PROC. INTERNAT. CONF. SCI. ENGIN.
Volume 3, April 2020 | Pages: 405-410

ISSN 2597-5250
E-ISSN 2598-232X

A Method for Precision Surgical Implantation and Probe Insertion in Sprague-Dawley Rat Brain

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Abstract. Motorized stereotaxic is an advanced tool for surgery and implantation of cannula and electrodes in neuroscience. Stereotaxic surgery and implantation has become increasingly important tool, applied in many experiments. The goal in this present study to determine the surgical implantation and probe insertion at the target location accurately and precisely using motorized stereotaxic. In this study, the method allowed to evaluate the DHEAS fluorescence level through *in vivo* imaging approach at the target region in the hippocampus rat brain. This present study also described the surgical implantation and probe insertion by motorized stereotaxic as the precise and accurate techniques than conventional stereotaxic procedures. With the rat brain atlas by Paxinos and Watson (2004), the imaging approach can be evaluated precisely at the target location corresponding surgical implantation and probe insertion techniques.

Keywords: surgical implantation, stereotaxic, probe insertion, imaging

INTRODUCTION

Stereotaxic technique has become widely used in neurosurgical implantation to investigate various vertebrate species in the brains location. The stereotaxic method relies on fact that the same species and strain of animals, gender, age and breeding conditions related to the brain position and its nuclei (Blasiak et al., 2010). Horsley and Clark (1908) was first introduced the stereotaxic technique with the bones of the cranium of the monkey and other experimental animals, which the landmark use to determine the position of brain structures precisely. Currently, as a gold standard method in neuroscience, the stereotaxic method in advance and commercial stereotaxic are available for many laboratory animals. The stereotaxic method is invaluable tool and applied in various type of experiments including surgery, implantation of cannula or electrodes, and microdialysis probes at the exact coordinate of the target area in the brain (Cetin et al., 2006).

There have several requirements to achieve the accuracy of stereotaxic placement as well as surgical implantation in the specific location in the brain (Blasiak et al., 2010). First, the anatomic brain structure of the surgery animals should be similar with the animal type of stereotaxic brain atlases. Rats are well-known applied in various experiments including surgery and imaging in the brain. The position of rats structures of the brain can be compared with stereotaxic atlas of the rat brain (Paxinos and Watson, 2004). Second, the stereotaxic equipment has performed and preferred in high level of stability and precision. Currently, motorized stereotaxic was used in surgical implantation along with three-dimensional Cartesian system. Third, the head of animal during surgical implantation procedures and imaging should be positioned and fixed in the stereotaxic frame. Fourth, determination and marked the stereotaxic reference point should be performed correctly and accurately. For the surgical implantation procedures of the rats, the simplest approach to assume the bregma point as stereotaxic reference point (Blasiak et al., 2010). According to Schuller et al. (1986) demonstrated that the coordinates of the target area was transferred from the brain atlas into the stereotaxic device coordinates position.

Motorised stereotaxic (Neurostar, Germany) is one of the commercial stereotaxic technique specially designed upgrading from conventional stereotaxic. The motorized stereotaxic instrument allows motorised and have a computer movement to control the coordinate system in 3 axes; X-axis (AP, anterio-posterior), Y-axis (ML, medio-lateral), Z-axis (DV, dorso-ventral). Moreover, this instrument was provided atlas intergration for rats and closely similar with stereotaxic atlas for the rat brain. Additionally, the stereotaxic microinjection has provided and essential used forfluorescence delivery, gene delivery, drug targeted lesions and dye tracers. With this approach, it would be helpful to determine the surgical implantation and probe insertion at the target area in research approach including genetic, cellular, and circuits functions in the brain precisely.

METHODOLOGY

Subjects

Adult male Sprague-Dawley rats of 8 weeks of age and weighing 250 – 280 g were procured from Animal Research and Service Centre (ARASC), Universiti Sains Malaysia, Kelantan, Malaysia. They were maintained under controlled conditions (27 ± 2ºC temperature; 40 ± 5% relative humidity). Animals were given standard rat pelleted feed and water *ad libitum* with a 12 h light-dark cycle with lights going on at 08 00h. The experimental protocols were approved by the Institutional Animal Ethics Committee.

Stereotaxic equipment and apparatus

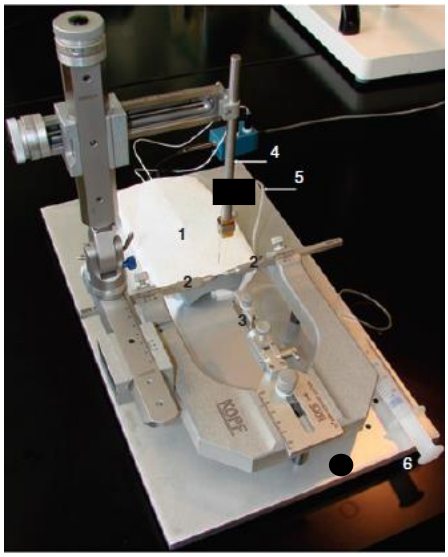

Figure 1. Motorized stereotaxic apparatus. 1) The rat is positioned on the heat-controlled blanket; 2) standard ear bars; 3) the incisor adaptor with the nose clamp; 4) microsyringe holder attached with stereotaxic arm. Adapted from Cetin et al. (2006).

- Motorized stereotaxic equipment (Neurostar, Germany)
- Surgical tools including surgical scalpel, scissors, forceps, fine sharp forceps, and small bone scraper, surgical blade
- Small animal stereotaxic apparatus including rodent adaptor, electrode/syringe holder, and standard ear bars
- Lead pencil
- Hair clipper
- Drilling tools (drill bit 0.5 mm, 0.7 mm & 1.2 mm of diameter)
- Guide cannula 22-gauge and screws
- Needles (25G & 27G)
- Dental cement
- Cotton swabs
- Surgical sutures
- Sterile phosphate buffer saline (PBS) solution
- Ethanol solution (70%) (disinfectant)
- Iodine
- Heating blanket
- Hamilton syringe (10 µL)
- Anaesthetics and analgesics (isoflurane, meloxicam)
- Antibiotic (Baytril)

Pre-operative surgery and anaesthesia preparation

The motorized stereotaxic equipment and apparatus was set up. The area of surgical implantation, all tools and reagents should be cleaned and sterilized. The rats were prepared for stereotaxic surgery and placed on the stereotaxic frame.A mixture of anaesthetic isoflurane-oxygen non-humidified (Dragon Vapor 2000, USA) was directed into the chamber at a rate of 4 l/min.The rats were anaesthetized and kept in a clear chamber by exposure to the inhalation anaesthetic isoflurane. Rat colonic temperature was monitored and maintained at 37ºC with a heating blanket (Harvard Apparatus, UK). Inhalation anaesthetic was carried out with 4% vaporized isoflurane. Surgical implantation procedures were conducted under deep anaesthesia to 2.5-3.0 % of isoflurane vaporization and ensured that no movements of the animal (toe pinch), stable blood pressure and respiratory rate. At the end of the surgical implantation procedures, the isoflurane vaporization was reduced to 0.8-2.0 % to maintain depth of anaesthesia until the dental cement dried.

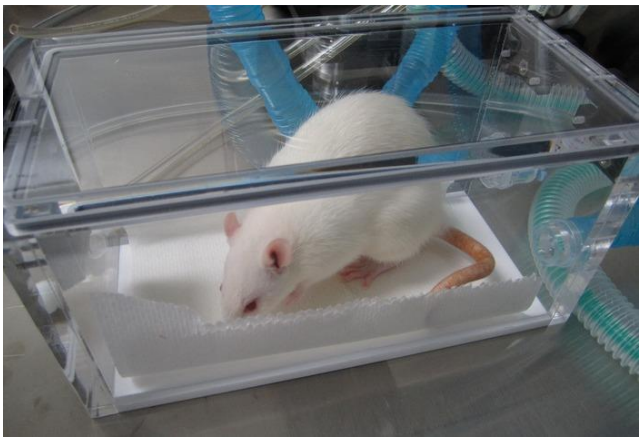

Figure 2. Inhalation isoflurane equipment in the chamber with mixture of anaesthetic-oxygen. Adapted from Godbey (2016).

Stereotaxic surgical implantation procedures

The stereotaxic apparatus, the area, tools and reagents were ensured clean and sterilized. Animals were then anaesthetised and positioned on the stereotaxic placement. The head was shaved of fur and cleaned with sterile PBS before incision. The animal was mounted onto the stereotaxic frame and then the ear bars were fixed in position to assure the head on the correct positioned to the stereotaxic frame. The incision was made down the midline using surgical blade and the top of the skull was exposed. After skin incision and removal of all soft tissue from the surface of the skull,

placement of the guide cannula was determined in relation to the bregma as a reference point. Care was taken so that the drill bit did not penetrate throughmeningeal membranes or blood vessels. After piercing the skull, the meninges were gently pierced with a 27G needle in order to allow unobstructed insertion of the microprobe. Another three holes made for insertion of screws to tight the anchor.

A guide cannula (22 gauge) was placed 2 mm above the intended site of target area according to the method described by Jafari-Sabet (2006). Stereotaxiccoordinates for the CA1 regions of the dorsal hippocampus were -3.00 mm posterior to bregma, -1.70 mm lateral to the midline and -3.00 mm ventral of the dorsal surface of the skull. Dental cement was applied around the implant base and the screws. To prevent clogging, stainless steel stylets (27 gauge) was placed in the guide cannula until the rats were given the injection and prior to the behavioural experiment. Once the dental cementwas dry, the incision was sutured around the implantation of the guide cannula. The rat was monitored until it regained consciousness and it was then returned to its home cage.

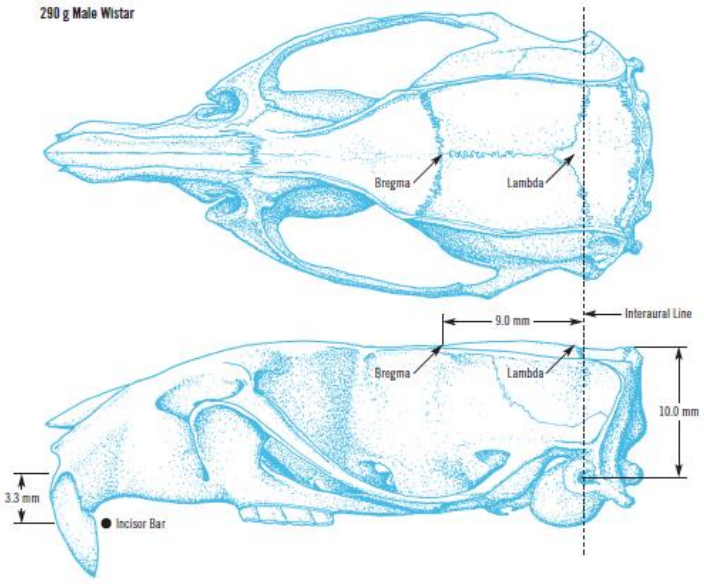

Figure 3. The positions of bregma. Lambda and interaural line on the skull are shown above the lateral view. The reference point was set at bregma on stereotaxic landmarks. Adapted from Paxinos & Watson (2004).

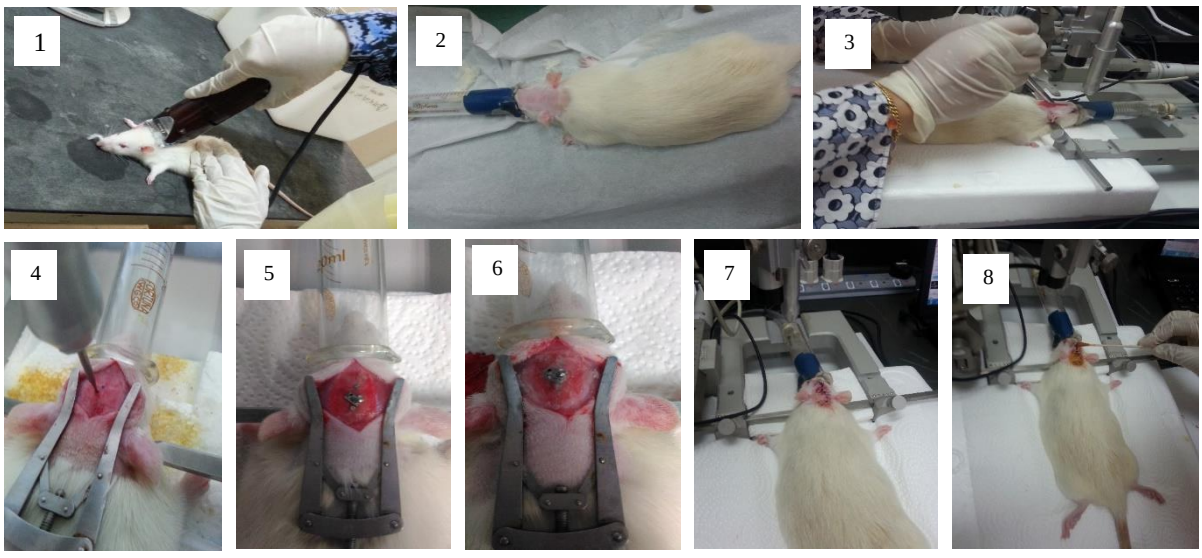

Figure 4. Surgical implantation procedures. 1) The fur on the head was shaved; 2) Rat was anaesthetized with a mixture of anaesthetic isoflurane-oxygen; 3) The incision was made with surgical blade and expose the skull; 4) The target location was drilled by drilling tools and drill bit; 5) Positioned the **guide** cannula and three screws to tight the anchor; 6) Dental cement was applied around the implant base and screws; 7) Once the dental cement dried, the incision was sutured by surgical suture; 8) Applied the iodine around the implantation of the guide cannula and injected the analgesic and antibiotic.

Stereotaxic microinjection

One week after implantation, the rat was anaesthetized using inhalation isoflurane and placed on the stereotaxis frame. The rats were gently restrained by hand and the stylets were removed from the guide cannula. The 10 µL Hamilton syringe was fixed on the holder of stereotactic frame and the distal part of the Hamilton syringe was used to verify the bregma. The Hamilton syringe was lowered to the appropriate area in the brain according to the rat brain atlas (Paxinos and Watson, 2004) coordinates (Z position). Next, the microinjection robot of the motorised stereotaxic apparatus was set up at 1.0 µL in 10 minutes (rate: 0.1 µL/minute). The CA1 hippocampal area was infused with 1.0 µL fluorescence labelled antibodies over a period of 10 minutes. The injection needles were left in place for an additional 10 minutes to allow and ensure diffusion and cell loading. The Hamilton syringe was then carefully removed from the brain. The imaging was recorded after one hour to ensure that the fluorescence labelled antibodies tagging the neurosteroid.

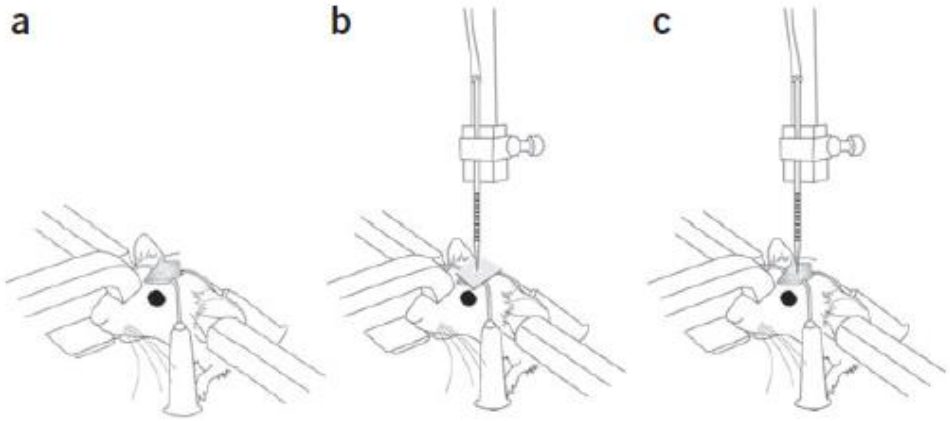

Figure 5. Stereotaxic microinjection to delivery fluorescence labelling as a tracer. a) The stylets of cannula implantation was kept open by short needles; b) positioned the 10 µL Hamilton syringe at the stereotaxic injection holder and the bregma point on top of guide cannula; c) delivery the fluorescence labelling at the desired location according to z coordinates at 1.0 µL in 10 minutes (rate: 0.1 µL/minute). Adapted from Cetin et al. (2006).

Post-operative care

After surgical implantation procedures, the rat was administered analgesic and antibiotic to ensure the rat absence in pain and stress. In this present study, meloxicam (analgesic) (0.1 ml / 300 g of body weight of rat) and baytril (antibiotic) (0.06 ml / 300 g of body weight of rat) were administered postoperatively. Following surgery, the rats were then individually housed with food and water available *ad libitum*. One week was allowed for recovery to become normal behaviour and active before start the behavioural experiments as well as imaging.

Probe insertion prior imaging

One week after recovery, individual rat was anaesthetized and placed on the stereotaxic frame to insert the probe for imaging approach. The rat internal temperature was monitored and maintained by heating blanket to prevent from hypothermia until the rat was fully awake. Anaesthetic isoflurane vaporization to 1.0-2.0 % to maintain while the probe was inserted into the CA1 region of hippocampus. Therefore, the anaesthetized ratwas imaged and monitored in real-time imaging with Fiber Fluorescence Microscopy connected to Cellvizio Lab system. The probe was attached to the stereotaxic holder and set the bregma point above of implantation. The probe was inserted into the target region corresponding to *z*-coordinate to monitor and measure brain activity.

DISCUSSION

The present study demonstrated that surgical implantations method was optimized to evaluate DHEAS fluorescence level in the CA1 region of hippocampus, hence DHEAS fluorescence labelling was carried out into the hippocampal CA1 region Sprague-Dawley rat brain by motorized stereotaxic.The optimization of surgical implantations methods has significantly easier as long as all the surgery animals in the same size and age. Therefore, the body weight was observed before, underwent and after surgical implantations to determine the exact and precise coordinates of the needle insertion, implantation of guide cannula and position of microprobe insertion location. Glanzman and Lasiter (1981) showed that the body weight is an indicator in relation to landmarks skull including bregma, lambda,

interaural line and upper incisor bar to control the variability in target location.

Following 24 hours after surgical implantation, the body weight loss was observed. The level of stress and pain associated with the surgical implantation may affected the loss of body weight. After 24 hours with the precision food intake and post-operative care surgery, the rats regain weight. The body weight of rat wasmeasured consecutive days until recovery.According to Ishida et al. (2017), body weight loss following surgery due to metabolism disruption, skeletal muscle and fat mass, and the changes of body composition. Moreover, precision and complete nutrition to regain weight underwent recovery (Pederson et al., 2018).

In this study, anaesthetic isoflurane was used in the surgical implantation method. As mentioned earlier, the anaesthetic isoflurane concentration was presented for induction, during surgical implantation and maintenance of anaesthesia while waiting dental cement dried around the implantation place and to suture the incision. Isoflurane is one of volatile anaesthetics which reliable to be used with mice or rats and performed faster induction, fast recovery, consistent survival and minimal pain and distress underwent surgical implantation (Farris & Snow, 1987; Messier et al., 1999; Stokes et al., 2009).

Consistent with the previous study, simplest approach to assume the reference point at the bregma point on the landmarks of skull. The bregma point which the positioned was crossing the coronal and sagittal sutures was markedly the zero point coordinates (0,0,0) (Blasiak et al., 2010). However, previous study has been shown that the different coordinates of the target location due to brain swelling/decompression or cortical surface damage during surgery. In this present study, delivery of fluorescence labelling and probe insertion prior imaging into the CA1 region of hippocampus were determined as follows: AP: -3.00 mm from bregma; ML: ±1.7 mm from the sagittal suture; and DV: 3.00 mm from the top implantation based on stereotaxic rat brain atlas by Paxinos and Watson (2004). The bregma point after surgical implantation was set up with syringe or probe on top of cannula implantation.

In order to define the precise coordinate, motorized stereotaxic was used in this study.The equipment is an applicable technique for surgery, implantation and injection, rapid, reliable and precise in comparison with conventional method (Asahi et al., 2003; Cetin et al., 2006; Messier et al., 1999). Studies showed that the stereotaxic technique is important to prevent the brain from injury while insertion the recording electrode into the target area and to avoid injury to the primary motor cortex causally paralysis (Asahi et al., 2003). Moreover, the stereotaxic coordinates can be important in determining the exact target area in the brain for neuronal recordings (Asahi et al., 2003). Thus, stereotaxic technique is a useful technique to determine accurate and precise the target area is located, to prevent brain disruption and drilled at the wrong area (Asahi et al., 2003).

Pre-operative, underwent surgery and post-operative should be considered in increasing the percentage of survival rate and reduce body weight loss. Several studies observed that the animals should be performed in physical such as active and healthy before start the surgical procedure (Rigalli and Loreto, 2009). Another important factors when underwent surgical implantation are continuously monitored of blood oxygenation and heart rate levels to avoid hypoxia (Fornari et al., 2012). Moreover, the animals always keep warm to prevent heat loss during the surgery and prolong the duration of anaesthetic uses. In this study, the results by post-operative mortality were achieved 0.3%, whereas the average body weight loss was 1% of Sprague-Dawley rats.

Consideration of post-operative care after surgical implantation to prevent occurrence of hyperthermia, signs of pain and stress, and poor grooming(Kamei et al., 1995; Rigalli and Loreto, 2009; Waynworth and Flecknell, 1994). The development of pain management, anaesthesia and post-operative care has been consistent in order to increase knowledge in optimizing stereotaxis surgical implantation methods (Fornari et al., 2012; Messier et al., 1999). In this present study, meloxicam (analgesic) (0.1 ml / 300 g of body weight of rat) and baytril (antibiotic) (0.06 ml / 300 g of body weight of rat) were used after surgical implantation procedure to prevent the rats from pain and weight loss and death as well.

In conclusion, motorized stereotaxic tools with the rat brain atlas by Paxinos and Watson (2004), the imaging approach can be evaluated precisely at the exact target location. Optimization of surgical implantation techniques and post-operative care may improve the survival rate of rats. The methods were establishedthat appropriate to rats and not involved in physiology and morphology in the rat brain as well as the target locationfor imaging can be determined.

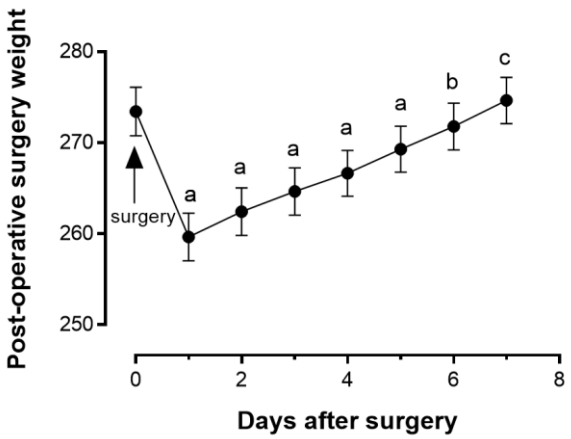

Figure 6. Body weight (g) loss following cannula implantation surgery on MWM task. (n=16). ($^a p < 0.0001$ when compared to day 0 starting of

surgery; [b]$p < 0.01$ when compared to 0 day; [c]$p > 0.05$ showed did not show any significant differences when compared to day 0).

ACKNOWLEDGEMENT

We thank to former supervisor, Professor Dr. Zalina Ismail on your guidance in this research. This study was supported by Fundamental Research Grant Scheme; FRGS (203/PPSK/6171153) and P3Neuro (304/PPSK/652204/K134).

REFERENCES

Asahi, T., Tamura, R., Eifuku, S., Hayashi, N., Endo, S., Nishijo, H. & Ono, T. (2003). A method for accurate determination of stereotaxic coordinates in single-unit recording studies in monkeys by high-resolution three-dimensional magnetic resonance imaging. *Neuroscience Research*, *47*, 255–260.

Blasiak, T., Czubak, W., Ignaciak, A., & Lewandowski, M. H. (2010). A new approach to detection of the bregma point on the rat skull. *Journal of Neuroscience Methods*, *185*(2), 199–203.

Cetin, A., Komai, S., Eliava, M., Seeburg, P. H., & Osten, P. (2006). Stereotaxic gene delivery in the rodent brain. *Nature Protocols*, *1*(6), 3166–3173.

Farris, H. E., & Snow, W. S. (1987). Comparison of isoflurane, halothane, or methoxyflurane with combinations of ketamine HCl and acepromazine for anesthesia of the rat. *Lab. Anim. Sci*, 37, 520.

Fornari, R.V., Wichman, R., Atsak, P., Atucha, E., Barsegyan, A., Beldjoud, H., Messanvi, F., Thuring, C.M.A., Roozendaal, B. (2012). Rodent Stereotaxic Surgery and Animal Welfare Outcome Improvements for Behavioral Neuroscience. *Journal of Visualized Experiments*, (59), 1–4.

Glanzman, D. L., & Lasiter, P. S. (1981). Fine Tuning Stereotaxi Surgery. *System*, *7*(1), 2–5.

Godbey, T. (2016). *Handbook of small animal imaging*. Routledge.

Horsley, V. & Clarke, R. H. (1908). The structure and functions of the cerebellum examined by a new method. *Brain*, 31, 45-124.

Ishida, J., Saitoh, M., Doehner, W., von Haehling, S., Anker, M., Anker, S. D., & Springer, J. (2017). Animal models of cachexia and sarcopenia in chronic illness: Cardiac function, body composition changes and therapeutic results. *International Journal of Cardiology*, *238*, 12–18.

Jafari-Sabet, M. (2006). NMDA receptor blockers prevents the facilitatory effects of post-training intra-dorsal hippocampal NMDA and physostigmine on memory retention of passive avoidance learning in rats. *Behavioural Brain Research*, *169*, 120–127.

Messier, C., Émond, S., Ethier, K. (1999). New Techniques in Stereotaxic Surgery and Anesthesia in the Mouse. *Pharmacology Biochemistry and Behavior*, *63*(2), 313–318.

Paxinos, G. & Watson, C. (2004). *The rat brain in stereotaxic coordinates*. Elsevier.

Pederson, S., Sniehotta, F. F., Sainsbury, K., Evans, E. H., Marques, M. M., Stubbs, R. J. & Lähteenmäki, L. (2018). The complexity of self-regulating food intake in weight loss maintenance. A qualitative study among short- and long-term weight loss maintainers. *Social Science and Medicine*, *208*, 18–24.

Rigalli, A., & Loreto, V. E. Di. (2009). *Experimental Surgical Models in the Laboratory Rat*. CRC Press.

Schuller, G., Radtke-Schuller, S., & Betz, M. (1986). A stereotaxic method for small animals using experimentally determined reference profiles. *Journal of Neuroscience Methods*, *18*(4), 339–350.

Stokes, E. L., Flecknell, P. A.& Richardson, C. A. (2009). Reported analgesic and anaesthetic administration to rodents undergoing experimental surgical procedures. *Laboratory Animals*, *43*(2), 149–154.