Thesis

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Behaviour analysis of the effect of fox pheromones on mice

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1. Introduction

"Transduction of olfactory information occurs when odorant molecules contacts the dendrites of olfactory receptor neurons (ORNs)." (Conn, 2003) The olfactory receptor neurons are found dorsally within the olfactory epithelium in the nasal cavity. These neurons extend to form the olfactory nerve. When the ORNs are stimulated they start the activation of olfactory G-protein, this is followed by several events leading to a generation of action potential which goes along the olfactory receptor neurons projections into the olfactory bulb. (Conn, 2003)

The vomeronasal organ (VNO) is another olfactory organ. This can be found in the midline of the nasal septum in many species, but not in all. Humans does not have this organ. VNO receptor neurons are structurally similar to the ones in the ORN, but they are mostly stimulated by non-volatile molecules like pheromones. These neurons extend to the accessory olfactory bulb (Conn, 2003).

Communication between individuals is done when the sender sends encoded information to the receiver which is decoding it (Green and Marler, 1979). Shorey states that "Communication is the mechanism through which these social animals interact with each other and by which they are organized according to their relative statuses and functions." (Shorey, 1976). Communication is highly essential and absolutely necessary for a species to survive. Communication can be chemical (smell and taste), mechanical (sense of touch or sound) or related to radiation (vision). Shorey states that chemical communication is actually the major way of transmitting information.

Pheromones are produces by the animal´s specific pheromonal glands. When an animal receives "releaser pheromones" it will respond with an instant behavioural change, induced by its nervous system. "Primer pheromones" on the other hand will cause long term changes in the receiver's hormonal system. Pheromones are secreted by specific glands localized mostly on the thorax, abdomen or the legs of the animals. Different species have different glands on species specific locations. The pheromones can either be released into the air, or they can be placed onto objects in the surrounding environment. The receiver animal then detects the pheromones trough the vomeronasal organ, as described above. (Shorey, 1976)

Laboratory animals, just like other prey animals, have an essential need for communication, especially for their survival. Vocalization can be used as primary alarm signal as this is the fastest message that can be sent and received, compared to odour

secretions, which has a delay between secretion and detection by another individual (Arakawa et al., 2008). However, the odours can provide valuable and long-lasting information to surrounding individuals about gender, reproductive status, dominance, identity, nutritional status and more. The mouse urine contains “major urinary proteins” (MUP) which are diverse, volatile proteins. MUP´s are excreted in high amounts in the urine of adult mice of both sexes, but mostly in males. MUP´s are individual and can help mice with distinguishing their own scents from other´s. A dominant male mouse will mark his territory by voiding many small amounts of urine around his area, resident males will then respond by counter marking (Brennan, 2001). The amount of urine marks can help with distinguishing aggressive and dominant males from the submissive ones. When it comes to reproductive relationships female urinary odours plays a huge role as this gives the males information about where the female is in her oestrus cycle (Arakawa et al., 2008).

2. A survey of literature

2.1 The olfactory system

Olfaction is most mammals´ main chemosensory tool. The olfactory system is made up of two main systems; the vomeronasal organ and the olfactory epithelium.

Firstly the vomeronasal organ (VNO) detects pheromones and other chemical substances (Lledo et al., 2005). It was detected by Ludvig Jacobson in the 1810´s. The VNO is placed in the nasal septum, and is a tube filled with mucous, and is enclosed by a capsule made of cartilage. It contains two classes of receptors; the V1R class, placed in the apical epithelium, and the V2R class found in the basal zone. The VNO sends the information detected by the sensory neurons located within it to different places within the accessory olfactory bulb, the V1R class receptors leads to the posterior part, and the V2R class receptors to the anterior part. (Trotier, 2011) The accessory olfactory bulb sends further stimuli to the hypothalamus which then evokes neuroendocrine or behavioural effects, depending on what type of pheromones that were detected. (Brennan and Keverne, 2004) The VNO is an important tool, especially in mammalian reproduction, as it can sense sexual pheromones. It is also essential when it comes to the animals social skills, and survival (Lledo et al., 2005). Lledo et al. states in "Information processing in the mammalian olfactory system" that "It has also been shown that the vomeronasal organ does not have an exclusive function with regards to pheromone recognition, but it responds also to molecules other than pheromones, at least in rodents."

The olfactory neuroepithelium is the other olfactory system. This organ detects all kinds of odorants and sends information to the main olfactory bulb. It is located dorsally in the nasal cavity. (Lledo et al., 2005)

The olfactory system is a complex system that is the middle joint between the outer environment and the animals' brain and nervous system. It must be able to distinguish many different senses and stimulus from each other and send the correct information further into the central nervous system (Lledo et al., 2005).

2.2 Pheromones

Pheromones are one or more molecules which can induce a certain response when detected. They are chemicals that contains a message, also called *"semiochemicals"*. Pheromones can be species-specific and is then called "*homechemicals*", or they can elicit

response between two animals of different species and is then called "*alloelcochemicals*." (Heath, 2007). There are two main types of pheromones: the "releaser pheromones" which leads to sudden behavioural responses, and the "primer pheromones" which elicits neuroendocrine effects, these will both be discussed later.

The author states that "pheromones were originally defined as substances secreted to the outside by an individual and received by a second individual of the same species in which they can release a specific reaction, for instance a definite behaviour or developmental process´" (Wyatt, 2003) Today we know that pheromones not only induces a response in individuals of the same species, but also in individuals of other species.

A Great example of a mammalian pheromone is the "rabbit nipple search" pheromone. The rabbit milk contains a pheromone made up of only one molecule. This molecule induces nipple search behaviour in the young rabbit pups. This pheromone is highly needed due to the fact that the adult rabbit doe only allows her pups to suckle for approximately 4 minutes per day! If a pup does not find the nipple in time, no milk will be ingested for the whole day. As mentioned, the main way for mammals to detect pheromones is through the vomeronasal system, but this is not the only pathway. The authors state that the "rabbit nipple search" pheromone will elicit a response in the pups, even without a working vomeronasal organ. (Brennan and Keverne, 2004)

Pheromones are mostly detected by sniffing it through the air, but can also be detected by direct contact; an example of this is fruit flies that deposit their pheromones into the female, mixed with the sperm while mating. (Wyatt, 2003)

Chemicals in the environment are detected by the receiver´s olfactory sensory neurons and transformed into information. This information can be used in several different ways; to avoid contact with a predator, to find food, and also to interact with other individuals, both sexually and also socially. If the detection of any chemical will lead to a significant increase in survival or success in any way, receptors sensitive to this specific chemical will be selected and expressed in increasing number. (Wyatt, 2003)

2.2.1 Releaser pheromones

Releaser pheromones will elicit an immediate behavioural response in the receiver. An example of this is the previously mentioned "rabbit nipple search" pheromone (Wyatt, 2003). These types of pheromones are recognized by sensory neurons

that sends the received information up to the nervous system which then stimulates motor neurons to cause a certain behavioural response in the animal (Shorey, 1976).

2.2.2 Primer pheromones

Primer pheromones have a long term physiological response by stimulating hormonal production that mostly leads to reproductive or development changes (Wyatt, 2003). An example of this type of pheromone is the fish sperm production stimulation, or the pheromones produced by the bee-queen that prevents development of ovaries in worker-bees (Shorey, 1976).

2.2.3 Pheromonal secretion

It is a huge diversity in types and forms of pheromonal secretory glands amongst different sexes and species. The glands are situated in species-specific places (Wyatt, 2003). They are often made up of modified epidermis. Most mammalian animals have a so called “scent-marking behaviour” where they place their pheromones onto objects. This allows them to leave their smell and message at places they no longer are nearby. Their pheromones are mostly deposited in the form of faeces, urine or skin glands (Shorey, 1976).

2.2.4 Alarm pheromones

A stressed prey often secretes so called alarm pheromones. The molecular structure of these alarm pheromones made by the mice are similar to that of the fox pheromones. Prey use their alarm pheromones to warn and protect their colony, which is absolutely crucial for their survival. Little is yet known about the mammalian´s alarm pheromones chemical structure, other than that they have a warning role and that they are hydrophilic, short lived and volatile. The detection of alarm pheromones and the predator’s pheromones will both take place in the Grueneberg ganglion neurons, which is located in the nasal cavity of rodents, and will induce a similar response in the prey. Both will lead to a certain fear response, that might save the prey´s life (Brechbuhl et al., 2013).

2.3 Inheritance of traits learned from pheromones

Epigenetics was first described by Conrad Waddington. The word "epigenetics" was originally made to explain genetic changes that couldn't be truly understood or explained by genetic principles. "Epigenetics, in a broad sense, is a bridge between genotype and phenotype – a phenomenon that changes the final outcome of a locus or chromosome without changing the underlying DNA sequence." (Goldberg et al., 2007).

Szyf (2014) states that "a study shows that when mice are thought to fear an odour, both their offspring and the next generation are born fearing it." Earlier it has been shown that when a specific pheromonal detection leads to success in any way the pheromonal receptors for this specific chemical will be increasingly expressed. This is not only true for the individual itself, but also for its offspring. This mechanism is crucial for the survival of prey in certain environments, like for example the survival of mice in areas where the fox population is high. (Szyf, 2014)

In an article published by the Nature Neuroscience journal an experiment was done where they subjected F0 generation mice to fear conditioning using acetophenone odour. This led to an increase in the amount of acetophenone sensitive olfactory neurons (Olfr 151). Not only the individuals themselves, but also F1 and F2 generations had an increase in sensitivity to the certain odour. They also found that the F1 generations had an increased size in the part of the olfactory bulb sensitive to the acetophenone. They discovered that the sperm of the fear conditioned mice had a CpG hypomethylation in the gene coding for the specific acetophenone sensitive olfactory neurons. It has long been a question whether the fear is genetically inherited or if it is learnt by the mother when growing up, but this question was answered when they did both in-vitro fertilization, and also cross-fostering, but still the F1 and F2 generations inherited the same fear (Dias and Ressler, 2014).

2.4 Predator odours

Avoidance of predator odours is a useful technique for prey animals to stay alive. These odours are mostly derived from the predator's faeces, urine or skin. Defensive behaviour and habitat shift are the most commonly seen behavioural changes in the prey. Defensive behaviour is unconditioned and differs from species to species, but good examples are the reduction in locomotion and reproduction. The main function of defensive behaviours is to increase the survival rate and reduce the animal's

defencelessness. Non defensive behaviours like foraging and grooming will decrease and only occur on places and at times during the day when it is safe (Yang et al., 2004).

Most prey animals will avoid places where traces of predators can be detected, and several studies previously made have confirmed this theory. One of these studies were described when hedgehogs were introduced to a predator odour in a certain area. These hedgehogs avoided that particular area for up to 4 days later (Ward et al., 1997).

Reproduction will, as mentioned, be significantly supressed during high risk periods. Experiments has shown that female voles generally avoid mating during these periods, and female hamsters shows an irregular oestrus cycle. (Apfelbach et al., 2005)

Hormonal effects will also occur. An experiment was done by File et al. (1993), where they presented a cloth full of cat fur pheromones to rats. This led to a significant increase in corticosterone.

Arakawa et al. (2008) conducted an experiment where they used 59 male mice and measured their scent-marking response to cat odour. Their results showed us that the mice scent marking behaviour was significantly reduced when the predator odour was present.

2.4.1 Predator calls

Hendrie and Neill (1991) states that "the antipredator defence depends on the ability of a prey species to recognise its predator, several studies have been conducted to test the hypothesis that the presence of a predator (signalling danger) activates endogenous (defensive) analgesia mechanism." They wanted to further examine this hypothesis, and they conducted an experiment exposing mice to owl calls. They proved that owl calls significantly increased the laboratory mice defensive behaviour. This suggests that not only odours can cause fear in prey but also the sounds of their predators elicit fear response.

2.4.2 Predator odours as natural repellents

Today many rodent and lagomorph species cause a major problem for the agricultural economy. Toxicants like rodent poisons has been used so far to keep them away, but this also has some disadvantages like for example resistance development and danger to non-target species (Sullivan et al., 1988).

Predator odours has several times been tried to use as repellents, but success has not always been achieved. Some types of prey have been successfully avoiding the areas, while on others the odour has had no effect at all. Experiments has shown that rodents that has already experienced the certain type of predator, from which the odour is derived, will take more care to avoid the areas sprayed with these scents than those who has never been in contact with such animals. Many factors play a role in the odorant's effectiveness as repellents, like for example the pheromonal concentration, from which animal it is derived, and also what kind of diet the predator has had. Another important factor is weather the odour is derived from the predator's faeces, urine or fur. In general success has mostly been achieved by using the fur pheromones (Apfelbach et al., 2005). Another possible way to success is by attracting other predators by the use of pheromonal smell, which will lead to them hunting the prey in the area and scaring them away (Sullivan et al., 1988).

An experiment was done investigating how the change in the predator's diet caused a change in its prey repelling effect. The results showed that a meat rich diet would lead to a strong volatile faecal odour, deriving from the sulphurous metabolites of the meat digestional breakdown, which kept the prey away (Nolte et al., 1994).

2.5 Rodents and pheromones

In the experiment described by Dielenberg and McGregor (2001) they treated rats with cat odours deriving from a piece of a collar earlier worn by a cat and analysed their behavioural response. This was done for several reasons. One of the reasons was to induce an anxiety response in the rat to make a study of human anxiolytics. Another reason for their experiment was to study the use of predatory odours as rodent repellents. In previous experiments it has been shown that the use of TMT elicited a "freezing behaviour" in rats, while in Dielenberg and McGregors experiment, using cat odours, freezing was rarely seen. However, "head out" and other defensive rat behaviours were only seen when cat odours were used, and this was also the only times the rats showed conditioned fear when they were put back into the odour-free test cages. The cat collar odour also caused an increase in the rat´s blood pressure but did not really change the heart rate. They summarized their experiment with concluding that the TMT seems to act like a noisome odour, while the cat odour is acting more like a specific predator odour (Dielenberg and McGregor, 2001).

2.5.1 The Whitten effect

The "*Whitten effect"* is the ability of the male mouse urine potentially has to induce oestrus cycle in the female mice. An environment consisting of only female mice might extend the length of the cycle, induce pseudopregnancies, or in general supress their oestrus cycles. The male mouse volatile substances that are excreted trough their urine alters the secretion within the female mouse of hormones like LH, Prolactin and also the steroids these two hormones regulate. The two volatile pheromones inducing this so-called Whitten effect are called "2-(sec-butyl)-4,5-dihydrothiazole" and "dehydro-exo-brevicomin" These two substances both attract females and also stimulates aggression between males. The pheromonal secretion significantly decrease in castrated males, but if they are treated with testosterone the production will again rise (Jemiolo et al., 1986). In the experiment done by Jemiolo et al. it was shown that groups of 8 females per cage showed a decrease in the oestrus cycle frequency. This fact did not change when the female mice were exposed urine from a castrated male mouse. The females that were exposed to an intact male mouse´s urine all showed a stable and similar oestrus cycle, under all housing conditions.

In Gangrade and Dominic´s experiments they exposed grouped females to a group of male mice and it induced oestrus in most of the females within 7 days, whereas the removal of the males once again lead to irregular and prolonged oestrus cycles (Gangrade and Dominic, 1984).

2.5.2 The Bruce Barton effect

The *"Bruce Barton effect"* is the effect intact male mouse has to induce implantation failure within mated females, elicited by the excretion of 17B-oestradiol (E2) in their urine (Guzzo et al., 2010). Whereas it is believed that the Whitten pheromones are airborne, volatile substances, the Bruce Barton pheromones are most likely not airborne, but acts trough physical contact (Gangrade and Dominic, 1984). In the experiment done by Gangrade and Dominic female mice were housed below a group of alien male mice and this led to failed implantations, while the ones housed above the alien males where not significantly affected. Just like in the Whitten effect, these pheromones can also only be produced by intact males, or castrated males treated with testosterone.

The implantation failure is induced by oestrogen. If male mice are introduced to females, their urinary E2:Creatinine ratio will drastically increase, and PU/PD will also be observed (Guzzo et al., 2010). The Bruce Barton effect was also proved by Dominic, when implantation failure was shown after exposure of newly mated females to both the male mouse urine, but also to the male itself. Stored male urine had a declining effect, and after it had been stored for 14 days, it no longer had any effect on the females at all (Dominic, 1965).

2.5.3 The Vandenberg effect

Colby and Vandenberg have proven that male mouse pheromones also influence the onset of female mouse puberty. Sexual maturation in weaned mice was hastened significantly in the females treated with an intact male´s urine, compared to the ones that were not. It was also seen that it led to a shortened time duration between the vaginal opening and their first oestrus. The use of urine from a castrated mouse was also here unsuccessful (Colby and Vandenberg, 1974).

An experiment was conducted by Drickamer and Murphy (1978) to evaluate the effect of prepubertal and also adult male mice on the sexual maturation of young female mice. They concluded their experiment with that the prepubertal male mouse´s urine has no effect on the induction of the female mouse sexual maturation, unless the males were presented in groups of several mice together. Furthermore, they discovered that the adult, intact, male mouse´s urine played a significant role in the induction of the sexual maturation in the females, both when taken directly from its bladder, and also when excreted naturally, due to its "maturation-accelerating pheromone" content. Lastly they also, surprisingly, discovered that the use of a castrated male mouse's urine leads to an earlier sexual maturation in the females, than what would be seen in females placed alone. This result was different from any other discovery earlier made.

2.6 Fox pheromones

Foxes also communicate by chemicals, like all other animals. They can mark their territory by urine and faces, but also by leaving general body scents. The fox has a tail gland called the "violet gland", due to its flower like smell. This is a multi-lobular, sebaceous gland that secrets 3 volatile apocarotenoids, which are the metabolites formed from breakdown of carotenoids. It will grow in size around the mating season. Complex

lipids have recently been discovered in the tail gland fur and is believed to serve as a protective matrix for the violet gland pheromones, to prolong their mode of action. Carotenoids and their apocarotenoids are believed to be crucial for both animals and plants survival and health. Foxes acquire carotenoids trough eating seeds and fruits, and also trough ingesting prey animals. Each fox has slight differences in their chemical amount and smell, which may tell us that each fox has their own signature smell (McLean et al., 2019).

2.6.1 TMT

TMT (2,5-dihydro-2,4,5-trimethylthiazoline) is a sulphur-containing component of the fox faeces. In addition to finding TMT in fox faeces, it can also be produced synthetically, which is beneficial for experimental studies. TMT is known to elicit fear response in rodents. Rampin et al. (2018) did an experiment using gas-chromatography-mass spectrometry to evaluate the presence of TMT in 24 samples of faeces from 3 different foxes. They compared pure faeces with the faces with an additional, known amount of TMT added. Only 1 out of 13 faecal samples was proven to contain TMT, while 10 out of 11 samples with added TMT were positive. This might be due to that the faecal trimethylthiazoline concentrations were under the threshold level of their gas-chromatography-mass spectrometry which is 50 nmol/g. Rats were then exposed to fox faeces, low quantity TMT, and a control group treated with 1-hexanol. The rats treated with fox faces showed the highest number and duration of freezing behaviour. Next, they exposed the rats to low quantity TMT, cadaverine, 2-phenylethylamine, fox faeces, rat faeces, and a control group with 1-hexanol. Again, the rats exposed to fox faeces showed the highest number of freezing episodes. They concluded with that the fear behaviour induced by fox faces most likely originates form the non-proven TMT content which in this case must be under 50nmol/g, or if the faeces really does not contain any TMT, the fear is elicited by another faecal compound (Rampin et al., 2018).

An experiment was done to check if TMT really stimulates unconditioned fear in rats. They measured the rats freezing behaviour in a medium sized cage where the rats had places to hide from the TMT odour, versus in a smaller cage where the rats had nowhere to escape. They saw that indeed the rats avoided TMT but also other foul odours like butyric acid and isoamyl acetate. In addition, they saw that the rats showed a higher duration of freezing behaviour than when they were treated with other odours. The

freezing behaviour increased significantly after 5 minutes, suggesting that it took several minutes for the TMT to be inhaled in sufficient levels before the response kicked in. In the small test cage they saw that freezing was the dominant behaviour, and freezing was shown at the rats very first meeting with TMT which indicates that this is an unconditioned fear rather than a learned trait (Wallace and Rosen, 2000).

Another experiment was done by Morrow et al. (2002), where they exposed rats to TMT and when they were placed in a large and brightly lit open-field cage it resulted in a fear response, in the form of freezing. This, however, did not occur in a small and familiar open field cage with dimmed lights. This may suggest that fear response also depends a lot on environmental exposure, and not just on the odour itself.

A study was done that showed a significant increase in activity in different locations of the brain which plays a role in the control of unconditional fear and endocrine stress response. Especially the bed nucleus of the stria terminalis (BNST) was thought to have an important relevance regarding activation of these responses. They concluded their experiment with confirming that BNST indeed did play a crucial role TMT elicited fear behaviour (Janitzky et al., 2015).

Genné-Bacon et al. (2016) states that "It is well documented that prey animals, particularly birds and mammals, reduce body size when predators are present in the environment." They explain this to be due to the fact that smaller and lighter animals can more freely move and run around, and hide in small places, compared to overweight ones. They conducted an experiment where they treated mice with long term threatening scents, where mT (an odour very similar to TMT) was one of them and undiluted butyric acid, being a noxious, non-threatening scent being another one. They saw a significant decrease in body weight gain in the mice exposed to mT daily, long term, compared to the ones that were exposed to the non-threatening but noisome odour. The Butyric acid group was actually very similar to the control group in body weight gain. Interestingly they did not see a significant difference in neither food intake, nor locomotion in the different groups. They stated that the underlying mechanism for the decreased weight gain still is unclear and that it should be considered to look more into it in future studies.

2.7 Humans and pheromones

Human body odour and pheromones are mostly excreted by the apocrine glands, found around the genitals, under the armpit, and on the chest. These glands only gets active at the time of puberty (Kohl et al., 2001).

The vomeronasal organ develops in the human *in utero* life. This is important for the reproductive system´s proper development, as the GnRH producing cells originates from the *olfactory placode*, which is a region which will later develop into the VNO and the olfactory region, and travels with the VNO neurons into the brain. Later the VNO is degenerated before the baby is even born. Nevertheless, the fact that humans do not have any functional vomeronasal organ does not mean that no human pheromones exist. (Trotier, 2011)

One of the believed pheromonal effects in humans is the pheromonal secretion by women, from their armpits, relating to what phase of the oestrus cycle they are in, affecting the LH and FSH levels of other women, resulting in the synchronisation of the oestrus cycles of a group of women living together (Brennan and Keverne, 2004). Another human pheromonal study showed that men smelling clothes previously worn by ovulating women had a higher increase in blood testosterone, compared to men smelling clothes of non-ovulating women. Observations has also been made that shows us for a fact that human mood generally improves, both in males and also in females, after smelling a satisfying odour. It seems like human response to odours is mostly psychological, in comparison with animals responses which often is physiological, like for example freezing behaviour in rats (Trotier, 2011).

2.8 Pheromonal use in practice

Cats detect pheromones by conducting a so called “Flehmen behaviour” while the dogs squeeze their tongue towards their hard palate rapidly (Hewson, 2014). Today several commercial cat and dog pheromones are on the market, said to have several beneficial effects, like for example having calming and anxiolytic effects, reducing urinary marking and also increasing food consumption in stressful surroundings, like at the veterinary clinic. Some are more effective than others.

2.8.1 Cat commercial pheromones

Cats leave their odours and pheromones around in their territory by rubbing and scratching objects, and also by urination and defecation (Silva et al., 2017). The cat´s sebaceous glands are mostly located on the head, digits, and in the perineal region. They feel comfortable when feeling the odour of themselves, and more tense and nervous if strong odours from other cats are present in the area. Salivary and plasma cortisol has been used to measure stress levels in cats. Five types of feline facial pheromones have been discovered until now, and two of them are widely used especially at veterinary clinics. The F2 pheromone is mostly used by the cats in a sexual context, F3 is mostly secreted when the cats are rubbing on objects and F4 is secreted when cats rub on others and mark each other, humans or other animals with their own scents. Lastly F1 and F5 are two other feline facial pheromones recently discovered but their purpose is still unknown (Vitale Shreve and Udell, 2017).

Several synthetic cat pheromones are on the market nowadays. One of them is the "*Felifriend*" spray, which contains a F4 fraction of the cat facial pheromone and is supposed to have an anxiolytic effect on cats when sprayed in the air and is therefore often used at veterinary clinics to calm the cat patients down. Studies have proved its effect in calming down aggressive cats during veterinary consults. This pheromone can also be applicated on the palms of the handler (Heath, 2007).

Another popular cat pheromone is the *"Feliway"* spray, which similarly to the Felifriend contains a synthetic analogue of the F3 fraction of the facial pheromone. This was the very first synthetic pheromone ever to be produced (Vitale Shreve and Udell, 2017). Cats mark areas with this pheromone when they rub their face and cheeks along objects. The cat feels safe when it smells its own pheromones, ensuring him/her that he/she is in its own territory (Hewson, 2014). Feliway is said to have a urine marking reducing effect, and is also used to decrease stress during transportation, and in other situations as it has a calming effect (Bakker, 2014).

Several studies have previously been made to investigate the true effect of both the F3 and the F4 facial pheromone fractions, some more successful than others. An experiment was conducted where they tested the effect of using a synthetic feline pheromone analogue containing gel at the veterinary clinic, during the physical examination of cats. Their results suggested that the gel indeed did calm the cats down successfully (Mills, 2005). In another study 30 FIV-positive cats, without any severe clinical signs, were used. Their saliva was collected, and salivary cortisol levels measured

on day 0 and then again after exposure to a synthetic F3 facial pheromone fraction on day 30. As much as 75% of the cats showed lower salivary cortisol levels after pheromonal exposure (Silva et al., 2017).

2.8.2 Dog commercial pheromones

The dog appeasing pheromone (DAP) is excreted post whelping by lactating bitches trough their sebaceous glands, and has a calming effect on the puppies (Landsberg et al., 2015). It is only excreted from day 3-4 post parturition until 2-5 days after weaning (Hewson, 2014). It has been used in several settings like at veterinary clinics, to treat separation anxiety, stress induced behavioural problems and during noise exposure (Landsberg et al., 2015).

Landsberg et al. (2015) states that "Noise induced fear and anxiety is a significant behaviour concern of dog owners." They conducted an experiment using 27 beagles. The dogs were divided into a placebo group and dog appeasing pheromone containing collar groups and exposed the dogs to a thunderstorm stimulation test. They analysed their behaviour, and examined their active, passive and global anxiety. A significant decrease was seen in both the active and the global anxiety in the dogs using the DAP collars compared to the placebo group. The collar using dogs also showed an increase in hiding behaviour during the thunder storms. They concluded their experiment with stating that "these findings support a possible use for DAP in the prevention and management of noise-related fear and anxiety" (Landsberg et al., 2015). Another experiment was conducted using 30 dogs, 16 males and 14 females, with previously known anxiety for fireworks. 22 of the 30 dog owners reported that the pheromones resulted in successful decrease in anxiety level, and 21 of the dog owners mentioned that they were either "very satisfied" or "mainly satisfied". The experiment was concluded with that dog appeasing pheromones indeed does have a successful effect on reducing anxiety levels in most cases. (Sheppard and Mills, 2003)

2.8.3 Horse commercial pheromones

Domesticated horses' instinct is to flee when meeting stress and danger. This is not advantageous for the rider. Neuroleptics have been tried for the treatment of horses with performance problems, but these have several side effects, and their use is also restricted by some anti-doping regulations (Falewee et al., 2006).

Falewee et al. (2006) conducted an experiment investigating the beneficial and stress reducing effects by using a synthetic equine appeasing hormone (EAP), this is a hormone excreted by the lactating mare, to calm down its foal. 40 horses were included in the experiment, and fear was elicited by moving the horses trough a fringed curtain. The horses that were treated with equine appeasing hormone did not stop as much as the control group, and also they had both a lower mean heart rate and also max heart rate. They resulted in that EAP is beneficial to use on some horses in stressful situations like veterinary treatments, horse shoeing, and other events, but it should be given 20 minutes before the stressful event, as it takes some time before the effect is observed

3. Aim of the Research

The behaviour analysis of the effect of fox pheromones on mice was conducted with the intention to induce smell related fear behaviour in laboratory mice, both males and females. Their anxious behaviour is important to evaluate and analyse for different reasons. It is important for us to understand what triggers fear response in laboratory animals, how it is triggered and also how to measure and control it.

When conducting this experiment, we were able to test the ability of fox pheromones, in this case artificially produced TMT, to act as a reliable fear inducing source, and if all mice responds to it in the same way, or if we have individual differences amongst the mice coming from different genetic lines with different epigenetics.

One of the reasons we induced fear and anxiety in these laboratory animals was so that we, in the future, possibly can be able to test human anxiolytic and anti-depressive drugs and their potential effects in live animal trials. Another reason for carrying out this experiment was to test TMT´s effect as rodent repellents that can possibly in the future be used to keep rodents and other pests away. We also wanted to know if the use of TMT is successful due to its actual pheromonal effects, or if the fear is simply caused by the high odour concentration causing a general reaction to a bad odour. This is why we treated the mice both with TMT 50% concentration, and also with TMT 100% concentration, as this is a crucial question to be answered.

Our main goal was to evaluate the effect of TMT pheromones on mice, while documenting and analysing how an anxious mouse responds to the fear, so that we in the future possibly can use this data for further experiments.

4. Materials and Methods

4.1 Animals, keeping, odours and climate

The experiment was conducted in the University of Veterinary Medicine Vienna. To conduct our experiment, we used all together 24 mice where 12 of these were females and 12 were males. This was intentionally done to examine if there are any gender differences when it comes to fear response. They were all B6D2 hybrids, F1 offspring of C5BL/6J (B6) female and DBA/2J (D2) male parents, and they were bought from the Jackson Laboratory. They were SPF mice, and all of them were 6 weeks old at the start of the experiment.

They were housed in 6 different conventional polycarbonate cages of 26x21x14 cm (**Figure 1**), 4 mice were placed in each cage, and separated randomly by gender. Here they were kept until the experiment started. The mice were given a breeding diet for mice and provided with ad libitum good quality tap water.

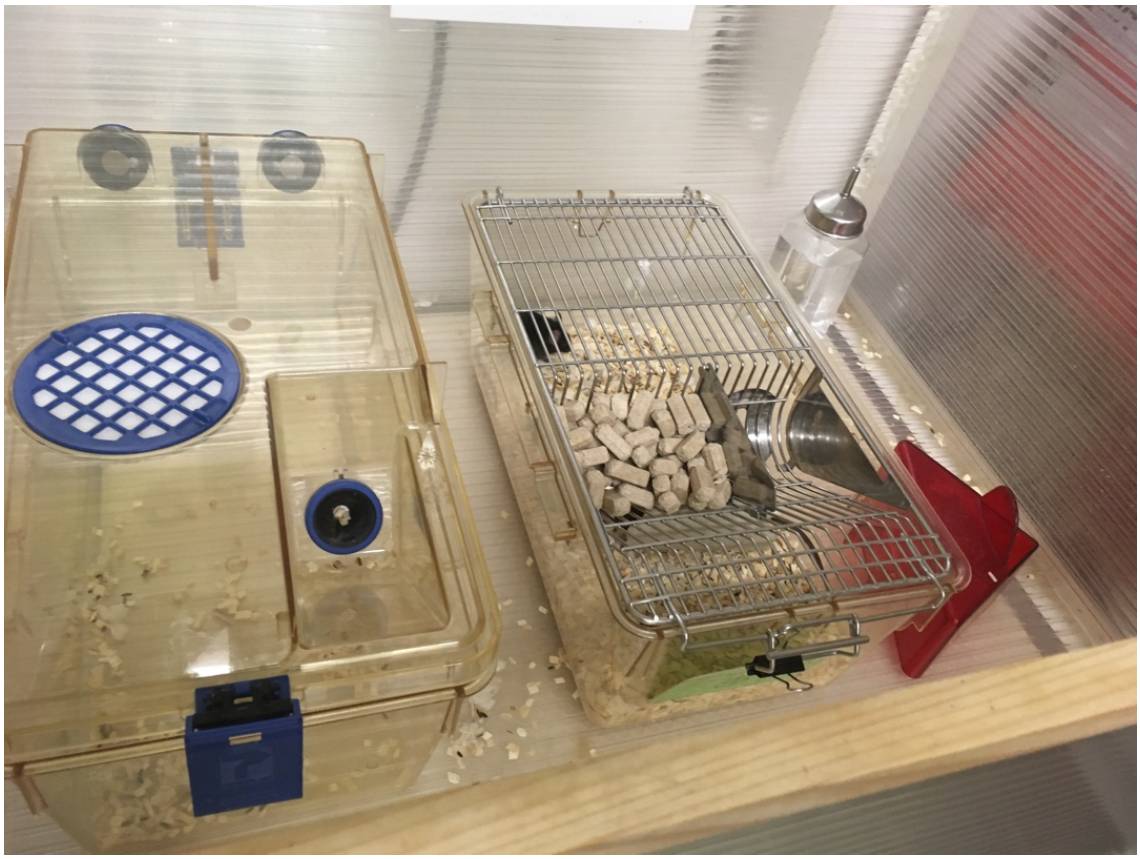

Figure 1 Conventional polycarbonate cage (on the right side of the picture) where the mice were placed. Four mice of the same gender in each cage.

The experiment was carried out during the daytime. Artificial lighting was used with a light cycle of 12 dark hours and 12 light hours. The light was turned on from 6 am until 6 pm every day.

The mice were acclimatized in the sex separated cages for 6 days after they were purchased. The testing itself was done in an open field cage which was completely separated from their normal environment. The mice were then gradually adapted to the test cage environment by placing them in the test cages for 15 minutes each on day 7, before starting the control part of the experiment on the next day.

All together 5 different odours were used to conduct this experiment. On day 1 of the experiment we treated the mouse with water on paper, used as a control odour, on day 2 they were exposed to citronella oil, and on day 3 they had a resting day. On day 4 50% concentration of TMT (2,5-dihydro-2,4,5-trimethylthiazoline) was used, followed by resting from day 5 until day 7. On day 8 they were treated with 100% concentration of TMT followed by rest on day 9. On the last day, day 10, Met-OH analogue was used on them.

The open field test cage was cleaned out and sterilized after every single mouse had been there. The open field had a 70 dB ventilator. The average temperature was 20-23 °C, and the relative humidity was 35-40%. The open field cage was completely ventilated after each trial in order to remove any leftover odour that may have still been there from the previous mouse trial.

4.2 Behaviour

In order to investigate the level of anxiety and fear in the mice we had to evaluate their movement and placement within the open field cage. The behaviours we aimed to check was their movement and location. A mouse shows fear by freezing behaviour, which is when the mouse stands still and is motionless. It will move a lot more when it feels safe in comparison to when it is nervous. The mouse will be a lot more in the edge, and especially in the corner of the box when it is anxious but will be more in the centre if it feels comfortable. We investigated the mice by filming them throughout the experiment. These behaviours are good indicators to the mouse´s wellbeing and comfort.

We divided the behaviours into two different groups. One of them being locomotion, where we examined if the mouse was 1) still or 2) moving, and the other one being location where we checked if the mouse was located in 1) the corner, 2) the edge

or 3) the centre. These groups were both studied and assessed in regard to the amount of time the mouse spent performing them.

4.3 Statistical analyses

All mice were recorded by video filming during the experiment within the open field cage. All of the 24 mice were treated with 5 minutes each of control, citronella, 50% TMT, 100% TMT and Met-OH odours, during the whole experiment. This was all recorded and analysed by using the Noldus Observer XT 14 software. This is a behavioural coding software that assesses reliability and calculates statistics.

One way ANOVA analysis with post-hoc Tukey HSD tests were used to measure the differences between treatments. Finally t-tests were done to compare the two different sexes, and if there is any significant gender differences. We used a threshold level of $p \leq 0.05$ for significance.

5. Results

5.1 Location

The duration of the time spent in the corner, edge and centre of the open field was videotaped and measured. In **table 1, 2** and **3** detailed results can be seen, in regards to the mean time where the mice were located within the open field in seconds, and also the gender differences can be seen.

CENTRE	Female	Male	All
Control	22.97±9.32	27.89±8.04	25.54±8.84
Citronella	28.62±12.20	32.49±36.32	30.56±26.57
TMT 50	25.72±9.98	21.24±14.39	23.48±12.32
TMT 100	22.10±8.17	25.19±14.53	23.78±11.90
MetOH	18.47±9.79	27.23±13.79	22.85±12.52
One-way ANOVA	Df=4,52 F=1.728 P=0.158	Df=4,55 F=0.506 P=0.731	Df=4,112 F=0.937 P=0.445

Table 1 The mean time duration (with standard deviation) that the mice spent in the centre of the open-field

CORNER	Female	Male	All
Control	124.57±16.18 **ab**	125.71±19.77 **ab**	125.17±17.75 **ab**
Citronella	134.51±18.88 **b**	143.46±32.09 **ab**	138.99±26.15 **bc**
TMT 50	137.65±20.01 **b**	152.28±34.03 **b**	144.96±28.31 **c**
TMT 100	107.27±22.56 **a**	117.11±32.57 **a**	112.64±28.26 **a**
MetOH	130.22±18.56 **ab**	121.63±20.58 **ab**	125.92±19.66 **ac**
One-way ANOVA	Df=4,52 F=4.105 P<0.01	Df=4,55 F=3.366 P<0.05	Df=4,112 F=6.241 P<0.001

Table 2 The mean time duration (with standard deviation) that the mice spent in the corner of the open-field. Different letters mark the significance ($p \leq 0.05$).

EDGE	Female	Male	All
Control	151.88±11.64 **ab**	146.01±19.95 **ab**	148.82±16.42 **b**
Citronella	136.41±11.58 **a**	123.58±32.37 **a**	129.99±24.66 **a**
TMT 50	136.13±15.29 **a**	126.03±32.30 **a**	131.08±25.25 **a**
TMT 100	167.89±17.17 **b**	156.92±25.09 **b**	161.91±22.08 **b**
MetOH	150.38±16.00 **a**	149.95±13.28 **ab**	150.16±14.38 **b**
One-way ANOVA	Df=4,52 F=8.956 P<0.001	Df=4,55 F=4.028 P<0.01	Df=4,112 F=9.723 P<0.001

Table 3 The mean time duration (with standard deviation) that the mice spent in the edge of the open-field. Different letters mark the significance ($p \leq 0.05$).

The mice were located at the edge of the open-field most of the time, regardless of which odour they were exposed to, and they always spent the least time in the centre. In general no significant gender differences were seen in regards to the location of the mouse, so here the most of our attention is put on all of the mice seen as one.

The mice all spent a very similar amount of time in the centre, regardless of what kind of treatments they were exposed to.

The mice spent a significantly larger amount of time in the corner when treated with citronella compared to when treated with TMT 100% ($t=-3.656$, $p<0.01$), also a difference was seen when the TMT 50 % treatment induced more time in the corner than the control treatment ($t=2.779$, $p<0.05$) The TMT 50 % treatment also led to a lot longer duration of time spent in the corner than the TMT 100% treatment ($t=4.486$, $p<0.001$)

At last the biggest behavioural differences were seen in time spent at the edge. The highest amount of time was spent at the edge when the mice were treated with TMT 100%. The control treatment induced more time spent in the edge than when treated with citronella ($t=3.066$, $p<0.05$) and also when we used MetOH the mice spent more time in the edge than when exposed to citronella ($t=3.321$, $p<0.01$.) The behaviours of mice when exposed to citronella versus TMT 100% was very different, as the TMT 100% exposure induced a lot more time in the edge than any other odour exposure, and especially more than the citronella treatment ($t=5.139$, $p<0.001$). TMT 50% exposure induced less time spent at the edge than when we used control odour ($t=-2.890$, $p<0.05$), and also less than

the MetOH treatment (t=-3.142, p<0.05). TMT 50% exposure also caused a much less duration of time at the edge than the TMT 100% (t=-4.964, p<0.001).

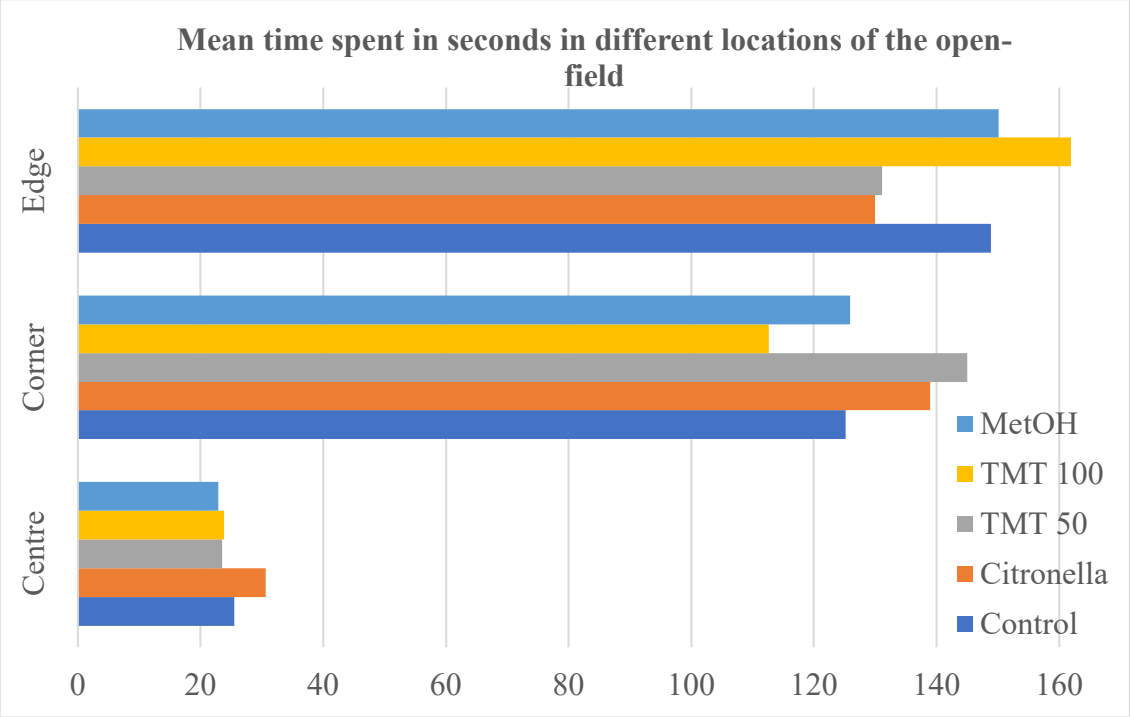

Figure 2 Graph showing the mean time spent on the edge, corner and centre of the open-field

As we can see in **figure 2** most time was definitely spent at the edge, a lot of time was spent in the corners, and the least amount of time was spent in the centre, regardless of which treatment was done. We can see that compared to any other treatment the TMT 100% showed the longest duration spent in the edge, while TMT 50% elicited the longest duration of staying in the corners, and citronella treated mice spent most time in the centre.

5.2 Locomotion

The duration of time when the mice were moving and when they were still was also videotaped and measured. **Table 4** and **5** shows all the detailed mean numbers of seconds the different mice spent moving or being motionless when exposed to different treatments.

MOVING	Female	Male	All
Control	270.13±26.38 **bc**	255.84±21.80 **ab**	264.89±24.64 **b**
Citronella	283.01±12.63 **c**	268.30±16.42 **b**	275.65±16.18 **b**
TMT 50	273.81±20.15 **c**	254.17±32.63 **ab**	263.99±28.35 **b**
TMT 100	245.31±25.41 **ab**	237.37±24.52 **a**	240.98±24.66 **a**
MetOH	225.75±24.03 **a**	232.14±26.82 **a**	228.95±25.11 **a**
One-way ANOVA	Df=4,52 F=13.34 P<0.001	Df=4,55 F=4.14 P<0.01	Df=4,112 F=14.67 P<0.001

Table 4 The mean time duration that the mice spent moving within the open-field. Different letters marks that there is a significant difference ($p \leq 0.05$)

STILL	Female	Male	All
Control	26.49±19.17 **a**	41.10±20.12 **ab**	35.11±20.62 **a**
Citronella	16.59±12.60 **a**	31.20±16.33 **a**	23.90±16.10 **a**
TMT 50	25.56±19.73 **a**	45.30±32.50 **ab**	35.43±28.16 **a**
TMT 100	54.03±25.49 **b**	62.24±24.27 **b**	58.51±24.59 **b**
MetOH	73.39±24.09 **b**	67.03±26.86 **b**	70.21±25.16 **b**
One-way ANOVA	Df=4,52 F=15.62 P<0.001	Df=4,55 F=4.407 P<0.01	Df=4,112 F=16.03 P<0.001

Table 5 The mean time duration that the mice spent still and motionless within the open-field. «a», «b», and «ab» mark the significance ($p \leq 0.05$)

Regarding the movement of all mice in general as one we see significant differences. The MetOH treatment induced less movement than when treated with citronella ($t=6.709$, $p<0.001$), and MetOH exposure also lead to less movement than when treated with the control odour ($t=-4.792$, $p<0.01$). The TMT 100% exposure lead to less time spent moving than when we conducted the citronella treatment ($t=-4.871$, $p<0.001$), and also less time than when we used the control odour ($t=-3.017$, $p<0.05$). The TMT 50% treatment induced longer duration of movement compared to the MetOH exposure ($t=5.034$, $p<0.001$) and also longer than when treating the mice with TMT 100% ($t=3.233$, $p<0.05$).

Looking at the still behaviour of the mice, all seen as one, we observe that the MetOH treatment showed the highest duration of freezing behaviour, both compared to the citronella exposure ($t=6.885$, $p<0.001$) and also compared to when exposed to the

control odour (t=5.309, p<0.001). The TMT 100% treatment of the mice also showed a long duration of motionless behaviour compared to the citronella treatment (t=5.032, p<0.001) and also compared to when using the control water (t=3.511, p<0.01). When using TMT 50% it caused the mice to stand less still than when the mice were exposed to MetOH (-5.170, p<0.001) and also less than the TMT 100% treatment. (t=-3.355, p<0.01)

As shown on **figure 3** the control, citronella and the TMT 50% treatments showed a significantly longer duration of movement and less duration of motionless behaviour than the TMT 100% and the MetOH treatments.

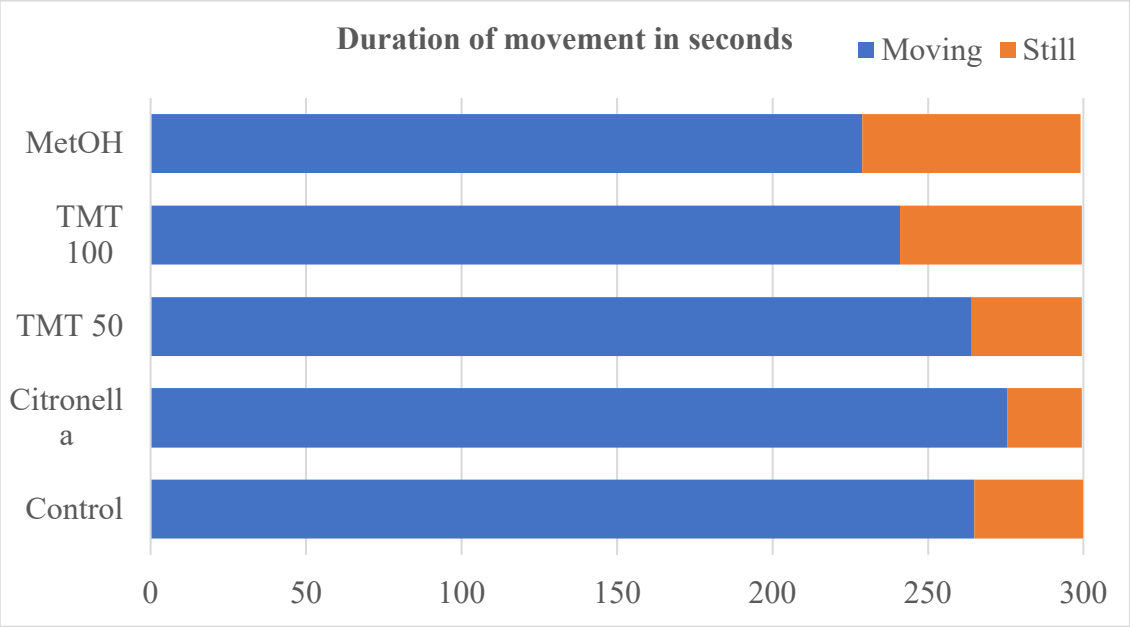

Figure 3 Graph showing the mean time in seconds spent moving or being still..

When we evaluated the genders the only significant difference between sexes we noticed was when the mice were treated with citronella. As **figure 4** shows, males were a lot less active and showed more freezing behaviour than what the females showed. We conducted a t-test to evaluate the difference between the genders. The still behaviour was higher in the males (t=-2.4521, P<0.05) and moving behaviour was lower in the males than in the females (t=2.4595, P<0.05)

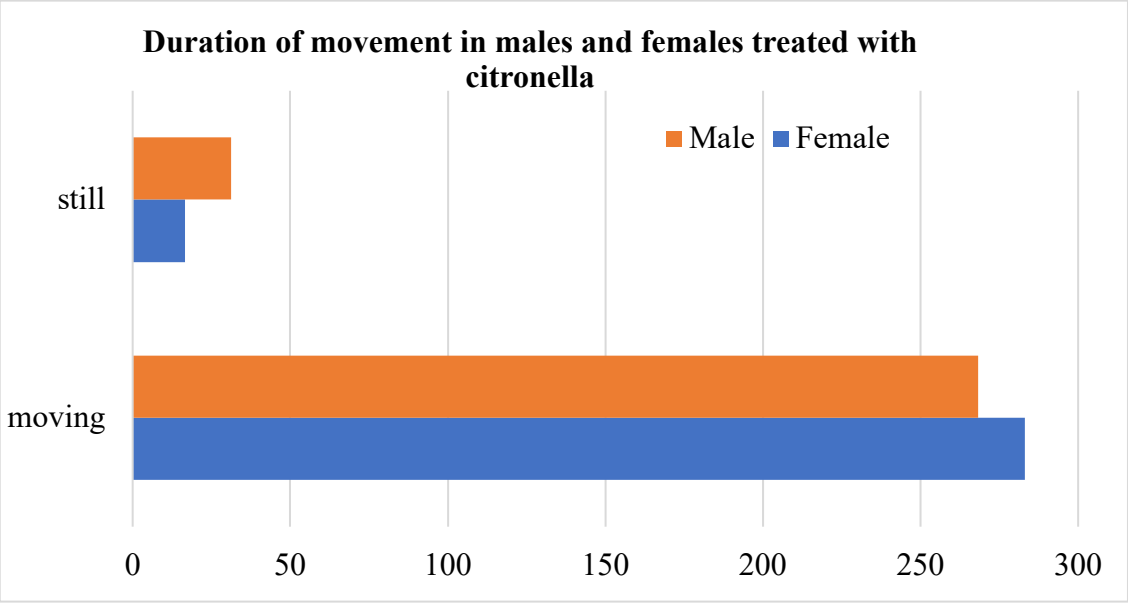

Figure 4 Mean duration in seconds of movement in males and females treated with citronella

6. Discussion

We wanted to evaluate if and how TMT stimulates fear in mice, and we also tried to evaluate the difference of low concentration versus high concentration TMT. We compared TMT to MetOH as this is working as a noisome odour and is not thought to have any pheromonal effect alone. In addition to evaluating the pheromonal effect itself, we also need to considered other factors that may play a part in triggering the fear response like for example the environment and for how long the pheromones are present.

To check the different odour´s ability to stimulate fear is crucial for future studies, especially to be able to study anxiolytic drugs, and also to decide whether predator pheromones can actually be used to trigger a reliable fear response or if their effect is too unreliable to be trusted. An animal welfare question which is important to consider is the different laboratory animals´ responses to predator pheromones, and if they feel comfortable in their own environment, or if they should be housed different places, far away from any possible predator that may induce any fear response.

Fear behaviour in the mice is most characteristically shown by a freezing behaviour. It is also shown by spending more time especially in the corners, but also in the edges, compared to being in the centre of the open-field. We videotaped and evaluated their movement and also their placement within the open-field cage, and we measured the duration of each behaviour. Ward et al (1997) has described defensive behaviour as seen in frightened mice as reduction in locomotion and reproduction, while non-defensive behaviours can be foraging and grooming but these activities we did not measure in our experiment. Defensive behaviour is absolutely crucial for the survival of all kinds of prey animals. Not only does it make the individual less vulnerable, but it is also important for the survival of the whole of the species.

In our experiment we could see a significant decrease in motion when the mice were treated with TMT 100% and MetOH, but we believe that these two odours work more as noisome odours rather than showing the pheromonal effect eliciting fear behaviour, as described in Dielenberg and McGregor (2001), when they concluded their experiment with that TMT seems to act like an irritating odour and not like a specific predator odour. When the mice were treated with TMT 50% their locomotion was very similar to the locomotion shown in the control group. This does not correlate with what we expected as TMT 50% should act as a pheromonal fear stimulator, and should in theory induce a much higher duration of fear behaviour than that of the control. Wallace

and Rosen (2000) showed that the highest duration of freezing was seen after 5 minutes exposure to the TMT to the rats, which may explain why we didn't get the freezing behaviour we expected by treating the mice with TMT 50%, as our treatments only lasted 5 minutes each. Another reason to why the mice did not show significant freezing behaviour when treated with TMT 50% may be that some studies suggests that unconditioned fear is only elicited in very low concentrations of TMT (<10%), which is more similar to that of the real concentration of TMT in fox faces (Hacquemand et al., 2012). Rampin et al. (2018) evaluated fox faeces and discovered that they could actually only prove that TMT was present in 1 out of 13 faecal samples that they collected from foxes. This was explained by them believing that the TMT concentration of the fox faeces is lower than 50 nmol/l, which was the threshold level for their gas-chromatography-mass-spectrometry measurement. Rampin et al. (2018) also proved that rats treated with real fox faeces showed a significantly higher duration of freezing behaviour in comparison to rats treated with artificially produced higher concentration of TMT.

Regarding the location of the mice within the open-field we clearly saw that the TMT 100%, MetOH but also the control water induced a much higher duration of time spent in the edges of the open-field than the other treatments. This does not give us any evidence that MetOH or TMT 100% induces fear, since animals while treated with tap water was expected to spend more time in the centre and less time in the edges than the other treatments. These results were not as we expected, and it is hard to understand why we got them. Interestingly the TMT 50% odour induced the longest duration of staying in the corners, followed by the citronella odour. This may give evidence that the TMT 50% odour caused the highest fear response, when it comes to their location, in comparison to the other treatments, just like what we expected them to. All of the odours induced a very little and similar amount of time spent in the centre, but we could clearly see that the citronella treatment caused the longest duration of staying in the centre. This is probably due to the fact that citronella is a neutral odour for rodents. We would expect both the control water and also the citronella treatment to induce the longest duration of time in the centre, as they are both neutral odours and should make the mice feel the most comfortable and secure, compared to the other odours.

When evaluating the genders we discovered that there were no significant difference in fear response between males and females, except when we looked at the movement of males and females treated with citronella, where we saw that females were a lot more active. The reason behind this is not clearly understood.

Other factors like the epigenetics can also be important for us to get an understanding of why the mice responded to the odours like they did. Studies shows that when prey learn to fear a specific predator odour, they will pass this fear down to their offspring. As mentioned earlier when pheromones are detected and gives the prey a reason for it to fear them this will lead to an increase in specific pheromonal receptors (Szyf, 2014). In our study we used B6D2 laboratory mice with no previous exposure to any pheromones. This may explain why we did not get successful results inducing fear with TMT.

Most of the experiments using TMT previously made has been done with rats, but these are all very interesting to compare to ours since both rats and also mice are natural prey which are supposed to show fear and flee from foxes and other predators. Most of the previously made experiments has shown that rats indeed does respond to TMT with a fear behaviour, like for example explained in Müller and Fendt (2006). Other authors like Dielenberg and McGregor (2001) concluded their experiment with that TMT acts more like a noisome odour rather than a specific predator odour. Morrow et al. (2002) showed us in their experiment that not only does fear depend on the fearful smell itself, but it also depends on the surroundings and the environment in which the animal is placed in. They proved that mice placed in a small, dimmed cage did not get as frightened as the ones placed in a large and bright cage.

It has earlier been suggested that TMT can be used as a reliable fear inducing substance in laboratory animals, which in the future can become important in several studies. Some of the reasons to why this odour is preferred is due to the possibility we have to produce it artificially in the laboratory and also the fact that its amount in the air can easily be measured and controlled.

In our study we did not find any reliable data to prove the fact that TMT induces anxiety and fear in mice. For future studies the environment in which the mice are kept should be put into consideration. Another important factor may be the TMT concentration and that it should probably be lower and more similar to the real concentration of the fox faeces. We should also keep in mind the duration of exposure to the different odours and how some of the odours may need to be inhaled for more than 10 minutes to show an effect. Another thing to keep in mind is the genetic line from where the mice is coming from and if they have inherited innate fear response to fox pheromones, or if they come from a line where no odour fear stimulation previously has been done.

Even though our results were not as reliable as we hoped for, and cannot be considered to be reliable, we could clearly see that when the mice were treated with TMT 50% they spent a lot more time in the corners, which tells us that even though they didn't show a long duration of freezing behaviour, like the ones treated with foul odours did, they did show some kind of fear response.

All in all we conclude this experiment with that it is very likely that TMT induces unconditional fear in mice, different from the fear induced by noisome odours, but that more studies will have to be done to be able to have reliable data about it.

8. Summary

In the current thesis we wanted to study the effect of synthetic TMT, similar to the TMT found naturally in fox faeces, on fear response in mice. Several studies have previously suggested them to have a good ability to induce fear in rodents, especially in rats. We wanted to see if these studies were reliable anxiety stimulators, also in mice.

For the experiment we used five different odours: distilled water as a control, citronella oil as a known neutral odour to rodents, TMT in 50% concentraion, TMT in 100% concentration, and also MetOH, which is a noisome odour without any specific relevance to rodents. We wanted to evaluate the difference between using a high concentration TMT versus using lower concentration TMT, as the high concentration one may act as an irritating odour rather than a fear-responsive odour.

We placed the mice in an open-field box and exposed them to each odour for 5 minute intervals. After each odour they had resting days. We videotaped and used Noldus Observer XT program to evaluate their behaviour.

We examined their locomotion – if they showed defensive behaviour by freezing, or if they were moving around suggesting that they felt comfortable. We also evaluated their location within the open-field – spending time in the edges and especially corners as a sign of fear and being in the centre shows that the mice feel confident and calm.

We found that with the TMT 100% and MetOH, both working as foul odours, the mice showed a significant increase in duration of freezing behaviour. With TMT 50% we saw a long duration of being in the corners, which suggests the TMT 50% to indeed be a fear-inducing odour as we expected it to. All in all our results did not provide a significant proof that TMT does cause unconditional fear in mice, but our data does suggest that the low dose TMT has a different effect than the high dose version.

We suggest that further studies should be made, with lower concentrations of TMT, for a longer duration of time, in a controlled environment. Epigenetics and previously ancestral meetings with foxes may also play a role in the unconditioned pheromonal fear of mice, which may be challenging for the future, especially if we want to use pheromones as rodent repellents, as this is very dependent on place and if the population in the specific area is well known to foxes as predators or not.

9. Összefoglaló

Jelen dolgozatban a rókák bélsarában természetes módon megtalálható TMT szintetikus változatának hatását vizsgáltuk egerek viselkedésére, elsősorban félelemérzetére. Számos korábbi kutatási eredmény utal arra, hogy a vegyület félelmet vált ki rágcsálókban, különösen patkányokban. Arra kerestük a választ, hogy hasonlóan megbízhatóan okoznak-e szorongást egér fajban.

A kísérlet során öt különféle szaganyagot használtunk fel: desztillált vizet kontrollként, citromfű olajat ami rágcsálók számára igazoltan semleges. 50%-os és 100%-os koncentrációjú TMT vegyületet és MetOH vegyületet, mely ismerten kellemetlen szagú, de biológiailag a rágcsálóknak nem jelentős vegyület. Célunk elsősorban a koncentrált és a hígabb TMT hatásának összehasonlítása volt, mivel gyanítható, hogy a tömény vegyület kellemetlen szaga elnyomja az esetleges specifikus, félelmet kiváltó hatást.

Az állatokkal porond-teszteket végeztünk, melynek 5 perce során a vizsgált szaganyag jelenlétének voltak kitéve. A porond-tesztekről videófelvételt készítettünk majd az állatok viselkedését a Noldus Observer Xt szoftver segítségével elemeztük ki.

Vizsgáltuk a mozgásaktivitásukat – hogy mutattak-e védekező viselkedést (lefagyást), vagy aktívak voltak, mely a jobb közérzet jele. Vizsgáltuk továbbá a porondon való elhelyezkedésüket, mivel a porond szélén és sarkaiban való tartózkodás a szorongás és félelem, míg a középen való helyeződés a magabiztosságé és nyugalomé.

Azt találtuk, hogy mind a 100%-os TMT és a MetOH hatására az állatok szignifikánsan több időt töltöttek mozdulatlanul. Az 50%-os TMT-vel való kezelés alatt az egerek több időt töltöttek a sarokban, ami igazolja annak félelmet kiváltó tulajdonságát. Eredményeink nem igazolják minden kétséget kizáróan a TMT félelmet és szorongást kiváltó tulajdonságát egereknél, de arra utalnak hogy a kisebb koncentrációjú vegyület valóban más hatással van az állatokra mint tömény változata.

A jövőben érdemes lenne további vizsgálatokat végezni alacsonyabb koncentrációkkal és hosszabb idejú kitettséggel kontrollált környezetben. Az epigenetika és a korábbi generációk esetleges találkozása ragadozókkal szintén befolyásolhatja a vegyület hatását, mely jelentősen bonyolíthatja a vegyület felhasználását, különösen ha esetleg rágcsálók elriasztására használnánk, hisz hatása nagyban függne az adott területen élő populáció rókáknak való kitettségétől.

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