Short Communication

NEOPHOBIA IN CAPTIVE WOLVES EVOKED BY SIMPLE, LOW-COST DISRUPTIVE STIMULI

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1. Introduction

Greater numbers of grey wolves (*Canis lupus*) on the landscape can lead to an increase in the number of livestock depredations (Mech, 1995). A multiplicity of methods exists to prevent livestock depredation by wolves and other carnivore species (Shivik and Martin, 2000; Shivik et al., 2003). Lethal predator control techniques have rarely reduced depredation to an acceptable level, and their use is disfavoured by the public (Shivik et al., 2003; Treves et al. 2016). In addition, traditional non-lethal methods to control predation, such as predator-proof fences, livestock guarding dogs and aversive devices, can be expensive and may not be suitable for every situation.

Novelty (such as novel objects and sounds) can evoke fear in animals (Corey, 1978). In the context of livestock protection, novel elements placed on the landscape can lead wolves to temporarily avoid a problematic area, such as livestock pastures. For example fladry, long ropes with hanging strips of material, has been used as a virtual barrier which wolves tend not to cross (Musiani and Visalberghi, 2001). In case of continuous exposure to a particular object, however, animals usually habituate to it (Corey, 1978).

Predators' responses to low-cost deterrents have seldom been studied. Zarco-Gonzalez and Monroy-Vilchis (2014) studied the effectiveness of low-cost felid deterrents to reduce predation. The effect of fladry on wolves' behaviour and its effectiveness to reduce predation have been assessed (e.g. Musiani and Visalberghi, 2001; Musiani et al., 2003), but little is known about wolves' behavioural response to other low-cost sensory stimuli, including novel objects, sounds and odours. Exploring the effect of various sensory stimuli on wolves' feeding behaviour may help the development of stronger deterrents. The aim of our study was to assess the relative effect of several low-cost, novel sensory stimuli on the feeding behaviour of sub-adult, captive and naïve wolves.

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2. Material and Methods

Tests were conducted on five orphan sibling wolves held in captivity at the Bubonitsy wolf rehabilitation centre, Tver region, Russia. These individuals (two males, three females) were taken from the wild by local people at one month of age and brought to the centre. They were eight months old at the start of our 2-month study. Their behaviour was more similar to free-ranging wolves compared to an adult living in the same enclosure. While the adult had been hand-reared and socialized with humans, contact between the young wolves and people had been minimized and they tended to avoid humans.

Experiments were conducted daily. The five young wolves were tested together in a 4,000 m^2 enclosure. Each day, the adult was isolated in an adjacent enclosure during the experiment and released back into the enclosure afterwards. Individual recognition of the siblings was not possible, so variables were recorded for the group.

2.1. Sensory stimuli tests

As an attractant, we used the same food usually given to the wolves (chicken heads, cow meat and fat), simultaneously spread at four feeding points inside the enclosure. Three points were associated with different sensory stimuli and one was used as a control (no stimulus). Stimuli were placed ≤2 m from the meat. We conducted 2-hour daily trials beginning at 10 am. Between trials, the location of each stimulus was rotated among the four feeding points to avoid site effect bias. After four trials, when each stimulus had been tested at each feeding point, we moved all feeding points to new locations within the enclosure and repeated the trials.

Stimuli tests were terminated when the meat had been consumed at least once for all the stimuli tested. We tested eight different low-cost sensory stimuli (Table 1). These devices were commercially available and were selected to represent a diversity of stimulus properties and activation modes (Fig. 1).

Table 1. Disruptive stimuli tested on five captive wolf siblings.

Stimulus	Characteristics	Referred to in text
Deodorant	Artificial smell, spread around the feeding point up to 1 m.	Artificial olfactory stimulus
Mole repeller	Defenders mega-sonic mole repeller. Aluminium cylindrical post, 50 cm long, 4 cm diameter. Placed next to the meat, hidden under leaves or snow.	Intermittent acoustic stimulus
Hanging aluminium leaves	7 to 10 leaves of 15 x 15 cm, hung on low branches (up to 50 cm above the ground) and spread around feeding point up to 1 m. In motion through wind activation.	Permanent visual stimulus
Radio	Constant background noise from the radio. Maximum volume.	Permanent acoustic stimulus
Motion-activated white light	Ovoid white light, 20 x 10 cm. Activation within 5 to 10 m, for 90 seconds. Intensity of 50 to 60 lm.	Movement-activated visual stimulus
Flashing red light	Headlamp; flashing point of red light.	Intermittent visual stimulus
Burnt sheep wool	Organic smell, spread in 4 points around the feeding point, up to 1 m.	Organic olfactory stimulus
Motion-activated ultrasound	Weitech WK0051 - Garden Protector. Activation up to 16 m, for 7 seconds. Re-activation after 5 seconds if motion still detected. Red light when activated. Frequency = 24 kHz.	Movement-activated ultrasonic stimulus

Fig. 1. Commercially available electronic devices tested for their influence on the behaviour of captive wolves: Weitech WK0051 Garden Protector (top left), radio (top right), motion-activated white light (bottom left) and Defenders mega-sonic mole repeller (bottom right).

In December 2015 we tested the reaction of wolves to deodorant, mole repeller and aluminium leaves. In January 2016 we tested the reaction of wolves to a radio, a motion-activated light and motion-activated ultrasound. After the first trial showed the motion-activated light to be unsuccessful in repelling wolves, we used this feeding point for exploratory testing of other new stimuli: a flashing red light and the smell of burnt sheep wool. A 2-week break was taken between the first experimental period in December and the second experimental period in January, in order to minimize the effect of stimuli testing in the first period on results from the second period.

Trials were conducted without human presence. Activity around each feeding point was recorded using remote cameras (Moultrie A-5 Digital Game Camera, Birmingham, USA; Tasco 119215C Digital Scouting Camera, Cody, USA). Cameras were set facing the feeding points. They were placed 5-6m away from the stimuli and 2-3 m above the ground to minimize their visibility and impact on the wolves' behaviour. Moreover, the wolves had been habituated to remote cameras prior to this experiment, as their behaviour was recorded throughout the rehabilitation process. Recordings made at the control feeding points also allowed us to confirm that cameras did not have any impact on wolves' feeding behaviour. Wolves were fed (2 kg per wolf, corresponding to the daily food requirements of sub-adult wolves) at the end of each trial if the attractant remained untouched.

For each feeding point and trial, we scored: whether the meat had been consumed; the number of wolves consuming it; the number of wolves approaching it; the number of approaches before consumption; times to first pre-sampling, first approach, and first consumption. A wolf was considered to have approached a feeding point when it was c. 1 m from the feeding point. Pre-sampling activities were defined as including looking at the meat, sniffing towards the meat and scratching the ground close to the meat.

2.2. Data analysis

Data were analysed in Microsoft Excel 2016 for Mac (version 15.25.1, Microsoft Corporation, Redmond, WA, USA) and R for Mac (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria). Time to first consumption, approach and pre-sampling in the sensory stimuli tests were given a maximum value equivalent to the full trial duration (i.e. 120 min) if the attractant was not consumed, approached or pre-sampled. Means and standard deviations of the time to first consumption and time to first approach for the first and second experimental periods were calculated. In addition, we used Principal Component Analysis (PCA) to assess which variables were correlated and to uncover factors associated with wolf response to novelty. We computed four PCAs in R using the FactoMineR package. One PCA was computed per feeding point of the first experimental period using six quantitative variables: time to first pre-sampling; time to first approach; time to first consumption; number of approaches; number of wolves approaching; and trial number.

3. Results

3.1. Sensory stimuli – Experimental Period 1

The first experimental period unfolded over 12 trials (Table 2). Attractants at the control feeding point and the feeding point associated with an artificial olfactory stimulus were consumed during every trial. The feeding point associated with a permanent visual stimulus was approached by a wolf during trial 2, and the feeding point associated with an intermittent acoustic stimulus was approached by a wolf during trial 3, but these approaches did not result in consumption (Fig. 2). Wolves approached these feeding points inconsistently during subsequent trials. The attractant associated with an intermittent acoustic stimulus was consumed after eight trials. The last feeding point at which the attractant was consumed, after 11 trials, was the one associated with a permanent visual stimulus. Following first consumption, wolves consumed attractants at these feeding points sporadically.

Table 2. Occurrence of consumption (C) and approach (A) across trials and by type of stimulus; consumption involves approach.

Trial number	Control	Artificial olfactory	Intermittent acoustic	Permanent visual
1	C	C	-	-
2	C	C	-	A
3	C	C	A	A
4	C	C	-	-
5	C	C	-	-
6	C	C	-	-
7	C	C	A	A
8	C	C	C	-
9	C	C	C	A
10	C	C	-	-
11	C	C	-	C
12	C	C	C	-
Total C	12	12	3	1
% of trials during which consumption occurred	100%	100%	25%	8%

Fig. 2. Juvenile wolf in captivity showing neophobic response to aluminium leaves.

Over the 12 trials, the average latencies to approach and consume attractants were the longest for the permanent visual stimulus ($\bar{X}=110.1\pm8.9$ and $\bar{X}=115.1\pm8.2$ respectively), followed by an intermittent acoustic stimulus ($\bar{X}=71.7\pm21.9$ and $\bar{X}=106.3\pm12.9$), the control feeding site ($\bar{X}=26.3\pm15.7$ and $\bar{X}=27.0\pm16.3$) and an artificial olfactory stimulus ($\bar{X}=15.5\pm6.5$ and $\bar{X}=15.8\pm6.7$).

PCAs with scores from 12 trials on six variables resulted in three components accounting for >80% of observed variance. For all feeding points, latencies to approach, consume and pre-sample were positively correlated. These latencies were negatively correlated with number of wolves approaching for the permanent visual stimulus and for the intermittent acoustic stimulus. Late trials were associated with fewer approaches for the permanent visual stimulus and with more wolves approaching for the control. Late trials were associated with shorter latencies to consume for the intermittent acoustic stimulus.

3.2. Sensory stimuli – Experimental Period 2

The second test period lasted for four trials (Table 3). After trial 4, the attractants at each feeding point had been consumed at least once.

Table 3. Occurrence of consumption (C) and approach (A) across trials and by type of stimulus; consumption involves approach.

Trial number	Control	New stimuli (light/odour)	Movement-activated ultrasounds	Permanent acoustic
1	C	C	C	-
2	C	C	C	-
3	C	C	C	C
4	C	C	C	C
Total C	4	4	4	2
% of trials during which consumption occurred	100%	100%	100%	50%

The last attractant to be consumed was associated with a permanent acoustic stimulus. Attractants at the control feeding point and at feeding points associated with new stimuli and ultrasound were consumed on every trial. The feeding point associated with a permanent acoustic stimulus was only approached by wolves and the attractant consumed on trials 3 and 4.

On average, wolves took longest to approach the feeding point associated with a permanent acoustic stimulus ($\overline{X}$=60.8±29.6), followed by new stimuli ($\overline{X}$=24.25±18.4), ultrasound ($\overline{X}$=16.5±8.9) and the control feeding point ($\overline{X}$=4.5±0.6). The attractant associated with a permanent acoustic stimulus was also the last to be consumed ($\overline{X}$=60.8±29.6), followed by ultrasound ($\overline{X}$=35±21.9), new stimuli ($\overline{X}$=25.5±18.4) and the control ($\overline{X}$=4.5±0.6).

4. Discussion

4.1. Wolf reaction to novel stimuli

Field investigations of uncombined visual or acoustic stimuli is almost non-existent for wolves, except regarding fladry tests, the results of which have been highly variable depending on test conditions. In penned experiments, wolves seemed to habituate to fladry after one day of exposure (Lance et al., 2010), whereas in free-ranging conditions it remained efficient for up to 90 days (Gehring et al., 2006). Regarding intermittent acoustic stimuli, tests on coyotes indicated that propane explosions could deter predation in free-ranging conditions for 1 to 180 days (Pfeifer and Goos, 1982).

Some authors have thought motion-activated stimuli to be more effective than permanent and intermittent stimuli (Shivik and Martin, 2000). However, we found that motion-activated and intermittent lights, as well as permanent acoustic stimulus (radio) and behaviour-contingent ultrasound were poor repellents compared to permanent visual and intermittent acoustic devices. This suggests that wolves' level of neophobic behaviour toward a stimulus may depend more on the properties of the stimulus rather than its activation mode (Harris and Knowlton, 2001). In our trials, the permanent visual stimulus (aluminium leaves) tested in the first experimental period elicited the longest neophobic reaction, with highest latencies to approach and consume, followed by the intermittent acoustic stimulus (mole repeller).

We observed that olfactory stimuli, either organic or artificial, were ineffective at evoking a neophobic reaction from wolves, which is in agreement with most previous studies (e.g. Harris and Knowlton, 2001 - for coyotes). We further observed that mean latencies to approach and to consume were even smaller for artificial olfactory treatment than for the control, supporting the suggestion that such stimuli might elicit approach instead of the intended avoidance (Harris and Knowlton, 2001). Attractiveness of olfactory stimuli might be related to the scent-rubbing behaviour of wolves. Manufactured odours such as deodorant or perfume were reported to elicit the strongest rubbing response by wolves (Ryon et al., 1985), which might explain the attractiveness of feeding points associated with artificial olfactory stimuli in our study.

Finally, many studies have found ultrasound to be ineffective as a repellent (e.g. Edgar et al., 2007 - for dingoes). The assertion that ultrasound is a stronger repellent than sounds audible to humans has yet to be confirmed and might be erroneous (Bomford and O'Brien, 1990). The use of ultrasound as a repellent has seldom been studied in wild canids. Our results indicate that 24 kHz motion-activated ultrasounds are ineffective at repelling captive sub-adult wolves.

4.2. Wild vs. captive individuals

Wolves seem to habituate to fladry faster in penned experiments than in free-ranging conditions (Gehring et al., 2006; Lance et al., 2010). This might be due to the fact that captive wolves are reared in an enriched environment, reducing their later level of neophobia (Corey, 1978; Greenberg, 2003). In addition, captive wolves in permanent contact with fladry during experiments have more opportunity to learn than wild wolves passing by the fladry line, and hence may habituate quicker. The young wolves in our study had known human handling, social interactions and visual variety from a young age, although such interactions were kept to a minimum within the rehabilitation process. Such early stimulation may have led to them being less fearful and more exploratory during tests (Corey, 1978). On the other hand, free-ranging juveniles may have to explore more unpredictable and dangerous territories, reducing their level of neophobia compared to captive individuals raised in safer conditions (Greenberg, 2003). Variation in wild wolves' level of neophobia depends on the interaction between their environment and juvenile exploration.

4.3. Neophobia vs. exploration

Our results indicate that wolves both investigated and avoided novel permanent visual and intermittent acoustic stimuli, as no consumption was undertaken despite variable latencies to approach or pre-sample over the trials. Novel objects can elicit animal reactions that range from curiosity to anxiety (Corey, 1978; Greenberg, 2003; Harris and Knowlton, 2001). Moretti et al. (2015) found that wolves displayed a greater interest in novelty, but also greater neophobia than domestic dogs. In the present study, wolves seemed to be more perseverant in approaching an intermittent acoustic stimulus than a permanent visual stimulus, as: 1) shorter latencies to approach were associated with a higher number of approaches for the acoustic stimulus, but not for the permanent visual stimulus; 2) consumption tended to occur faster over the trials for the acoustic stimulus; and 3) the number of approaches tended to decrease over trials with the visual stimulus. It has been shown that juveniles tend to have a spontaneous attraction to novel objects and a lower neophobic response (Greenberg, 2003). The young age of our experimental animals might thus have led them to adopt more exploratory behaviours than adult wolves.

4.4. Individual variation

We were not able to study individual variation in response to novelty, but this could be an important aspect to consider when studying neophobia. Each individual tends to react differently to novelty due to differences in personality and experience. Behavioural differences are also related to the social status of individuals within a group. For example, during pairs' tests, dominant coyotes were found to be less neophobic, thus taking more risks, than subordinates coyotes in novel settings (Mettler and Shivik, 2007).

In addition, the presence of an experienced adult may facilitate learning (Galef and Laland, 2005). The young wolves involved in the present study were not exposed to novelty alongside an experienced adult (i.e. an adult already habituated to the devices tested). Free-ranging juveniles, on the other hand, usually have the opportunity to learn from experienced parents.

4.5. Group effect

Longer latencies to approach tended to be associated with fewer wolves approaching for the permanent visual stimulus and for the intermittent acoustic stimulus, implying a possible group effect in wolves' neophobic reaction. Moretti et al. (2015) observed that wolves manipulated a novel object more when in a group than alone. Such social facilitation was effectively observed throughout the tests, but no definitive conclusion can be drawn from our group sample size (n=1).

4.6. Use of senses during predation

Wells and Lehner (1978) suggested that the most significant senses used during predatory behaviour might be more susceptible to the corresponding aversive stimuli. They asserted that vision was the most important sense during coyote predation, followed by audition. This would be consistent with our own findings regarding wolves' level of neophobia towards auditory and visual stimuli. In addition, different stimuli may be more effective during different phases of the predation sequence. Visual cues are the strongest releaser of prey capture in foxes, but they rely mostly on audition to locate prey (Osterholm, 1964). We found that novel visual stimuli placed close to an attractant evoked the strongest neophobic reaction in wolves. By ordering wolves' sensory importance during the hunt, and understanding at which scale of the anthropogenic landscape these senses intervene, it might be possible to create discomfort areas around livestock using low-cost stimuli and thus selectively reduce wolf predatory activity on the landscape.

4.7. Recommendations for further research

Caution should be exercised when interpreting results from experiments in captivity, as captive conditions differ from free-ranging conditions. Biases caused by socialization, exposure to an enriched environment, restrained group dynamics and regular feeding should be taken into account. Thus, the results of our study cannot be extrapolated to free-ranging conditions. Nevertheless, our results help clarify hypotheses and topics for further research and possible field trials. Studying wild wolves' behaviour requires time and, in many cases, expensive equipment. Experiments in captivity are easier to implement and can give primary indications on general wolf behaviour that could subsequently be applied to experiments in the wild.

Explorative studies of various deterrents are quite challenging. Indeed, it is difficult to test large numbers of novel stimuli on a single wolf group, as they may gradually habituate to novelty and integrate it as part of their environment. This is especially true in captive conditions, as wolves are continuously exposed to novel objects and their responses may therefore quickly diminish. We decided to test eight stimuli that were quite different in nature and made broad conclusions on the relative effect on wolf behaviour of various visual, olfactory and acoustic stimuli. For future research, however, we recommend focusing on comparing specific stimulus properties, such as comparing the effect of visual stimuli size or sound stimuli volume, in order to refine conclusions. Various factors, unrelated to stimulus properties, should also be investigated to clarify their effect on wolves' neophobia, such as rotating stimuli to delay habituation, the influence of conspecifics and familiarity with the environment.

5. Conclusions

We believe there is potential for low-cost deterrents to effectively repel predators and alleviate the cost of livestock protection. We found that neophobic responses of wolves were highly dependent on the properties of the novel stimuli to which they were exposed. In our trials, simple permanent visual and intermittent acoustic stimuli evoked stronger neophobic responses from a group of captive wolves than permanent acoustic stimuli, lights, olfactory and ultrasonic cues. We suspect a group effect and individual boldness to have influenced wolf behaviour in this study. Many other variables are important to consider in relation to free-ranging wolves' neophobic response to simple stimuli. We recommend conducting further explorative studies of wolf deterrents to help elucidate key properties for low-cost disruptive devices.

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