The effect of handling and training on measures of the affective state of farmed mink (*Neovison vison*)

P.M. Svendsen
Group of Animal Behaviour and Stress biology, Department of Animal Science, Aarhus University, Blichers Allé 20, P.O. Box 50, 8830 Tjele, Denmark; pernille.svendsen@agrsci.dk

Abstract

The present study aimed to examine if handling and training female mink on a learning task, as preparation for a cognitive bias test, had an effect on measures of their affective state. Adult female farm mink were used based on measures of approach/avoidance behaviour, and categorised as explorative (E, n=10) and fearful (F, n=10). Affective state was assessed on the basis of approach/avoidance behaviour in two contexts (stick-tests, novel object tests) and adrenocortical activity (faecal cortisol metabolites, FCM). The results showed that the F mink changed to being more explorative after handling and training for the cognitive bias test. Furthermore, the handling and training also had an effect on the E mink, but in the opposite direction. This study showed that preparing mink for a cognitive bias test had a marked effect on measures of their affective state, which has to be considered when interpreting the results from experiments using training to assess welfare.

Keywords: American mink, assessment of welfare, behaviour, cognitive enrichment, FCM

Introduction

The assessment of positive experiences and affect in animals has become a growing focus of animal welfare research. Boissy *et al.* (2007) have pointed out that animal well-being is not only the absence of negative experiences and affective states (e.g. fear) but indeed also the presence of positive ones, such as pleasure. Affective or emotional states can be defined as subjective states that are characterised by valence (how positive or negative they are) and arousal (how activated the animal is) (Mendl *et al.*, 2010b). Whilst it is currently not possible to directly assess the subjective experiences of other species, or to know for certain whether they have such experiences, it is possible to measure other components of affective states including behavioural, physiological, and cognitive responses (Paul *et al.*, 2005). The avoidance of a novel object or stick may indicate a general 'fearfulness' because approach/avoidance behaviours are thought to be important correlates of affective valence (Paul *et al.*, 2005). The hypothalamic – pituitary – adrenal (HPA) axis responds and cortisol is released from the adrenal cortex when confronted with a stressor which may correlate to fear and anxiety, but the HPA axis is also responsive to other types of activity that are associated with emotional arousal (Touma and Palme, 2005).

Cognitive bias refers to the influence that affect has on a range of cognitive processes in animals, including attention, memory and judgement (Mendl *et al.*, 2009). It is known from humans that individuals in a negative affective state pay more attention to threatening stimuli, retrieve more negative memories and judge ambiguous stimuli more negatively (being 'pessimistic') than happier individuals (e.g. Mendl *et al.*, 2009; Paul *et al.*, 2005). Harding *et al.* (2004) have developed a cognitive bias method for determining affective state in animals, based on measuring emotionally induced biases in decision making. Their findings indicated that rats in unpredictable housing, inducing a mild negative affective state (Willner, 1997), were more pessimistic in response to ambiguous stimuli than controls. The method has been applied successfully to a number of other species, including starlings (Matheson *et al.*, 2008), sheep (Doyle *et al.*, 2010) and dogs (Mendl *et al.*, 2010a). The method is suggested to be a useful indicator of animal emotion and it is a promising indicator of animal welfare but it requires a lot of intensive training (Mendl *et al.*, 2009).

Cognitive enrichment is based on instrumental learning directed to a highly motivating consummatory act that may have the capacity to increase animal welfare and it may initiate positive emotions (Manteuffel *et al.*, 2009). On the farm, cognitive enrichment usually refers to the provision of a specific and short-term problem solving activity or device (Puppe *et al.*, 2007). The possibility that mastering a problem-solving task is rewarding is suggested by Hagen and Broom's (2004) finding that cattle show an affective reaction (e.g. increased heart rate and behavioural vigour (indicating increased arousal, though valence is not entirely clear)) when they increase performance on a simple instrumental task.

The study aimed at investigating the effect of preparation for a cognitive bias test in mink, i.e. handling, habituation to the test chamber and training on a positive instrumental learning task, on measures of the affective state of female farmed mink. The study used traditional indicators of affective state, being measures of approach/avoidance behaviour in two contexts (stick test, novel object test) and adrenocortical activity (faecal cortisol metabolites, FCM), assessed before and after handling and training, as the preparation for a cognitive bias test.

Materials and methods

Animals and housing

The start population comprised 200 adult female farm mink (*Neovison vison*) of the color type wild, born around May 1, 2009. Based on approach/avoidance categorization using a stick test (see below) in the home cage, 60 animals (30 explorative and 30 fearful) were selected for a novel object test also in the home cage. Ten explorative mink with very short latencies to make contact and 10 fearful mink with very long latencies to make contact or no contact at all in the novel object test were selected and allocated to two groups, explorative (E) and fearful (F) respectively.

The selected mink were moved to new housing facilities where they were habituated for 7 days before the first faecal sampling, followed by the onset of the handling and training procedure. The mink were individually housed in standard-sized wire cages (W: 30 cm, H: 45 cm, L: 90 cm; Hedensted-Gruppen, Hedensted, Denmark) connected to one wooden transportable nest box (W: 25 cm, H: 30 cm, L: 21 cm), at the mink farm at Aarhus University, Research Centre Foulum, Denmark. Food was provided once daily at 12:00±15 min in amounts to keep the animals at maintenance level, water and straw was provided *at libitum*, and all mink had access to either a shelf or a tube in their cage. The study was performed in September – December 2010.

Handling and training procedure

The animals were shut into their transportable nest box and moved 50 m to another building, where they were let into a standard-sized wire cage and trained on a positive instrumental learning setup. The mink had to press a lever on a sound cue to obtain a food reward (positive reinforcement). The mink were returned to their home cage after approx. 20 min. All animals were handled 2-3 times per week for 12 weeks continuously. Once every 2 weeks faecal samples were collected from the backside of the cages (first and last collection described here). Human movement behind the cages is unusual.

Stick test

The approach/avoidance behaviour of the mink was characterized by the stick test (Hansen and Jeppesen, 2006; Malmkvist and Hansen, 2002) prior to and after 12 weeks of handling and training. The mink were excluded from the nest box using a metal shutter and were tested individually. A tongue spatula was put through the wire netting in the upper part of the lid of the cage and the immediate reaction as well as the position (front or back of the cage) of the mink after 30 s was registered. The reaction was allocated to one of four categories: (1) explorative if the mink approached and sniffed the spatula persistently; (2) fearful if the mink withdrew and did not touch the stick; (3) aggressive if the mink attacked and bit the stick and (4) uncertain if the mink did not respond or showed a mixture of responses and so could not be placed in one of the first three categories.

Novel object test

The first test was performed on two testing days one week after the stick test and prior to the handling and training. The second test was performed on one single test day following the handling procedure and stick test. The mink were shut into the nest box using a metal shutter placed at the entrance. A wooden cube (10×10×10 cm) was placed centrally approx. 60 cm into the cage from the entrance to the nest box as a novel object. A new cube was used in all cages. The duration of the test was 180 s and began when the metal shutter was removed and the mink had full view and access to the cage. Latency to approach and to contact, as well as the duration of manipulation of the novel object was recorded using a handheld computer (Psion workabout Copyright Psion PLC 1995). The wooden cube was removed after each test. Two mink (one from each group) were excluded from the analysis due to human error.

Sampling and analysis of cortisol metabolites

Concentrations of FCM are a validated measure in mink, reflecting circulating cortisol in female mink (Malmkvist *et al.*, 2011). Fresh faecal samples were collected non-invasively following habituation and after the intensive handling period between 10:30 h and 14:30 h. Fresh faecal samples (0.5 g) were frozen within 20 min after delivery at -21 °C until analysis. A total of 0.5 g of each sample was extracted with 5 ml methanol (80%) and concentrations of FCM were measured in an aliquot of the supernatant with two different enzyme-immunoassays (EIAs) described by (Palme and Mostl, 1997) (11-oxoaetiocholanolone EIA) and (Frigerio *et al.*, 2004)

(11β-hydroxyaetiocholanolone EIA). One E mink did not defecate during the sample collection hours in the sampling after the intensive handling period and was excluded in the further analysis. Only FCM values measured by the 11β-hydroxyaetiocholanolone EIA are presented, as both measures were highly correlated.

Statistical analysis

Differences between each of the approach/avoidance behaviour categories and position in the stick-test before and after handling within the two groups (E and F) were tested using a paired t-test. A Wilcoxon test for matched pairs was used to analyze latencies to approach (leave the nest box) and to touch the novel object in the novel object tests, between the first and second test for both groups. The duration of contact with the novel object before and after handling in both groups was analyzed using a paired t-test. The difference in pre- and post-handling on FCM concentrations within groups was analyzed using a Wilcoxon test for matched pairs. All analyses were performed according to (Siegel and Castellan, 1988) using the statistical software R 2.13.0 (R Core Development Team, 2012). Differences were considered significant if $P<0.05$.

Results

After the handling period, significantly fewer of the E mink were classified as explorative in the stick test (40%, t=3.67, df=9, $P<0.01$) and more as uncertain (40%, t=-2.45, df=9, $P=0.04$) compared to all being explorative in the first test. There were no significant differences in aggressive ($P=0.34$) or fearful reactions ($P=0.34$) between the two tests. In contrast, the F mink were significantly more explorative after the handling and training (60%, t=-3.67, df=9, $P=0.005$) with a reduction in fearful mink (20%, t=6, df=9, $P<0.001$) compared to all mink being fearful in the first test. There were no differences in aggressive ($P=0.34$) or uncertain ($P=0.34$) between the two tests. The position of the mink in the back of the test cage immediately after the stick test indicates an active movement to avoid the stick and the testing person. The F mink showed a significant change in position, from 1 mink being at the front in the first test to 8 mink in the second test (t=-4.58, df=9, $P=0.001$). There was no difference in position before and after handling in the E mink ($P=0.17$) with 10 mink being at the front in the first test and 8 mink in the second.

Median latency to approach and make contact with the novel object in the novel object test changed in both groups, illustrated in Figure 1. The change in the median in the F mink was significant (approach, W=72.5, $P=0.03$; contact, W=80.5, $P<0.001$), but not in the E mink (approach, $P=0.23$; contact, $P=0.77$).

All mink touched the novel object placed in their home cage in both tests, except for one F mink that did not touch the novel object in the first test. Mink from both groups spent significantly more time in contact with the object after the handling procedure (F: t=-4.32, $P<0.01$; E: t=-3.06, $P=0.02$).

Figure 2 illustrates the median concentrations of the FCM from the samplings prior to and after the handling period for both groups. There was a significant rise in FCM concentrations in the E group (W=9, $P=0.004$) with higher concentrations in samples after the intensive handling period. No difference was found in the F group ($P=0.43$).

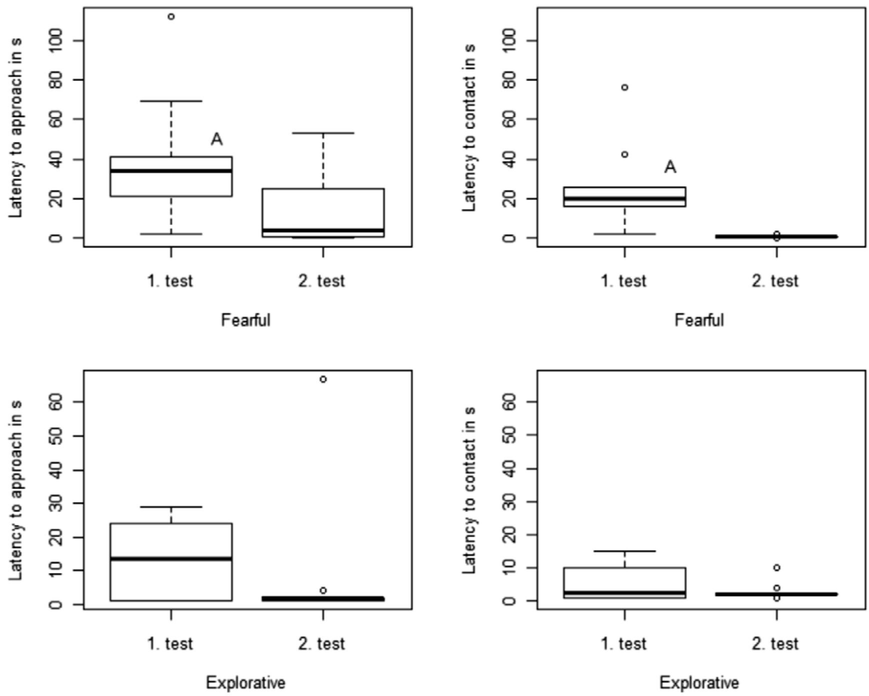

Figure 1. Median latencies to mink's reaction in two novel object tests in fearful and explorative mink, before and after handling. The horizontal lines show the median and the bottom and top of the box the 25th and 75th percentiles. Points are outliers and are plotted individually. When there are outliers the dashed lines show the interquantile range of the data and otherwise they show the max and min. An A indicates that the tests differ significantly (P<0.05).

Discussion

The purpose of handling and training the explorative (E) and fearful (F) mink was to prepare them for a cognitive bias test, a novel indicator of animal welfare. However, the handling and training had an effect on (1) measures of the affective state of the mink and (2) these effects were not in the same direction for the two types of mink. The F mink became less fearful and more explorative as measured in the stick test after a period of handling and training, and they were faster at approaching and touching the novel object as well as spending more time in contact with it. Contrary, even though the E mink spent more time in contact with the novel object in the second test and the fearfulness did not increase significantly, fewer were scored as explorative and more as uncertain in the stick test after the handling procedure. Additionally the E mink reacted to the handling procedure with an increased concentration of FCM. Thus the training and handling had an apparently positive effect on the affective state of F mink, becoming less fearful and an apparently negative effect on the E mink, becoming less explorative.

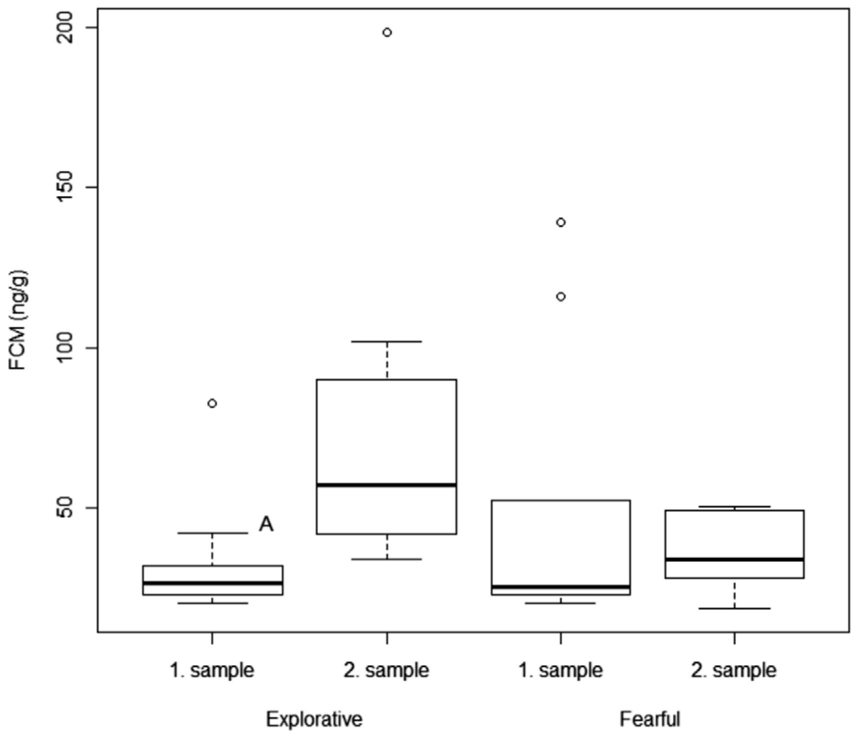

*Figure 2. Median concentrations of FCM (ng/g) from one sampling before and one after handling in explorative and fearful mink. The horizontal lines show the median and the bottom and top of the box the 25th and 75th percentiles. Points are outliers and are plotted individually. When there are outliers the dashed lines show the interquantile range of the data and otherwise they show the max and min. An A indicates that the samples within groups differ significantly (*P*<0.05).*

The handling in this study included training on an instrumental learning setup using positive reinforcement (food), i.e. a type of cognitive enrichment, likely to influence the affective state (Manteuffel *et al.*, 2009). The F mink showed reduced fearfulness, possibly due to a combination of the learning setup and habituation i.e. experience with the handling and training procedure as well as the ability to generalize across tests. Malmkvist and Hansen (2002) have showed that within two groups of mink, genetically selected for being either explorative or fearful, the animals generalized their fear responses across an exposure of six different tests becoming more explorative.

The elevated level of FCM concentration in the E mink suggests that they (1) perceived the handling and training as aversive or (2) that they were frustrated as an anticipated reward (in the following learning task) was unexpectedly absent (Burman *et al.*, 2008). The shift of some E mink from being categorized as explorative in the first test to uncertain in the second supports an aversive reaction. Classified as 'uncertain' in the stick test reflect that the observer is not able to classify the response unambiguously, but not necessarily that the mink experience an aversive situation. An aversive reaction would probably involve increased fear, whereas frustration more likely would be categorized by different motivational reactions in the stick test (uncertain). It would be interesting to investigate the behaviour of the animals prior to each handling and training session to determine whether they anticipate a reward (in the following learning task). Expectation of a positive reward has been found to increase the activity of mink in the cage (Hansen and Jeppesen, 2006) possibly causing inattentiveness towards the stick.

Fear and anxiety are negative affective states and in this study, F mink became less fearful thus moving towards a more positive affective state after handling and training. E mink showed the opposite, being less explorative. The handling and training procedure thus had a different effect on the animals depending on their initial affective state. Even though the two groups were subjected to the exact same treatment, they responded differently. There are some factors that need to be investigated further such as the inclusion of a non-handled control group and an investigation of anticipatory behaviour prior to each handling session.

Taken together, studies that involve induced affective states and a lot of training of the animals to assess their welfare, such as the cognitive bias method, need to be interpreted carefully as the handling and training has a different effect on animals in different affective states. The addition of established welfare indicators would support the interpretation.

References

Boissy, A., Manteuffel, G., Jensen, M.B., Moe, R.O., Spruijt, B., Keeling, L.J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I. and Aubert, A., 2007. Assessment of positive emotions in animals to improve their welfare. Physiology and Behavior 92: 375-397.

Burman, O.H.P., Parker, R.M.A., Paul, E.S. and Mendl, M., 2008. Sensitivity to reward loss as an indicator of animal emotion and welfare. Biology Letters 4: 330-333.

Doyle, R.E., Fisher, A.D., Hinch, G.N., Boissy, A. and Lee, C., 2010. Release from restraint generates a positive judgement bias in sheep. Applied Animal Behaviour Science 122: 28-34.

Frigerio, D., Dittami, J., Mostl, E. and Kotrschal, K., 2004. Excreted corticosterone metabolites co-vary with ambient temperature and air pressure in male Greylag geese (*Anser anser*). General and Comparative Endocrinology 137: 29-36.

Hagen, K. and Broom, D.M., 2004. Emotional reactions to learning in cattle. Applied Animal Behaviour Science 85: 203-213.

Hansen, S.W. and Jeppesen, L.L., 2006. Temperament, stereotypies and anticipatory behaviour as measures of welfare in mink. Applied Animal Behaviour Science 99: 172-182.

Harding, E.J., Paul, E.S. and Mendl, M., 2004. Animal behavior – Cognitive bias and affective state. Nature 427: 312.

Malmkvist, J. and Hansen, S.W., 2002. Generalization of fear in farm mink, *Mustela vison*, genetically selected for behaviour towards humans. Animal Behaviour 64: 487-501.

Malmkvist, J., Jeppesen, L. and Palme, R., 2011. Stress and stereotypic behaviour in mink (*Mustela vison):* A focus on adrenocortical activity. Stress-the International Journal on the Biology of Stress 14: 312-323.

Manteuffel, G., Langbein, J. and Puppe, B., 2009. Increasing farm animal welfare by positively motivated instrumental behaviour. Applied Animal Behaviour Science 118: 191-198.

Matheson, S.M., Asher, L. and Bateson, M., 2008. Larger, enriched cages are associated with 'optimistic' response biases in captive European starlings (*Sturnus vulgaris*). Applied Animal Behaviour Science 109: 374-383.

Mendl, M., Brooks, J., Basse, C., Burman, O., Paul, E., Blackwell, E. and Casey, R., 2010a. Dogs showing separation-related behaviour exhibit a 'pessimistic' cognitive bias. Current Biology 20: R839-R840.

Mendl, M., Burman, O.H.P. and Paul, E.S., 2010b. An integrative and functional framework for the study of animal emotion and mood. Proceedings of the Royal Society B-Biological Sciences 277: 2895-2904.

Mendl, M., Burman, O.H.P., Parker, R.M.A. and Paul, E.S., 2009. Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. Applied Animal Behaviour Science 118: 161-181.

Palme, R. and Mostl, E., 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Zeitschrift fur Saugetierkunde-International Journal of Mammalian Biology 62: 192-197.

Paul, E.S., Harding, E.J. and Mendl, M., 2005. Measuring emotional processes in animals: the utility of a cognitive approach. Neuroscience and Biobehavioral Reviews 29: 469-491.

Puppe, B., Ernst, K., Schon, P.C. and Manteuffel, G., 2007. Cognitive enrichment affects behavioural reactivity in domestic pigs. Applied Animal Behaviour Science 105: 75-86.

R Core Development Team, 2012. R version 2.13.0. In: www.r-project.org.

Siegel, S. and Castellan, N.J., 1988. Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York, N.Y.

Touma, C. and Palme, R., 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: The importance of validation. Ann N Y Acad Sci. 1046: 54-74.

Willner, P., 1997. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology 134: 319-329.