 51/59.

Eating

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		1			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim					

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		2			



When stimulation is on eating is not disturbed 8/8.

When control stimulation is on, the mouse keeps eating 3/3.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1		0	1
# control stim	1	1		1	0

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2			1	
# control stim	1			0	

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1			1
# control stim	1	0			1



When stimulation is on, both hands are at the mouth and no face washing occurs 8/9.
 When control stimulation is on, the mouse keeps grooming 6/6.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	1	0	0	0
# control stim	3	1	1	1	1

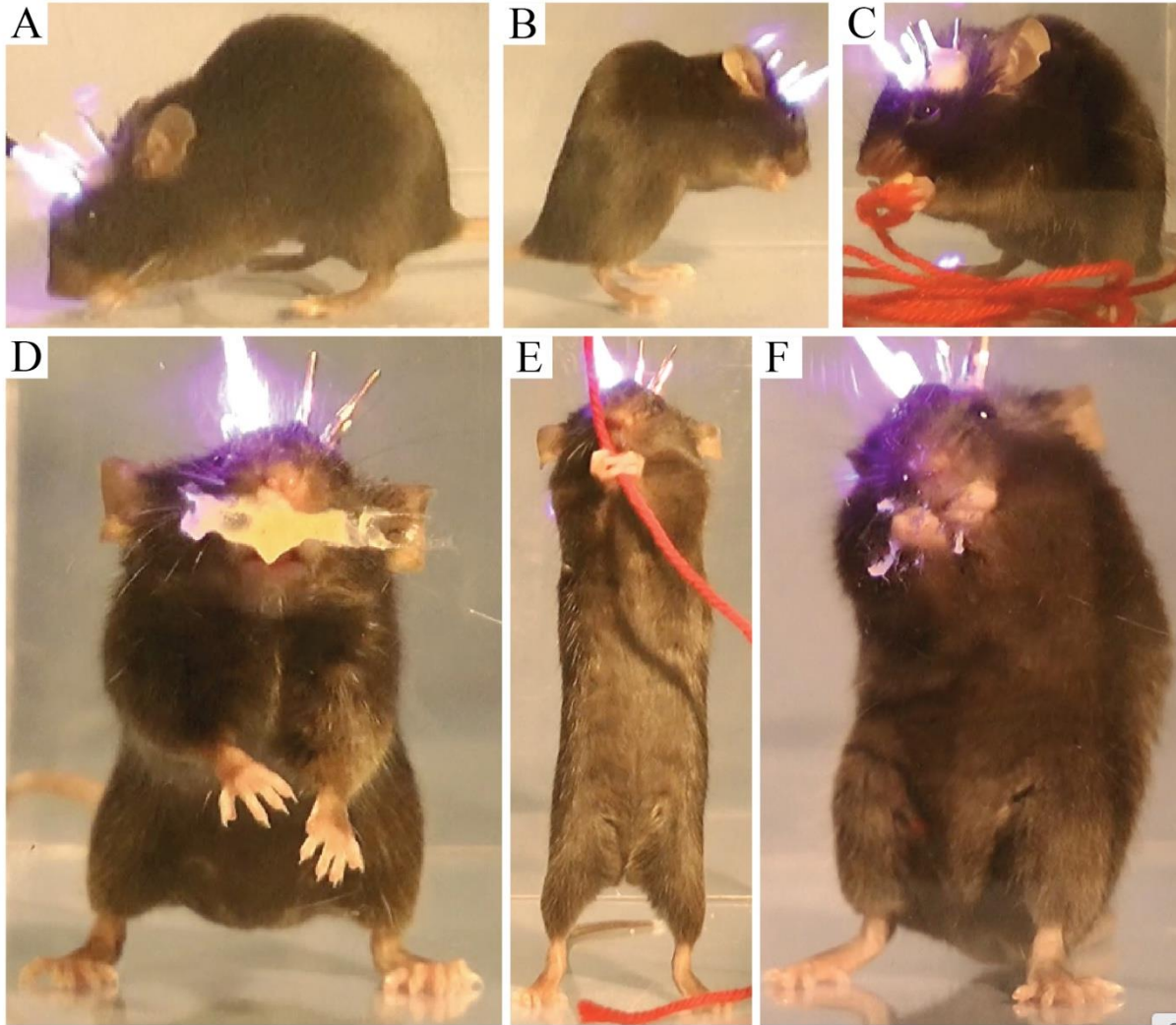
20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		0	1		
# control stim		1	0		

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	0	0	0	1
# control stim	2	2	2	1	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					



When stimulation is on this behaviour is seen 3/4: while standing up mouth to both hands
 When control stimulation is on, the mouse keeps rearing 10/16.



Common behaviours that occur during optogenetic stimulation of ALM area in mouse #5F. A) walking, B) rearing, C) eating, D) peanut butter licking, E) string-pulling, and F) grooming. (Note that the stimulation affected walking, rearing, licking peanut butter, and string-pulling).

Mouse #6F

- ALM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 217 stim + 148 control stim = 365

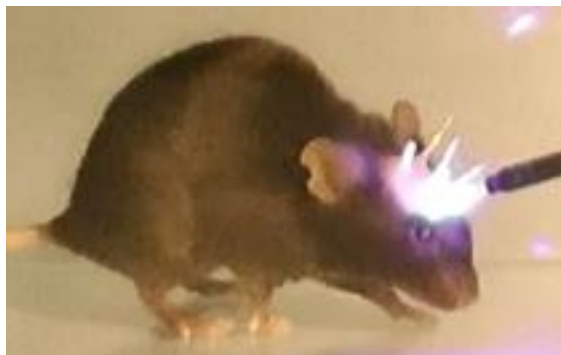
Walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	9	5	4	5
# control stim	6	4	3	3	4

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	7	5	5	4	3
# control stim	5	4	6	3	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	2	3	3
# control stim	2	1	1	2	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	2	2	2
# control stim	2	2	2	1	2



When walking stimulation is on this behaviour is seen 50/79: stopping, left hand forward and mouth to floor.

When control stimulation is on, the mouse keeps walking 45/55.

String-pulling

10 Hz	0.7 sec
# stim	8
# control stim	3

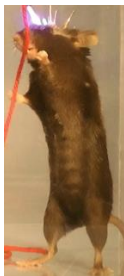
20 Hz	0.7 sec
# stim	6
# control stim	1

30 Hz	0.7 sec
# stim	3
# control stim	2

40 Hz	0.7 sec
# stim	9
# control stim	3



When stimulation is on these behaviour is seen 18/26: left hand makes grasping movement but is unable to grasp the string (no sitting down) 7/26, biting the string (no sitting down) 4/26.



When stimulation is on at 40Hz this behaviour is seen 4/26: biting the string (no sitting down)
When control stimulation is on, the mouse keeps pulling the string 9/9.

Licking peanut butter

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	3	4	3	5
# control stim	5	3	3	2	3

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	3	3	4
# control stim	1	2	4	3	3

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	7	6	5
# control stim	2	3	5	3	2

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	5	4	5	6
# control stim	2	2	2	2	3



When stimulation is on this behaviour is seen 63/88: mouth away from peanut butter, left hand raised making grasping movements at the wall (no sitting down).

When control stimulation is on, the mouse keeps licking 49/55.

Eating

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		2			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		4			

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		4			



When stimulation is on eating is not disturbed 10/10.

When control stimulation is on, the mouse keeps eating 8/10.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1	2	0	0
# control stim		0	3	1	1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2			2	
# control stim	2			0	

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim			1		
# control stim			1		



When stimulation is on, it seems grooming is not disturbed 5/8.
 When control stimulation is on, the mouse keeps grooming 8/8.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		0		0	0
# control stim		1		2	1

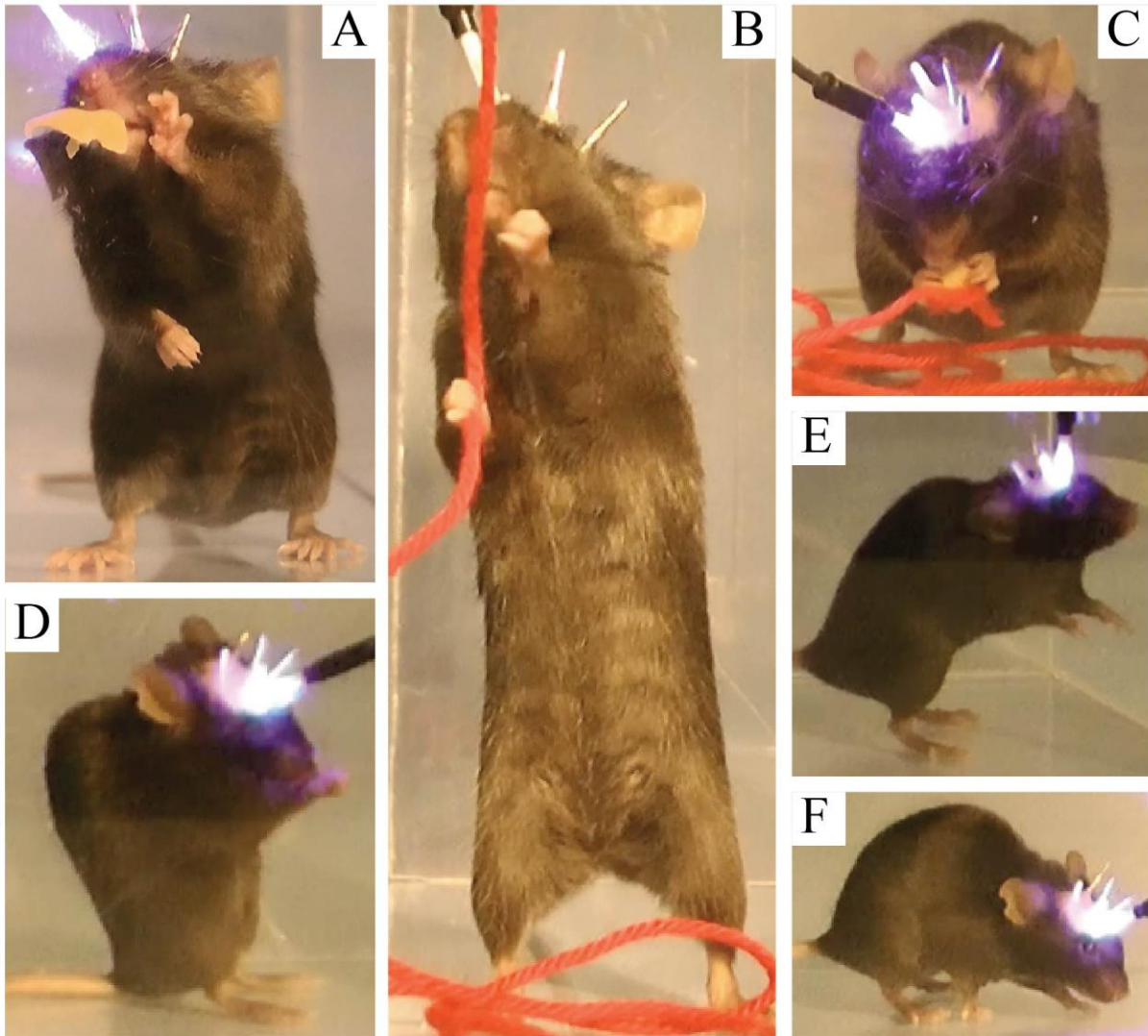
20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1		0	0
# control stim		1		1	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		1		
# control stim	0		1		

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	0	1	1	
# control stim	1	1	0	1	



When stimulation is on this behaviour is seen 4/6: rearing then sitting down.
 When control stimulation is on, the mouse keeps rearing 7/11.



Common behaviours that occur during optogenetic stimulation of ALM area in mouse #6F A) licking peanut butter, B) string-pulling, C) eating, D) grooming, E) rearing, and F) walking. (Note that the stimulation affected licking peanut butter, string-pulling, rearing, and walking).

Mouse #7M

- ALM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 177 stim + 128 control stim = 305

Walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	7	5	1	4	3
# control stim	4	3	3	1	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	5	6	4	4
# control stim	4	4	6	3	4

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	3	2	2	3
# control stim	0	0	0	0	1



When walking stimulation is on this behaviour is seen 57/60: stopping, mouth to floor with mouth movements.

When control stimulation is on, the mouse keeps walking 32/35.

String-pulling

10 Hz	0.7 sec
# stim	3
# control stim	1

20 Hz	0.7 sec
# stim	5
# control stim	3

30 Hz	0.7 sec
# stim	8
# control stim	2

40 Hz	0.7 sec
# stim	5
# control stim	3



When stimulation is on this behaviour is seen 21/21: both hands at the mouth level, biting the string.

When control stimulation is on, the mouse keeps pulling the string or does not show hand to mouth 9/9.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	2	0	-	-
# control stim	4	2	2	-	-

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	5	3	2	2
# control stim	2	4	4	2	5

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	3	4	3	5
# control stim	3	3	2	4	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	5	5	5	4
# control stim	3	2	2	2	3



When stimulation is on this behaviour is seen 61/62: mouth away from peanut butter, left hand raised making grasping movements at the wall (no sitting down).

When control stimulation is on, the mouse keeps licking 39/52.

Eating

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		6			
# control stim		4			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		1			

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		7			
# control stim		2			

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		6			
# control stim		6			



When stimulation is on eating is not affected 20/20.

When control stimulation is on, the mouse keeps eating 13/13.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1				
# control stim		2			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1			1	
# control stim		1		1	

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					



When stimulation is on, there is no face washing 2/3.

When control stimulation is on, the mouse keeps grooming 3/4.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	2	0	3
# control stim	0	2	1	3	4

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1		0	
# control stim	2	1		1	

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim				1	1
# control stim				0	1

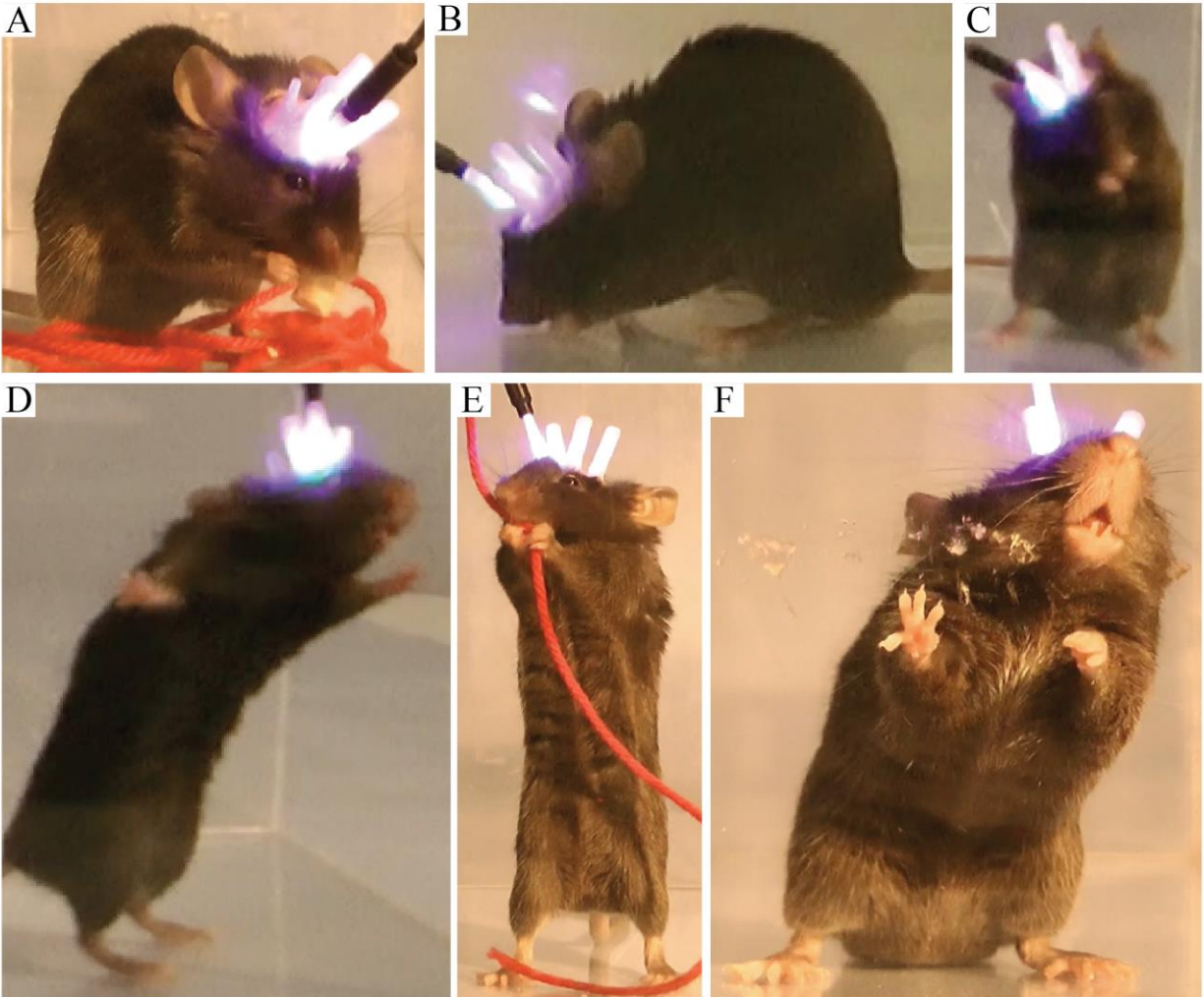
40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					



When stimulation is on this behaviour is seen 5/11 when standing up in the middle of the box: head towards the floor, mouse sits on haunches.



When stimulation is on this behaviour is seen 5/11 when rearing at the wall: mouth movement
When control stimulation is on, the mouse keeps rearing 12/15.



Common behaviours that occur during optogenetic stimulation ALM area in mouse #7M A) eating, B) walking, C) grooming, D) rearing, E) string-pulling, and F) licking peanut butter. (Note that the stimulation affected walking, grooming, rearing, string-pulling, and licking peanut butter).

Mouse #10M

- ALM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- total # observations = 223 stim + 151 control stim = 374

Walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	2	2	3
# control stim	5	2	3	0	1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	4	2
# control stim	5	3	1	1	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	5	3	2	2
# control stim	2	4	3	3	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	2	2	2	4
# control stim	2	2	4	3	2



When walking stimulation is on this behaviour is seen 45/58: walking.
When control stimulation is on, the mouse keeps walking 45/49.

String-pulling

10 Hz	0.7 sec
# stim	4
# control stim	4

20 Hz	0.7 sec
# stim	6
# control stim	3

30 Hz	0.7 sec
# stim	4
# control stim	5

40 Hz	0.7 sec
# stim	4
# control stim	4



When stimulation is on all or part of the following is seen 14/18: left hand had difficulty grasping the string or hands make short reaches or pause.

When control stimulation is on, the mouse keeps pulling the string 9/16.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	5	2	4
# control stim	2	2	2	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	5	4	3
# control stim	2	3	2	2	3

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	6	6	4
# control stim	2	1	3	3	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	5	4	5	3
# control stim	2	3	3	2	2



When stimulation is on all or part of the following is seen 23/81: away from peanut butter with mouth movement.

When stimulation is on all or part of the following is seen 42/81: away from peanut butter with left hand movement or waving.

When control stimulation is on, the mouse keeps licking 42/46.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1			
# control stim		2			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		2			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1			
# control stim		1			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		2			



When stimulation is on eating is not affected 5/6: eating.

When control stimulation is on, the mouse keeps eating 7/7.

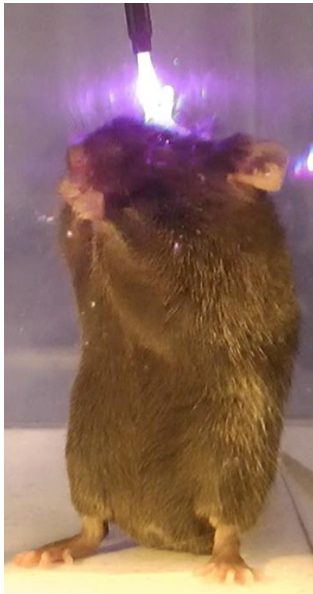
Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	1	0	
# control stim	0	0	1	1	

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		1		1
# control stim	0		0		0

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	2		2
# control stim	1	0	1		1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	1	1	1	
# control stim	2	0	0	1	



When stimulation is on this behaviour is seen 12/19: hands stopped at the mouth level.
 When control stimulation is on, the mouse keeps grooming 8/8.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	2	2	1
# control stim	0	1	0	1	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1	1	2
# control stim	0	1	3	1	2

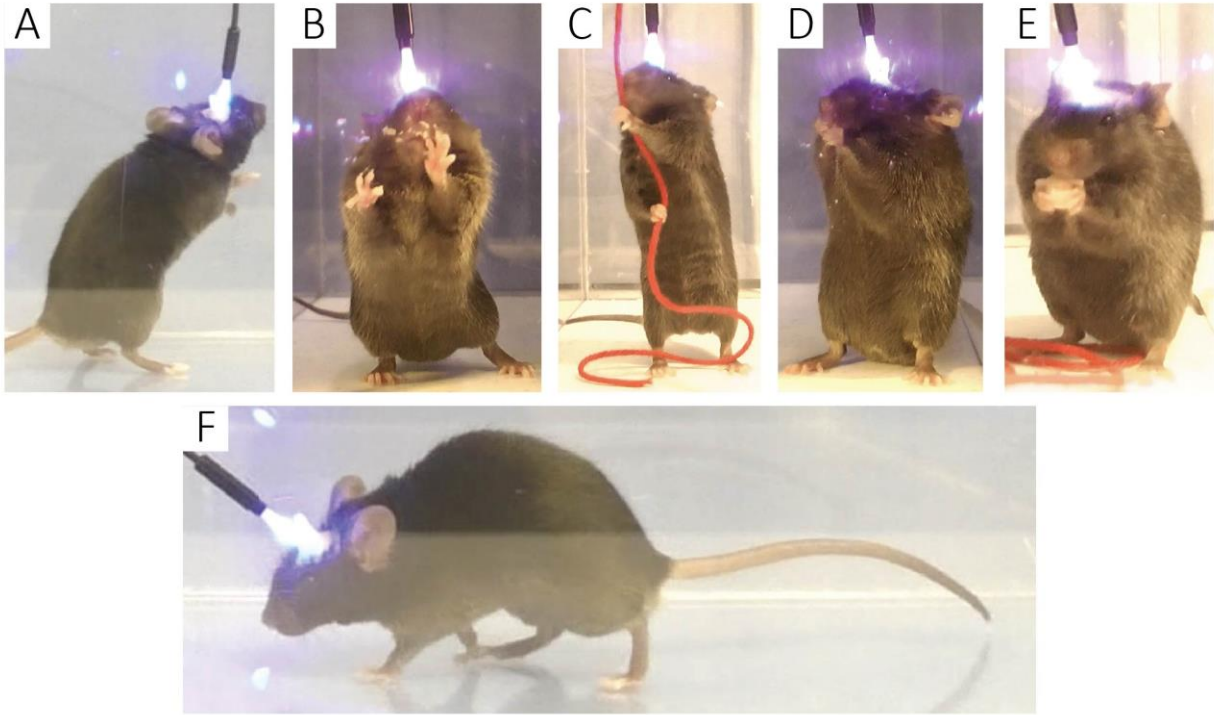
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	4	3	2	3
# control stim	1	2	1	1	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	5	4	3	1
# control stim	1	2	0	3	2



When stimulation is on all or part of the following is seen 23/41: came down, hands on the floor and hand movement.

When control stimulation is on, the mouse comes down 19/25.



Common behaviours that occur during optogenetic stimulation of ALM area in mouse #10M. A) rearing, B) licking peanut butter, C) string-pulling, D) grooming, E) eating, and F) walking. (Note that the stimulation affected rearing, grooming, licking peanut butter and string-pulling).

Mouse #11F

- ALM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- total # observations = 227 stim + 162 control stim = 389

Walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	1	4	3	2
# control stim	3	1	5	1	3

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	3	2	3
# control stim	1	2	3	0	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	2	2	3
# control stim	3	1	2	3	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	3	1	3	0
# control stim	2	2	3	3	2



When walking stimulation is on this behaviour is seen 39/49: continues walking.
When control stimulation is on, the mouse keeps walking 34/43.

String-pulling

10 Hz	0.7 sec
# stim	2
# control stim	3

20 Hz	0.7 sec
# stim	4
# control stim	3

30 Hz	0.7 sec
# stim	5
# control stim	4

40 Hz	0.7 sec
# stim	6
# control stim	5



When stimulation is on all or part of the following is seen 12/17: stopped string-pulling, lowered the body.

When control stimulation is on, the mouse lowered body 9/15.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	5	6	4
# control stim	3	3	3	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	4	3	4
# control stim	2	3	3	2	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	4	3	4	4
# control stim	2	2	3	3	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	4	6	4
# control stim	3	3	4	3	2



When stimulation is on all or part of the following is seen: 34/78 continues licking, 22/78 away from peanut butter, 13/78 digits extended, 9/78 other.

When control stimulation is on, the mouse keeps licking 42/52.

Eating

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		2			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		2			

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		1			

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		1			



When stimulation is on eating is not disturbed 9/10: continues eating.
When control stimulation is on, the mouse keeps eating 5/6.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	1		1	
# control stim	1	1		0	

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	1	1	2	0
# control stim	1	0	0	0	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2		1	
# control stim	0	1		0	

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	2	1	1
# control stim	0	0	1	0	0



When stimulation is on this behaviour is seen 18/19: continues grooming.
 When control stimulation is on, the mouse keeps grooming 6/6.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	4		0	3
# control stim	1	3		2	1

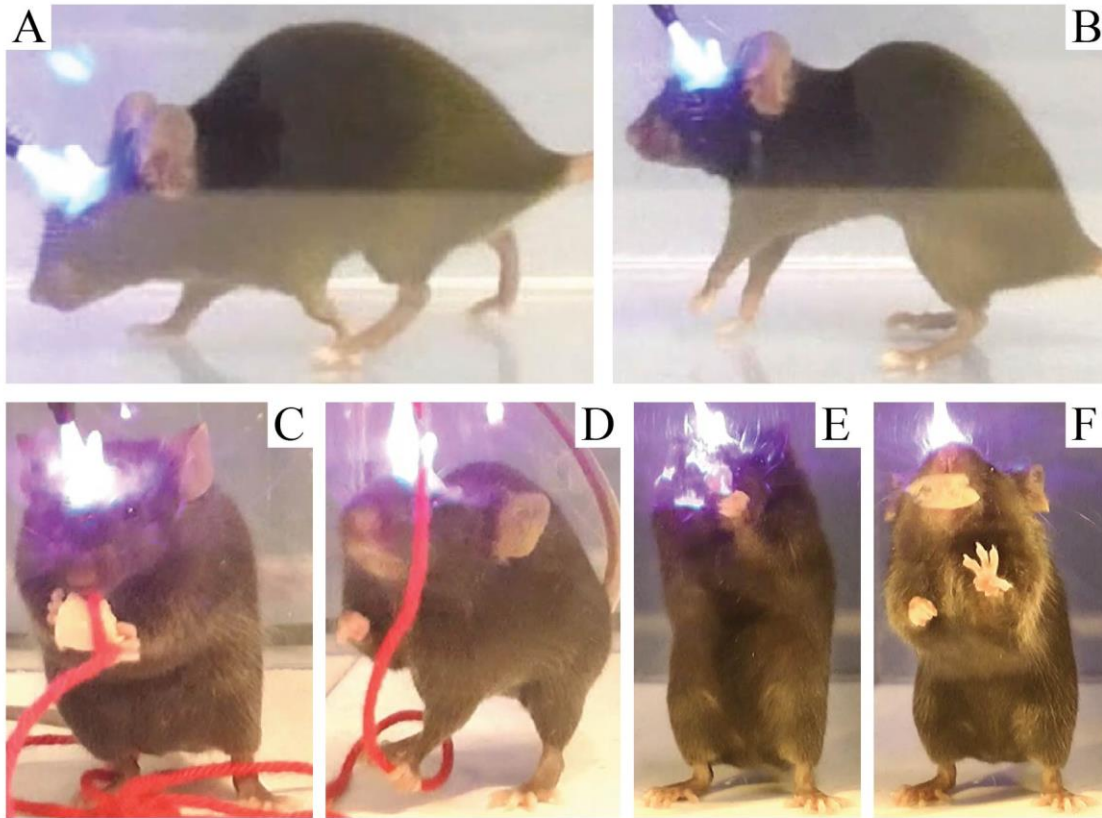
20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	2	2	1
# control stim	1	2	1	3	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	2	4	4	4
# control stim	3	4	3	2	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	4	3	5
# control stim	2	2	1	2	2



When stimulation is on all or part of the following is seen 33/54: came down on the floor.
 When control stimulation is on, the mouse comes down 22/40.



Common behaviours that occur during optogenetic stimulation of ALM area in mouse #11F. A) walking, B) rearing, C) eating, D) string-pulling, E) grooming, and F) licking peanut butter. (Note that the stimulation affected rearing, licking peanut butter, and string-pulling).

APPENDIX 2: SUMMARY OF AMM ANIMALS

Mouse #13F

- AMM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 200 stim + 141 control stim = 341

Walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	3	3	1	3
# control stim	1	1	2	1	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	3	1	2
# control stim	1	2	1	1	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	2	3	2	3
# control stim	2	1	2	2	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	3	2	3	1
# control stim	0	1	1	2	1



When stimulation is on, it seems walking is not affected 31/47.
 When control stimulation is on, the mouse keeps walking 16/28.

String-pulling

10 Hz	0.7 sec
# stim	5
# control stim	2

20 Hz	0.7 sec
# stim	5
# control stim	3

30 Hz	0.7 sec
# stim	4
# control stim	4

40 Hz	0.7 sec
# stim	4
# control stim	4



When stimulation is on, it seems string-pulling is not affected 11/18.
When control stimulation is on, the mouse keeps pulling the string 11/13.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	3	5	4
# control stim	3	2	3	3	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	4	4
# control stim	3	2	2	3	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	4	3	4
# control stim	2	1	2	4	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	4	4	5
# control stim	3	3	2	3	4



When stimulation is on, it seems peanut butter licking is not affected 71/78.
 When control stimulation is on, the mouse keeps licking 49/51.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		4			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		4			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		3			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		9			
# control stim		8			



When stimulation is on eating is not affected 21/21.

When control stimulation is on, the mouse keeps eating 19/19.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	3		1	
# control stim	1	3		1	

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1		1	
# control stim	1				

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1			1	
# control stim	1				

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3			
# control stim	2	3			



When stimulation is on, it seems grooming is not affected 14/17.
When control stimulation is on, the mouse keeps grooming 10/11.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	1	1		1
# control stim			1	1	1

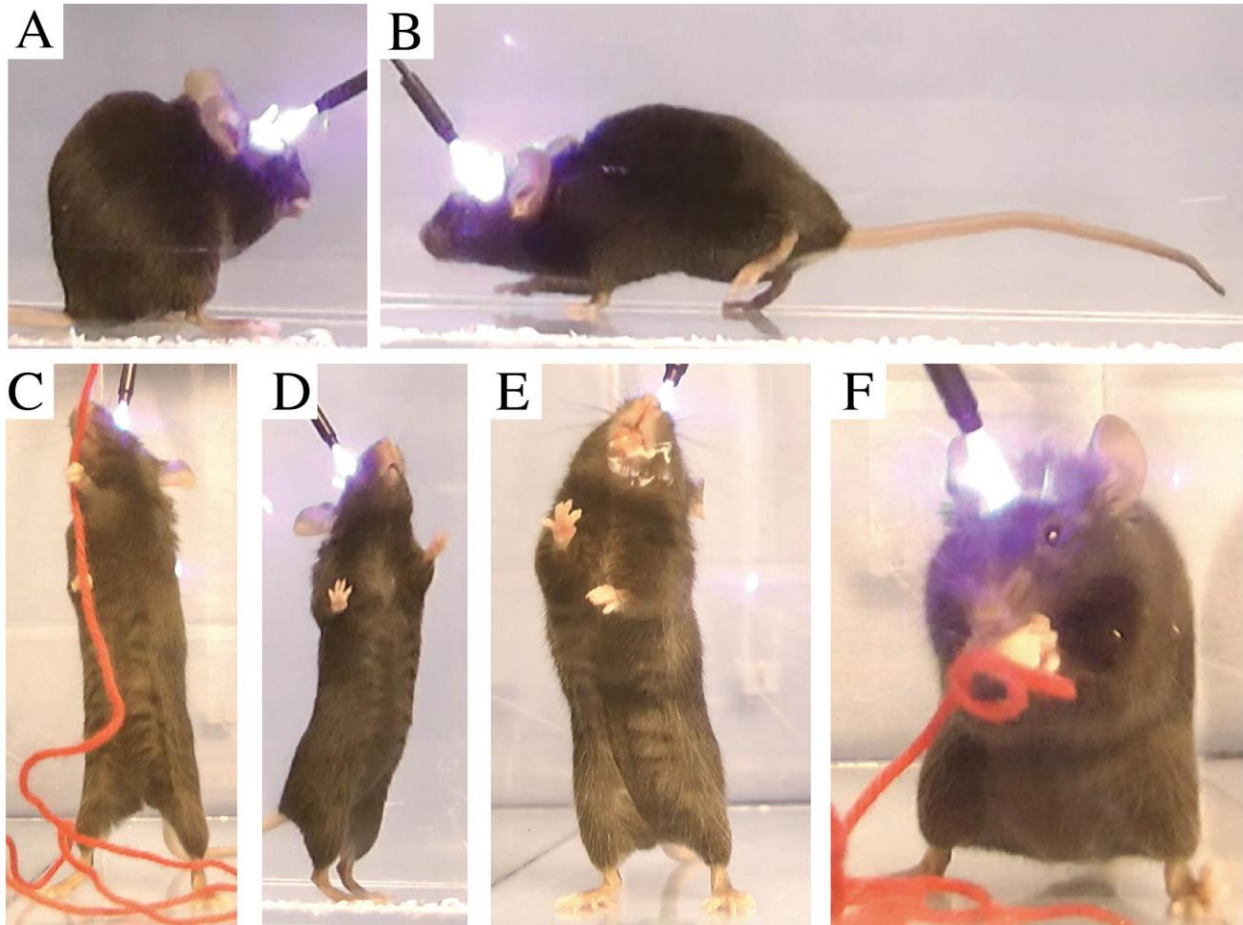
20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			1
# control stim	2	1			

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4	2		1
# control stim	1	2	1	2	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1		2	1
# control stim	1	1		2	2



When stimulation is on, it seems rearing is not affected 14/19.
 When control stimulation is on, the mouse keeps rearing 14/19.



Common behaviours that occur during optogenetic stimulation of AMM area in mouse #13F A) grooming, B) walking, C) string-pulling, D) rearing, E) licking peanut butter, and F) eating. (Note that the stimulation did not affect any of the behaviours).

Mouse #14F

- AMM
- histology to be done
- summary in following behaviours: string-pulling, licking peanut butter, eating, grooming, and rearing.
- Total # observations = 176 stim + 124 control stim = 300

String-pulling

10 Hz	0.7 sec
# stim	4
# control stim	3

20 Hz	0.7 sec
# stim	4
# control stim	3

30 Hz	0.7 sec
# stim	3
# control stim	3

40 Hz	0.7 sec
# stim	5
# control stim	3



When stimulation is on, left hand is unable to grasp the string and lowers the body 16/16.
When control stimulation is on, the mouse keeps pausing or miss grasping the string 7/12.

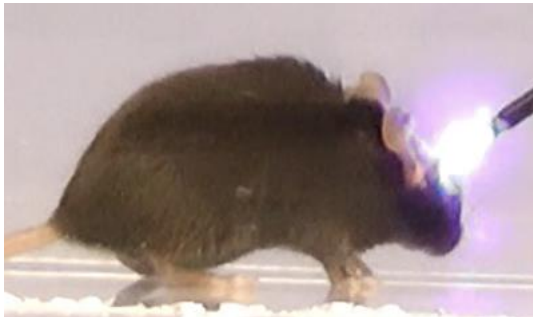
Walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	1	1	2	3
# control stim	1	3	2	1	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	2	0	1
# control stim	2	0	1	1	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	4	2	3
# control stim	1	1	1	0	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	1	3	2
# control stim	2	1	2	1	2



When stimulation is on, the mouse stopped walking 35/43.

When control stimulation is on, the mouse keeps walking 16/27.

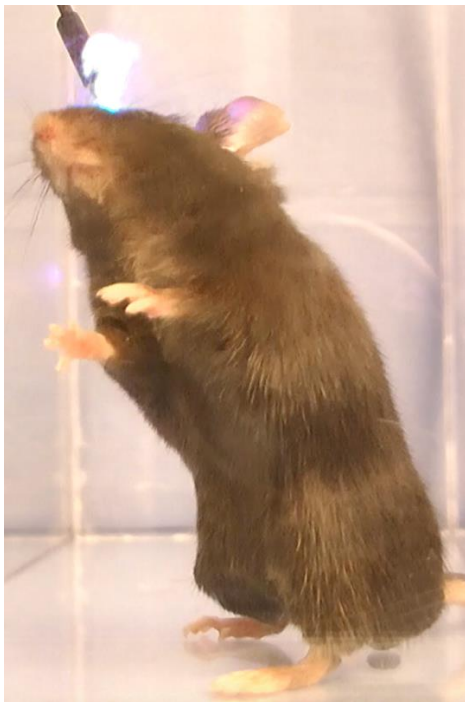
Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	3	2	3
# control stim	2	2	4	3	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	5	4	3
# control stim	2	2	2	2	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	3	4	4
# control stim	2	2	2	2	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	6	4	4
# control stim	2	2	3	3	2



When stimulation is on, the mouse is away from peanut butter and lowers body 54/75.
 When control stimulation is on, the mouse keeps licking 42/45.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		7			
# control stim		5			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		7			
# control stim		6			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		6			
# control stim		4			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		7			
# control stim		7			



When stimulation is on, it seems that the mouse holds the food or is not able to bite the food 19/27.

When control stimulation is on, the mouse keeps 18/22.

Grooming

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1				
# control stim					

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1				
# control stim		1			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1			
# control stim	1	0			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	3		1	
# control stim	2	4		1	



When stimulation is on, the mouse stops grooming 7/9.

When control stimulation is on, the mouse keeps grooming 6/9.

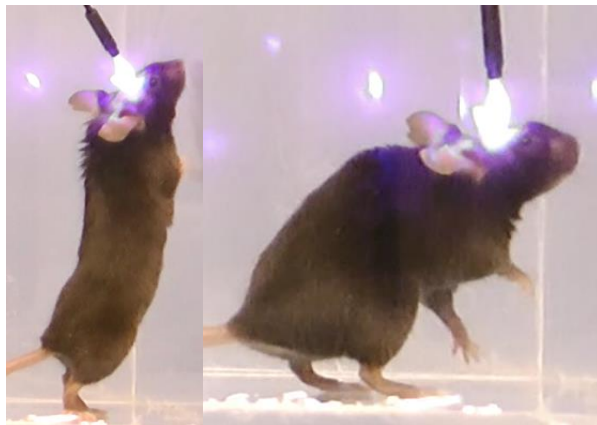
Rearing or standing up on hindfeet

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim				1	

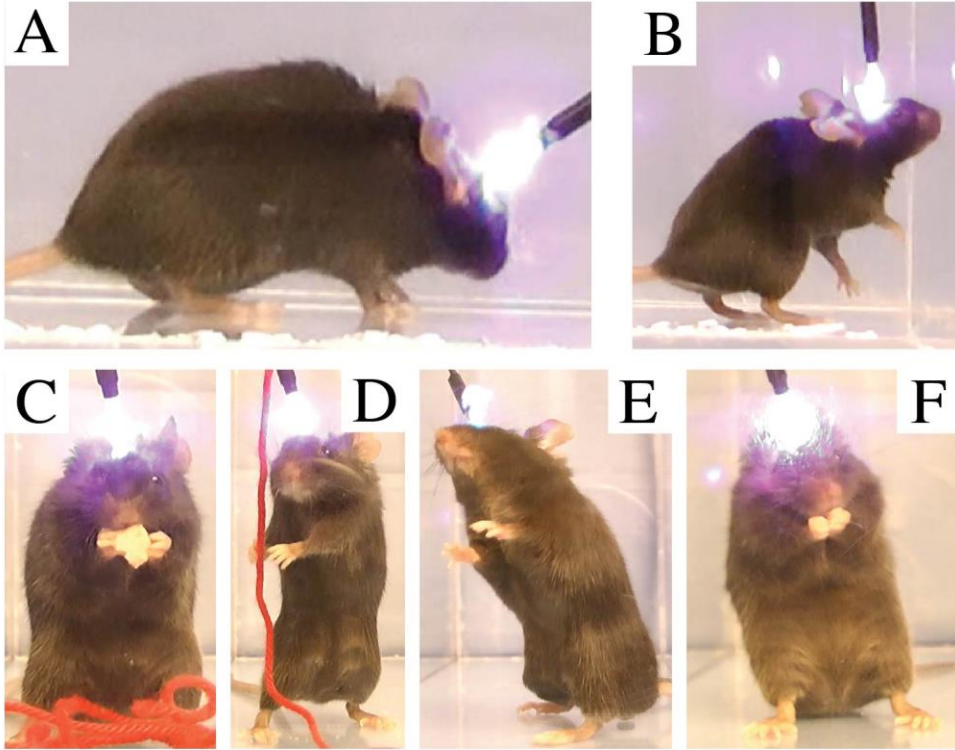
20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					1

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim			1		1
# control stim	1		1	3	

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1	1	1	1
# control stim				1	1



When stimulation is on the mouse either continues rearing 3/6 or comes down 3/6.
 When control stimulation is on, the mouse came down 8/9.



Common behaviours that occur during optogenetic stimulation of AMM area in mouse #14F A) walking, B) rearing, C) eating, D) string-pulling, E) licking peanut butter, and F) grooming. (Note that Stimulation affected all the behaviours).

Mouse #15F

- AMM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 183 stim + 133 control stim = 316

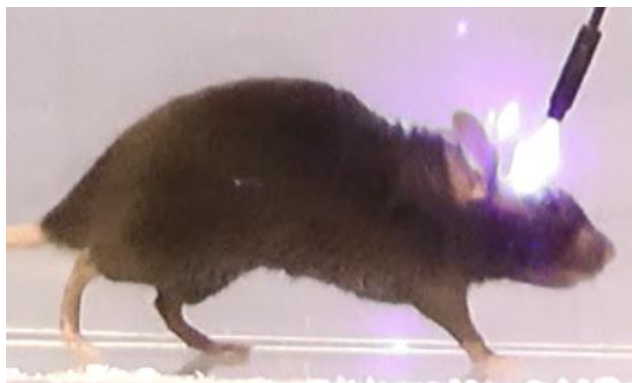
walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	2	3	3
# control stim	1	1	1	2	1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	3	1	2	3
# control stim	1	2	3	2	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	3	2	2
# control stim	3	2	2	1	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	1	3	2	2
# control stim	2	2	2	3	2



When stimulation is on, it seems walking is not affected 24/48.
When control stimulation is on, the mouse keeps walking 26/38.

String-pulling

10 Hz	0.7 sec
# stim	4
# control stim	4

20 Hz	0.7 sec
# stim	4
# control stim	4

30 Hz	0.7 sec
# stim	5
# control stim	3

40 Hz	0.7 sec
# stim	5
# control stim	4



When stimulation is on, the mouse lowers the body and has trouble grasping the string 15/18.
When control stimulation is on, the mouse keeps pulling the string 10/15.

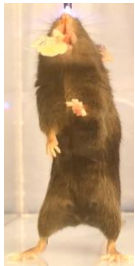
Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	4	3	3
# control stim	2	2	2	3	3

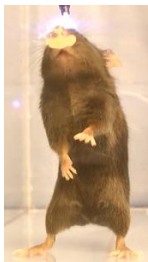
20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	5	4	5
# control stim	2	2	3	2	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	4	2	4
# control stim	2	2	2	3	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	4	5
# control stim	3	2	2	2	3



When stimulation is on, the mouse keeps licking peanut butter 41/78.



When stimulation is on, the mouse is away from peanut butter 37/78.
When control stimulation is on, the mouse keeps licking 38/46.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		2			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		3			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		3			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		6			



When stimulation is on, the mouse seems not affected 13/16.
When control stimulation is on, the mouse keeps eating 13/14.

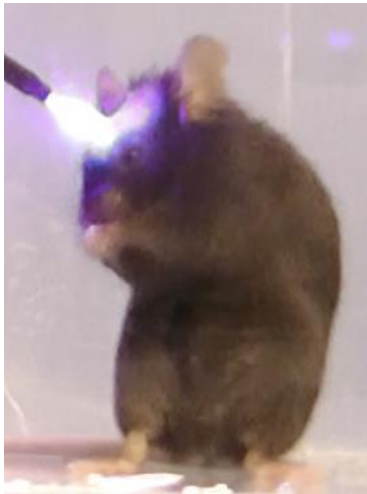
Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		1		
# control stim	0		1		

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1		
# control stim	0	0	1		

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1			1	
# control stim	1			1	

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1		
# control stim	1	1	1		



When stimulation is on, it seems grooming is not affected 8/10.
When control stimulation is on, the mouse keeps grooming 7/7.

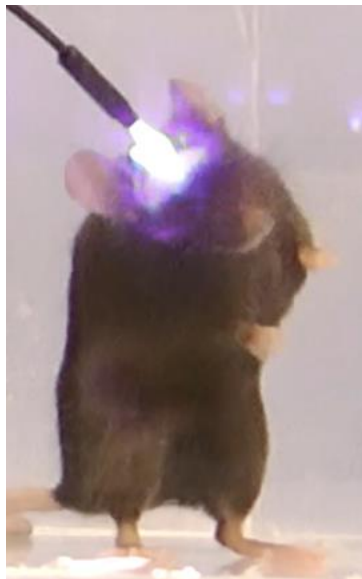
Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	0		2
# control stim	1	1	2		1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	2	0	1
# control stim	1	1	0	1	0

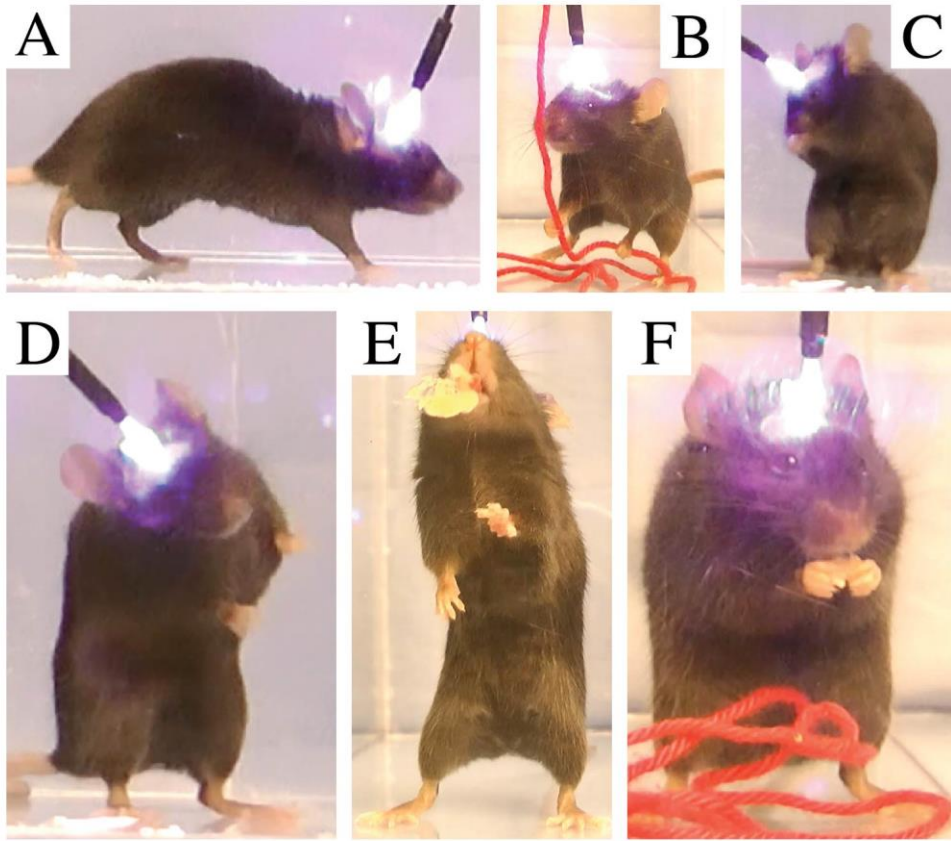
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	0	0	0	2
# control stim	1	1	1	1	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1			
# control stim	0	0			



When stimulation is on, the mouse keeps rearing 8/13. The rest of the time the mouse came down 5/13.

When control stimulation is on, the mouse comes down 7/13.



Common behaviours that occur during optogenetic stimulation of AMM area in mouse #15F A) walking, B) string-pulling, C) grooming, D) rearing, and E) licking peanut butter, and F) eating. (Note that the stimulation affected string-pulling).

Mouse #16M

- AMM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 188 stim + 119 control stim = 307

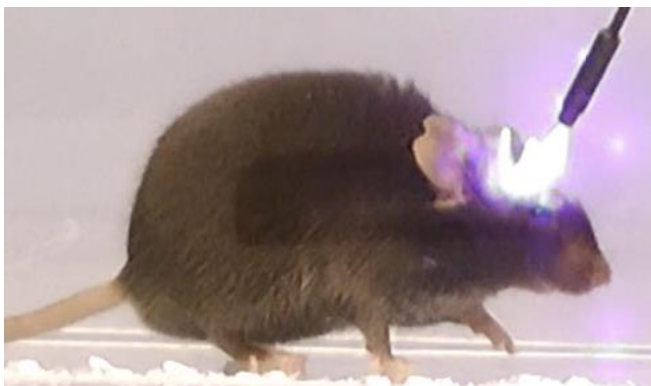
walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	2	2	2	2
# control stim	2	1	3	1	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	2	3	2	1
# control stim	0	0	0	2	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	3	3	1	3
# control stim	2	1	1	1	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	3	3	1
# control stim	2	3	1	2	2



When stimulation is on, the mouse pauses 42/50.

When control stimulation is on, the mouse keeps walking 18/28.

String-pulling

10 Hz	0.7 sec
# stim	4
# control stim	2

20 Hz	0.7 sec
# stim	4
# control stim	2

30 Hz	0.7 sec
# stim	5
# control stim	3

40 Hz	0.7 sec
# stim	3
# control stim	3



When stimulation is on, the mouse sits down or lowers the body 13/16.
When control stimulation is on, the mouse keeps pulling the string 10/10.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	3	3	3
# control stim	2	1	1	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	4	3	2
# control stim	3	2	2	2	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	5	3	2
# control stim	3	3	3	2	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	3	4
# control stim	2	2	3	2	2



When stimulation is on, it seems licking is not affected 58/72.
 When control stimulation is on, the mouse keeps licking 40/42.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		4			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		4			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		2			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		6			
# control stim		4			



When stimulation is on, it seems eating is not affected 8/16.
When control stimulation is on, the mouse keeps eating 14/14.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1		0	
# control stim		2		1	

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2			1	
# control stim	0			0	

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	0			1
# control stim	2	1			1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		1		
# control stim	1		0		



When stimulation is on, it seems grooming is not affected 7/9.
 When control stimulation is on, the mouse keeps grooming 7/8.

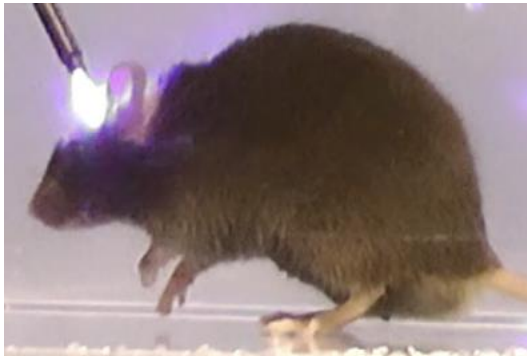
Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	2	2	1	2
# control stim	1	1	0	0	1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3		0	2
# control stim		0		1	2

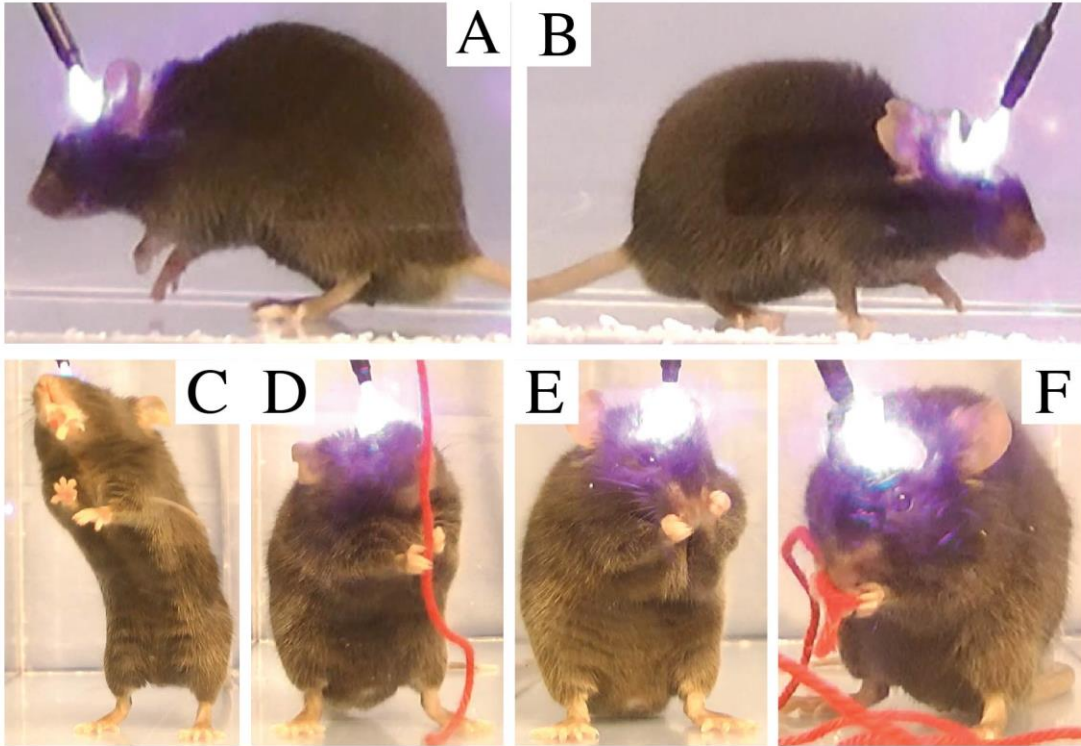
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2	1	4	0
# control stim		1	1	1	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	0	1	3
# control stim	1	1	2	1	2



When stimulation is on, the mouse comes down 18/25.

When control stimulation is on, the mouse keeps rearing 9/17.



Common behaviours that occur during optogenetic stimulation of AMM area in mouse #16M. A) rearing, B) walking, C) licking peanut butter, D) string-pulling, E) grooming, and F) eating. (Note that the stimulation affected walking, rearing and string-pulling).

Mouse #17M

- AMM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 210 stim +146 control stim = 356

walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	3	2	2
# control stim	3	2	2	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	2	2	2	1
# control stim	1	2	1	0	0

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	4	4	0
# control stim	3	2	3	2	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	4	2	2
# control stim	2	2	1	2	2



When stimulation is on, it seems walking is not affected 34/52.

When control stimulation is on, the mouse keeps walking 26/37.

String-pulling

10 Hz	0.7 sec
# stim	3
# control stim	3

20 Hz	0.7 sec
# stim	6
# control stim	2

30 Hz	0.7 sec
# stim	4
# control stim	4

40 Hz	0.7 sec
# stim	4
# control stim	4



When stimulation is on, it seems string-pulling is not affected 11/17.
When control stimulation is on, the mouse keeps pulling the string 9/13.

Licking peanut butter

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	5	5	4	2
# control stim	1	3	2	2	1

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	5	4	4	4
# control stim	2	3	3	2	2

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	6	4	4	4
# control stim	3	3	3	3	2

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	6	4	4	5
# control stim	4	4	3	3	4



When stimulation is on, it seems licking is not affected 76/86.
 When control stimulation is on, the mouse keeps licking 46/53.

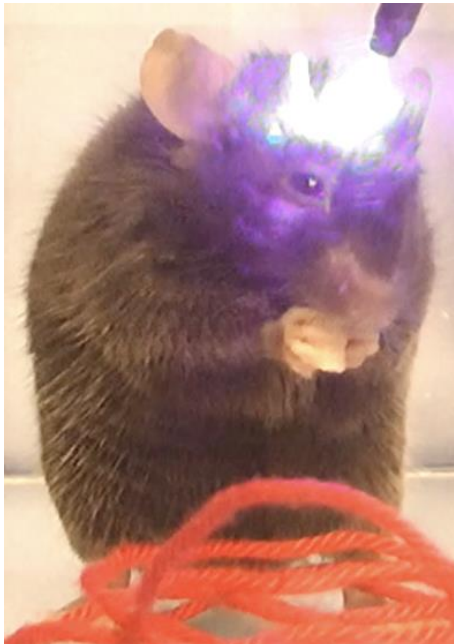
Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		5			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		4			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		3			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		6			
# control stim		5			



When stimulation is on, it seems eating is not affected 11/20.
When control stimulation is on, the mouse keeps eating 13/17.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1	0	
# control stim	2	1	1	1	

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1			1
# control stim		1			0

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1			1
# control stim	1	0			0

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2	1		
# control stim		1	1		



When stimulation is on, it seems grooming is not affected 11/11.
 When control stimulation is on, the mouse keeps grooming 9/9.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	1	0	2
# control stim	1	1	1	0	1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	1	1	1	1
# control stim	1	2	1	0	0

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	2	1	3
# control stim	1	1	1	0	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	1	0	1	1
# control stim	1	1	1	1	1

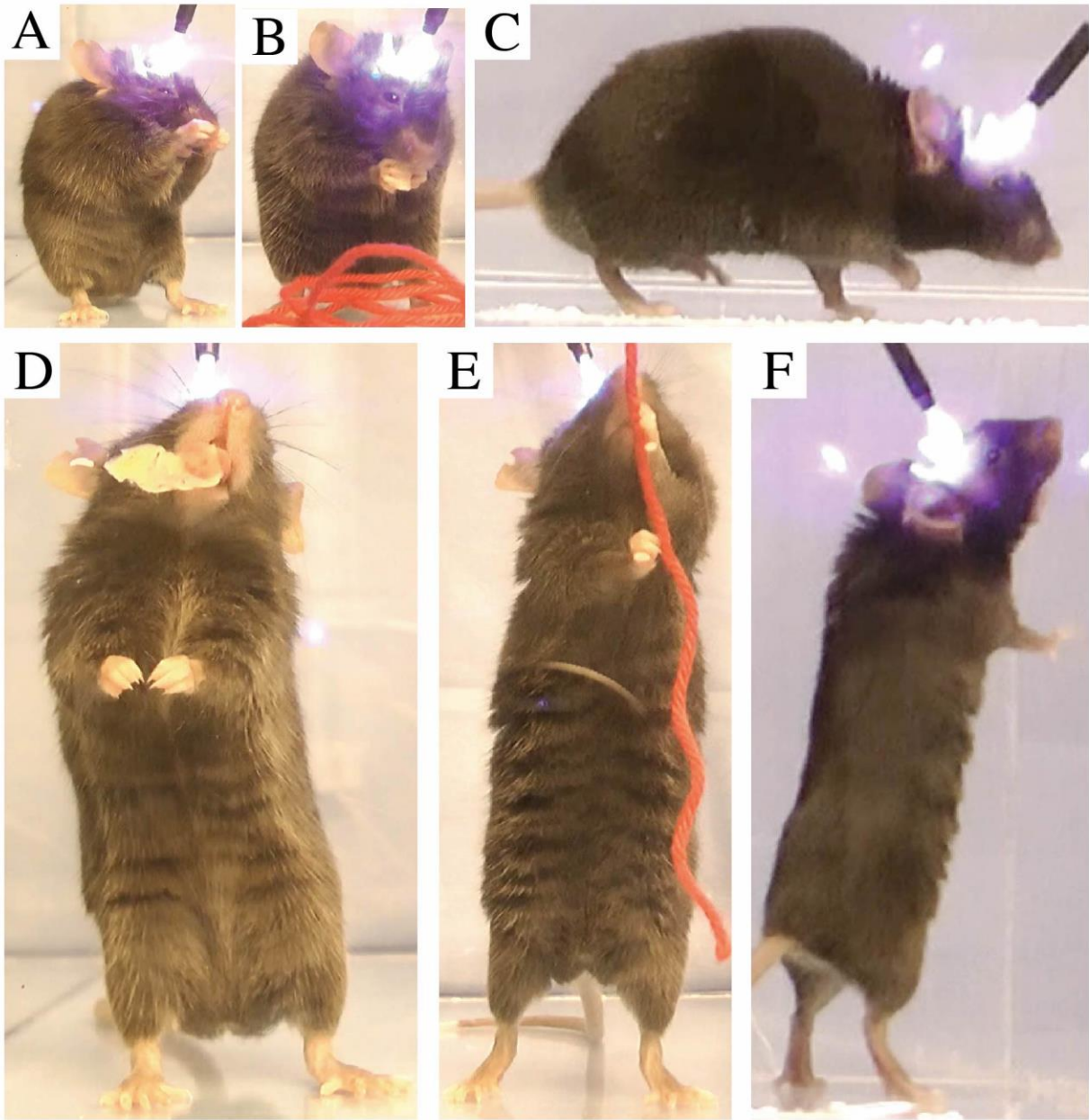


When stimulation is on, the mouse keeps rearing 12/24.



When stimulation is on, the mouse comes down 12/24.

When control stimulation is on, the mouse keeps rearing 15/17.



Common behaviours that occur during optogenetic stimulation of AMM area in mouse #17M. A) grooming, B) eating, C) walking, D) licking peanut butter, E) string-pulling, and F) rearing. (Note that the stimulation did not affect any of the behaviours).

Mouse #18M

- AMM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 204 stim + 141 control stim = 345

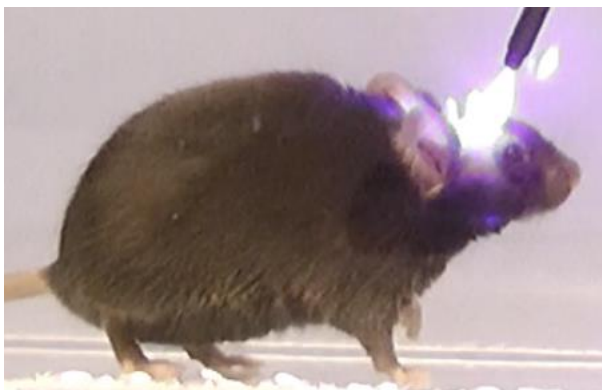
walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	3	3	3	3
# control stim	2	3	2	1	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	1	1	3
# control stim	2	1	3	0	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	5	1	2	2
# control stim	2	3	1	1	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	2	3	2
# control stim	4	3	0	1	2



When stimulation is on, the mouse pauses 30/51.

When control stimulation is on, the mouse keeps walking 25/36.

String-pulling

10 Hz	0.7 sec
# stim	3
# control stim	3

20 Hz	0.7 sec
# stim	5
# control stim	2

30 Hz	0.7 sec
# stim	4
# control stim	4

40 Hz	0.7 sec
# stim	5
# control stim	4



When stimulation is on, the mouse either lowers the body or makes short pull and push 13/17.
When control stimulation is on, the mouse keeps pulling the string 9/13.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	6	6	5	4
# control stim	3	3	3	2	5

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	5	5	4	4
# control stim	1	2	3	2	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	5	4	3
# control stim	3	2	2	2	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	5	4	3	8
# control stim	2	2	3	3	4



When stimulation is on, it seems licking is not affected 58/91.
 When control stimulation is on, the mouse keeps licking 42/52.

Eating

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		3			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		2			

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		5			

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1			
# control stim		1			



When stimulation is on, the mouse holds the food and does not bite 9/12.
When control stimulation is on, the mouse keeps eating 10/11.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	0	0		
# control stim	0	1	1		

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1		1	
# control stim		1		1	

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1		
# control stim	0	1	1		

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1	1		
# control stim		1	1		



When stimulation is on, it seems grooming is not affected 7/8.
When control stimulation is on, the mouse keeps grooming 8/8.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	2	2	2	1
# control stim	3	2	1	1	0

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	0	3	1	1
# control stim	0	1	0	1	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	2	1	1
# control stim	1	0	1	2	0

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	1	3	1	2
# control stim	0	2	1	2	1

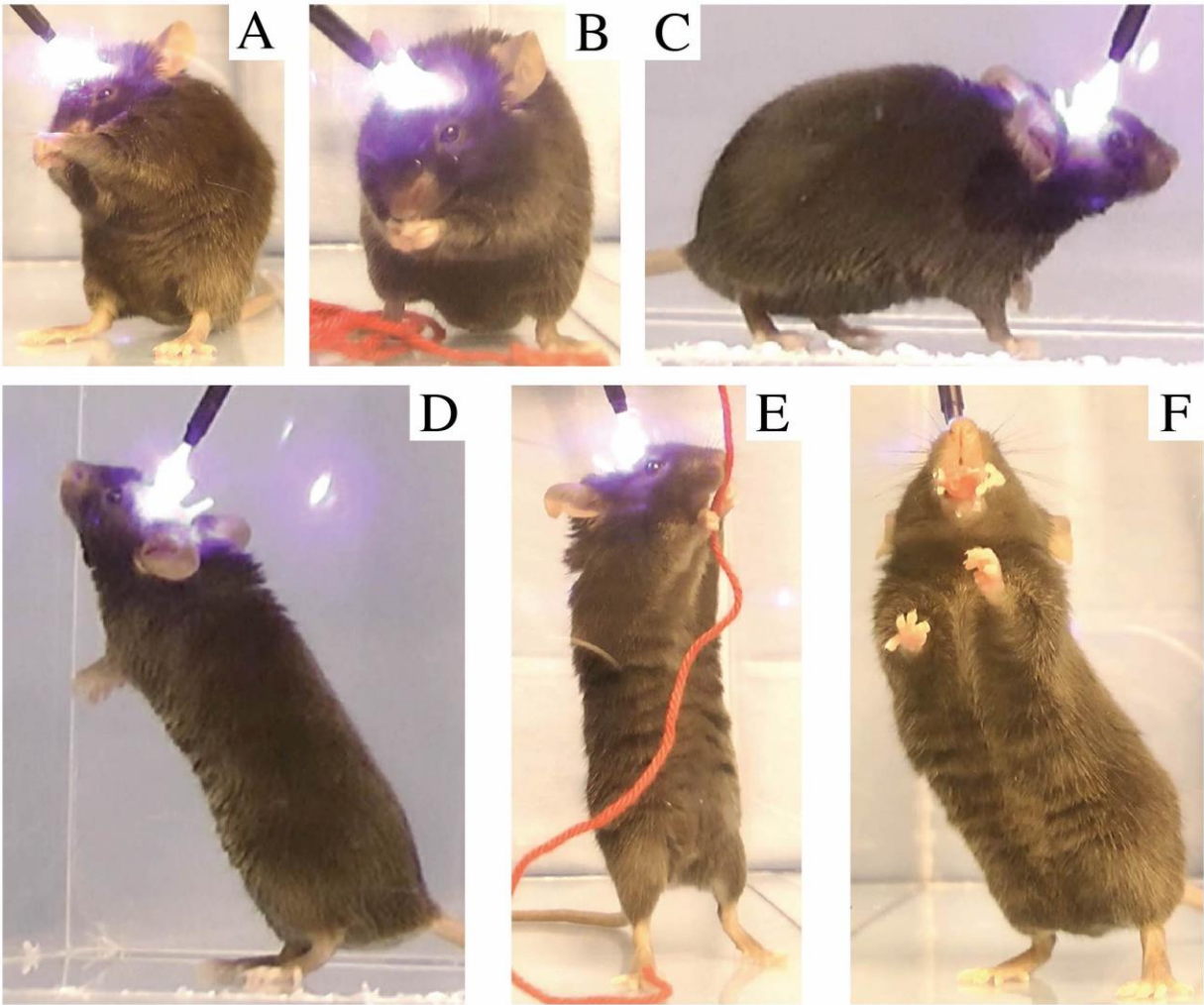


When stimulation is on, it seems rearing is not affected 14/25.



When stimulation is on, the mouse lowers body 11/25.

When control stimulation is on, the mouse keeps rearing 12/21.



Common behaviours that occur during optogenetic stimulation of AMM area in mouse #18M. A) grooming, B) eating, C) walking, D) rearing, E) string-pulling, and F) licking peanut butter. (Note that the stimulation affected walking, string-pulling, and eating).

Mouse #19M

- AMM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 199 stim + 136 control stim = 335

walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	3	4	4
# control stim	3	3	3	3	1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	2	2	2	3
# control stim	3	1	3	1	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	1	4	3
# control stim	3	3	3	2	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	2	2	3
# control stim	3	2	1	2	1



When stimulation is on, the mouse keeps walking 44/59.

When control stimulation on, the mouse keeps walking 30/45.

String-pulling

10 Hz	0.7 sec
# stim	6
# control stim	3

20 Hz	0.7 sec
# stim	4
# control stim	3

30 Hz	0.7 sec
# stim	4
# control stim	4

40 Hz	0.7 sec
# stim	4
# control stim	4



When stimulation is on, the mouse keeps pulling the string 12/18.
When control stimulation on, the mouse keeps pulling the string 8/14.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	7	4	4	4
# control stim	2	3	2	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	4	3
# control stim	3	2	3	3	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	4	4	4	4
# control stim	3	2	3	2	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	3	3	3
# control stim	2	2	3	2	3



When stimulation is on, it seems licking is not affected 62/80.
 When control stimulation on, the mouse keeps licking 47/48.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		4			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		3			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		2			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		6			
# control stim		5			



When stimulation is on, it seems eating is not affected 13/16.
When control stimulation is on, the mouse keeps eating 13/14.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1			
# control stim	1	1			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	1		0	
# control stim	0	1		1	

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	1		
# control stim	1	1	0		

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1		
# control stim	1	1	1		



When stimulation is on, it seems grooming is not affected 11/12.
When control stimulation is on, the mouse keeps grooming 9/9.

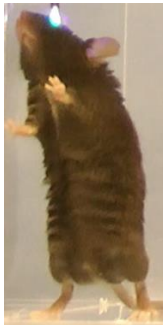
Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3	0		1
# control stim		0	1		0

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1	2	
# control stim	0	0	0	0	

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1	2	0	
# control stim		0	1	1	

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim			1	1	0
# control stim			1	0	2

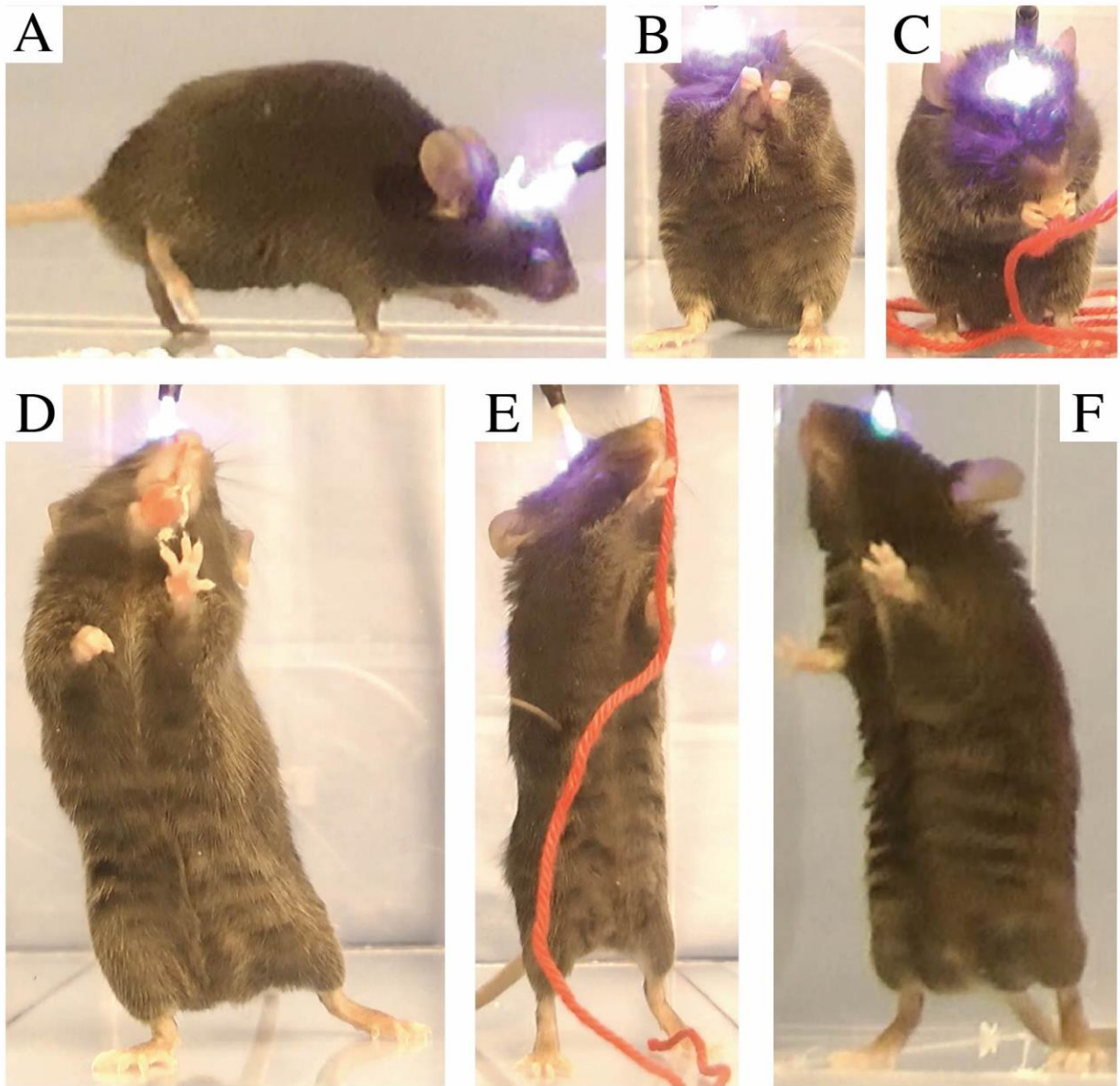


When stimulation is on, the mouse keeps rearing 8/14.



When stimulation is on, the mouse comes down 6/14.

When control stimulation is on, the mouse keeps rearing 3/6.



Common behaviours that occur during optogenetic stimulation of AMM area in mouse #19M. A) walking, B) grooming, C) eating, D) licking peanut butter, E) string-pulling, and F) rearing. (Note that the stimulation does not affect any of the behaviours).

APPENDIX 3: SUMMARY OF PLM ANIMALS

Mouse #13F

- PLM
- histology to be done
- summary in following behaviours: string-pulling, licking peanut butter, eating, grooming, and rearing.
- Total # observations = 162 stim + 104 control stim = 266

String-pulling

10 Hz	
	0.7 sec
# stim	6
# control stim	1

20 Hz	
	0.7 sec
# stim	4
# control stim	4

30 Hz	
	0.7 sec
# stim	6
# control stim	2

40 Hz	
	0.7 sec
# stim	4
# control stim	4



When stimulation is on, the mouse keeps pulling the string 12/20.
When control stimulation is on, the mouse keeps sitting down 7/11.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	4	4	4
# control stim	3	3	3	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	4	4	3
# control stim	3	3	3	3	3

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	4	4	6
# control stim	2	2	3	3	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	4	4	5
# control stim	2	2	5	3	3



When stimulation is on, it seems licking is not affected 56/79.
 When control stimulation is on, the mouse keeps licking 50/56.

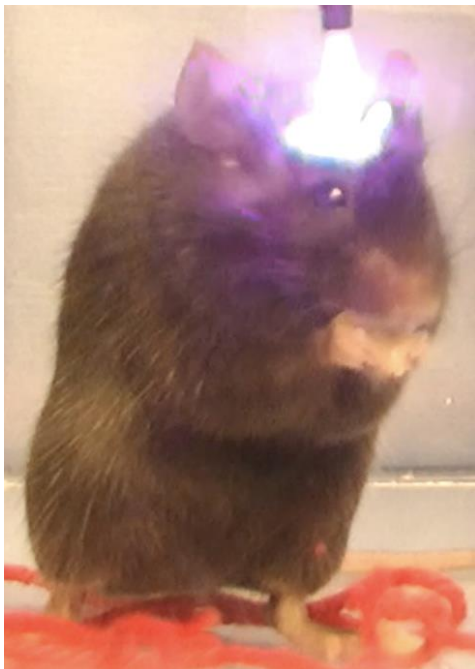
Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		2			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		11			
# control stim		6			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		13			
# control stim		6			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		6			
# control stim		4			



When stimulation is on, it seems eating is not affected 33/34.
When control stimulation is on, the mouse keeps eating 18/18.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	1		1
# control stim	1	1	1		1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	3		1	
# control stim	1	3		1	

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1	1	
# control stim	1	1	1	1	

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4	1	2	
# control stim		2	1	1	



When stimulation is on, it seems grooming is not affected 15/22.
When control stimulation is on, the mouse keeps grooming 16/17.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0			2	
# control stim	1			0	

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		1			

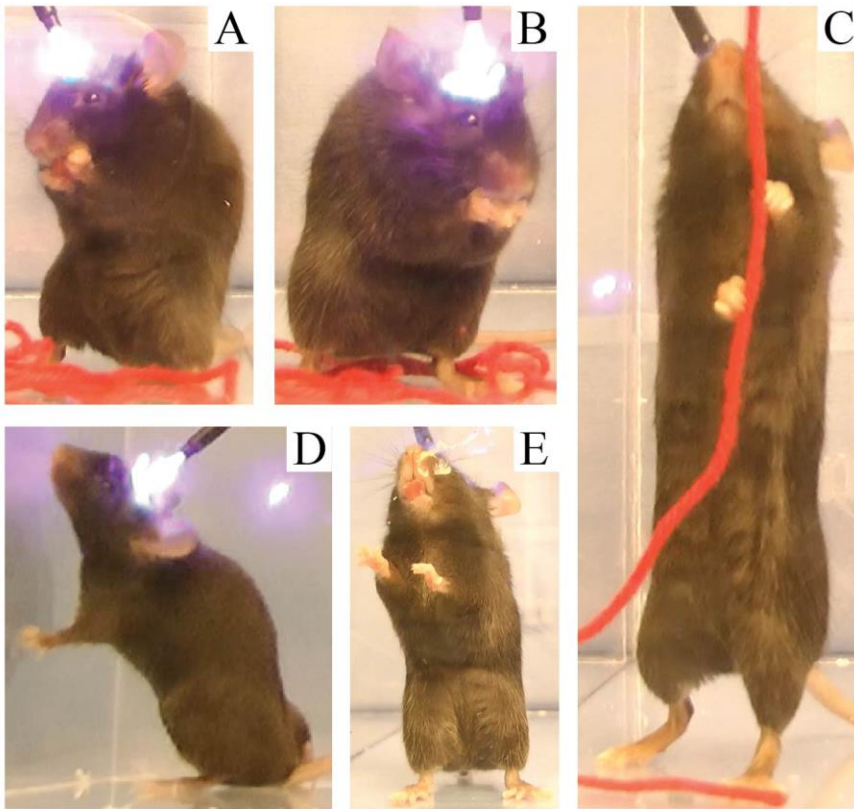
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		0			

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1			
# control stim		0			



When stimulation is on, the mouse keeps rearing 5/7.

When control stimulation is on, the mouse keeps rearing 2/2.



Common behaviours that occur during optogenetic stimulation of PLM area in mouse #13F. A) grooming, B) eating, C) string-pulling, D) rearing, and E) licking peanut butter. (Note that the stimulation did not affect any of the behaviours).

Mouse #14F

- PLM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing.
- Total # observations = 196 stim + 126 control stim = 322

Walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	1	4	2
# control stim	3	0	2	1	1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	3	2	2
# control stim	2	1	1	1	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	0	1	1	1
# control stim	2	0	1	1	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	1	1	0	0
# control stim	0	0	0	0	0



When stimulation is on, the mouse keeps walking 15/36.



When stimulation is on, the mouse stops 21/36.

When control stimulation is on, the mouse keeps walking 13/18.

String-pulling

10 Hz	0.7 sec
# stim	5
# control stim	3

20 Hz	0.7 sec
# stim	5
# control stim	3

30 Hz	0.7 sec
# stim	4
# control stim	3

40 Hz	0.7 sec
# stim	4
# control stim	3



When stimulation is on, the mouse lowers the body 17/18.

When control stimulation is on, the mouse keeps pausing and lowering its body but it is not the same as the effect of stimulation 12/12.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	4	5	4	5
# control stim	3	3	3	3	3

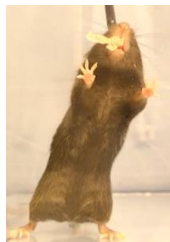
20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	4	3	4
# control stim	2	3	3	2	3

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	3	3	4
# control stim	3	3	3	2	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	1	4	4	3
# control stim	3	3	3	3	2



When stimulation is on, the mouse lowers the body 38/74.



When stimulation is on, it seems peanut butter licking is not affected 36/74.
When control stimulation is on, the mouse keeps licking 53/55.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		12			
# control stim		6			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		11			
# control stim		8			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		3			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		14			
# control stim		7			



When stimulation is on, it seems the mouse cannot bite 27/42.
When control stimulation is on, the mouse keeps eating 23/24.

Grooming

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim			1		
# control stim			1		

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2		1	
# control stim		2		1	

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	1		
# control stim	3	1	1		

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	1	1	
# control stim	1	2	1	1	



When stimulation is on, hands are at the mouth 16/18.

When control stimulation is on, the mouse keeps grooming 10 /14.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1	1		
# control stim		1	0		

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim			2		1
# control stim			1		1

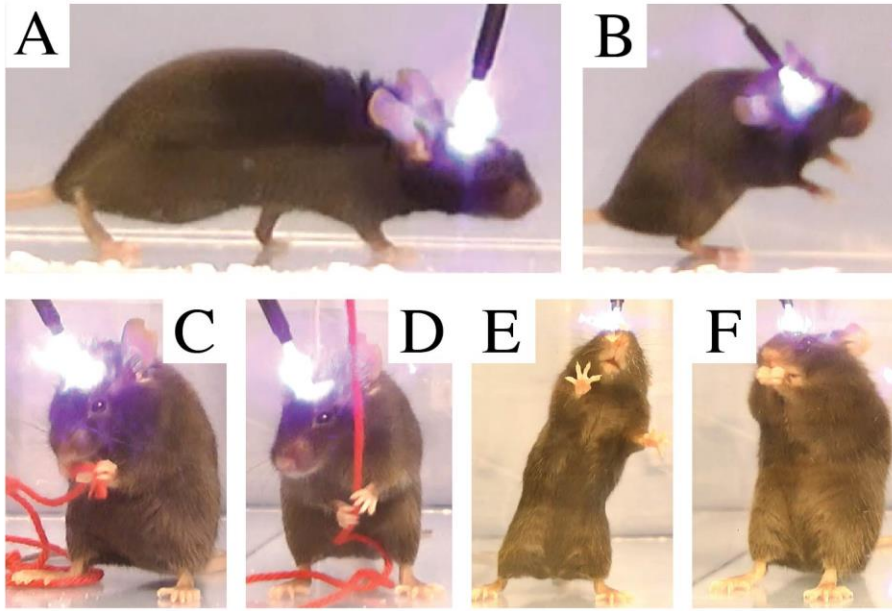
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1			
# control stim		0			

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		1		
# control stim	0		0		



When stimulation is on, the mouse comes down 6/8.

When control stimulation is on, the mouse comes down 2/3.



Common behaviours that occur during optogenetic stimulation of PLM area in mouse #14F A) walking, B) rearing, C) eating, D), string-pulling, E) licking peanut butter, and F) grooming. (Note that the stimulation affected all behaviours).

Mouse #15F

- PLM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 200 stim + 138 control stim = 338

walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	2	3	2
# control stim	3	3	2	1	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	3	3	2
# control stim	1	2	2	1	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	3	3	3	2
# control stim	2	2	2	1	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	4	4	4	3
# control stim	3	3	4	3	2



When stimulation is on, the mouse pauses when walking 34/61.
When control stimulation is on, the mouse keeps walking 28/43.

String-pulling

10 Hz	0.7 sec
# stim	5
# control stim	3

20 Hz	0.7 sec
# stim	4
# control stim	4

30 Hz	0.7 sec
# stim	4
# control stim	4

40 Hz	0.7 sec
# stim	4
# control stim	4



When stimulation is on, the mouse keeps string-pulling 11/17.



When stimulation is on, the mouse lowers body 6/17.

When control stimulation is on, the mouse keeps pulling the string 15/15.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	5	5	2
# control stim	3	3	3	3	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	4	5	4
# control stim	2	2	3	3	3

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	4	3
# control stim	3	2	3	2	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	5	3	4
# control stim	3	2	3	2	3



When stimulation is on, it seems that licking is not affected 63/79.
 When control stimulation is on, the mouse keeps licking 48/53.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		3			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		7			
# control stim		3			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		4			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		6			
# control stim		3			



When stimulation is on, it seems that eating is not affected 19/23.
When control stimulation is on, the mouse keeps eating 13/13.

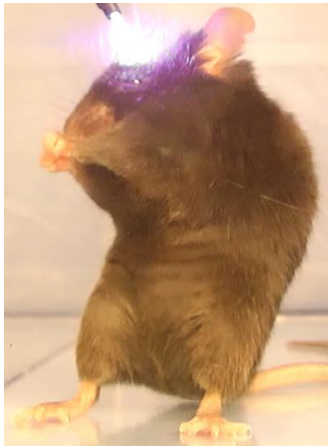
Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	2		
# control stim	1	2	1		

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1		
# control stim	1	1	1		

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1			
# control stim		1			

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		1		
# control stim	0		0		



When stimulation is on, it seems that grooming is not affected 10/11.
When control stimulation is on, the mouse keeps grooming 8/8.

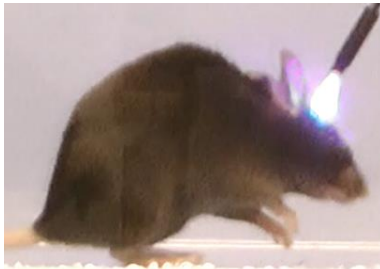
Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1		1	1
# control stim		1		1	0

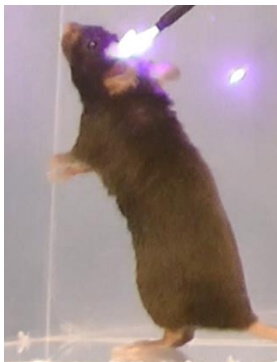
20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		0			1
# control stim		1			0

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim			1	1	1
# control stim	1		0	0	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1				1
# control stim	0				1

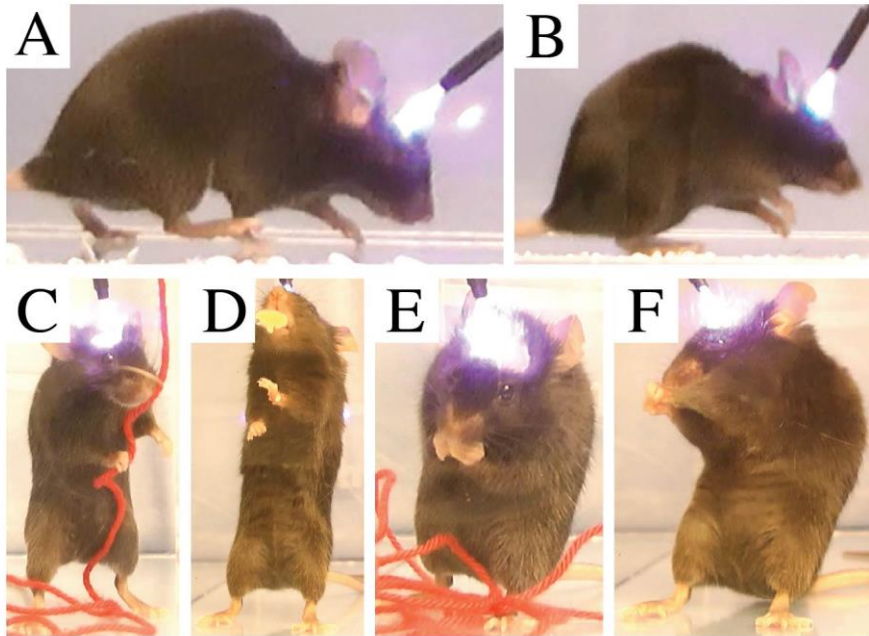


When stimulation is on, the mouse comes down 5/9.



When stimulation is on, the mouse keeps rearing 4/9.

When control stimulation is on, the mouse keeps rearing 4/6.



Common behaviours that occur during optogenetic stimulation of PLM area in mouse #15F A) walking, B) rearing, C) string-pulling, D) licking peanut butter, E) eating, and F) grooming. (Note that the stimulation affected walking and rearing).

Mouse #16M

- PLM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 199 stim + 125 control stim = 324

walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	5	3	2	4
# control stim	3	1	1	1	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	1	3	4
# control stim	1	2	2	1	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	3	3	4
# control stim	3	4	1	1	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	4	2	3
# control stim	1	2	2	2	2



When stimulation is on, the mouse pauses 41/63.

When control stimulation is on, the mouse keeps walking 24/35.

String-pulling

10 Hz	0.7 sec
# stim	5
# control stim	4

20 Hz	0.7 sec
# stim	4
# control stim	4

30 Hz	0.7 sec
# stim	5
# control stim	3

40 Hz	0.7 sec
# stim	5
# control stim	4



When stimulation is on, the mouse struggles grasping the string with left hand 17/19.
When control stimulation is on, the mouse keeps pulling the string 14/15.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	4	3	5
# control stim	2	2	2	2	3

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	4	2	4	5
# control stim	1	3	2	2	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	3	3	4
# control stim	3	3	2	3	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	3	4
# control stim	2	3	3	2	2



When stimulation is on, it seems licking is not affected 51/74.
 When control stimulation is on, the mouse keeps licking 44/47.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		8			
# control stim		4			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		2			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		7			
# control stim		4			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		2			



When stimulation is on, the mouse chews but cannot bite 13/24.
When control stimulation is on, the mouse keeps eating 12/12.

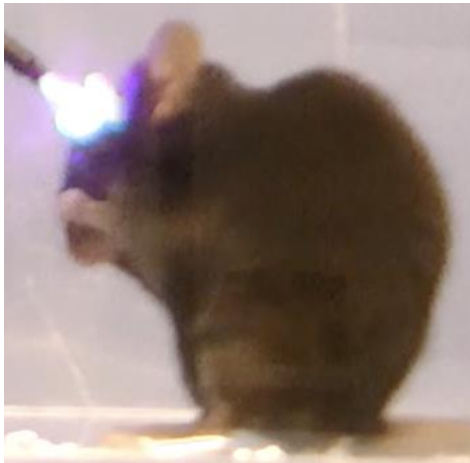
Grooming

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1		
# control stim	1	1	1		

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1			
# control stim	1	0			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1		1	
# control stim	1	1		1	



When stimulation is on, it seems grooming is not affected 8/8.
When control stimulation is on, the mouse keeps grooming 7/7.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	0	0	2	
# control stim	0	1	2	0	

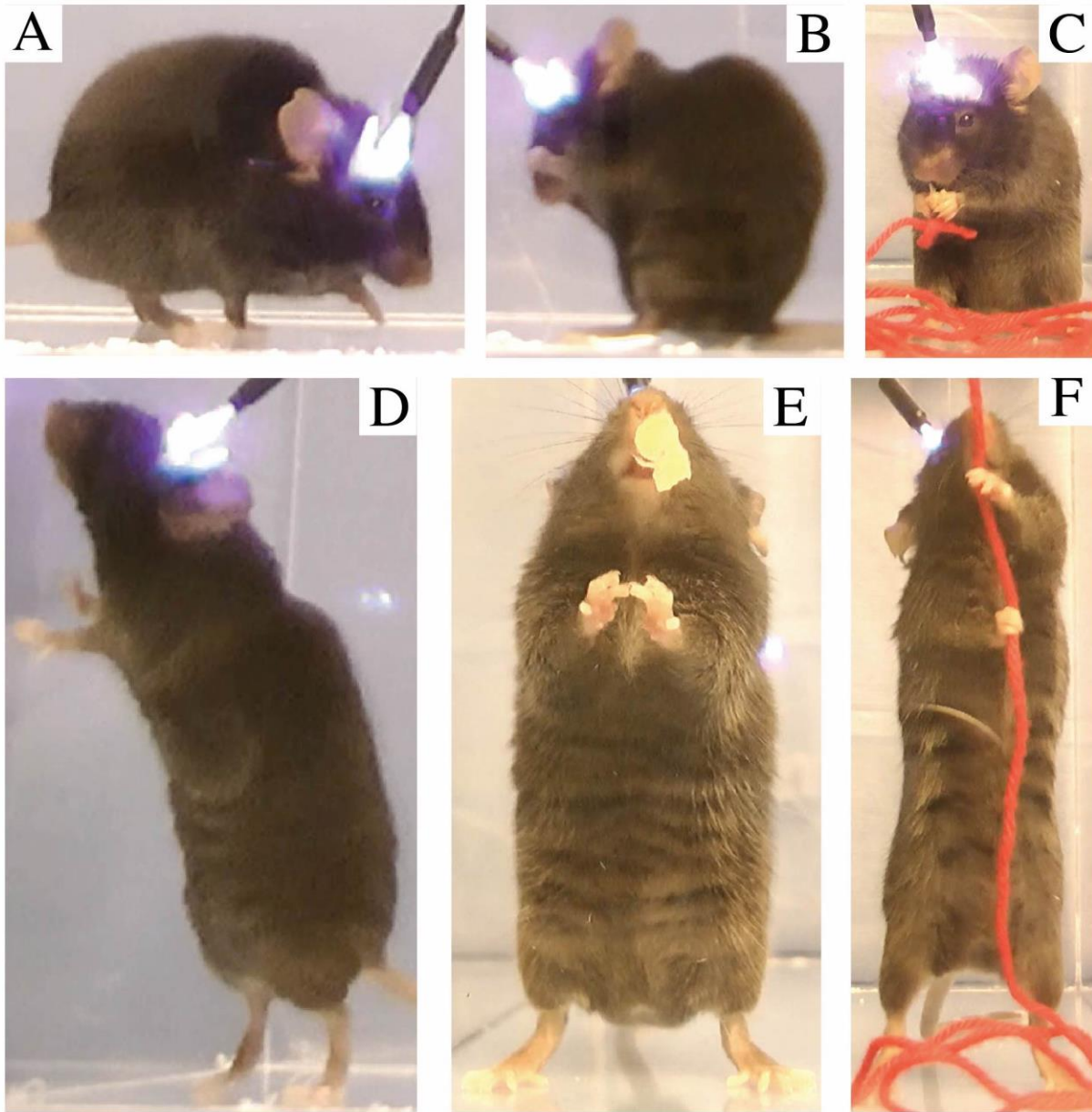
20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1	1	0	0
# control stim		0	1	1	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1	1	1	0
# control stim		0	1	1	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1		1	1
# control stim		0		0	0



When stimulation is on, it seems rearing is not affected 9/11.
 When control stimulation is on, the mouse keeps rearing 6/9.



Common behaviours that occur during optogenetic stimulation of PLM area in mouse #16M. A) walking, B) grooming, C) eating, D) rearing, E) licking peanut butter, and F) string-pulling. (Note that the stimulation disturbs walking, eating and string-pulling).

Mouse #17M

- PLM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 203 stim + 143 control stim = 346

walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	3	3	2
# control stim	2	2	1	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	2	2	1
# control stim	2	3	1	2	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	2	4	1
# control stim	3	2	2	3	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	2	3	2
# control stim	2	3	1	2	2



When stimulation is on, it seems walking is not affected 32/53.
When control stimulation is on, the mouse keeps walking 33/40.

String-pulling

10 Hz	0.7 sec
# stim	4
# control stim	3

20 Hz	0.7 sec
# stim	3
# control stim	3

30 Hz	0.7 sec
# stim	4
# control stim	4

40 Hz	0.7 sec
# stim	4
# control stim	4



When stimulation is on, it seems string-pulling is not affected 10/15.
When control stimulation is on, the mouse keeps pulling the string 9/14.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	5	4	3
# control stim	3	2	2	4	3

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	5	4	4	4
# control stim	3	3	4	3	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	7	4	4	4	4
# control stim	4	2	3	3	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	4	5	4	4
# control stim	3	3	2	3	3



When stimulation is on, it seems licking is not affected 78/88.
 When control stimulation is on, the mouse keeps licking 53/57.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		3			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		7			
# control stim		4			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		2			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		6			
# control stim		3			



When stimulation is on, the mouse chews but cannot bite 17/21.
When control stimulation is on, the mouse keeps eating 12/12.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2			
# control stim	2	1			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	0	1	1	1
# control stim	1	2	0	1	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			1
# control stim		2			1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		1		1
# control stim	1		1		1



When stimulation is on, it seems grooming is not affected 11/14.
 When control stimulation is on, the mouse keeps grooming 14/14.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim			2		1
# control stim			1		1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1			
# control stim	0	1			

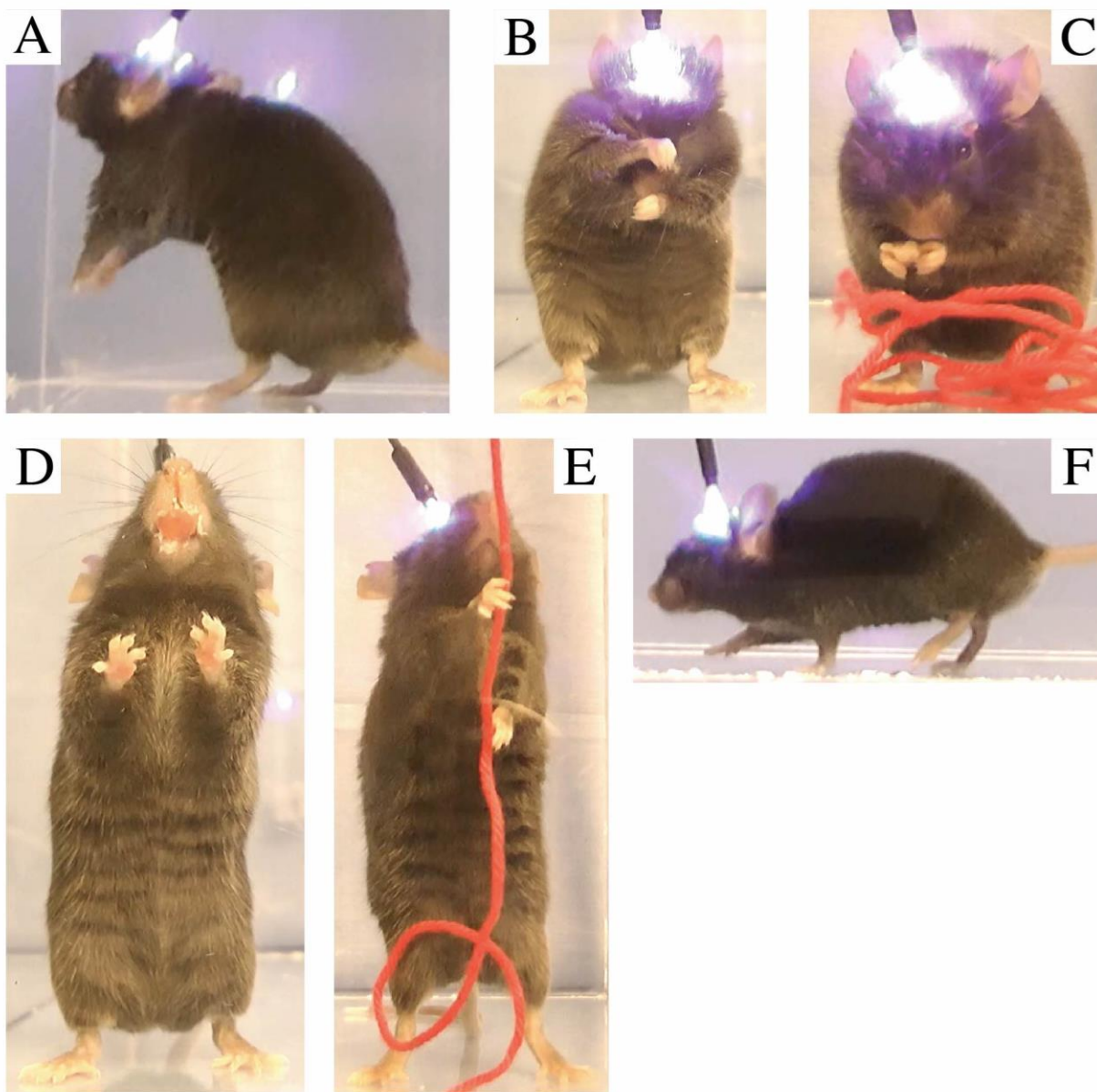
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0		1	1	2
# control stim	1		0	0	0

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		0	2	0
# control stim	0		1	0	1



When stimulation is on, the mouse comes down 8/12.

When control stimulation is on, the mouse keeps rearing 3/6.



Common behaviours that occur during optogenetic stimulation of PLM area in mouse #17M. A) rearing, B) grooming, C) eating, D) licking peanut butter, E) string-pulling, and F) walking. (Note that the stimulation affected eating and rearing).

Mouse #18M

- PLM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 203 stim + 139 control stim = 342

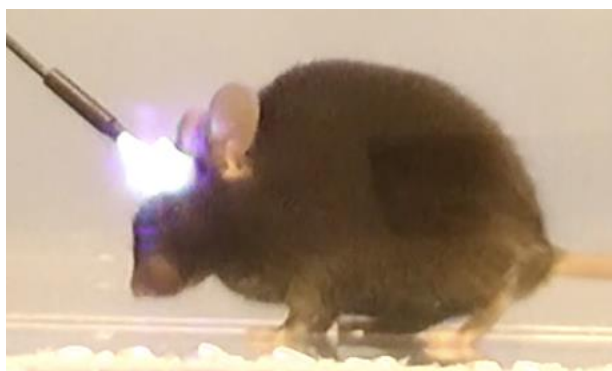
walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	2	3	2	2
# control stim	2	3	1	2	0

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	3	1	4	2
# control stim	3	1	2	2	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	3	2	4	4
# control stim	3	3	2	1	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	1	3	3	3
# control stim	2	2	1	2	1



When stimulation is on, the mouse pauses 43/58.

When control stimulation is on, the mouse keeps walking 24/36.

String-pulling

10 Hz	0.7 sec
# stim	4
# control stim	3

20 Hz	0.7 sec
# stim	5
# control stim	4

30 Hz	0.7 sec
# stim	4
# control stim	4

40 Hz	0.7 sec
# stim	4
# control stim	4



When stimulation is on, left elbow is extended and left hand makes grasping movements 12/17.
When control stimulation is on, the mouse keeps pulling the string 15/15.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	5	4
# control stim	3	1	2	3	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	4	4	3
# control stim	3	3	3	3	3

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	5	5	4	3
# control stim	2	3	3	3	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	4	3	3
# control stim	3	2	2	2	2



When stimulation is on, digits extended while licking 46/76.
 When control stimulation is on, the mouse keeps licking 42/50.

Eating

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		7			
# control stim		2			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		9			
# control stim		5			

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		3			

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		2			



When stimulation is on, the mouse holds the food in hand 18/26.
When control stimulation is on, the mouse keeps eating 10/12.

Grooming

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1				
# control stim	1				

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1			
# control stim	1	1			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2		0	
# control stim	1	1		1	

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	2	1		
# control stim	1	1	1		



When stimulation is on, it seems grooming is not affected 6/11.
When control stimulation is on, the mouse keeps grooming 9/9.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	1	1	2	2
# control stim	1	0	1	1	3

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	0	0	1	1
# control stim	0	1	1	0	0

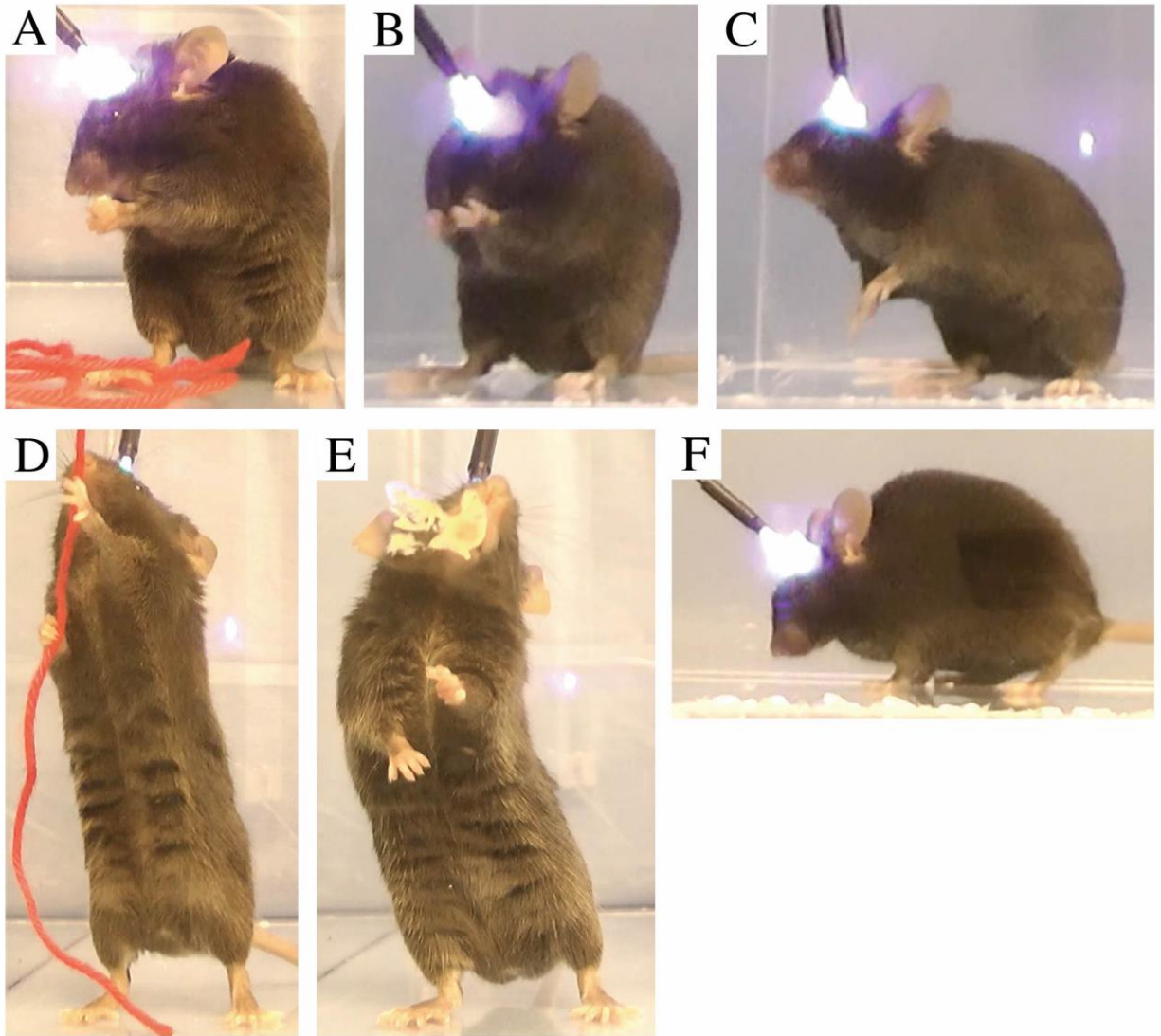
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	2	1	0
# control stim	0	2	1	0	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	1	0	0	1
# control stim	1	1	1	0	2



When stimulation is on, the mouse comes down 9/15.

When control stimulation is on, the mouse keeps rearing 11/17.



Common behaviours that occur during optogenetic stimulation of PLM area in mouse #18M. A) eating, B) grooming, C) rearing, D) string-pulling, E) licking peanut butter, and F) walking. (Note that the stimulation affects all behaviours except for grooming).

Mouse #19M

- PLM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 202 stim + 135 control stim = 337

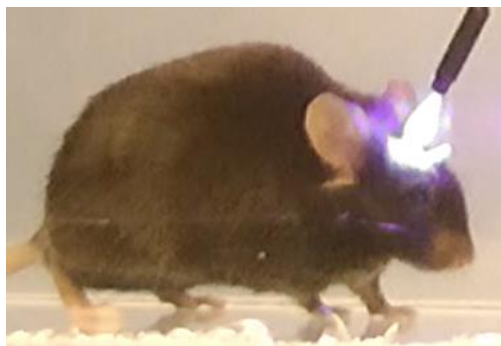
walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	2	3	1
# control stim	2	2	2	1	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	4	3	3
# control stim	3	1	0	1	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	1	2	2	1
# control stim	2	1	2	2	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	2	4	2
# control stim	2	2	1	2	1



When stimulation is on, the mouse pauses 32/51.

When control stimulation is on, the mouse keeps walking 20/32.

String-pulling

10 Hz	0.7 sec
# stim	4
# control stim	2

20 Hz	0.7 sec
# stim	5
# control stim	4

30 Hz	0.7 sec
# stim	4
# control stim	4

40 Hz	0.7 sec
# stim	4
# control stim	4



When stimulation is on, the mouse lowers the body and left hand is unable to grasp 12/17.
When control stimulation is on, the mouse keeps pulling the string 13/14.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	4	3	4
# control stim	3	3	3	3	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	4	4
# control stim	3	3	2	3	3

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	3	3	4
# control stim	3	3	3	2	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	4	4	5
# control stim	3	2	3	2	2



When stimulation is on, right digits extend while licking 53/83.
 When control stimulation is on, the mouse keeps licking 53/54.

Eating

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		1			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		6			

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		3			

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		6			
# control stim		2			



When stimulation is on, the mouse holds the food 11/18.

When control stimulation is on, the mouse keeps eating 10/12.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2			1
# control stim	2	2			0

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	1	1	
# control stim	1	1	1	0	

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1			1
# control stim	1	0			1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	2		
# control stim	2	1	0		



When stimulation is on, it seems grooming is not affected 12/18.
 When control stimulation is on, the mouse keeps grooming 9/12.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	0	1	0	1
# control stim	1	1	0	1	0

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1			0	1
# control stim	0			1	0

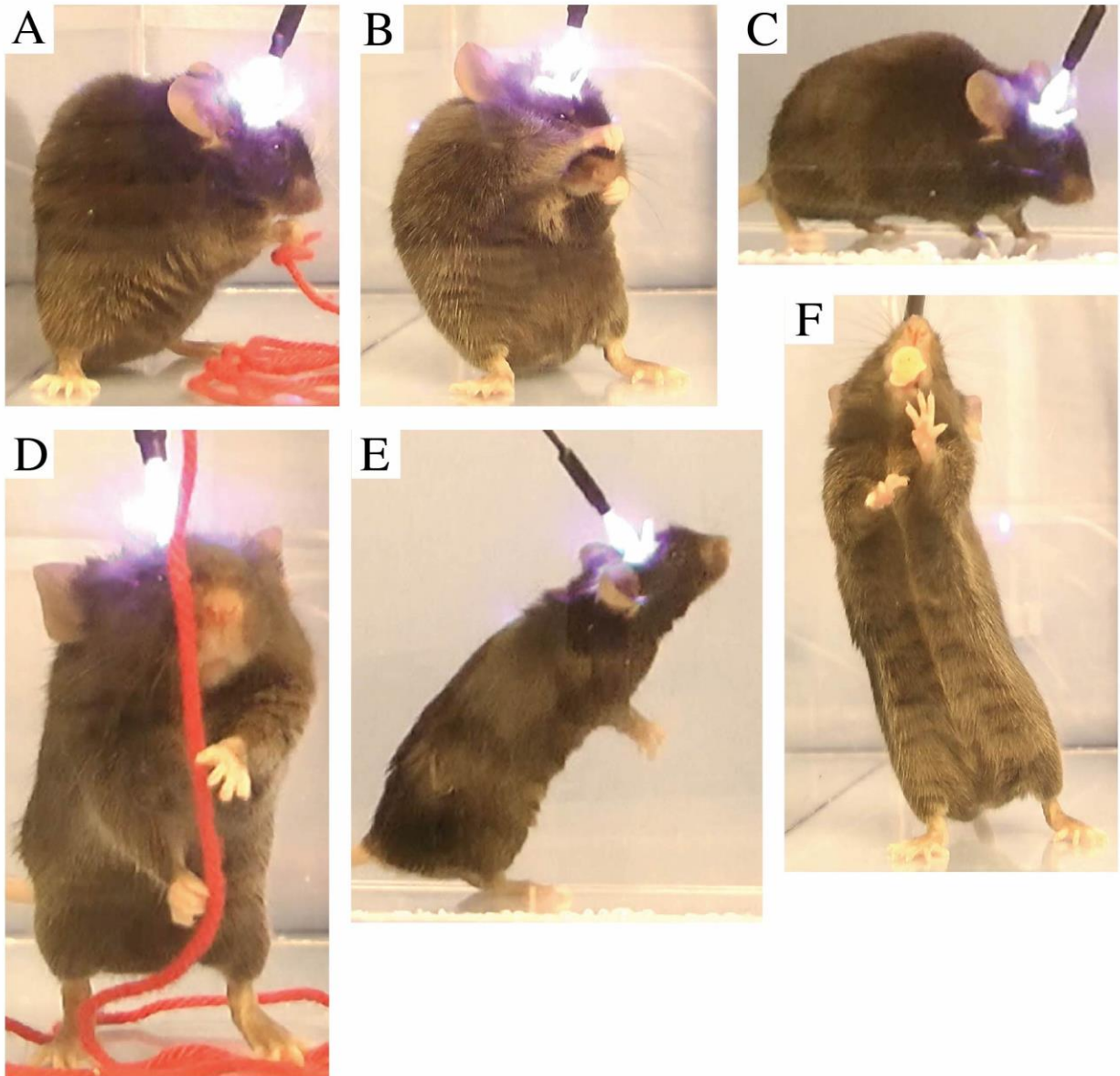
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	2	1	
# control stim	1	2	2	1	

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim				1	1
# control stim				0	1



When stimulation is on, it seems rearing is not affected 9/15.

When control stimulation is on, the lowers body and comes down 8/11.



Common behaviours that occur during optogenetic stimulation of PLM area in mouse #19M. A) eating, B) grooming, C) walking, D) string-pulling, E) rearing, and F) licking peanut butter. (Note that stimulation affects all behaviours except for grooming and rearing).

APPENDIX 4: SUMMARY OF PMM ANIMALS

Mouse #5F

- PMM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- observed sequence (seq): 1) hands open and extend 2) elbow extends 3) hands on the floor. [x means no sequence observed]
- total # observations = 225 stim + 102 control stim = 327

Walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	7	2	5	5
# control stim	2	3	5	7	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	5	4	3	2
# control stim					

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	3	3	3
# control stim					

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	4	3	5
# control stim					



When walking stimulation is on this behaviour is seen 63/78: continued walking
 When control stimulation is on, the mouse keeps walking 13/19.

String-pulling

10 Hz	0.7 sec	1,2,3 seq	1,2 seq	1 seq	x
# stim	7	3	1	1	2
# control stim	1				

20 Hz	0.7 sec	1,2,3 seq	1,2 seq	1 seq	x
# stim	8	2	2	2	2
# control stim	3				

30 Hz	0.7 sec	1,2,3 seq	1,2 seq	1 seq	x
# stim	8	2	2	3	1
# control stim	2				

40 Hz	0.7 sec	1,2,3 seq	1,2 seq	1 seq	x
# stim	6	0	0	3	3
# control stim	2				



When stimulation is on all or part of the following is seen 21/29: 1) hands open and digits extend, 2) elbow extends, 3) and hands on the floor. String-pulling is affect 24/29.

When control stimulation is on, the mouse keeps pulling the string 8/8.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	6	4	5	5
# control stim	2	4	3	3	4
1,2,3 seq	3	6	3	3	5
x	1	0	0	0	0

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	3	7	4	5
# control stim	3	3	3	3	3
1,2,3 seq	4	1	5	2	
1,2 seq	1	2		2	4

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	6	6	4	5
# control stim	4	4	4	3	1
1	2		6	1	3
1,2 seq		1		1	
x	4	4		2	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	3	4	3
# control stim	1	1	2	2	2
1	1	2	1		3
x	1	2	2	3	



When stimulation is on all or part of the following is seen 62/92: 1) hands open and digits extend, 2) elbow extends, 3) and hands on the floor especially in the left hand.

When control stimulation is on, the mouse keeps licking 54/55.

Eating

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim		2			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim					



When stimulation is on eating is not affected 2/3.

When control stimulation is on, the mouse keeps eating 2/2.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	0		1
# control stim	1	1	1		1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1				
# control stim	0				

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim			2		
# control stim		1			

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					



When stimulation is on this behaviour is seen 5/6: stopped grooming, hands on the floor (the sequence was not seen).

When control stimulation is on, the mouse keeps grooming 5/5.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	4	2	0
# control stim	2	5	2	3	1

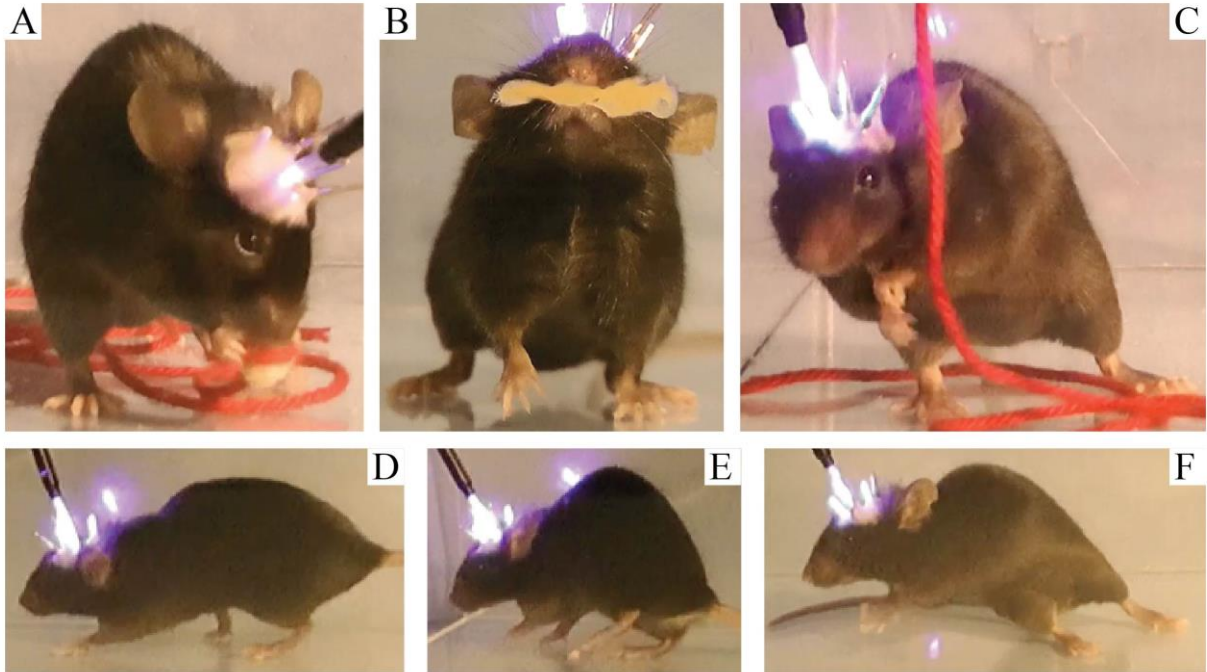
20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		1		
# control stim	0		0		

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim				1	
# control stim				0	

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3		1	1	
# control stim	0		0	0	



When stimulation is on all or part of the following is seen 14/17: 1) hands open and digits extend, 2) elbow extends, 3) and hands on the floor especially in the left hand. When control stimulation is on, the mouse keeps rearing 11/13.



Common behaviours that occur during optogenetic stimulation of PMM area in mouse #5F. A) eating, B) licking peanut butter, C) string-pulling, D) walking, E) grooming, and F) rearing. (Note that the stimulation affected string-pulling, licking peanut butter, grooming, and rearing).

Mouse #8M

- PMM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 242 stim + 151 control stim = 393

walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	5	4	5	2
# control stim	3	3	2	4	1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	4	3	3
# control stim	1	2	3	1	5

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	2	2	3	2
# control stim	3	0	0	2	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	2	3	3	2
# control stim	4	0	0	0	1



When stimulation is on, the mouse keeps walking 41/65.

When control stimulation is on, the mouse keeps walking 25/36.

String-pulling

10 Hz	0.7 sec
# stim	4
# control stim	4

20 Hz	0.7 sec
# stim	2
# control stim	3

30 Hz	0.7 sec
# stim	4
# control stim	3

40 Hz	0.7 sec
# stim	3
# control stim	4



When stimulation is on, the mouse keeps string-pulling 8/13. The mouse let go of string but still standing 5/13.

When control stimulation is on, the mouse keeps string-pulling 11/14.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	5	4	4	5
# control stim	3	3	2	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	5	3	2
# control stim	2	2	2	2	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	3	5	3
# control stim	2	2	2	1	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	5	4
# control stim	3	2	2	1	2



When stimulation is on, it seems licking is not affected 53/80. In 30/80 digits on either one or both hands open and extend.

When control stimulation is on, the mouse keeps licking peanut butter 38/41.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		1			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		1			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		0			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		3			



When stimulation is on, it seems eating is not affected 7/12.
When control stimulation is on, the mouse keeps eating 5/5.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	1	0	1	0
# control stim	1	1	0	1	1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1	0	0
# control stim	1	0	0	0	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	0	2	0	2
# control stim	0	0	1	0	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	0	1	0	1
# control stim	0	1	1	0	1



When stimulation is on, it seems grooming is not affected 8/9.

When control stimulation is on, the mouse keeps grooming 10/10.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	3	1	0	7
# control stim	0	3	3	0	4

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	5	5	3	4
# control stim	2	3	3	4	1

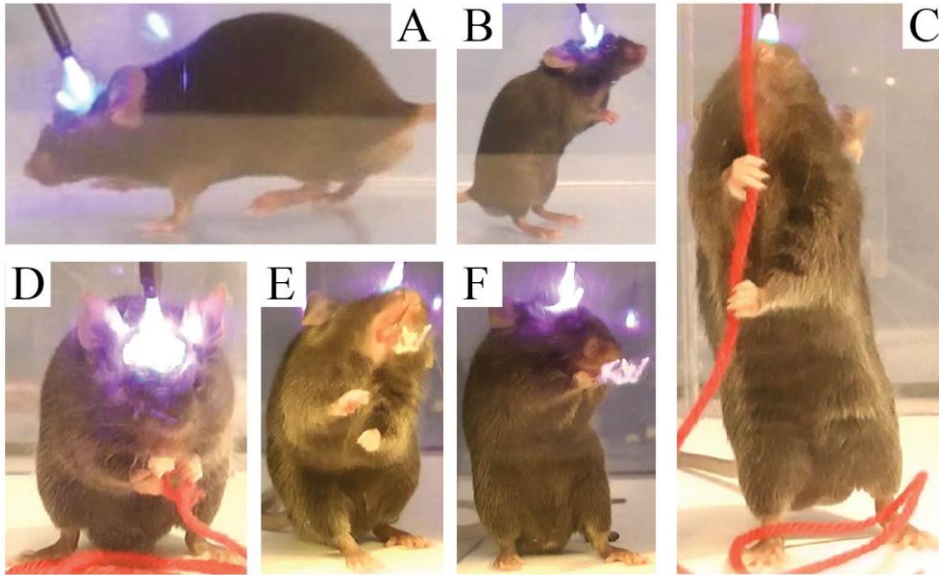
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	3	2	4
# control stim	2	3	3	4	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	4	3	2
# control stim	2	4	0	0	1



When stimulation is on, the mouse keeps rearing 34/63.

When control stimulation is on, the mouse keeps rearing 20/45.



Common behaviours that occur during optogenetic stimulation of PMM area in mouse #8M. A) walking, B) rearing, C) string-pulling, D) eating, E) licking peanut butter, and F) grooming. (Note that stimulation did not affect any of the behaviours).

Mouse #9M

- PMM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 255 stim + 165 control stim = 420

walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	6	5	3	5
# control stim	3	4	5	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	4	3	5	5
# control stim	1	0	2	2	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	2	1	4	2
# control stim	1	1	2	1	4

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	6	4	3	5
# control stim	1	0	1	2	1



When stimulation is on, the mouse keeps walking 76/84.

When control stimulation is on, the mouse keeps walking 28/36.

String-pulling

10 Hz	0.7 sec
# stim	5
# control stim	3

20 Hz	0.7 sec
# stim	5
# control stim	3

30 Hz	0.7 sec
# stim	3
# control stim	4

40 Hz	0.7 sec
# stim	4
# control stim	2



When stimulation is on, the mouse keeps biting the string 15/17. 7/15 the mouse lowered its body.

When control stimulation is on, the mouse bites the string 9/12. 4/9 the mouse lowered its body.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	4	5	4
# control stim	2	3	3	3	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	4	5	3
# control stim	3	2	3	3	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	3	4	4
# control stim	3	3	1	2	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	3	4	4
# control stim	3	2	3	2	2



When stimulation is on, it seems licking is not affected 64/73.

When control stimulation is on, the mouse keeps licking peanut butter 48/49.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		0			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1			
# control stim		1			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		2			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		2			



When stimulation is on, it seems eating is not affected 6/7.
When control stimulation is on, the mouse keeps eating 5/5.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	0	1	1	0
# control stim	1	0	1	1	0

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	2	0	0	2
# control stim	1	2	0	0	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	1	1	0	2
# control stim	1	1	0	0	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	1	0	0	1
# control stim	1	1	0	0	1



When stimulation is on, it seems grooming is not affected 19/20.
When control stimulation is on, the mouse keeps grooming 14/14.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	3	3	5	1
# control stim	1	2	0	3	3

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	3	6	4	2
# control stim	4	2	4	3	4

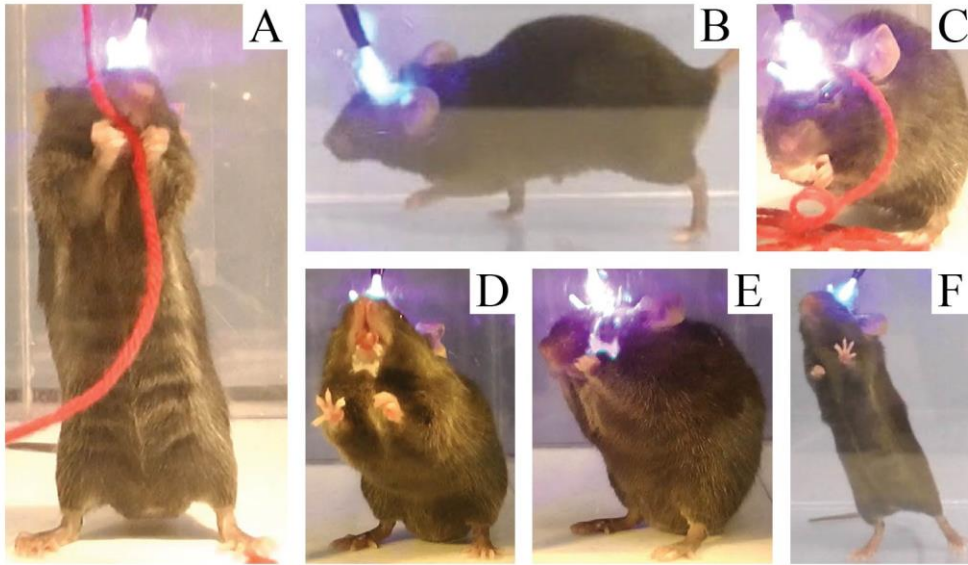
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	5	3	3	2
# control stim	2	5	1	1	0

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	3	3	0
# control stim	2	3	5	2	2



When stimulation is on, the mouse keeps rearing 45/54.

When control stimulation is on, the mouse keeps rearing 28/49.



Common behaviours that occur during optogenetic stimulation of PMM area in mouse #9M. A) string-pulling, B) walking, C) eating, D) licking peanut butter, E) grooming, and F) rearing. (Note that the stimulation affected only the string-pulling behaviour).

Mouse #10M

- PMM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 246 stim + 169 control stim = 415

walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	4	5	6	5
# control stim	2	3	3	3	1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	7	8	3	1
# control stim	2	4	4	1	4

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	4	3	2
# control stim	3	1	2	3	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	2	4	4	3
# control stim	4	2	3	1	3



When stimulation is on, the mouse keeps walking 54/79.

When control stimulation is on, the mouse keeps walking 39/52.

String-pulling

10 Hz	0.7 sec
# stim	4
# control stim	4

20 Hz	0.7 sec
# stim	4
# control stim	3

30 Hz	0.7 sec
# stim	3
# control stim	3

40 Hz	0.7 sec
# stim	5
# control stim	3



When stimulation is on, the mouse bites the string or comes down 13/16.
When control stimulation is on, the mouse keeps pulling the string 11/13.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	3	4	2
# control stim	3	2	2	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	3	4	3
# control stim	4	2	2	2	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	5	2	5
# control stim	2	2	2	2	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	4	4	4
# control stim	4	1	3	3	2



When stimulation is on, it seems licking is not affected 63/70.
 When control stimulation is on, the mouse keeps licking 45/45.

Eating

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1			
# control stim		1			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		1			

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		1			

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		3			



When stimulation is on, it seems eating is not affected 6/8.
When control stimulation is on, the mouse keeps eating 4/6.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0		1		
# control stim	1		0		

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			3
# control stim		0			1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		1		
# control stim	1		1		

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1			1	
# control stim	1			0	



When stimulation is on, it seems grooming is not affected 9/10.
 When control stimulation is on, the mouse keeps grooming 3/5.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	7	2	5
# control stim	5	1	2	2	5

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	2	6	4
# control stim	1	3	3	5	4

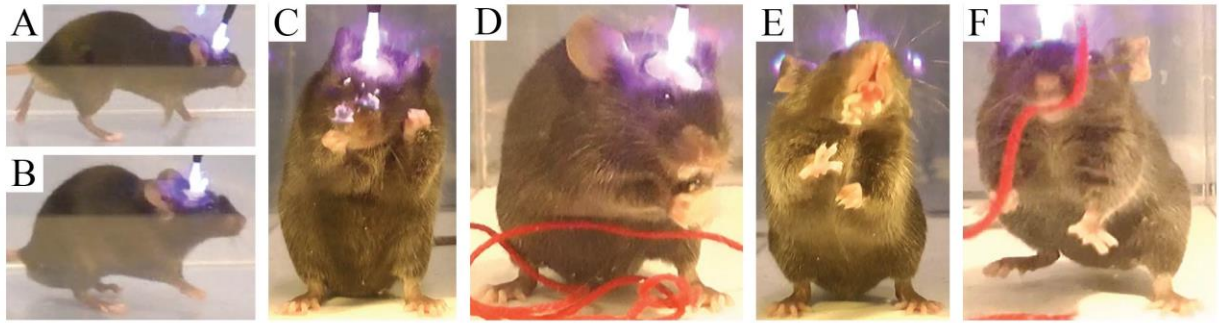
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	3	2	2	2
# control stim	2	3	1	1	0

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	4	4	3	0
# control stim	1	2	3	3	1



When stimulation is on, the mouse comes down 52/63.

When control stimulation is on, the mouse comes down 38/48.



Common behaviours that occur during optogenetic stimulation of PMM area in mouse #10M. A) walking, B) rearing, C) grooming, D) eating, E) licking peanut butter, and F) string-pulling. (Note that stimulation affected rearing and string-pulling).

Mouse #11F

- PMM
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- total # observations = 254 stim + 151 control stim = 405

Walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	7	6	3	6	3
# control stim	4	2	3	2	5

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	6	5	4	3
# control stim	1	2	2	3	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	2	4	4	2
# control stim	2	1	3	1	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	4	4	3
# control stim	0	0	0	0	0



When walking stimulation is on this behaviour is seen 69/82: continued walking.
When control stimulation is on, the mouse keeps walking 27/35.

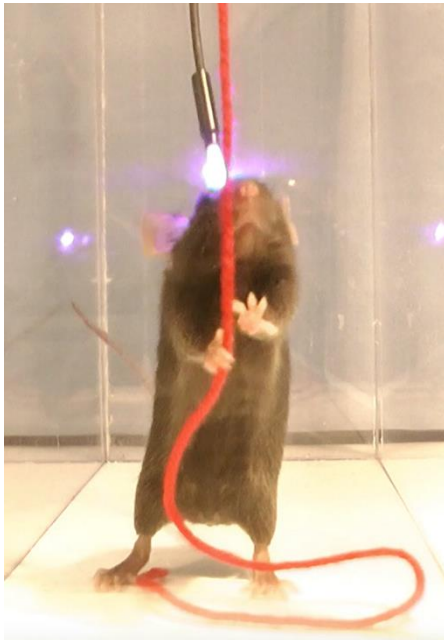
String-pulling

10 Hz	0.7 sec
# stim	5
# control stim	4

20 Hz	0.7 sec
# stim	5
# control stim	5

30 Hz	0.7 sec
# stim	4
# control stim	4

40 Hz	0.7 sec
# stim	3
# control stim	5



When stimulation is on all or part of the following is seen 17/17: left hand unable to grasp.
When control stimulation is on, the mouse keeps pulling the string 18/18.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	3	4	3
# control stim	2	3	3	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	5	4	4	2
# control stim	3	3	3	3	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	5	5	4	3	4
# control stim	2	2	2	2	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	4	4	5
# control stim	1	2	3	3	3



When stimulation is on all or part of the following is seen 70/77: left digit movement or being away from peanut butter.

When control stimulation is on, the mouse keeps licking 45/47.

Eating

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		0			
# control stim		2			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		2			

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1			
# control stim		1			

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1			
# control stim		3			



When stimulation is on eating is not disturbed 3/4: left hand has difficulty grasping food.
When control stimulation is on, the mouse keeps eating 7/8.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1		1	1	
# control stim	0		0	1	

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1	1	1
# control stim	1	0	1	0	0

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1		1	1
# control stim	0	1		0	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			1
# control stim		0			0



When stimulation is on this behaviour is seen 12/15: continues grooming.
 When control stimulation is on, the mouse keeps grooming 4/4.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	2	3	3
# control stim	2	3	2	3	0

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	5	4	3	4
# control stim	2	4	4	3	5

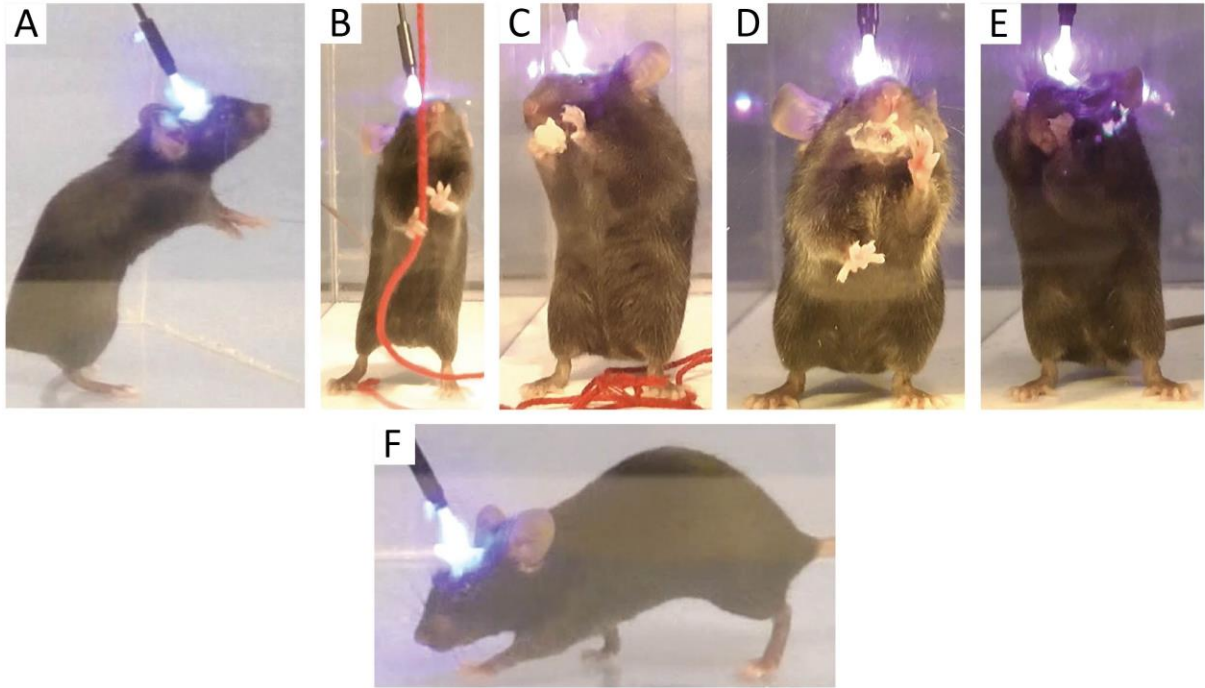
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	6	1	2	2
# control stim	3	4	0	3	0

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	2	1	3
# control stim	1	0	0	0	0



When stimulation is on all or part of the following is seen 42/59: left digits movement or extension or came down.

When control stimulation is on, the mouse keeps coming down 27/39.



Common behaviours that occur during optogenetic stimulation of PMM area in mouse #11F. A) rearing, B) string-pulling, C) eating, D) licking peanut butter, E) grooming, and F) walking. (Note that the stimulation affected rearing and string-pulling).

APPENDIX 5: SUMMARY OF V ANIMALS

Mouse #5F

- V
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 161 stim + 128 control stim = 289

walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	7	4	2	5	5
# control stim	5	3	5	4	4

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	1	3	1	3
# control stim	2	2	2	2	1

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	1	2	2
# control stim	1	2	2	1	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	1	0	2
# control stim	1	2	1	1	2



When stimulation is on, the mouse keeps walking 37/44.

When control stimulation is on the mouse keeps walking 32/34.

String-pulling

10 Hz	0.7 sec
# stim	3
# control stim	3

20 Hz	0.7 sec
# stim	6
# control stim	1

30 Hz	0.7 sec
# stim	6
# control stim	2

40 Hz	0.7 sec
# stim	7
# control stim	3



When stimulation is on, the mouse keeps pulling the string 20/22.
When control stimulation is on the mouse keeps pulling the string 9/9.

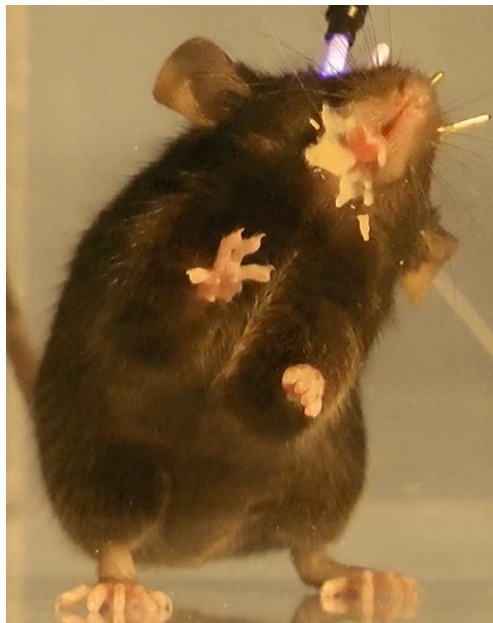
Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	3	4	2
# control stim	3	2	4	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	4	4	4
# control stim	4	3	3	2	3

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	5	6	3
# control stim	3	3	3	4	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	5	5
# control stim	3	4	4	3	3



When stimulation is on, the mouse keeps licking 77/77.

When control stimulation is on the mouse keeps licking 61/61.

Eating

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		4			



When stimulation is on, the mouse keeps eating 5/5.
When control stimulation is on the mouse keeps eating 4/4.

Grooming

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		0	1	1	
# control stim		2	0	0	

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					
# control stim					

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0		2		
# control stim	1		1		



When stimulation is on, it seems grooming is not affected 2/4.
 When control stimulation is on the mouse keeps grooming 4/4.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	0	3	0	0
# control stim	1	1	1	2	1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	1	0	0	0
# control stim	1	2	0	1	0

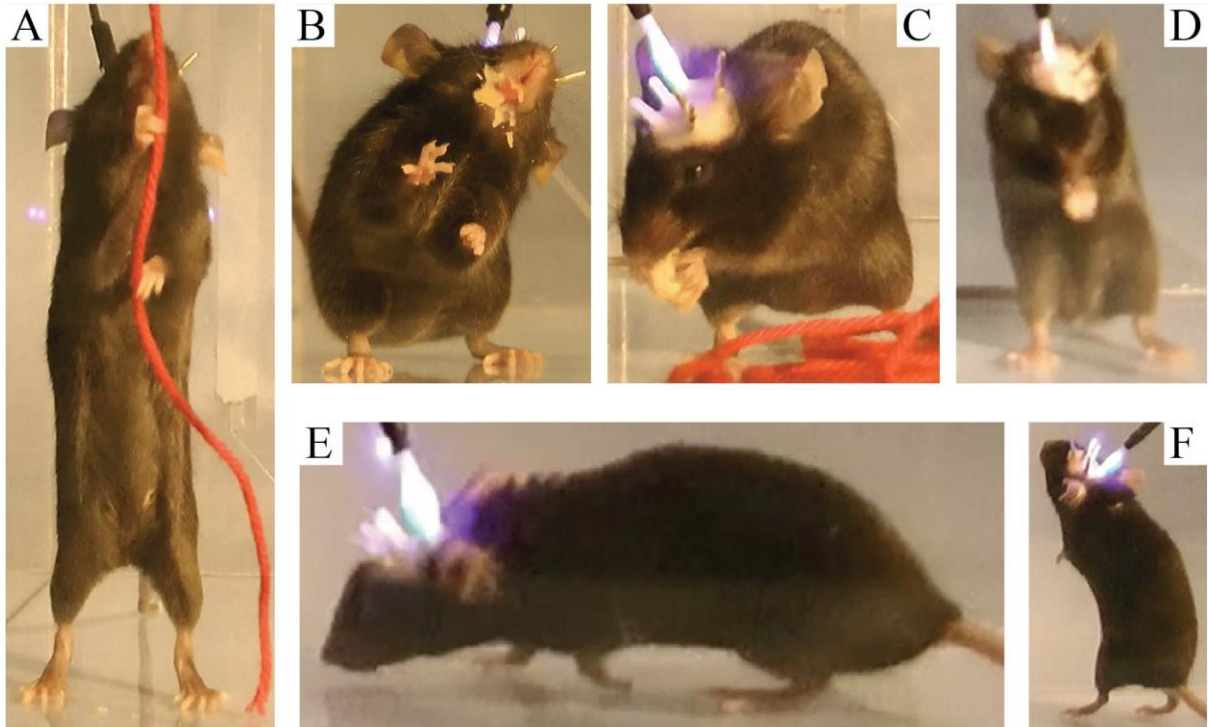
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	0	1	0	0
# control stim	1	2	0	1	0

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	0	1	1
# control stim	0	0	1	0	1



When stimulation is on, the mouse keeps rearing 8/9.

When control stimulation is on the mouse keeps rearing 15/16.



Common behaviours that occur during optogenetic stimulation of Visual cortex (V) in mouse #5F. A) string-pulling, B) licking peanut butter, C) eating, D) grooming, E) walking, and F) rearing. (Note that the stimulation did not affect any of the behaviours).

Mouse #6F

- V
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 186 stim + 134 control stim = 320

walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	6	5	3	5	3
# control stim	5	7	4	5	4

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	2	4	5	3
# control stim	7	5	4	0	3

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	0	1	3	3
# control stim	1	0	0	3	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	1	4	3	2
# control stim	1	0	3	0	2



When stimulation is on, the mouse keeps walking 56/61.

When control stimulation is on, the mouse keeps walking 53/55.

String-pulling

10 Hz	0.7 sec
# stim	4
# control stim	1

20 Hz	0.7 sec
# stim	6
# control stim	2

30 Hz	0.7 sec
# stim	8
# control stim	1

40 Hz	0.7 sec
# stim	9
# control stim	3



When stimulation is on, the mouse keeps string-pulling 25/27.
When control stimulation is on, the mouse keeps string-pulling 6/7.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	4	4	3	3
# control stim	3	5	3	3	3

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	2	3	4	3
# control stim	3	2	2	3	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	3	4	4
# control stim	3	2	4	2	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	4	3
# control stim	3	2	2	2	3



When stimulation is on, it seems licking is not affected 68/68.
 When control stimulation is on, the mouse keeps licking 54/54.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		1			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		1			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		1			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		6			
# control stim		4			



When stimulation is on, it seems eating is not affected 14/14.
When control stimulation is on, the mouse keeps 7/7.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim			0	1	
# control stim			1	0	

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1			2
# control stim	0	0			0

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	2		
# control stim	0	0	2		

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2			1
# control stim	2	1			0



When stimulation is on, it seems grooming is not affected 11/11.
 When control stimulation is on, the mouse keeps grooming 6/6.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim			1		1
# control stim			1		1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2		0	0
# control stim		0		1	1

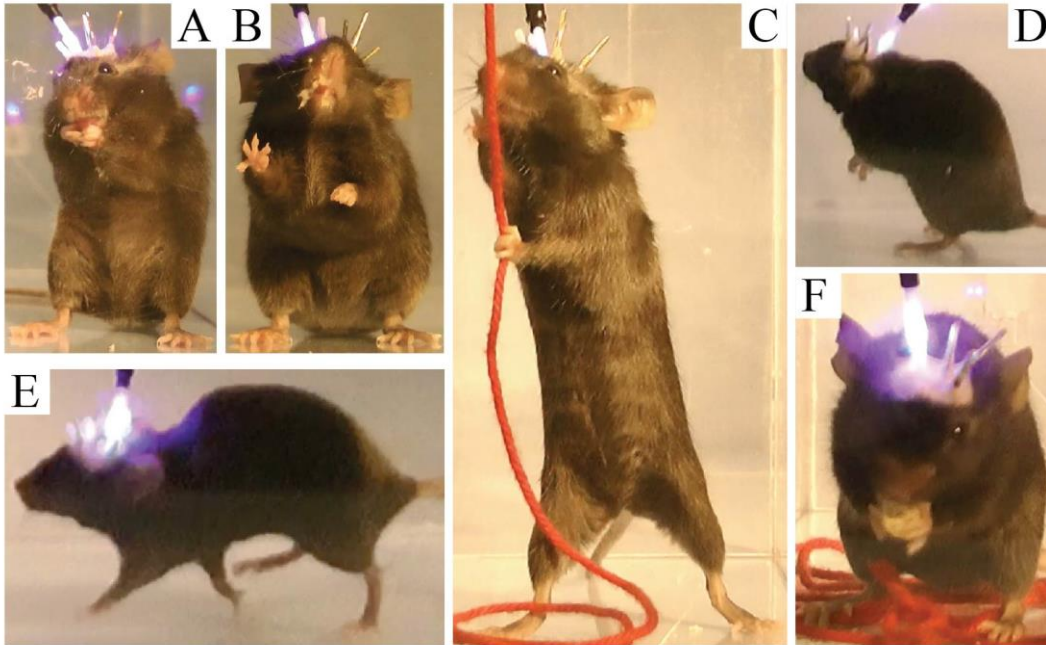
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					0
# control stim					1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim					1
# control stim					0



When stimulation is on, the mouse keeps rearing 5/5.

When control stimulation is on, the mouse keeps rearing 5/5.



Common behaviours that occur during optogenetic stimulation of Visual cortex in mouse #6F. A) grooming, B) licking peanut butter, C) string-pulling, D) rearing, E) walking, and F) eating. (Note that the stimulation did not affect any of the behaviours).

Mouse #16M

- V
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 176 stim + 132 control stim = 308

walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	1	1	2	3
# control stim	3	2	0	1	0

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	3	3	3
# control stim	2	3	2	1	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	2	2	2
# control stim	3	2	1	2	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	3	3	1
# control stim	3	2	2	2	2



When stimulation is on, the mouse keeps walking 50/53.

When control stimulation is on, the mouse keeps walking 35/38.

String-pulling

10 Hz	0.7 sec
# stim	4
# control stim	3

20 Hz	0.7 sec
# stim	5
# control stim	3

30 Hz	0.7 sec
# stim	4
# control stim	4

40 Hz	0.7 sec
# stim	4
# control stim	4



When stimulation is on, the mouse keeps string-pulling 17/17.
When control stimulation is on, the mouse keeps string-pulling 12/14.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	4	2
# control stim	3	2	2	3	3

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	3	4	4
# control stim	2	2	2	3	3

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	3	3	3
# control stim	2	2	3	1	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	3	4	4	4
# control stim	3	3	4	2	2



When stimulation is on, it seems licking is not affected 68/69.

When control stimulation is on, the mouse keeps licking peanut butter 49/50.

Eating

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		3			

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		2			

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		3			

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2			
# control stim		2			



When stimulation is on, it seems eating is not affected 15/15.
When control stimulation is on, the mouse keeps eating 10/10.

Grooming

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		0	1		
# control stim		1	1		

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1		1	
# control stim		1		1	

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1				
# control stim	1				

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1				
# control stim	1				



When stimulation is on, it seems grooming is not affected 5/5.
When control stimulation is on, the mouse keeps grooming 6/6.

Rearing or standing up on hindfeet

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	3	1	1	2
# control stim	0	1	1	1	1

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	0	0	
# control stim	1	1	1	1	

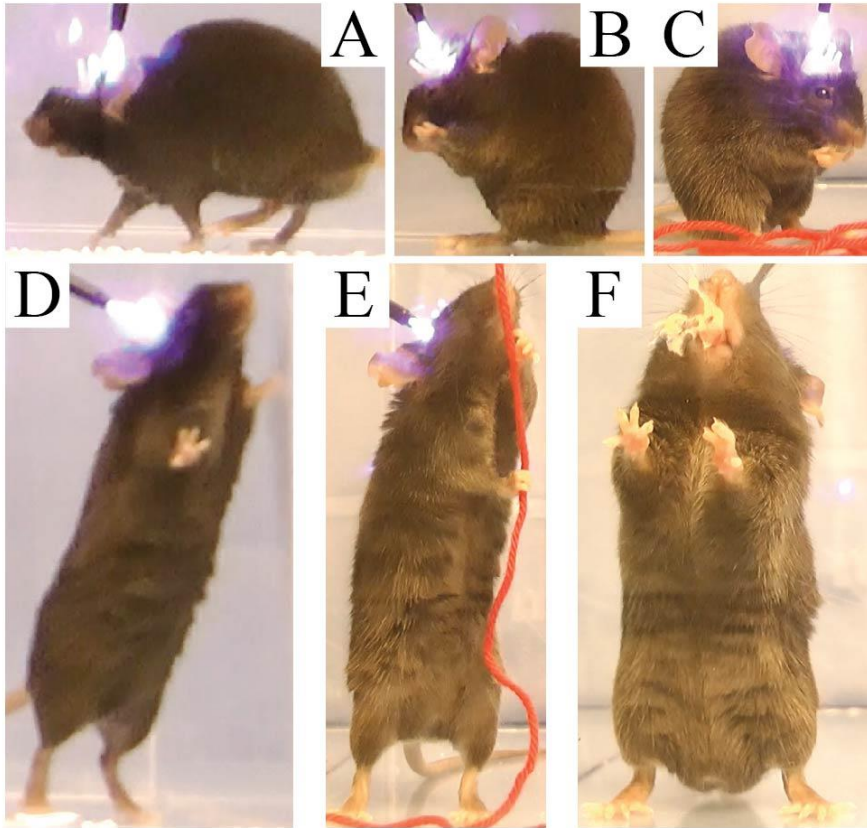
30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	1	1	1	0
# control stim	0	1	1	1	0

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	0	1	1	1	2
# control stim	0	1	1	0	1



When stimulation is on, the mouse keeps rearing 10/17.

When control stimulation is on, the mouse keeps rearing 7/14.



Common behaviours that occur during optogenetic stimulation of Visual cortex in mouse #16M. A) walking, B) grooming, C) eating, D) rearing, E) string-pulling, and F) licking peanut butter. (Note that the stimulation did not affect any of the behaviours).

Mouse #17M

- V
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 188 stim + 149 control stim = 337

walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	2	1	2
# control stim	2	2	2	1	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	1	2	1
# control stim	3	2	1	1	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	2	4	2	1
# control stim	4	2	1	2	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	2	3	3	3
# control stim	3	1	1	3	3



When stimulation is on, the mouse keeps walking 42/43.

When control stimulation is on, the mouse keeps walking 35/39.

String-pulling

10 Hz	0.7 sec
# stim	4
# control stim	1

20 Hz	0.7 sec
# stim	4
# control stim	3

30 Hz	0.7 sec
# stim	2
# control stim	6

40 Hz	0.7 sec
# stim	4
# control stim	3



When stimulation is on, the mouse keeps string-pulling 12/14.
When control stimulation is on, the mouse keeps string-pulling 10/13.

Licking peanut butter

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	4	4	4	4
# control stim	2	3	3	3	3

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	4	3	4
# control stim	3	2	4	3	3

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	4	4	3
# control stim	3	3	3	3	2

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	4	6	6	4
# control stim	4	3	4	4	4



When stimulation is on, it seems licking is not affected 77/78.

When control stimulation is on, the mouse keeps licking peanut butter 62/62.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		7			
# control stim		6			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		2			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		5			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		2			



When stimulation is on, it seems eating is not affected 21/21.
When control stimulation is on, the mouse keeps eating 15/15.

Grooming

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1		1	
# control stim		1		1	

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1	1		
# control stim		1	1		

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1			
# control stim		1			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1			
# control stim	1	1			



When stimulation is on, it seems grooming is not affected 7/7.
When control stimulation is on, the mouse keeps grooming 7/7.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	2	1	1	1
# control stim	1	2	1	1	0

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1	1	2
# control stim	1	1	1	1	1

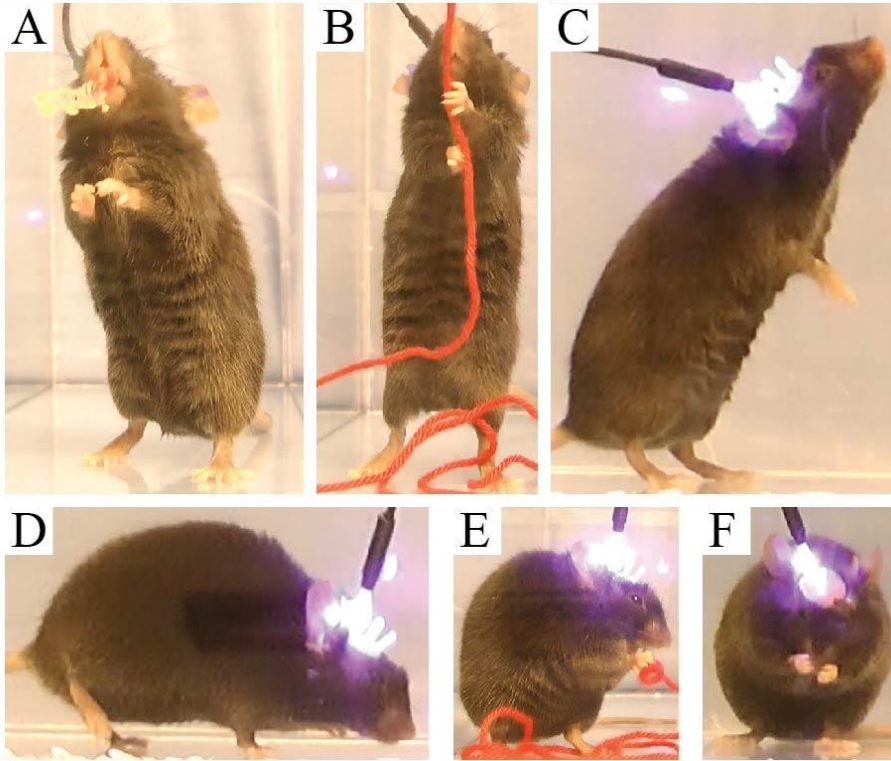
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1	1	3
# control stim	0	0	0	0	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	2	1	0	1
# control stim	0	1	1	0	0



When stimulation is on, the mouse keeps rearing 19/25.

When control stimulation is on, the mouse keeps rearing 13/13.



Common behaviours that occur during optogenetic stimulation of Visual cortex in mouse #17M. A) licking peanut butter, B) string-pulling, C) rearing, D) walking, E) eating, and F) grooming. (Note that the stimulation did not affect any of the behaviours).

Mouse #18M

- V
- histology to be done
- summary in following behaviours: walking, string-pulling, licking peanut butter, eating, grooming, and rearing
- Total # observations = 193 stim + 134 control stim = 327

walking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	4	2	3	2
# control stim	2	2	1	1	1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	2	3	2
# control stim	3	2	2	1	2

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	1	2	2
# control stim	2	1	2	1	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	2	3	3	2
# control stim	2	1	2	2	1



When stimulation is on, the mouse keeps walking 47/55.

When control stimulation is on, the mouse keeps walking 28/32.

String-pulling

10 Hz	0.7 sec
# stim	4
# control stim	4

20 Hz	0.7 sec
# stim	4
# control stim	3

30 Hz	0.7 sec
# stim	4
# control stim	4

40 Hz	0.7 sec
# stim	4
# control stim	4



When stimulation is on, the mouse keeps string-pulling 15/16.

When control stimulation is on, the mouse keeps string-pulling 10/15.

Peanut butter licking

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	3	2	4	4	4
# control stim	2	1	2	2	2

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	5	4	4	4
# control stim	3	3	2	3	3

30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	4	4	4
# control stim	3	2	3	3	3

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	4	3	5	3	4
# control stim	3	2	3	3	2



When stimulation is on, it seems licking is not affected 75/76.

When control stimulation is on, the mouse keeps licking peanut butter 49/50.

Eating

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		4			
# control stim		3			

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		5			
# control stim		4			

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		3			
# control stim		3			

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		7			
# control stim		4			



When stimulation is on, it seems eating is not affected 19/19.
When control stimulation is on, the mouse keeps eating 14/14.

Grooming

10 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim				1	
# control stim				0	

20 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1			1
# control stim	1	2			1

30 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	1	1	1		
# control stim	1	0	1		

40 Hz					
	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim	2	1	1		1
# control stim	2	1	1		1



When stimulation is on, it seems grooming is not affected 12/12.
 When control stimulation is on, the mouse keeps grooming 11/11.

Rearing or standing up on hindfeet

10 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim			0	1	0
# control stim			1	1	1

20 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1	1	0	1
# control stim		0	1	1	0

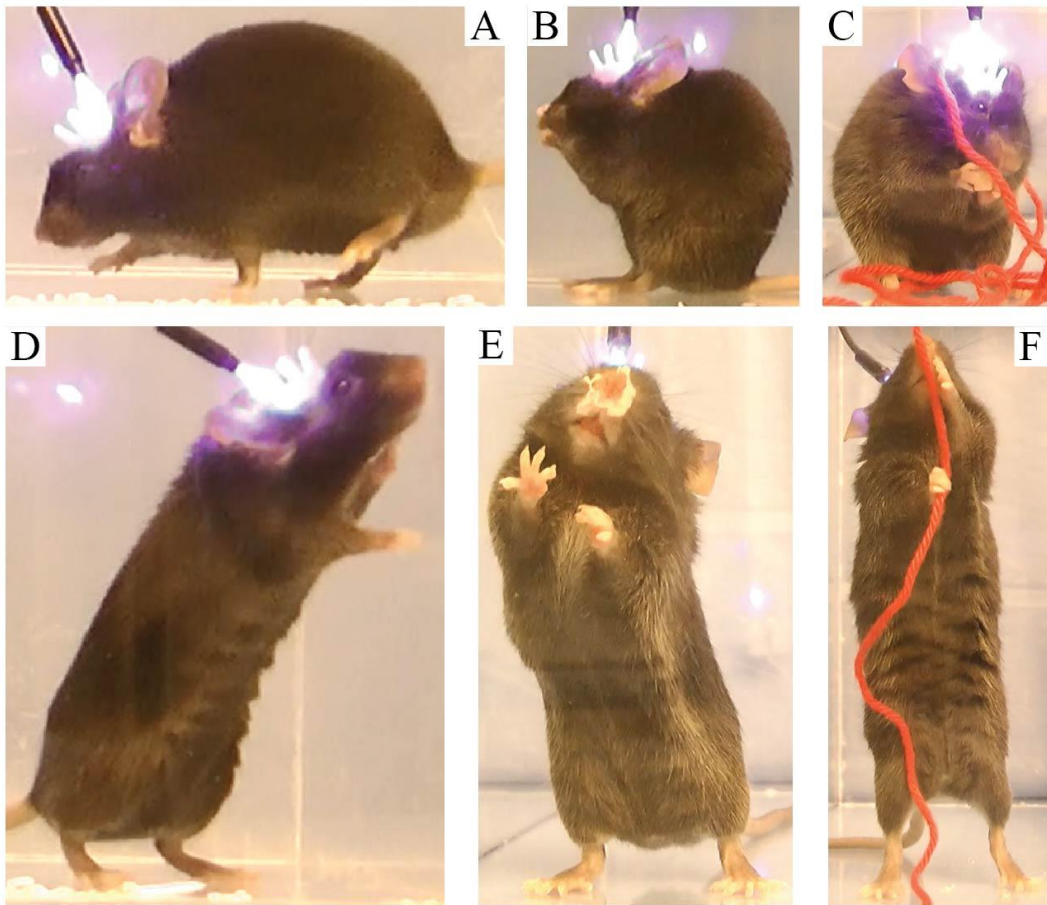
30 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		1	2	1	1
# control stim		1	1	1	1

40 Hz	0.5 sec	0.7 sec	1 sec	1.5 sec	2 sec
# stim		2	1	1	2
# control stim		2	0	0	1



When stimulation is on, the mouse keeps rearing 15/15.

When control stimulation is on, the mouse keeps rearing 12/12.



Common behaviours that occur during optogenetic stimulation of Visual cortex in mouse #18M. A) walking, B) grooming, C) eating, D) rearing, E) licking peanut butter, and F) string-pulling. (Note that the stimulation did not affect any of the behaviours).