UNIL | Université de Lausanne
Faculté de biologie et de médecine

Centre hospitalier universitaire vaudois

Mémoire de Maîtrise en médecine No 2540

An animal model of Eye Movement Desensitization and Reprocessing (EMDR)

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Lausanne, le 15 Janvier 2016

Abstract

Background

Eye Movement Desensitization and Reprocessing (EMDR) is a psychotherapy for Post-Traumatic Stress Disorder (PTSD), which has the particularity of using alternating stimulation on both sides of the patient, such as eye movements, tones or taps. EMDR has been proven to be one of the most effective treatments for PTSD and can show signs of improvement in fewer hours of therapy than other therapies. While EMDR has been investigated through physiological and imaging studies on humans, its method of action and the neural pathways it employs remain unknown. In this context, the elaboration of an animal model of EMDR would permit the use of more invasive methods of investigation which could provide new and more precise data on the subject.

Aims:

The aim of the thesis was to develop an animal model of EMDR, which was carried out in two parts. The aim of part A was to develop and validate a device for rats capable of alternatingly stimulating the rat bilaterally. The aim of part B was to study the difference of fear extinction in fear conditioning experiments between rats receiving EMDR-like stimulations during testing versus those with no stimulation of such.

Method:

Part A: A chronic implant with detachable earphones, inspired by a device developed by Nodal et al. in ferrets, was adapted to rats to produce the alternating stimulation. To prove that the rodent could hear tones coming from the device and could distinguish tones coming from one side or the other, we used a fear conditioning experiment (n=3) in which tones coming from one side of the earphone device were paired with a shock, while tones coming from the other side of the device were not.

Part B: To study the effect of alternating stimulation on fear extinction, we used a fear conditioning experiment in which light was used as a CS and alternating stimulation was delivered to the rat through the earphone device during CS presentation in the testing phase.

Results:

Part A: Maximal levels of freezing to tones coming from the earphones were measured in the testing phases of the experiment, however no discrimination of the origin of the tones was observed as maximal levels of freezing were seen in reaction to tones originating from both earphones.

Part B: While an appropriate protocol was designed and tested, technical difficulties which occurred during the experiment prevented the production of analyzable results. Nevertheless measures to avoid such technical difficulties were successfully implemented and only limited time prevented production of further results.

Conclusion

The development of a device to deliver audio EMDR-like stimulation was successful as rats were able to hear the tones given through both earphones. However, I was unable to prove that the rats were able to distinguish the origin of the tone. While technical difficulties and time limitation prevented the acquirement of results in the study of the effect of EMDR-like stimulation on fear extinction, measures to avoid the technical problems were successfully implemented.

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1.0 Introduction

1.1 Post Traumatic Stress Disorder (PTSD)

PTSD is a disorder which affects people who have been exposed to an extreme stressor or traumatic event, such as exposure to actual or threatened death, serious injury, or sexual violence. The traumatic event at fault can be experienced directly, or indirectly by witnessing it, learning about a close friend or family member who has experienced it or being repeatedly exposed to such events (police officers, first responders)(American Psychiatric Association, DSM V, 2013)

This traumatic experience induces debilitating symptoms which are categorized in different groups in the DSM V, the first of which relates to the re-experiencing of the event, in which patients unwillingly re-experience the event in a distressing, intrusive way. This is experienced through flashbacks, nightmares, psychological and physiological reactions to cues/reminders of the event which then leads to the deliberate avoidance of such reminders. The second group of symptoms relates to hyperarousal, which is conveyed in irritable behavior, hypervigilance, exaggerated startle responses and problems with concentration and sleep. Lastly a third group refers to negative changes in mood and cognition, such as feelings of emotional numbness, estrangement, amnesia in regards to the traumatic event and persistent negative emotions towards oneself.

PTSD usually manifests in the first month following the traumatic event, and a significant percentage of patients will recover in the following years without treatment (N.I.C.E, 2005). However at least a third are still symptomatic for 3 years or more (N.I.C.E, 2005). Common secondary problems of PTSD are drug abuse, depression and anxiety disorders (N.I.C.E, 2005).

In a large survey conducted in the USA, the lifetime prevalence of traumatic exposure to at least one event was 60.7 % in men and 51.2% in women and the risk of developing PTSD after experiencing a traumatic event was 8.1% in men and 20.4% in women (Kessler et al., 1995). With a life time prevalence of 5 to 6 % in men and 10 to 14 % in women in the USA, PTSD is the 4th most common psychiatric disease there (Yehuda et al., 2002).

1.2 Eye Movement Desensitization and Reprocessing (EMDR)

1.2.1 Introduction to EMDR

EMDR is relatively new treatment of PTSD, having been introduced by Shapiro in 1989, consisting of an 8-step procedure which has the particularity of using alternating bilateral stimulation (BLS) such as horizontal eyes movements, tapping on opposite sides of the body or tones being alternatively played in both ears, at precise moments of the therapy session (Shapiro, 2012).

EMDR therapy follows the Adaptive Information Processing (AIP) model which considers that the primary basis of clinical pathology is inappropriately stored memories (Oren et al., 2012). In this model, all relevant information relating to a memory is stored in specific networks in a way that enables an adaptive, functional use of the memory. A problem arises when a distressing, overwhelming memory occurs, which leads to an inappropriately stored memory, in isolation and out of the normal adaptive memory (Oren et al., 2012). Current events may then trigger it and cause a re-experiencing of the memory, and thus cause the symptomatology of the pathology. It is this model which has led to the design of the current EMDR protocols (Shapiro, 2001).

1.2.2 Summary of an EMDR protocol

The 8-step protocol is summarized by Elofsson et al. (2008) in 3 phases: a target assessment phase, a desensitization phase and an installation phase. In the target assessment phase, the clinician obtains a history of the patient's complaints, helps the patient assess a target memory, the most vivid one, evaluates the extent of distress it produces and finally asks the patient to identify a positive cognition for the same memory. Self-reported scales are used to quantify the level of anxiety the target memory causes and to estimate the validity of the new positive cognition, which are respectively the Subjective Units of Distress (SUDS) (0 to 10, 0=no distress, 10= maximum distress) and a Validity of Cognition (VoC) scale (0 to 7, 0=completely false, 7= completely true).

These measures are first made during the target assessment phase, which is followed by the desensitization phase. During this phase, the patient focuses on the target memory while BLS is given during short sets and the clinician guides the patient through the session. At the end of each set the SUDS are measured and the sets are continued until a SUDS score of 0 or 1 is obtained.

Next comes the installation phase in which the positive cognition is focused upon while sets of BLS are given. The sets are continued until the patient has a VoC score of 6 or 7. To finish the session, a "body

scan" is done in which the patient identifies and processes with BLS any residual feelings of distress, tension in the body until they disappear if possible.

1.2.3 EMDR in PTSD treatment

Since its introduction in 1989, EMDR has been shown to be an effective (Watts et al., 2013 ; Bisson et al., 2009) treatment of PTSD and has been recommended with a high rating of evidence in a number of national and medical association's clinical guidelines, such as the UK National Institute of Clinical Excellence PTSD guidelines (Forbes et al., 2010). Other commonly recommended treatments of PTSD are trauma focused cognitive behavior therapy (TFCBT) and pharmacotherapy (Forbes et al., 2010).

TFCBT is recommenced as the first line treatment of PTSD in most clinical guidelines (Forbes et al., 2010) and has been the most researched form of PTSD therapy (Watts et al., 2013). TFCBT and EMDR have been shown to be equally efficacious in different meta-analysis comparing both forms of therapy (Seidler et al., 2006 ; Bisson et al., 2009) however the lesser number and size of EDMR studies has led TFCBT to have a higher rating of evidence in certain guidelines (Bisson et al., 2009 ; Forbes et al., 2010).

Pharmacotherapy has also been shown to be effective, with no clear evidence as to which class of medication is most effective, although Selective serotonin reuptake inhibitors (SSRI) have been the most studied (Stein et al., 2006). Pharmacotherapy is recommended in most clinical guidelines, but recommendations differ as to whether they should be used initially in combination to psychotherapy or as an alternative to it (Forbes et al., 2010). A single study compared pharmacotherapy to EMDR, which found no differences in efficacy immediately after treatment but did show higher remission rate in the 6 months follow up in EMDR (Jonas et al., 2013).

While its efficacy has been shown to be similar to other treatments, one of the advantages of EMDR is that it can show signs of improvement in fewer hours of treatment and without the need of homework which is required in TFCBT (Oren et al., 2012; Ironson et al., 2002). This which makes EMDR a more user-friendly, better tolerated treatment by both patients and clinicians (Oren et al., 2012). While the efficacy of the treatment is commonly acknowledged, the main controversy has been whether the bilateral stimulation is necessary to the treatment and if so, why? (Jeffries et al., 2013).

1.2.4 Eye Movements, proposed effects and controversy

Shapiro initially included eye movements (EM) in her therapy in 1989 after having noticed that her eyes would spontaneously have saccadic bursts to the upper right when thinking of distressing thoughts (Shapiro, 1995). This led her to gradually develop the protocol that we now know.

Despite initial research providing some substance to the claim of the necessity of EM (Jeffries et al 2013), more recent research has found conflicting results: In a meta-analysis conducted in 2001, Davidson and Parker found no additional benefits to EM on the outcome of EMDR treatments (Davidson et al., 2001) while other meta-analysis found a significant moderate additive effect (Lee et al., 2013). Nonetheless, EM has also been shown to reduce vividness and emotionality of memories (Gunther et al., 2008), induce a parasympathetic activity and psychophysiological dearousal (Eloffson, 2008) and increase cognitive flexibility and episodic memory (Maxfield, 2008). However, these findings were mostly found in non-pathological subjects and it is difficult to evaluate their contribution to EMDR therapy (Maxfield, 2008)

1.2.5 Different models of EMDR

As research has grown in the field, so has the number of models theorizing the mechanism of action of EMDR, with no clear front-runner. However the Orienting Response has been cited in a number of speculative models and neurobiological studies on EMDR (Bergmann, 2010). It is a model in which the BLS is theorized to elicit an orienting response (OR), a behavioral response to a novel stimulus which was first described by Pavlov as an immediate reflex to the changes in the environment so as to orient the appropriate receptor organ (Barrowcliff et al., 2003). It involves information processing as the novel information is compared to familiar information, to assess it as a threat or not for example (Bergmann, 2010). It is theorized that the OR, elicited by BLS, enables the processing of the traumatic event. One theory is that, as the OR induced by BLS doesn't identify a threat, it causes a functional parasympathetic dearousal in the patient which enables the deconditioning of cues in therapy. The study of sympathetic and parasympathetic activity during eye movements have been undertaken to give credence to this theory, but inconsistencies in measurements across studies have been problematic (Gunter, 2008).

A different theory is that BLS brings forth a mind state similar to Rapid Eye Movement (R.E.M) sleep, one of the phases of sleep. R.E.M sleep is thought to play a crucial role in memory processing, enabling traumatic memories to be integrated in general semantic memory networks, a mechanism thought to be lacking in PTSD patients (Stickgold, 2002). Through research on eye movements during EMDR, it has been shown that increased activity in cholinergic, parasympathetic activity and decreased sympathetic systems

have similarities with physiological patterns of REM sleep which gives credence to this theory but no studies have been undertaken to directly study it (Stickgold, 2002).

1.2.6 Imaging studies of EMDR

EMDR neuroimaging has been undertaken in various forms such as SPECT, fMRI and EEG imagery, of which the most consistent finding is an activation of the prefrontal cortices (Stickgold, 2006; Bergmann, 2010)). Among this literature, a noteworthy study used EEG monitoring in symptomatic patients during EMDR sessions and during autobiographical script reading of their respective traumatic event. This showed an increased activity in the orbito-frontal, prefrontal and anterior cingulate cortex in the initial EMDR session and script listening which shifted to the fusiform gyrus and visual cortex at the last session of EMDR and the script reading (Pagani et al., 2012). This shift from limbic related areas to cortical areas suggest a shift from emotion related areas to associative area, an important concept in the AIP model. This study among others suggests an important interplay between different brain structures in EMDR (Bergmann, 2010).

1.3 An animal model of EMDR

Past research on the mechanisms of EMDR has found some promising yet inconclusive results as detailed above. Multiple theories on the mechanism of action exist, none of which have been entirely demonstrated. The absolute necessity of BLS in EMDR therapy has been scrutinized as well. Although insight on the neural structure involved in EMDR has been gained through imaging studies, the exact neural pathways remain unknown. Research has been limited by lack of homogeneity in patient groups, small samples sizes and lack of control conditions (Bergmann, 2010).

The goal of this thesis is the elaboration of an animal model of EMDR which would enable us to bypass these problems. It would permit the design of better controlled experiments with larger, homogenous study groups and the use of more invasive methods of investigation. The use of imagery and electrophysiological experiments would allow the deeper investigation of the exact neural pathway involved in EMDR. Using optogenetics stimulation or direct electrode stimulations would allow testing of whether the same effects of EMDR can be achieved by activating specific brain regions. And through sections of neural pathways, the crucial interconnections necessary for EMDR could be discovered. The development of an animal model would thus permit new and better controlled experiments and could provide a breakthrough in EMDR research.

1.3.1 Proposed animal model of EMDR

To create an animal model of EMDR, we planned to use an EMDR stimulation producing device in conjunction with a fear conditioning experiment in rats. Classical fear conditioning consists of a protocol in which a conditioned stimulus (CS), such as a tone or light signal, is paired with an unconditioned stimulus (US) such as an electric shock in a conditioning phase. This leads to the association of the CS and US and as a result the presentation of the CS alone (in the testing phase) produces a fear response, which is called the conditioned response (CR). Overtime the presentation of the CS alone gradually ceases to elicit a fear response, a phenomenon called fear extinction. Fear conditioning has been used in animal models of PTSD, usually with additional stressors to create a CS-US association that doesn't extinguish and a neurobiological state close to those found in PTSD (Pitman et al., 2012).

Using such models as a basis, we planned to study the effect of EDMR-like stimulation on fear extinction. To deliver the EDMR-like stimulation, we planned to develop a device which produces audio-based EMDR-like stimulation. This device was a chronic implant with detachable earphones, which was inspired by a device developed by Nodal et al in 2010 to study sound localization in ferrets.

2.0 Hypothesis

I hypothesize that rodents process traumatic memories in a similar fashion to humans. As such EMDR-like stimulation should also have an effect on the processing of traumatic memories in rats. Using Pavlovian fear conditioning to emulate a traumatic event and using the conditioned stimulus (CS) as a reminder of the event, I hypothesize that EMDR-like stimulations given during presentation of the CS should have an effect on the extinction of fear expression in rats.

3.0 Aims of the thesis

The aim of the thesis is to develop an animal model of EMDR. I aim to achieve this by going through these two steps:

- Part A: Develop and validate an appropriate EMDR-like bilateral stimulation device for rats. To validate the device, we plan to prove that the rodent can hear tones coming from the device and can distinguish tones coming from one side or the other. We plan to prove this using a fear conditioning experiment in which tones coming from one side of the earphone device are paired with a shock, while tones coming from the other side of the device are not.

- Part B: Study the difference in fear extinction in rats which receive EMDR-like stimulations in fear conditioning experiments. To study the effect of EMDR stimulation on fear extinction, we plan to set up a fear conditioning experiment in which light is used as a CS and EMDR stimulation is delivered to the rat during CS presentation in the testing phase.

4.0 Part A: Development of an EMDR stimulation producing device and experiments on its validity

4.1 Methods and materials

4.1.1: Introduction

In part A of the thesis, we discuss the design of the device as well as Experiment A in which the device is tested in its ability to appropriately deliver sound to the rat using a fear conditioning experiment in which a tone produced through the earphones is paired with a shock.

4.1.2 Design of the detachable earphones device

The device developed by Nodal et al. in 2010 was made of two components: a "chronic implant" which rested on the head of the animal and a "detachable earphone holder". The chronic implant was made of dental cement and rested on top of a layer of dental adhesive applied to the skull of the animal. The upper part of the implant had 2 nuts (*Figure 1A*) embedded in it which allowed the attachment of the earphone holder with 2 screws (*Figure 1B, n°1*). To reinforce the strength of my build of the chronic implant, the two bolts were replaced with 2x2 bolts which were soldered together.

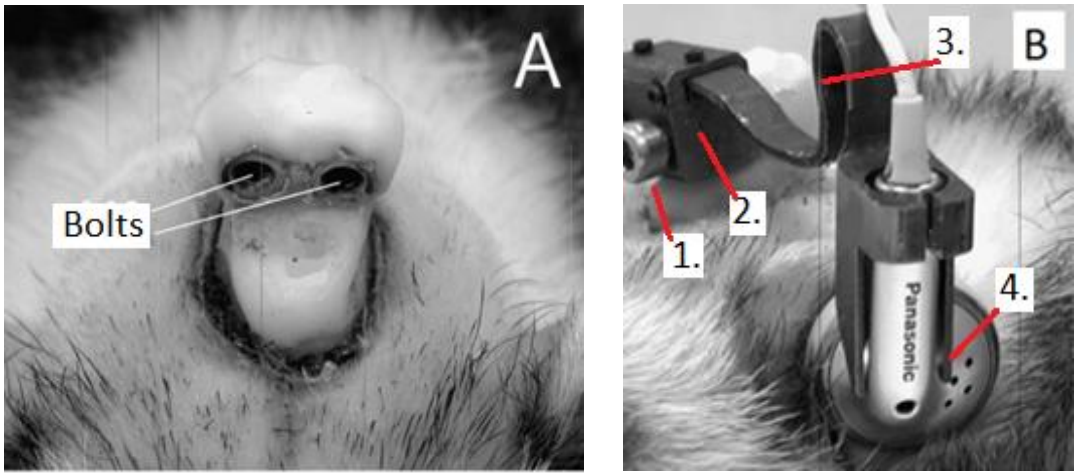

Figure 1: A. Chronic part of device originally used in ferrets. B: Complete assembly 1.Screws 2. Central block 3. Earphone holder arms 4. Earphone (Nodal FR, J Neurosci Methods. 2010)

The headphone holder was composed of a central block (*Figure 1, B, n°2*) and two arms (*Figure 1, B, n°3*) which held the earphones (*Figure 1, B, n°4*). The central block (*Figure 2,A*), originally a block of 13x8x10 mm made in titanium was replaced by a 8x5x6 mm block made with lighter and easier to work aluminum. The size of the block was planned using the skull of a rat of the appropriate age to estimate the ideal size of the block.

The central block contained two holes (*Figure 2A,a)* on its anterior face through which two M2 screws allowed it to be attached to the chronic implant. On both sides of the central block there is a hole (*Figure 2B, 1)* through which the arms of the holder are fixed. These are secured in place by two M1 screws which are placed on the anterior face of the block (*Figure 2A,b*) , instead of four M2 screws in the original.

The original titanium arms were replaced with stainless steel arms to allow easier fine tuning of the position of the earphones. The other end of the arms, the holder which held the earphones, were made in plastic instead of titanium to reduce the weight of the device (15g in total).

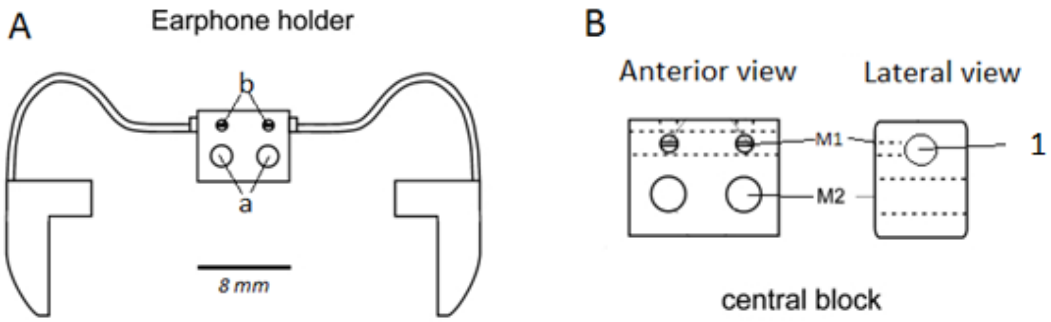

Figure 2: A: Design of detachable earphone holder. a: holes for m2 screws which allowed fixation to the chronic implant. b: holes for m1 screws which allowed fixation of the arms of the earphone holder. B: central block of headphone holder. 1.Hole for an arm of the earphone holder (Nodal FR, J Neurosci Methods. 2010 May 30)

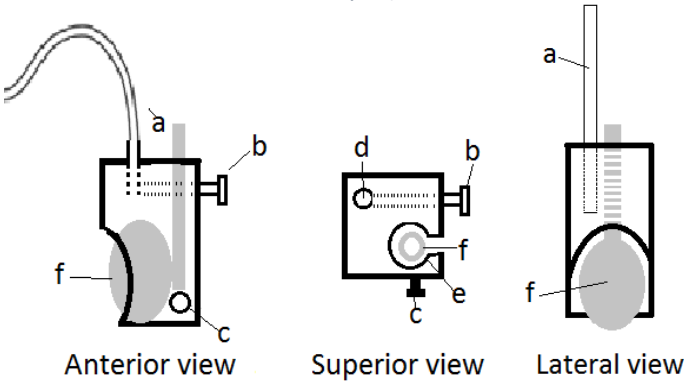

Figure 3: Design of the holder end of the earphone holder arm: a: arm of earphone holder, b: M1 screw to secure arm, c: M1 screw to secure earphone in holder, d: hole in which arm is fixed, e: hole for earphone wire, f: earphone

The aluminum arm (*Figure 3, a*) was inserted in a hole in the holder (*Figure 3, d*) and secured by a M1 screw (*Figure 3, b*). The earphone was placed in the holder from below and secured by a M1 screw *(Figure 3, c).*

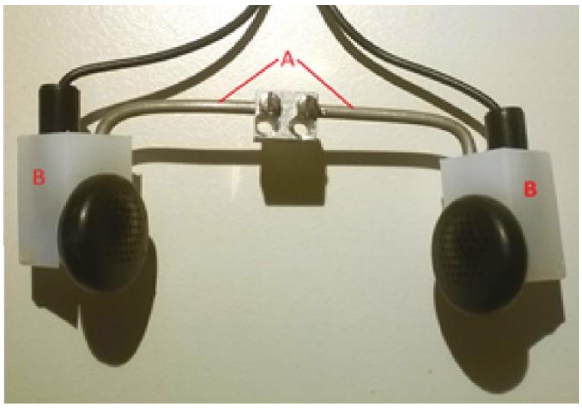

Figure 4: Rat-Adapted earphone holder: A: aluminum arms of the holder, B: Plastic earphone holder piece

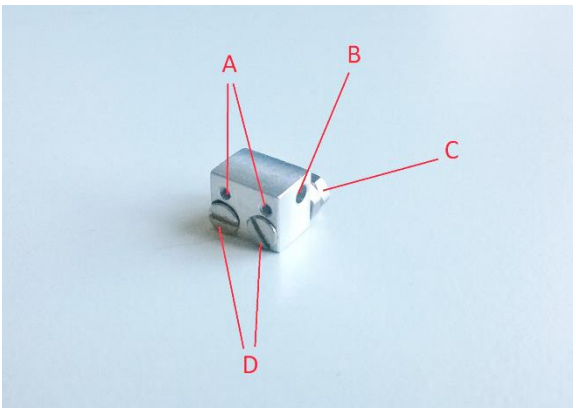

Figure 5: Central block of earphone holder: A: holes for M1 screws to secure arms in place hole in which arms of earphone holder are inserted, B: hole in which arms of earphone holder are inserted, C: M2 bolts which are soldered together, which will be placed in the chronic implant. D: M2 screw for fixation to chronic implant.

4.1.3 Implantation of device on rats

4.1.3.1 Rat rearing and Housing

Male Sprague Dawley rats aged 70 to 84 days were used for the implantation and were housed before surgery in groups of 2 to 4 in Plexiglas rat cages with raised lids, in an enriched environment consisting of straw and wooden chips, in a temperature-controlled room. They were kept in 12h dark/light cycles and given food and water ad libitum. Three days prior to the surgery, the rats were handled once a day for 15 minutes by the operator to reduce operator-induced stress.

4.1.3.2 Surgical procedure

On the day of the surgery, the animal was anesthetized with isoflurane. It was then placed on a heating pad, which kept its temperature around 36 C°. The head of the rat was then fixed in place in a stereotaxic frame (Model 900, KOPF Instruments, USA) which had a built-in link to the isoflurane source. Once the rat was secured in place, lubricant eye anointment that prevents corneal drying during the operation was applied (Viscotears, Novartis, Switzerland). Subsequently, the top of the rat's head was locally anesthetized with a lidocaine cream (Emla® 5%, AstraZeneca AG, Switzerland) and cleaned with a Povidone-iodine solution (Betadine®, Mundipharma Medical Company, Bermuda)

A midline incision was performed and clamps were used to pull apart the edges of skin to expose the dorsal part of the skull. Using a 1% oxygen peroxide solution, the exposed skull was washed and any soft tissue was pushed aside mechanically. Two holes were drilled in the skull, in which two shortened M2 screws were screwed (Figure 6). To increase the abrasiveness of the surface, the drill was used to lightly scratch the surface of the skull.

A ~1mm thick layer of dental adhesive was applied to the skull in an area as large as possible. Once the adhesive had hardened completely and special care had been taken to exclude any bleeding or blood on the surface, bone cement was added layer by layer until it had reached the height of the skin (Figure 7). The area of the implant was reduced gradually to allow a maximum bonding area and a minimum externalized area above the scalp. To avoid damaging tissue with the heat released through the exothermic hardening of the bone cement, the cement was added layer by layer, letting it harden and cool before adding the next.

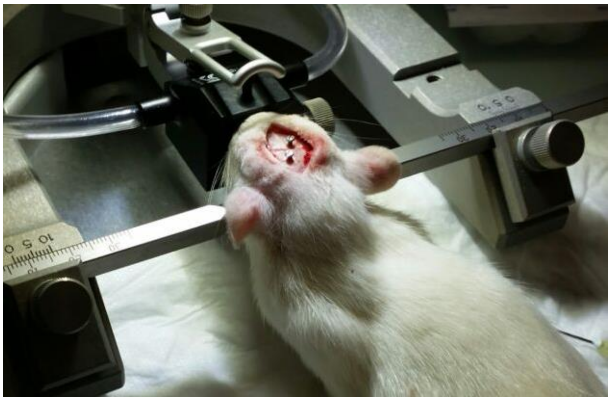

Figure 6: Once the skull was exposed and cleaned, two shortened M1 screw were drilled in the skull.

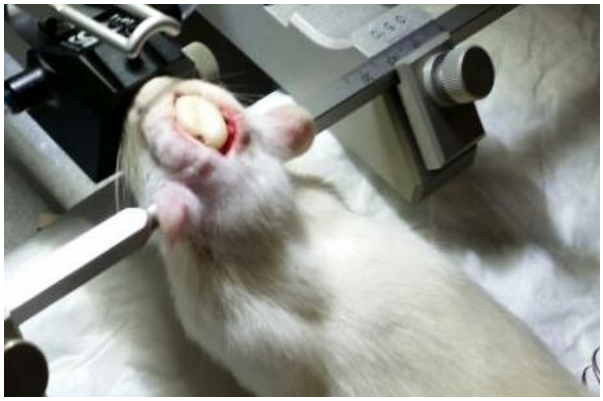

Figure 7: Dental cement is later applied, layer by layer until reaching the height of the skin.

Once the part of the implant lying under the skin was finished, the skin was sutured in front and behind the implant. Once sutured, the external part of the implant was constructed layer by layer and the nuts which had been aligned and soldered together beforehand, was added to it (Figure 8). Special attention was given during this process to build a strong base for the bolts.

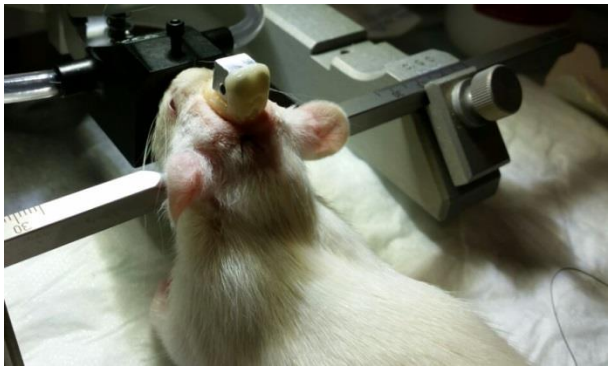

Figure 8: completed chronic implant with central block attached to it to test proper alignment

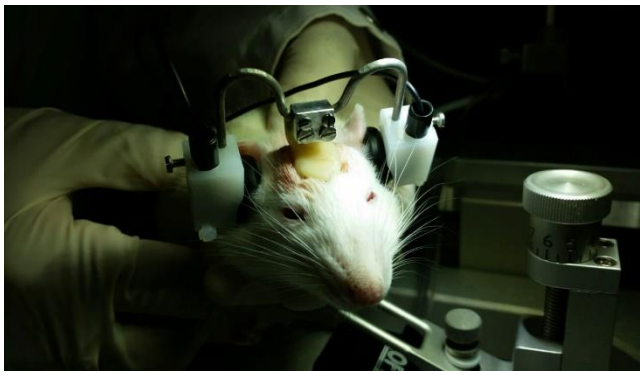

Figure 9: completed chronic detachable earphone device, with earphone holder fixed to central block

At the end of surgery, the rat was removed from the stereotaxic frame, and following recovery from anaesthesia, was placed in isolation in a Plexiglas cage of 42x42x50 cm, in order to prevent other rats from inflicting wounds to it. A post-operative analgesic protocol consisting of 500 mg of paracetamol (Dafalgan, Bristol-Myers Squibb SA, Switzerland) dissolved in the drinking water of the rat was used for 3 to 5 days. Following surgery, the wounds of the rats were controlled, cleaned and disinfected for 3 days with a Povidone-iodine solution (Betadine®), a Dexpanthénol spray (Bepanthen®, Bayer, Switzerland) and lidocaine cream (Emla® 5%). This was sometimes done after anesthetizing the rat with isoflurane when the wound needed extensive care.

4.1.5 Fear conditioning protocol.

4.1.5.1 Introduction to protocol

The main goal of experiment A is to test whether the chronic detachable earphone holder is an appropriate EMDR stimulation delivery system. To achieve this, we designed a protocol which would pair a tone (CS) given through the earphones with a shock (US). The CR and UR in this experiment, is a stereotypical behavior observed in rats, called freezing.

As the notion of stimulating the patient on both sides of the body is essential in EMDR, we wanted to test if the rat was able to differentiate from which earphone the tone was coming. To do this, we designed a protocol in which the rat would be exposed to tones coming from either the left or right earphone and only tones coming from a specific side were paired with the US.

As we apprehended an indiscriminate fear reaction to the tones irrespective of their origin we decided to further differentiate the tones coming from the right or the left earphone by differentiating them in frequency. As such all tones coming from one earphone were at 5 kHz or 15 kHz while those from the left were of the other frequency. The testing phase was designed to be able to assess which modality of the tone was associated with the shock, the laterality, the frequency, or both.

4.1.5.2 Pre-experimental phase

Prior to experiment A, the rats were handled for three days by the experimenter to reduce experimenter-induced stress. The earphone device was placed on the rats, the earphone position adjusted next to ears and the rats were placed in the experimental cage for 15 minutes the two days before the experiment. As it was time consuming to successfully attach the earphone holder to the implant in awake rats, the rats were shortly anesthetized in an induction chamber with isoflurane, until unconscious, and then removed from it and quickly fixed with the earphone holder. The maximum anesthesia duration was approximately

90 seconds. After anesthesia, a fifteen minute waiting period after full recovery of motor functions was put in place before placing the rat in the experimental cage.

4.1.5.3 Experimental setup

The experimental setup consisted of a 30.5 x 24.1 x 21.0 cm fear conditioning box (Med Associates Inc, Model: ENV-008, USA) with a floor made of metal bars through which the electrical shock was given. A custom-made Plexiglas roof was designed consisting of a Plexiglas sheet with a 1 cm wide gap along its length at the midline, through which the earphone cable was connected. The cable of the earphones was taped with adhesive tape to make it stiff to avoid entanglement. This combined to the custom roof proved efficient in preventing entanglement. The experimental cage was placed in a wooden box to screen the animals off from unwanted external stimuli and a webcam was placed in the box, filming the cage from the top, for visual and audio recordings of the experiments. The cage was cleaned with a disinfecting solution (Deconex®, Borer Chemie AG, Switzerland) in between every step of the experiments to reduce contaminants which could influence animal behavior. To differentiate the context of the conditioning and testing phases, the beams on the floor of the cage were covered and a new scent (basil powder) was added during the testing phase. However following an apparent high level of freezing on the day of testing, an additional day of testing was conducted in a plastic box containing wooden chips, with raised walls and no roof (35 x 22 x 35 cm). The plastic box was placed in the same wooden box as in first day of testing. The use of the plastic box was done in hope of avoiding a fear reaction to the context which seemed apparent in the first day of testing.

4.1.5.4 Habituation phase

During the habituation phase, the earphone device was mounted on the rat, and the animal was placed in the experimental cage. A first series of ten tones lasting for 5 seconds each, all at the same frequency (all at 5 kHz or all at 15 kHz) was played in the right earphone. A second series of ten, 5 seconds tones of a different frequency (5 kHz or 15 kHz, depending what was used in the first series) was then played from the left earphone with a random delay of 90 to 180 seconds in between each tone. This allowed the rat to get habituated to tones being played in the earphones without associating them with the shock.

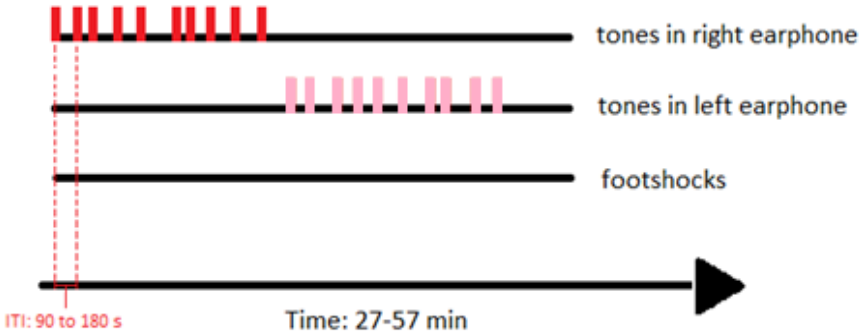

Figure 10 : Habituation protocol: ten tones (red rods) coming from the right and then ten tones (pink rods) coming from the left were played with an inter-trial interval (ITI) of 90 to 180s. Tones coming from the right were of a specific frequency, either 5 kHz or 15 kHz, while those coming from the left were of the other frequency (5 kHz or 15 kHz).

4.1.5.5 Conditioning phase

During the conditioning phase, eight tones of five seconds, of respective frequency and origin as in the habituation phase, were played in a random sequence with random delay of 90 to 180 seconds. Only tones from the left earphones were paired with a co-terminating shock of 0.5 mA.

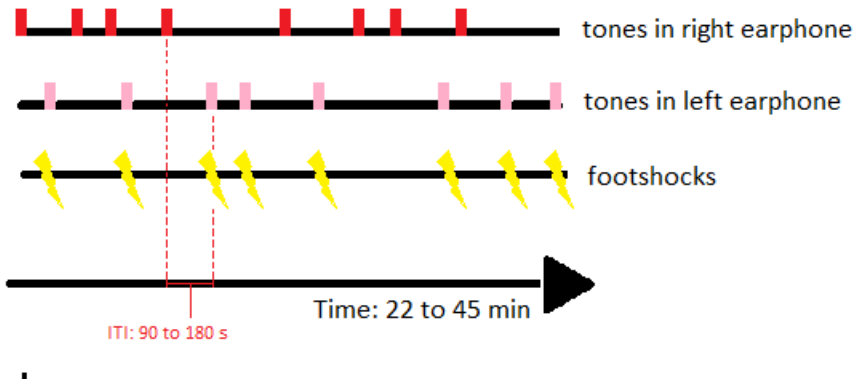

Figure 11: Fear conditioning protocol: eight tones (red rods) were played in each earphone in a random sequence with a inter-trial interval (ITI) of 90 to 180 s. Only tones coming from the left side (pink rods) were paired with a shock (yellow thunder).

4.1.5.6 Testing Phase

The testing phase was designed to test which modality of the CS was learned. The CS had two characteristics, its origin (from which earphone it came) and its frequency. For example, the CS could come from the right earphone, and be 5 kHz.

Thus the testing phase consisted of 16 presentations of tones with a random delay of 60 to 180 seconds, divided in four sequences (Figure 12): the first to fourth presentation tested the CS which was paired with the US, with both the correct origin and frequency. The fifth to eight presentation were of different origin and frequency as the CS. The ninth to twelfth were of same frequency but wrong origin. And finally the thirteenth to sixteenth were of the same origin but wrong frequency as the CS. A two minute pre-trial phase preceded the CS presentation during testing to measure levels of freezing before CS presentation.

As mentioned earlier, the initial plan was to test the rats with one sequence of 16 presentation of tones during one day. However following the day of testing and having observed a very high level of freezing throughout the phase (even before tone presentation), an additional day of testing was added in a different context (detailed in chapter 4.1.5.3). Overall the rats underwent two days of testing, with one sequence of 16 tones presentations per day.

	Same frequency as CS	Same origin as CS
Trials 1-4	✓	✓
Trials 5-8	✗	✗
Trials 9-12	✓	✗
Trials 13-16	✗	✓

Figure 12: Table summary of testing phase trials: modalities of the different trial sequences are compared to the CS which was paired with the US.

4.1.6 Behavior Analysis

To measure the level of freezing during the experiment, all phases of the experiment were video recorded and analyzed in their entirety. The thirty seconds period following CS presentation was also specifically analyzed. The time expressing the following behaviors was measured: freezing, grooming, and exploration. Freezing was defined as the complete absence of movement with the exception of respiratory movement. Exploration included any movement not involving freezing or grooming. All videos were analyzed by an experimenter using a Mathlab program while viewing the videos to add up the time in which each behavior was observed.

4.2 Results

The values which will follow are presented in a mean ± standard deviation of mean format. I used three rats for experiment A and during the habituation phase, I measured an average freezing level of 77 ± 15 % (Figure 13). A plot of the behavior following each tone presentation (Figure 14) did not show an overall trend (downward or upward).

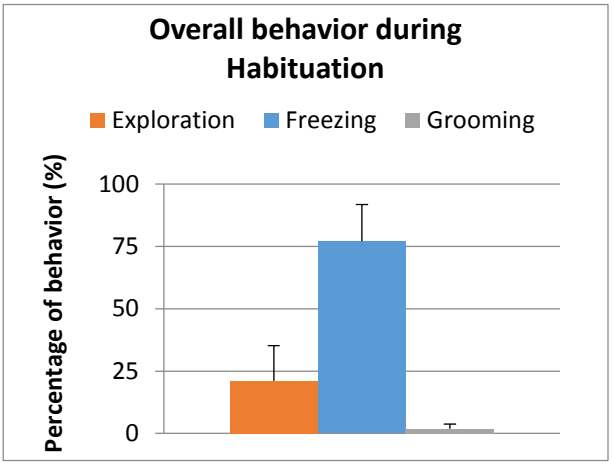

Figure 13: Bar plots of percent of time spent exhibiting a behavior throughout the habituation phase

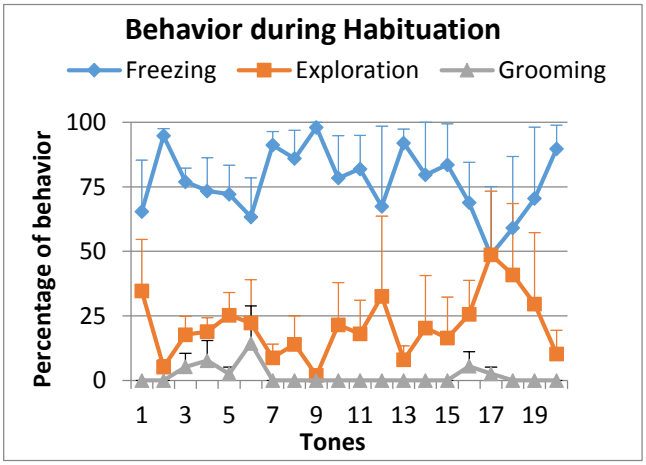

Figure 14 : Behavior during habituation: Plot of the percent of time spent exhibiting a specific behavior through the 20 tons of habituation. Each measuring period started at the beginning of the tone presentation and ending at the beginning of the next of the tone.

On the first day of testing, I measured a percentage of freezing of 77 ± 11 % in the pre-trial phase. In trials 1-4, I measured a level of freezing of 99 ± 1% , 99 ± 1 % for trials 5-8, 100 ± 0 % for trials 9-12 and 90 ± 9 % for trials 13-16 (Figure 16). A breakdown of the behavior for each trial is displayed below (Figure 15), followed by the behavior per modalities of the trial/CS (Figure 16) :

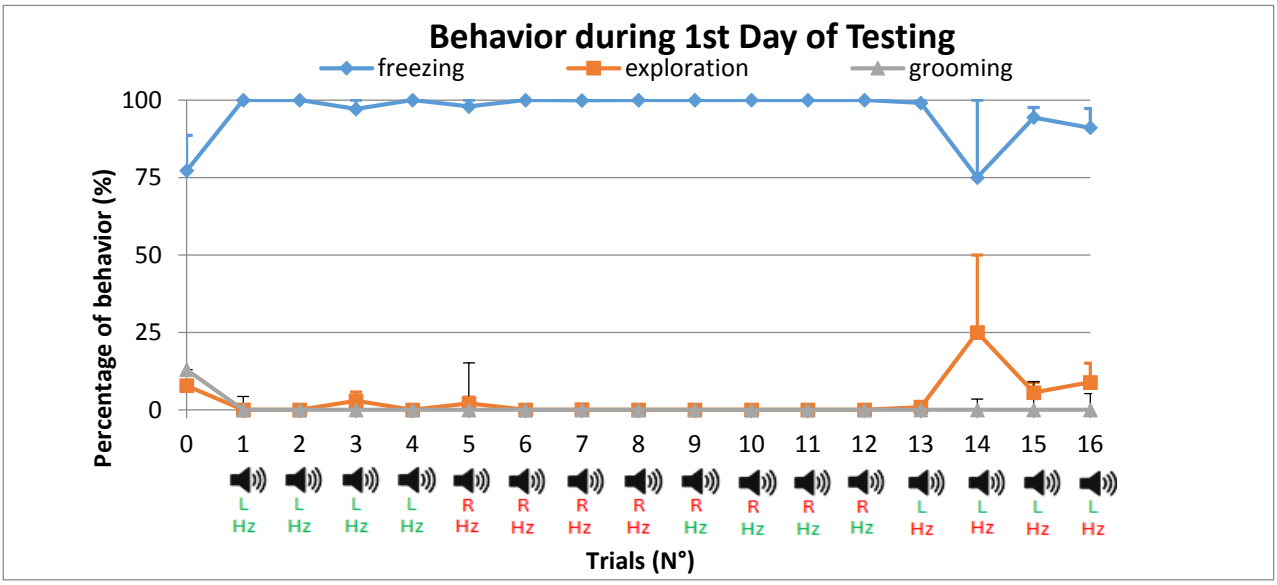

Figure 15 : Plot of behavior during the first day of testing: Percentage of time spent exhibiting a specific behavior during pre-trial phase (point 0) and 30s following CS presentation for each trial. (L=Left earphone, R=Right earphone, Hz = frequency, Green= same as conditioned CS. Red=different to conditioned CS

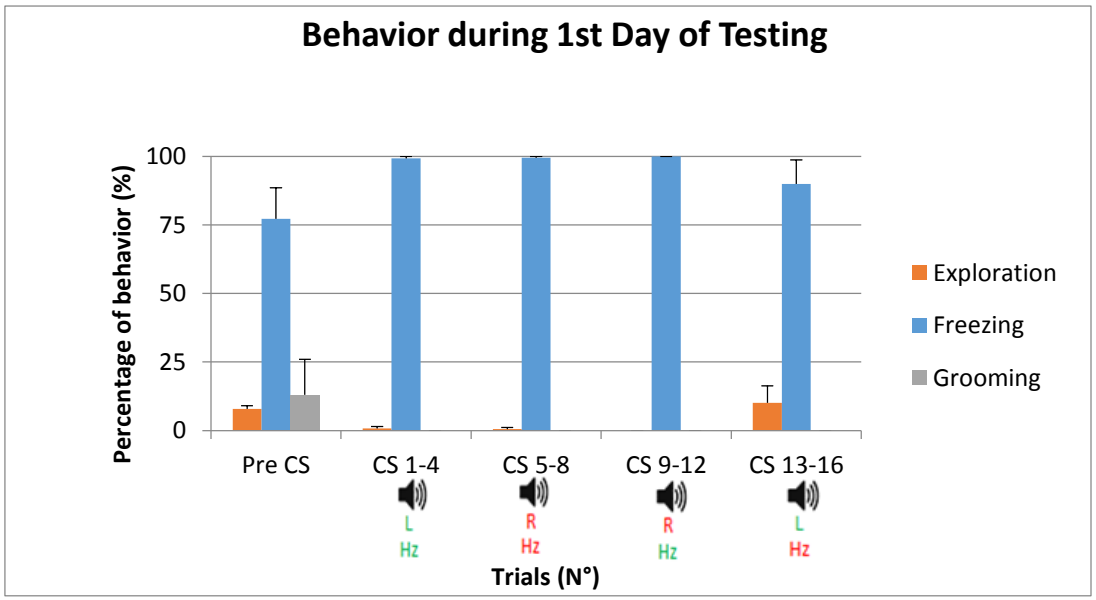

Figure 16: Comparison of behavior in different phases of testing: Bar plots of percent of time spent exhibiting a behavior during the pre- trial (pre-CS) phase and 30 s following CS presentation, per type of the CS (L=Left earphone, R=Right earphone, Hz = frequency, Green= same as conditioned CS, Red=different to conditioned CS)

On the second day of testing, which took place in a different context, I measured a freezing percentage during the pre-trials of 30 ± 14 %, 93± 4% for trials 1-4, 84 ± 11 % for trials 5-8, 98 ± 2 % for trials 9-12 and 85 ±14 % for trials 13-16 (Figure 18). Below is a breakdown of the behavior for each trial (Figure 17), followed by the averages per modality of the CS (Figure 18):

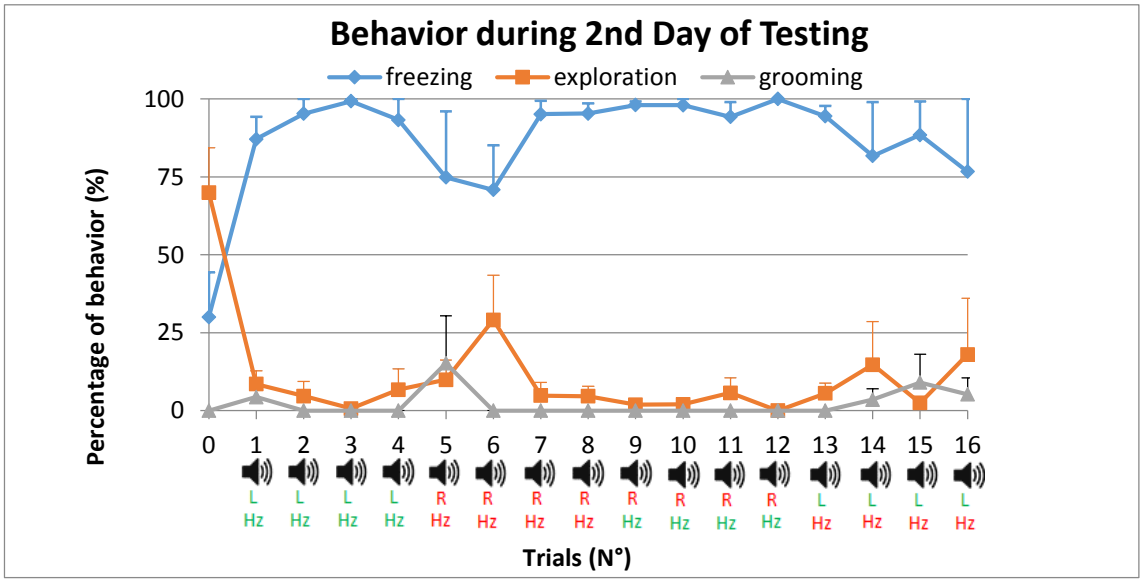

Figure 17: Plot of behavior during the second day of testing: Percentage of time spent exhibiting a specific behavior during pre-trial phase (point 0) and 30s following CS presentation for each trial. (L=Left earphone, R=Right earphone, Hz = frequency, Green= same as conditioned CS, Red=different to conditioned CS

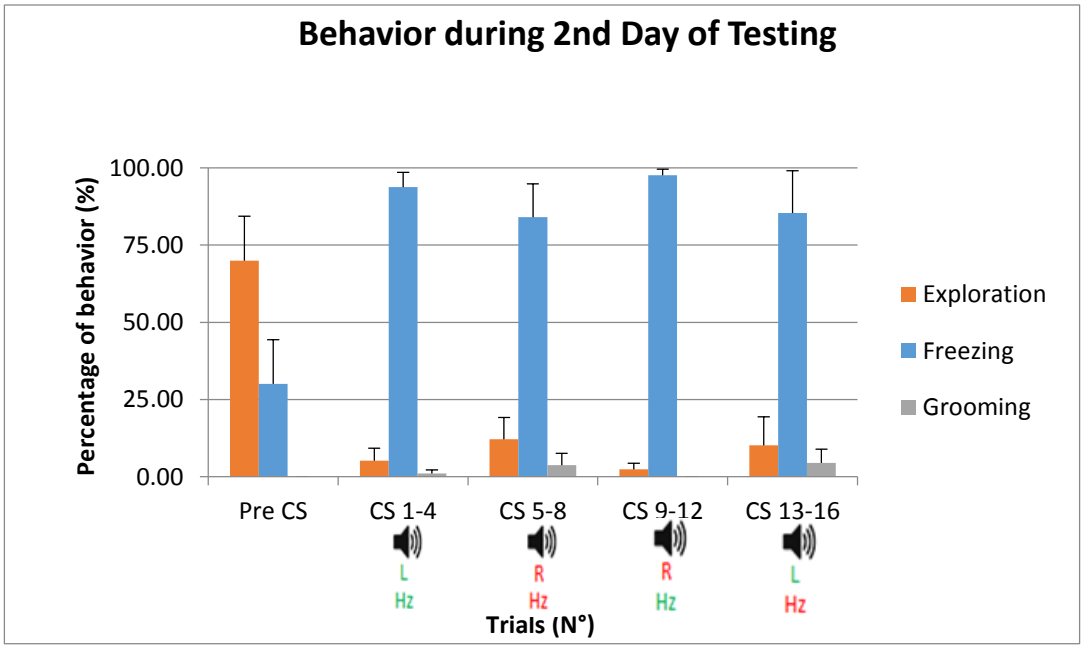

Figure 18: Comparison of behavior in different phases of testing: Bar plots of percent of time spent exhibiting a behavior during the pre- trial (pre-CS) phase and 30 s following CS presentation, per type of the CS (L=Left earphone, R=Right earphone, Hz = frequency, Green= same as conditioned CS, Red=different to conditioned CS

The high level of freezing I observed during the pre-CS phase (77 ± 11 %) on the first day indicated a generalized fear conditioning to the context. In this situation, I couldn't interpret the results of the trial phase, as the high level of freezing could be in reaction to the trials as well as the context. However, on the second day of testing, when the context was further differentiated, I measured a relatively low pre-CS level of freezing (30 ± 14 %) and high levels of freezing in the trials (between 84 to 98%), which clearly indicated fearful reactions to tones coming from the earphones. This confirmed the validity of the sound delivery system. Nevertheless the high level of freezing during habituation (77 ± 11 %) made the interpretation of the results problematic and brought forth the possibility that the tone itself caused freezing, and no CS-US association had occurred.

I did not find substantial differences in the freezing levels of the different modalities of the CS (Figure 16, Figure 18). I did find a very slight decrease of freezing (84 %, 85 % vs. 93 %, 97 %) on the second day of testing, in trials 5-8 and 13-16, both of which are of a different frequency to the conditioned CS. I could interpret this as a higher discrimination of the frequency of the CS, and no discrimination of the origin of the CS. However, the difference in the numbers are too minor to be able to confirm such observations and again the high level of freezing during habituation renders the interpretation of CS-US association problematic.

To conclude, it is undoubted that the rats heard the tones being played in earphones but it was difficult to conclude whether CS-US association had entirely occurred taking into account the high level of freezing during habituation. No discrimination of origin or frequency were brought to light, but the earphone device was confirmed in part to be an appropriate sound delivery system.

4.3 Discussion

Considerations on the level of freezing measured during habituation:

During the habituation phase, a mean of 77% of freezing was measured, making the interpretation of the results in the testing phase difficult. The high level of freezing during habituation could be interpreted as a fearful reaction to the tones irrespective of shocks. While this would prove that the rat can hear the tones, this would make it difficult to observe whether the CS-US association has occurred and would render the testing of the different modalities of the CS impossible. Furthermore using tones for EMDR-like stimulations would become problematic for following experiments. The decibel level of tones coming out of the earphones was measured at 60 dB (usual dB levels of tones used in fear conditioning experiments are between 70 to 85 dB (Buccafusco et al., 2009)), which should exclude a fearful reaction to the intensity of the tone.

Ideally, measuring freezing levels in a control group, going through the same habituation and testing protocols and through a control "conditioning" protocol without shocks, would enable us to see whether CS-US association has occurred or only fear reaction to tones is observed. Additional habituation sessions could also be planned before starting the testing phase, permitting better habituation and thus lower freezing levels. This would consequently allow clearer interpretation of results during the testing phase.

Considerations regarding design of the chronic detachable earphones:

Though I successfully adapted the detachable earphone to rats, a few improvements could be made to improve its ease of use. Indeed, I had difficulties in relation to the fixing of the earphones. As detailed in the Methods section, a short anesthesia was used to simplify the process of fixing the earphones. While its effect on memory was found to be minimal in young rats in literature (Culley et al., 2003), it could have been a source of stress to the animal, even if precautions were taken to minimize it (i.e. Methods), it would be best to be able to avoid this.

The use of anesthesia is a consequence of both the lack of my experience as an experimenter as well as an imperfect design of the chronic detachable earphones. Indeed screwing the earphone holder block to the implant proved to be challenging, as the rat had to be held totally still to screw two screws. Using a system which would enable an initial fixing of the earphone holder with a guide instead of a screw, and which could later be secured with a single screw, would be ideal.

A guide consisting of an aluminum bar which would be placed in the implant beforehand would enable this (Figure 19). Adding a protrusion to the guide would avoid rotational movement of the central block and keep the hole and the underlying bolt of the implant in line to allow easy screwing of the block. This improvement would not require a lot of additional work, as discussed with the biomedical maintenance technician at CHUV.

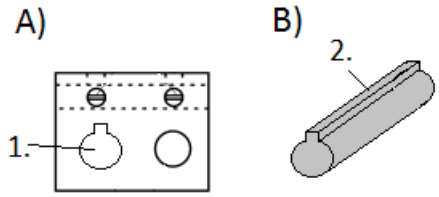

Figure 19: Schematic representation of proposed changes to central block design. A) Central block with hole (1.) in which the guide could be fitted. B) Design of guide which would be placed in the implant, with a protrusion (2.).

Considerations on the inconclusive results on which modality of CS was learnt:

One of the goals of experiment A was to prove that the rat was able to differentiate a tone coming from the left or right earphone. Bilateral stimulation is essential to EMDR and I believed that proving discrimination of laterality when using the earphone device was as an important step in validating it as an appropriate EMDR stimulation producing device. The use of fear conditioning enabled the precise testing of which modality of the CS was associated with the US in a short three day protocol. The drawback of fear conditioning was the risk of generalized fear conditioning to tones irrespective of their laterality. In an attempt to avoid this, the tones were further differentiated by using different frequencies for each earphone. However during the testing phase, freezing levels to different modalities of the CS were similarly high, proving inconclusive as to which one was learnt. Considering the high level of freezing throughout the experiment, I believe a protocol not based on fear conditioning might be better adapted.

I propose an instrumental conditioning experiment, in which an instrumental behavior such as pulling a lever or pushing a button when receiving a CS (such as a tone) is rewarded with food or water. Only rewarding a tone coming from one earphone (CS+) while not rewarding a tone coming from another earphone (CS-), we could study and quantifying the amount of instrumental behavior occurring when presented with the CS+ and compare it with behavior occurring when presented with the CS-. This would avoid the risk of a generalized fear reaction to tones and would measure an easily quantifiable behavior. A Pavlovian incentive learning experiment nevertheless would require a longer amount of time in training than fear conditioning, which wasn't available for this project.

5.0 Part B: Effect of EMDR-like stimulation on fear extinction in fear conditioning experiments

5.1 Introduction

The second part of the thesis focuses on elaborating a way of studying the effect of EMDR-like stimulation on fear memory in rats. In fear conditioning experiments, once a CS-US association has been learnt, the presentation of the CS alone elicits a fear response. Overtime the presentation of the CS alone ceases to elicit the response. This is called fear extinction. The goal of this experiment was to study the effect of EMDR-like stimulation on fear extinction in rats. The first step to achieve this was to find a fear conditioning protocol that would have an appropriate fear extinction curve (Experiment B1 and B2) and then study the effect of the EMDR stimulation on it (Experiment B3).

5.1.1 General methods and materials

In these conditioning experiments, I used a light signal as a CS in order to avoid using the same sensory modality for fear conditioning as for EMDR stimulation (auditory). The CS lasted 20 seconds and co-terminated with the US, a two second long 0.5 mA shock. To maximize visibility of the CS while still being able to see the rat in the video recordings, the experimental cage was lighted with a red LED, a color wavelength rats are less sensitive to (Burn, 2008), while the CS was white light. The rats used in these experiments were reared, housed and operated in the same way as in the 1st part of the thesis. They were also handled for 1 week before experiments to reduce experimenter induced stress. To differentiate the context of the conditioning and testing cages, the beams on the floor of the conditioning cage were covered and a new scent (basil powder) was added to the testing cage.

5.2 Experiment B1

5.2.1 Fear conditioning protocol

The protocol for experiment B1 was a four day protocol with two days of conditioning with three US/CS pairings per day with a two minute fixed interval between pairings (Figure 20). This was followed by two days of testing with three CS presentations per day with two minutes interval between presentations. A five minutes habituation period in which the rat is placed in the cage preceded the start of the conditioning protocol and a three minute pre-trial phase preceded the CS presentation during the testing.

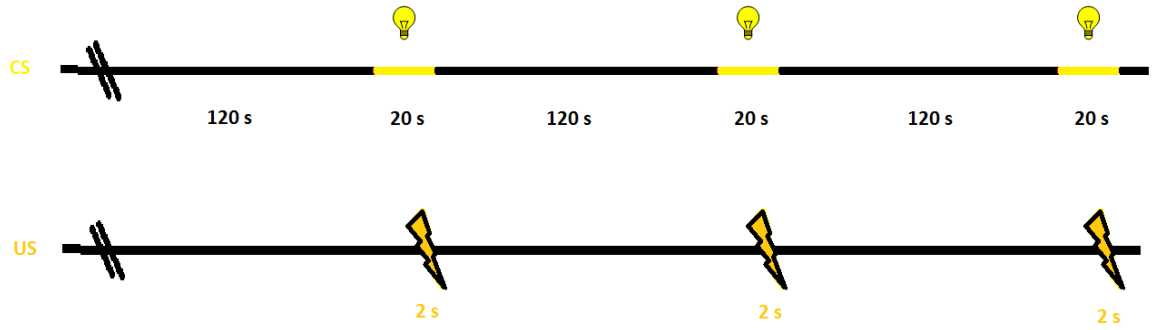

Figure 20: Fear conditioning protocol for experiment B1 which consists of two days of conditioning with three US/CS pairings per day with a two minute fixed interval between pairings

5.2.2 Results

I used one rat for this experiment. During the habituation phase, I measured a freezing percentage of 2.6 %. In the time period including the twenty seconds of CS presentation and the following ten seconds, I measured a freezing level on the first day of 41 %, 69 %, and 27 % for 1st, 2nd, and 3rd CS presentations respectively and on the second day, I measured levels of 47 %, 47 % and 32% respectively (Figure 21).

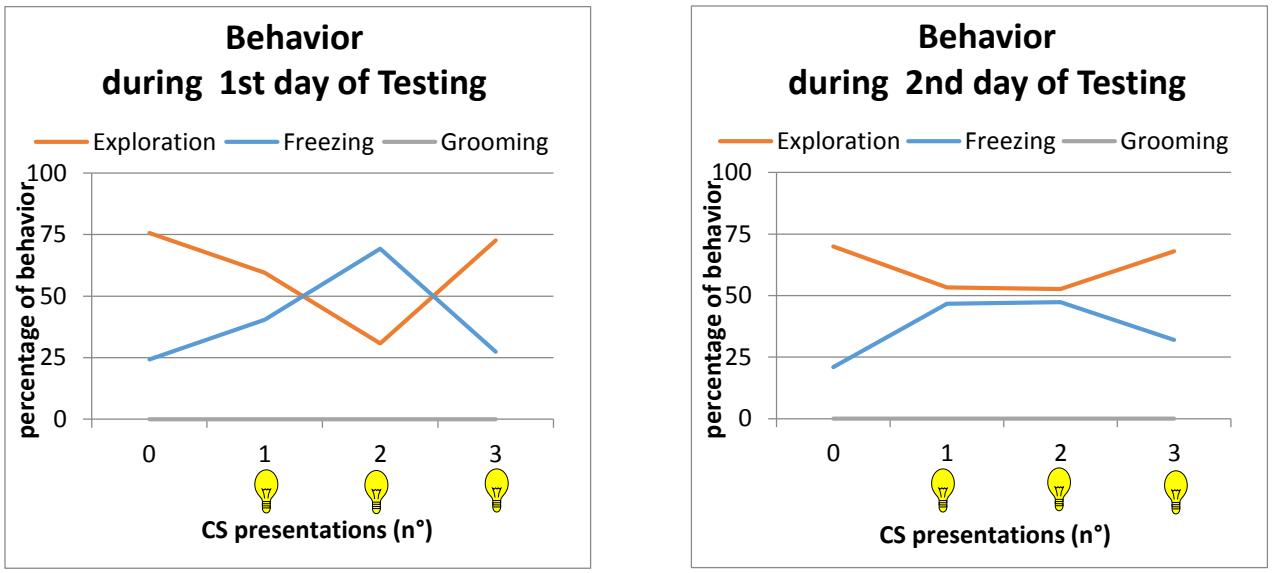

Figure 21: Plot of behavior during the testing: Percentage of time spent exhibiting a specific behavior during the pre-trial phase (point 0) and 30s following CS presentation for each trial.

5.2.3 Discussion

While an increase in freezing was seen during CS presentation, I found this to be insufficient and not long-lasting enough, as I wished for a fear extinction curve that would allow a clear observation of the effect of EMDR. I thus proposed a different protocol and tested it in experiment B2.

5.3 Experiment B2

5.3.1 Fear conditioning protocol

The protocol for experiment B2 consisted of a four day experiment with two days of conditioning with six US-CS parings each day (Figure 22), and which was followed by two days of testing with six presentations of the CS per day. A five minute habituation period in which the rat is placed in the cage preceded the start of the conditioning protocol and a three minute pre-trial phase preceded the CS presentation during the testing.

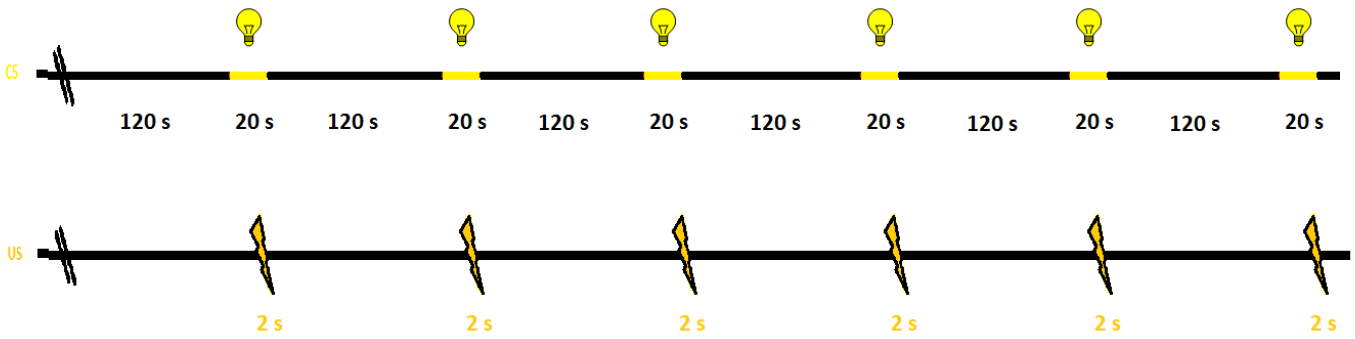

Figure 22: Fear conditioning protocol for experiment B2 which consists of two days of conditioning with six US-CS parings each day and fixed inter-trial interval of 120 s.

5.3.2 Results

In this experiment, a better conditioning was observed. Indeed I measured a freezing level of 3 % during habituation, and during the 20 seconds CS presentation and the following 10 seconds, I measured levels in the 1st to 6th CS presentation of 80 %, 74 %, 62 %, 46 %, 41 % and 50 % respectively on the first day and 55 %, 44 %, 27 %,31 %, 22 %, 6 % on the second day (Figure 23). I measured a pre-trial level of freezing of 31 % and 30 % during the day first and second day respectively.

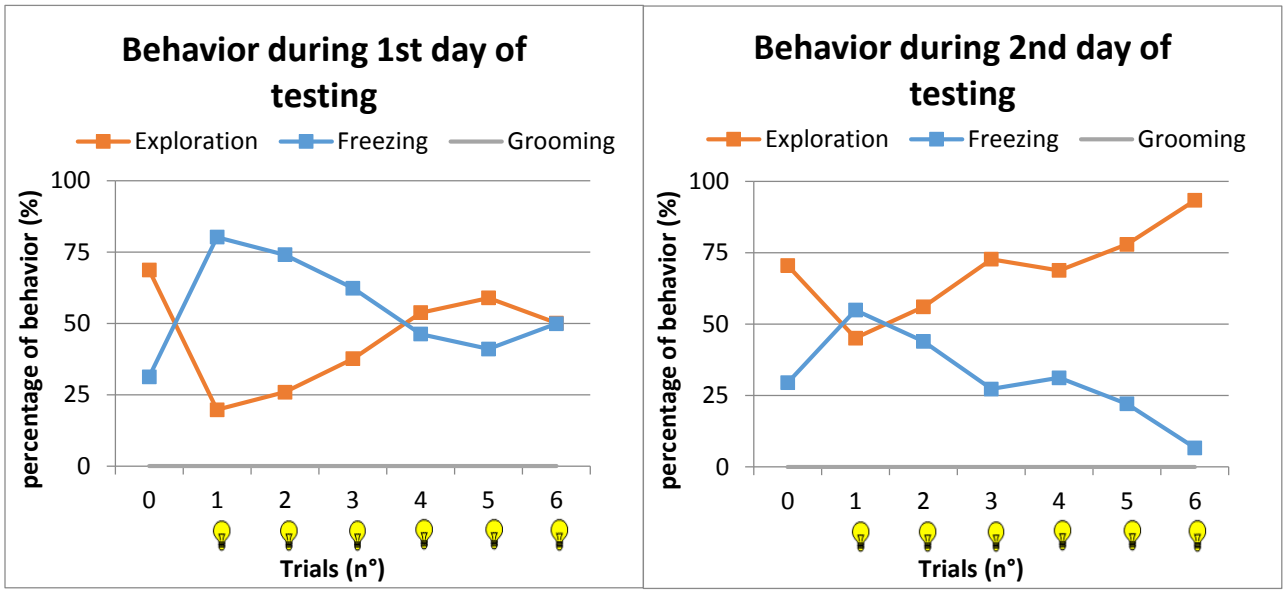

Figure 23: Plot of behavior during the testing: Percentage of time spent exhibiting a specific behavior during the pre-trial phase (point 0) and 30s following CS presentation for each trial.

5.3.3 Discussion

The results of this experiment were promising, I measured a high level of freezing following the CS presentations and a gradual fear extinction curve could be seen over the two days of testing. I deemed this protocol to be appropriate and selected it for EMDR testing.

5.4 Experiment B3

5.4.1 Fear conditioning and testing protocol

In experiment B3 the EMDR-like stimulations were added to the previous protocol. Indeed after two days of conditioning with six US-CS pairings per day, the rat was exposed to two days of testing with six CS presentation per day (Figure 24). During the twenty seconds of CS presentation, the rat received an EMDR stimulation through the earphones (Figure 25). The EMDR stimulation consisted of 1 second 5 kHz tones played alternatively in both earphones at 60 kHz.

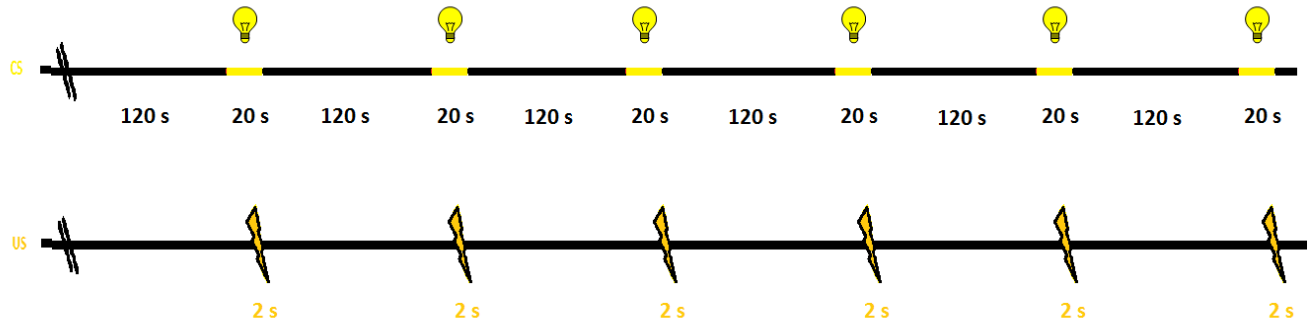

Figure 24: Fear conditioning protocol for experiment B3 which consists of two days of conditioning with six US-CS pairings per day with an inter-trial interval of 120 seconds.

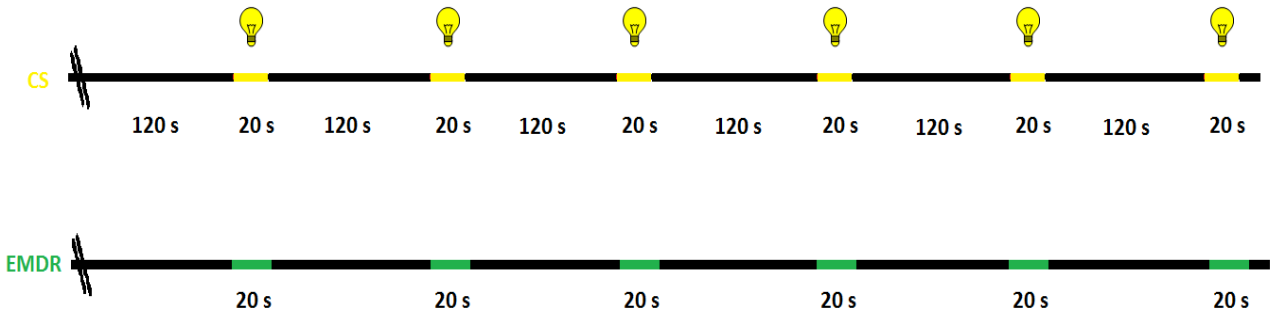

Figure 25: Testing protocol for experiment B3 which consists of two days of testing with six CS presentation per day (inter-trial interval of 120 seconds) with a simultaneous presentation of EMDR-like stimulation.

5.4.2 Results

The results in this experiment were studied taking into account that during the conditioning phase of the protocol, the experiment had to be stopped twice as the rat got tangled in earphone wire. As this was chronologically the first experiment in which conditioning was done with the earphone device, efficient measures were later implemented to avoid this. This includes the construction of the special roof used in experiment A and tapping the wires of the earphones to avoid entanglement. In total the rat received, 4 CS-US pairings on the first day of conditioning and 3 pairings on the second of day of testing.

I measured a level of freezing of 83 % during habituation before conditioning on day 1. During testing, I measured levels of 37 %, 57 %, 73 %, 87 %, 89 % and 100 % on the 1st to 6th CS presentation respectively on the first day. On the second day, I measured levels of 36 %, 98 %, 34 %, 47 %, 29 % and 27 % respectively. In the pre-trial phases of first and second day, I measured levels of 34 % and 51 % respectively.

These results were problematic to interpret because of the circumstances surrounding the experiment, nevertheless it was certain no CS-US pairing has occurred, which means we were unable to study the effect of EMDR stimulation on fear extinction.

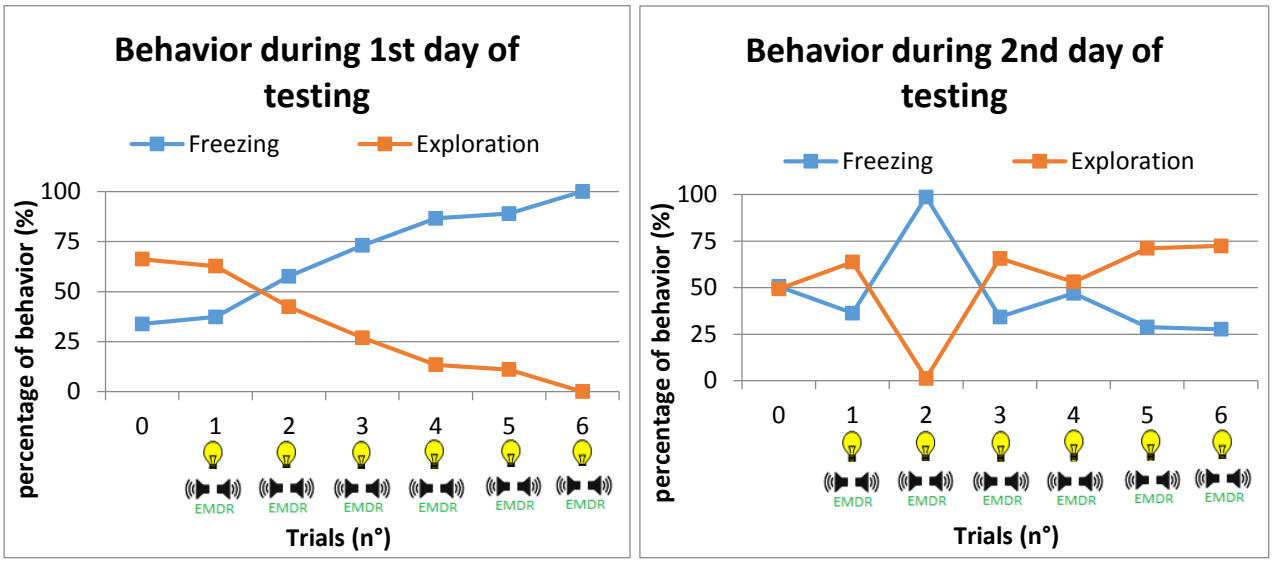

Figure 26: Plot of behavior during the testing: Percentage of time spent exhibiting a specific behavior during the pre-trial phase (point 0) and 30s following CS presentation for each trial.

5.4.3 Discussion

While the results of this experiment were inconclusive, the previously underlined problems with the setup, namely changes to the conditioning box setup and earphone wire (detailed in chapter 4.1.5.3), were corrected for experiment A. All the pre-requisite work to continue the study of EMDR on fear extinction was completed, but because of time limitations on the project, I was unable to continue. I had planned to experiment on 10 implanted rats, divided in a control group and experimental group of 5 rats each. I would have used the same protocol as experiment B3, without the EMDR stimulation for the control group.

<u>Future applications of the animal model</u>

Testing different modalities of EMDR stimulation such as non-alternating intermittent bilateral tones and continuous bilateral tones would be of interest. This would enable to clarify whether the alternating feature of EMDR is crucial to its efficacy.

The long lasting effect of EMDR on fear extinction will also need to be studied and ultimately tested on animal models of PTSD. Multiple animal models of PTSD exist in which "PTSD specific" variables have been measured such as exaggerated startle responses or enhanced glucocorticoid negative feedback (Pitman et al., 2012). Such models include the Predex model, in which an animal is exposed to a predator in an inescapable context, or fear conditioning with additional stress, in which repeated stress introduced before or after fear conditioning enhances fear conditioning (Wurtz et al., 2015). Testing the effect of EMDR on such fear conditioning protocols would further substantiate our EMDR model.

To further understand the mechanism of EMDR, using an fMRI to study which regions are active during EMDR-like stimulation would be interesting. With a few modifications, the chronic detachable headphone could be made MRI friendly: the replacement of metallic components with plastic material such as PEEK , POM or PMMA and the use of MRI friendly earphones which can be found commercially.

A further step could be taken into discovering the pathways of EMDR by using electrodes or optogenetics to stimulate specific brain regions in which activity has been shown during EMDR stimulation and try to replicate the effect EMDR-like stimulation on fear extinction.

Considerations on a different EMDR animal model found in literature

During the final stages of this thesis an article was published in Neuroscience (Wurtz et al., 2015) describing the development of their own animal model of EMDR. The main basis of their model was similar, using fear conditioning as a model of PTSD in mice. On the other hand they used somatosensory stimuli in the form of an alternating electrical impulse to the eyelids. They found significant reductions in the fear extinction rate of freezing and long lasting retention of the fear extinction. This is a major step in creating an animal model of EMDR and should spur even more research in the field. This is encouraging as well as it means we were not far from achieving our goals.

The method used in the article is of similar invasiveness and simplicity as our method, continuing with our model would however enable us to verify if auditory stimuli also have an effect on fear extinction, furthering the notion that it is the alternating left-right application and not the modality of the stimulus which is relevant. If fMRI studies are undertaken, comparing the activity recorded using either the somatosensory or auditory stimuli would enable us to see whether a specific region is activated during EMDR stimulation other than the respective sensory pathways of audition and somatosensation.

6.0 Conclusion

The aims of this thesis were to develop an EMDR stimulation producing device and to study its effect on fear extinction. A previously developed chronic detachable earphone device was successfully adapted to rats and its capacity to appropriately deliver sound was confirmed through a fear conditioning experiment using auditory tones played from the earphones. However a high level of freezing in the habituation phase of the experiment, brought forth the possibility that the tones given through earphones caused a fearful reaction on their own. This will have to be further investigated to fully validate the device. Differentiation of the origin of the tone, which is an important feature of EMDR, was also unclear. Using a fear conditioning protocol to test this might have not been appropriate as a generalized fear reaction to the tones was observed, even if precautions to avoid this were made.

The study of the effect of EMDR stimulation on fear extinction following fear conditioning experiments was not completed in satisfactory manner because of time restrictions, nonetheless an appropriate fear conditioning protocol was found and all practical considerations for further experimenting have been accounted for.

Acknowledgements:

I wish to sincerely thank Professor Ron Stoop for accepting to tutor my thesis, providing a thought-provoking subject matter and of course for his expert advice and guidance through its redaction.

I would also like to thank Mr Danilo Gubian, biomedical maintenance technician at CHUV, for his expert knowledge, assistance and availability.

I also wish to thank my fellow colleagues from the lab, for their companionship and their help in understanding and setting up the required experiments. Special thanks go to Dr. Chloé Hegoburu, Dr. Diego Scheggia, M. Marios Abatis, Mme Ruifang Niu for their time and advice.

Special thanks also goes to my family and friends for their support and encouragement throughout the elaboration of this thesis.

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