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Abstract

According to the Swedish welfare act, cattle stables must have inlet of natural light and artificial light that do not disturb the animals' natural behaviour and diurnal rhythm, dairy cows should also be provided with light at night. However, there are no specific recommendations on light programs for calves.

In this study, the behaviour and movement of 12 female dairy calves were assessed in four different light intensities (225 lx, 5 lx and 0.5 lx in white light and 0.5 lx in red coloured light) during two weeks. The experiment had a changeover design with three calves allocated to four groups. The calves within one group followed the same test order but were individually tested. On average, the calves were 11.5 weeks of age and 3.5 weeks after weaning.

The response was evaluated by first enticing the calves to pass through an obstacle course where time in the obstacle course, number of steps and number of contacts were measured, and secondly by a novel object test were six different behaviours, time until first contact, number of contacts and mean length of contacts were recorded.

In the obstacle course the calves took significantly ($p<0.05$) more steps in 0.5 lx red light compared to 5 lx. In the novel object test, the calves moved the novel object significantly more in 225 lx compared to 5 lx ($p<0.01$) and 0.5 lx red light ($p<0.05$) and significantly less in 5 lx compared to 0.5 lx white light ($p<0.05$). The results also showed a tendency that the calves moved the novel object more when they were tested in their third or fourth treatment.

In conclusion, only few significant effects were found between the treatments. This suggests that calves can cope with intensities down to 0.5 lx without large significant effects on their behaviour or movement. There were no significant diffences in willingness to pass the course or on the behaviour in the NOT between white light and red coloured light in 0.5 lx.

Sammanfattning

Enligt svensk djurskyddslag måste all nötkreatur ha tillgång till naturligt ljus samt artificiellt ljus som inte stör djurens naturliga beteende eller dygnsrytm. Mjölkkor ska dessutom ha nattbelysning. Det finns dock inte beskrivet något om kalvars ljusbehov och lagstiftningen saknar för närvarande specifika rekommendationer om ljusprogram till kalvar.

I denna studie undersöktes beteende och rörelsemönster hos 12 kvigkalvar av mjölkras i 4 olika ljusintensiteter (225 lx, 5 lx, 0,5 lx i vitt ljus och 0,5 lx i rött ljus) under två veckors tid. Försöket följde en change-over design med fyra grupper och tre kalvar i varje grupp Kalvarna inom samma grupp följde samma testordning men testades individuellt. Kalvarna var i medel 11,5 veckor gamla och avvanda sedan 3,5 veckor.

Kalvarna utvärderades genom två tester. I det första testet fick de gå igenom en hinderbana där antal steg, tid och antal kontakter mättes. Därefter fick de genomgå ett så kallat novel object test (NOT), där 6 olika beteenden, tid till första kontakt, antal kontakter och medellängden av kontakterna studerades.

I hinderbanan tog kalvarna signifikant fler steg ($p<0,05$) i 0,5 lx rött ljus jämfört med 5 lx. I novel object testet flyttade kalvarna objektet signifikant fler gånger i 225 lx jämfört med 5 lx ($p<0,01$) och 0,5 lx rött ljus ($p<0,05$) samt signifikant färre gånger i 5 lx jämfört med 0,5 lx vitt ljus ($p<0,05$). Resultaten visar även en tendens till att kalvarna flyttade objektet mer i sin tredje eller fjärde behandling.

Som slutsats pekar dock resultaten på att kalvarna inte visar nämnvärda förändringar i varken beteende eller rörelser av ljusintensiteter ner till 0,5 lx. Inga signifikanta skillnader i beteende eller rörelser kunde påvisas mellan rött och vitt ljus i 0,5 lx.

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1 Introduction

Although there are some studies that indicate that light is an important factor to consider in calf rearing (Petitclerc et al., 1983; Danneman et al., 1995), this is not reflected in the national legislation, where only cattle as a main group is mentioned. The Swedish Welfare Act states that all cattle must have inlet of natural light and artificial light that does not disturb their natural behaviour and diurnal rhythm. Additionally, dairy cows should be provided with light at night. There are, however, currently no specific recommendations on light programs for calves (Swedish board of agriculture, 2015). The technical specification (SIS-TS 37:2012) recommends 100-150 lx daytime and 5 lx at night for cattle. Internationally, there are large variations of recommended minimum light intensity for cattle, ranging from 20 to 120 lx (Phillips & Weiguo, 1991).

For adult dairy cows there are a vast number of studies describing the effect of light intensity and light hours on production parameters and behaviour. Common measurements such as milk yield (Dahl et al., 1997; Hjalmarsson et al., 2014), cow traffic (Hjalmarsson et al., 2014) and locomotion (Philips et al., 2000) have been proven to depend on the light conditions in the barn. Based on this, it is possible that the light conditions used in barns for calves may affect the behaviour and locomotion patterns in a similar way. For example, beef calves exposure to low light intensities (20 or 2 lx) compared to high light intensities (100 and 130 lx) was associated with less playing behaviour. In the lowest intensity of 2 lx stereotypical licking and increased resting behaviour was also shown (Danneman et al., 1985). Also, studies have shown that short days (8 h light and 16 h dark) compared to long days (16 h of light and 8 h dark) resulted in impaired physiological measurents such as growth and onset of puberty in pre-pubertal heifers (Petitclerc et al., 1983). However, having barns with too bright light conditions may also cause adverse animal welfare, since a certain amount of darkness is needed in order to stimulate the release of melatonin, a sleep regulating hormone (Sjaastad et al., 2010). In fact, studies have shown that reduced melatonin levels at night may occur already in 50 lx (Muthuramalingam et al, 2006). The importance of correct light conditions also include colour, as cattle may be reacting differently in various wavelengths of light. In literature, there are different ideas whether or not cattle, as an animal with dichromatic colour vision, are able to distinguish the colour red from blue or green light (Sjaastad et al, 2010; Philips, 2010; Moran and Doyle, 2015). There is also a suggestion that cattle show more activity in red light (Philips, 2010; Philips and Lomas, 2001). It is obvious from literature, that there is a lack of knowledge on how cattle, especially calves, perceive light and that more studies need to be conducted on this subject in order to find out the correct balance of light to match their needs.

1.1 Aim

The aim of this experiment was to find out how low light intensities affect the dairy calves behaviour during a novel object test and their willingness to move through an obstacle course. It was also to find out if there was a difference in reaction in red light compared to white light in 0,5 lx. The results of this experiment aim to provide scientific information that can be used as guidance when conducting further experiments in this area.

2 Literature background

2.1 Light

2.1.1 Light intensity

There are a vast number of measurements used to describe light properties. In radiometry, the focus is to measure light in all wavelengths, whereas photometry focuses on measuring the visible part of the light spectra as it is perceived by the eye. Light intensity, or brightness, is measured as illuminance and is the photometric equivalent to irradiation. The unit for illumination is lux (lx), defined as lumens per square meter (Commission on illumination, 1983). As the eye perceives wavelengths with different sensitivity, radiologic instruments measuring the objective value of radiated energy will give deceptive results (HS engineering, 2015). A lux meter is however designed to measure illumination from a human eye perspective and is commonly used for this purpose. Important to remember is that humans and cattle have different vision and may also have different sensitivity to wavelengths, which might result in a different perception of the illumination (Hörndahl et al., 2013).

2.1.2 Light settings in the barn

According to the Swedish Welfare Act, the stables for all cattle must have inlet of natural light and have lighting that does not disturb their natural behaviour and diurnal rhythm. Additionally, dairy cows shall have a lit stable at night (Swedish Board of Agriculture, 2015). The Technical specification (SIS-TS 37:2012) further recommends that the light intensity should be 100-150 lx daytime and 5 lx at night time. In Europe, the recommendations for minimum light intensities in cattle barns range from 20 to 120 lx (Philips & Weiguo, 1991).

Little is known about the need of daily light for calves; however there are studies that describe the relationship between day length, growth and onset of puberty, which are important aspects to consider in calf rearing. An experiment conducted by Petitclerc et al. (1983), showed that 16 h of light and 8 h of dark compared to 8 h of light and 16 h of dark increased the daily weight gain and feed efficiency in pre-pubertal Holstein heifers. Also, it is further implied that these heifers had an earlier induced puberty at a lower weight than those from 8 h of daylight (Petitclerc et al., 1983).

There are also some advice on the source and distribution of light for cattle. For example, high intensity light sources, such as halogen light, are not recommended due to their tendency to be perceived as too strong or even blinding. Instead, energy efficient light such as fluorescent tubes or sodium lights should be used (Philips, 2010).

2.1.3 Light and circadian rhythm

The 24-hour rhythm (circadian rhythm) is mostly associated with the regulation and variation of hormone concentrations and could be described as the body's internal clock. Melatonin, produced by the pineal gland behind the hypothalamus, is the hormone responsible for sleep regulation in mammals and birds. It is also believed to affect the estrus cycle. Melatonin is formed from the amino acid tryptophan. When light is present, it is captured by the retina and information is transmitted through nerves to the pineal gland, where the secretion of melatonin is inhibited. When darkness falls, and the amount of light suppressing the secretion is lowered, the level of melatonin increases. The level will continue to increase until midnight, where it reaches a peak. After midnight, the melatonin level will slowly start to decline (Sjaastad et al., 2010).

If light conditions in the barn are too bright at night, the secretion of melatonin will be suppressed and the sleeping patterns of the animals may be disturbed. Muthuramalingam et al. (2006) showed that a light intensity of 50 lx suppressed half of the night plasma melatonin levels in the first two hours of the night in pre-pubertal heifers. However, no such effect could be observed in 5 or 10 lx in the same experiment. Thus, intensities of 50 lx or higher should be avoided at night, instead 10 lx or less can be used (Muthuramalingam et al, 2006).

2.1.4 Light influence on behaviour and locomotion

It is known that cattle are more active in high light intensities due to a better visual capacity in bright light (Philips, 2010). Consequently, there may be behavioural differences and different locomotion patterns in light compared to dark. A study made by Philips and Arab (1998) investigated the preference to perform certain behaviours in light compared to dark on single housed bullocks. The four bullocks in the experiment were trained to switch the lights on and off in a stable with no other light inlet. The results show that there was a weak preference to feed, stand and lie in the light but no difference in preference when ruminating and sleeping. The authors conclude that the weak preference of light during the performance of feeding, standing and lying may be linked to their need of a higher alertness when performing these behaviours. The authors further speculate that there may be a stronger preference in group housed cattle to perform certain behaviours in the light since they are likely to be influenced by the behaviour of other members in the group, and therefore require better vision. Another study on beef calves measured the behaviour in low light intensities (2 and 20 lx) compared to high intensities (100 or 130 lx). In the lowest light intensity (2 lx) the calves showed prolonged resting behaviour and behaviour of stereotypical nature, furthermore the calves showed less playing behaviour in both 2 and 20 lx. The prolonged resting behaviour shown in 2 lx may be caused by the insignificant difference between night and day, and the stereotypic behaviours are believed to be a reaction to the poor vision and compromised welfare generated by the darkness (Dannenmann et al., 1985).

In Philips et al., (2000) the locomotion of dairy cows in different light intensities was measured in two experiments. In the first experiment, the cows step length, step rate and speed was recorded as they passed through a lit (259 lx) or a dark (no artificial or supplementary light) aisle in their way back after milking. In the second experiment, another group of cows was tested as they walked through a passage to get feed. In the passage, 6 different light intensities, between 0 and 265 lx, were applied on six different occasions. In this experiment the step length, stepping rate and the angle of the leg joints was measured. The results in the first experiment showed that the locomotion patterns in darkness tended towards shorter but more rapid steps compared to the locomotion in the lit aisle; however the walking rate (speed) was the same in both treatments. In the second experiment, the cows showed no changes in step length but a faster walking- and stepping rate in 0 lx, and the lowest in 32 lx. Furthermore the cow's position of the fore- and hind feet were more vertical in the dark. The authors believe that the faster stepping rate, shorter steps and altered position of the feet were ways to find stability and avoid slipping in the dark and that at least some level of lighting should be present at night in cow barns.

2.2 Cattle behaviour

The behaviour of cattle can be divided into three categories; the instinctive behaviours, the sensory behaviours and the learned behaviours. The instinctive behaviours are those that the cows are naturally driven to do and that are fully developed at birth. The sensory behaviours are the behaviours performed after a sensory stimulation, e.g. a certain smell or sound. The learned behaviour is often motivated by instinctive or sensory behaviour but is not correctly performed the first time, for example the need of drinking milk is instinctive but drinking from a bucket needs practicing. Often, the behaviours can be a mix of these three categories (Moran and Doyle, 2015).
As prey animals, cattle are dependent on their social structure in big herds as a way of reducing the risk of predication on individual animals. If left alone, cattle may therefore show distress and fear. Furthermore, if cattle are disturbed by a predator or during handling, they often stick together making it harder to divide or move individual animals. By nature, cattle are fearful of unfamiliar things in their environment, such as objects or smells and of sudden sounds and movement. To minimize the risk of fearful animals it is therefore good to implement a daily handling routine (Moran and Doyle, 2015).

2.2.1 Fear and fear evaluation in cattle

Fear can be described as the response to a real or perceived threat and is a vital emotion for survival in pray animals such as cattle, as it motivates them to stay alert. As previously mentioned, cattle are often fearful of unfamiliar things and environments (Moran and Doyle, 2015). Hence, there are many situations in their everyday life where cattle can experience fear. For example changes in light intensity, new enclosures or isolation (Klindworth et al., 2003). The strength and nature of the fear reaction is individual and there are many behavioural signs that could be interpreted as fear related, which might lead to misleading conclusions about the actual welfare of the animals (Boissy and Bouissou, 2005).

Welp et al. (2004) suggested that fear or fearfulness could be evaluated by observing the vigilance, defined as “head raised” in different situations and environments. They found that the cows spent more time alert when put under fear inducing situations, such as the presence of a dog or an unfriendly human and that the behavioural response was proportional to the believed fearfulness. However, this is not consistent with other fear related behaviours described in more recent literature, where changes in vocals (Moran and Doyle, 2015; Westin et al., 2009), freezing, moving backwards and running (Westin et al., 2009) are described. Even inactivity can be a sign of fear in cattle (Boissy and Bouissou, 2005).

One commonly used method to evaluate stress or fearfulness is the Novel object test (NOT) (Forkman et al., 2007). In the novel object test, one single animal is introduced to an object either by human or mechanical placing. The behaviour of the animal is then recorded during the testing time, which is usually no longer than 15 minutes. The types of behaviour usually measured are for example exploration behaviour, number and duration of contacts, vocalizations, body posture and distance to the novel object. The NOT is often performed together with other stress tests, with good correlation. The open field test, also commonly used fear test in cattle, has shown low correlation to other fear test results, and are therefore not recommended as a method for fear evaluation. The forced and voluntary approach tests are focused on measuring the fearfulness towards humans, rather than as a general indicator of fear (Forkman et al., 2007).

2.3 Vision

The vision is the most important sense in cattle for perceiving the surroundings, accounting for half of the total sensory information (Moran and Doyle, 2015). In most mammals the visible light consists of light particles (photons) emitted from electromagnetic waves of 400-700 nm. This is however only a fraction of the total electromagnetic spectrum. Long wavelengths, emitting red and orange light, have photons with less energy than waves of short length, such as violet and blue light (Sjaastad et al., 2010). In cattle, the maximum wavelength perceived has been estimated to be 620 nm (orange light) (Philips and Lomas, 2001).

Due to special receptors located in the retina, called photo pigments, light can be captured in the eye. In the photo pigments, a chain of reactions causes the sensory cells to hyperpolarize, resulting in a reduced release of neurotransmitter. The reduced release of neurotransmitter results in either inhibition or stimulation of the ganglion cells of the optical nerve that ultimately sends visual information to the brain. When a photo pigment is used up, it has to be resyntesised in order to once again be useful. The rate of resynthesation is dependent on the light conditions. In bright conditions, very little photo pigments is needed, whereas more is needed in the dark. This explains why obtained vision is delayed after moving from bright to dark (Sjaastad et al., 2010). The estimated time for complete dark adaptation is 15-30 minutes in cattle (Philips, 2010).

Herbivores such as cattle have eyes positioned on the side, rather than in the front of the head. This enables them to have good monocular vision (vision covered by one eye) and a total visual field of 330 degrees (Philips 2010; Moran and Doyle, 2015). The remaining 40 degrees, located directly behind the cow is a blind spot (Moran and Doyle, 2015). The wide visual field is of vital importance when watching out for predators. However, the location of the eyes means that they have poor binocular vision (overlapping visual field), limiting their ability to determine depth (Sjaastad et al., 2010). Cattle and most other animals have a reflective layer behind the retina, called tapetum lucidum, often seen as “glowing eyes” in the dark (Olliver et al., 2004). The tapetum duplicate the light as it passes the retina, which enables good vision even in low light intensities (Philips 2010). Some animals, including primates (Olliver et al., 2004) and humans, lack this layer and it is therefore hypothesised that cattle are better adapted to low light intensities than humans (Philips, 2010).

2.3.1 Colour vision

The two types of sensory cells, located in the retina of the eye, are rods and cones. The cones supply colour vision but are only efficient in higher light intensities, the rods, however, allow vision even in low light intensities. Since the cones are insufficient in low light intensities, the colour vision disappears and only black and white can be distinguished. There are different types of cones, all with different sensitivity to light of specific wavelengths; this is what enables the eye to discriminate between colours. The colour of an object is determined by the wavelength of reflected light. If no light is reflected, the object will appear black. The opposite is when all light is reflected and the colour is then perceived as white (Sjaastad et al., 2010).

Most domestic animals have dichromatic colour vision, meaning that they have two types of cones. Unlike humans, who possess three types of cones, animals with dichromatic colour vision are not able to distinguish between green and red light. Interestingly, some literature states that cattle have some ability to discriminate red from green or blue light (Moran and Doyle, 2015), however, this is suggested to appear as shades of gray rather than in colour as perceived by humans (Hörndahl et al, 2013). Studies have also shown that cattle are more active in red light (Philips 2010; Philips and Lomas, 2001). According to Philips (2010), the capacity to distinguish the colour red may have developed to enable them to detect blood or a female in oestrous.

3 Material and methods

The experiment was conducted at Lövsta research centre in Uppsala, Swedish University of Agricultural Sciences. All animal handling was approved by the Uppsala ethics board (C89/15).

3.1 Animals and housing

For the experiment a total number of 12 female calves of two different dairy breeds were used. Eight of the calves were from the Swedish red breed (SRB) and the remaining four from the Swedish Holstein (SLB). Average age of the calves was 11.5 (range 8-20 weeks) weeks and average time since weaning was 3.5 weeks (range 0-12 weeks) during the experiment. More information about the calves is expressed in table 1.

Table 1. *Breed, date of birth and week of testing presented for the calves*.

Calf ID	Breed	Date of birth	Experimental Week
0562*	SLB	2015-04-04	1
0569	SLB	2015-05-11	1
0571	SRB	2015-05-25	1
0573	SRB	2015-05-27	1
0574	SLB	2015-05-28	1
0575	SRB	2015-06-07	1
0578**	SRB	2015-06-11	2
0579	SLB	2015-06-15	2
0580	SRB	2015-06-18	2
0581	SRB	2015-06-22	2
0582	SRB	2015-06-27	2
0583	SRB	2015-06-29	2

*Calf nr 0562 had a low birth weight for unknown reasons.
** Calf nr 0578 was approximately 1 month prematurely born

In the beginning of each experimental week, the calves were moved from their usual group housing, to the experiment stable where the test arena and three boxes were located. The three boxes were 3 x 3 meters and held two calves per box; the experiment was therefore performed in two batches of six calves, one batch per week.

During the experimental week, each calf had ad lib access to silage and a total of 1 kg concentrate (Lantmännen IDOL) per day. The concentrate was distributed in two allowances per day, one morning and one evening allowance. In connection to the feeding, the boxes were cleaned from faeces and urine. After cleaning, the bedding material that consisted of wood shavings was refilled. By the end of each week the calves were moved back to the calf unit when the experiment was finished. Additionally, the boxes were cleared out and cleaned between week one and two in preparation for the next batch.

3.2 Experiment material and test arena

In order to conduct the obstacle course test, a test arena was built inside the experiment stable by using the original interior with addition of some portable metal gates (figure 1). The gates were tied together to create a confined passage. The passage, 13.5 m long and 2.8 meters wide was then made into an obstacle course with tree obstacles consisting of white plastic bars (light weight cavaletti bar, Safety-system, Enköping, Sweden) and blocks (cavaletti block small, Safety-system, Enköping, Sweden) The edge of the passage had slattered floors; these were covered with rubber mats, otherwise the floors were of concrete. The obstacle course was built deliberately to give the calf two opportunities; either to jump the obstacles or to walk around them. This was done by leaving a gap of at least 0.5 m between the obstacle and the passage wall. To make sure that the "obstacle free way" was not just a straight line the gaps were created on different sides of the obstacles. The obstacle course was also built so that when the calf was entering the obstacle course it was facing the boxes and other calves. This was deliberately done to use the calf's need of social interaction as a motivator to finish the course.

A confined area of 1.5 x 2.5 meters was created in the aisle along the obstacle course. This area was used as a test arena for the novel object test (figure 1).

In the NOT a number of different novel objects were used; a pink box, an inflatable beach ball, an inflatable beach frog, a colourful pinwheel, a traffic cone and a black plastic cat.

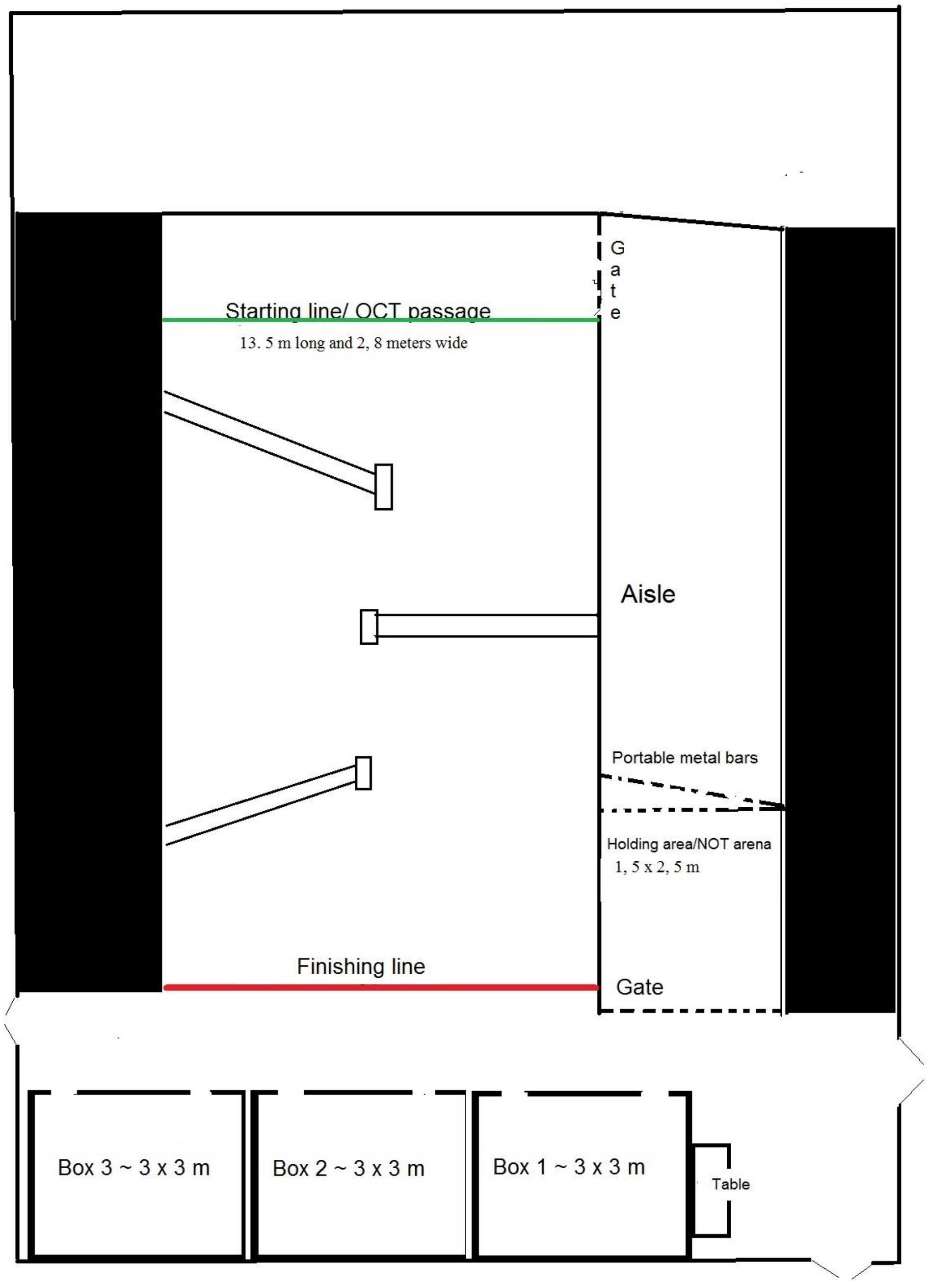

Figure 1. Illustration of experiment stable. The figure is not to scale.

3.3 Test procedure and measurements

3.3.1 Light intensity settings

In the experiment, four different light intensities were tested; 225 lx, 5 lx, 0.5 lx white light and 0.5 lx red coloured light. To ensure that the light was evenly distributed in the testing arena, the light intensity was measured on three different points in the obstacle course test (OCT) passage and the NOT arena using a lux meter, inaccuracy level ± 5% + 10 digit (Lux meter Standard ST-1300, Clas Ohlson, Insjön, Sweden) with ability to measure in four ranges; 0.01-200, 200-2000, 2000-20000 and 20000-50000 lx. In this experiment only the 0.01-200 lx range was used. In the OCT passage, the light intensity was measured at approximately 30 cm over above floor level at the starting line, halfway through the course and at the finishing line. In the test arena for NOT, the light intensity was measured at both ends and in the centre of the arena.

In order to get the right light intensity and to get the light evenly distributed in the test arena, light strands with LED lights of white light (Glänsa light strand 12 meters, Rusta, Upplands Väsby, Sweden) and of red coloured light (light strand 10 meters, Konstsmide, Gnosjö, Sweden) were put up along both sides of the OCT passage wall, and switched on and off depending on trial. Additional to this, fixed light fittings were covered with plastic bags to various extent depending on desired light intensity. All the emergency signs were covered with plastic bags. Before starting a trial the calves were given 30 minutes to adjust to the new light intensity.

3.3.2 Pre test procedure

Before starting the trials, a number of test runs were performed the first day of every week. In the first week some additional runs were made to practice the test procedure and calf handling. The purpose of the test runs was to acclimatize the calves to the new environment and to make sure they had all walked through the aisles and passages before the start of the experiment. The number of runs per calf was not fixed, the test runs were terminated when all the calves had learned to move though the different steps. Also, a small amount of feed concentrate was given in connection to this as a positive reinforcement. For the same reason, the calves that did not want to eat concentrate were gently patted after and during each test run.

3.3.3 Experimental design

The calves were divided into a total of four groups with three calves in each group. The experiment was conducted as a changeover study, where all calves in one group were subjected to the treatments in the same order, but tested individually. The treatment order for each group is presented in table 2. The groups of calves were tested 1-2 times per day depending on time use of each calf.

Table 2. *Order of testing, 225 lx =225 lx intensity light (full intensity), 5 lx= 5 lx intensity light, 0.5 lx (r or w) = 0.5 lx intensity light in r = red or w = white.*

Group	**Order**			
	1	2	3	4
A	225 lx	0,5 lx w	5 lx	0.5 lx r
B	0,5 lx w	225 lx	5 lx	0.5 lx r
C	225 lx	0.5 lx r	5 lx	0.5 lx w
D	0.5 lx w	225 lx	0.5lx r	5 lx

3.3.4 Obstacle course test

At the start of a trial, one calf was moved to the NOT arena at the start of the aisle next to the OCT passage (figure 2). This was done by enticing the calf with a bucket of concentrate and some gentle pushing to move forward. After some measurements had been taken, belonging to a simultaneous study, the calf was released and could proceed to the end of the aisle where one person was waiting with the concentrate bucket. The calf was enticed to walk out in to the OCT passage using the concentrate bucket and mild pushing. When the calf passed the starting point, a digital timer able to count seconds (Kitchen Timer, Philips, Amsterdam, The Netherlands) was started and the number of steps from this point to the finishing line was counted. If the calf did not continue the course by its own, the person with the bucket continued to walk approximately 0.5-2 meters ahead of the calf, motivating it to finish the course. To keep the calf from turning back, another person walked approximately 2 meters behind and gave the calf a push if it did not want to move forward. If the calf had any type of physical contact with the obstacles this was noted.

The parameters registered in the OCT were number of steps, time from start to finish and number of contacts with the obstacles.

3.3.5 Novel object test

When the calf had passed the finishing line the timer was stopped and the calf was once again brought to the NOT arena. After a few more measurements belonging to the other study, the novel object study was initiated. This was done by firmly putting a novel object in the NOT arena, making sure the calf was noticing. As the object touched the ground, the timer was started. During the test, the time until first contact, the number of contacts and the length of each contact were measured. The study progressed for 15 minutes. During the 15 minutes, 6 types of behaviours were counted, see table 3 for ethogram. The novel object and the obstacle course were only used once per group, after that the object was changed and the obstacle course was rebuilt.

Table 3. *Ethogram of scored behaviours in the NOT.*

Behaviour	Definition	Description
Licking, smelling or biting (LSB)	Licking: The calf's tongue touches the object. Smelling: The calves muffle is lightly touching (but not moving) the object or is close (< 10 cm) to the object. The nostrils are moving or sounding. Biting: The calf touches the object with its teeth, but is not moving the object.	One count for each behaviour respectively. The behaviour was manually counted
Unintentional contact	Contact without awareness, for example tripping over the object or touching the object with one or more hoofs as they are moving.	Manually counted
Moving	The calf either pushes the object or lifts it up and moves	Manually counted
Retreat	The calf is retreating or moving away in a fearful manner immediately after (or before) making contact with the novel object	Manually counted
Vocalization	Sound made by the calf's vocal cord, Note: Not in connection to urination or defecation	Manually counted
Lie down	The calf lies down on the chest or side in the test arena	Manually counted

3.4 Data handling

The data was manually collected during the trials and thereafter transferred, sorted and organized (Microsoft Office Excel 2007, Microsoft Corp. Albuquerque, NM, USA). The effect of light intensity, group, order in which light intensities were applied, previous light intensity and breed was tested with a linear mixed model (SAS 9.4, SAS Inst. Inc., Cary, NC, USA). Breed and previous light intensity was found non-significant. The statistical model included the random effect of calf(ID), and the fixed effects of light intensity (TREAT), group and order. Repeated measures on calf nested within group with autoregressive covariance structure was included as a random effect using the statement 'repeated order/subject=ID(group) type=AR (1)'. For the fixed effects, least-squares means (LSMEAN) were calculated and differences between them were tested for significance using t-tests. Normality and equality of variance were checked by visual inspection of the residuals. Values given are mean(SD) or LSMEAN±SEM.

4 Results

4.1 Obstacle Course Test

No significant difference in time passing the course or number of contacts with the obstacles in the OCT was found between groups, intensity or order of testing. However, there was a significant increase ($p<0.05$) in number of steps taken in 0.5 lx red light (39.9), compared to 5 lx (28.9). For further information, please see table 4.

4.2 Novel Object Test

In the novel object test, no significant difference was found in time of first contact, length of contact, licking, smelling or biting (LSB) and number of contacts between groups, intensity or order. There were, however, significantly more movements of the novel object in 225 lx compared to 5 lx ($p<0.01$) and 0.5 lx red light ($p<0.05$), and significantly less moving in 5 lx compared to 0.5 lx white light ($p<0.05$). The results also show a strong tendency ($P=0.0582$) for more moving when calves were tested in their third or fourth treatment compared to the second, and more in the fourth treatment compared to the first. More information can be found in table 4.

4.3 Other results

Three of the measured behaviours in the NOT; retreat, vocalization and lie-down, did not have enough observations to secure any statistical relevance. However, they gave some interesting information that could be useful in future experiments with more animals.

The lie down behaviour was only observed in 5 lx and in 0.5 lx red light. There mean value of lie downs were twice as high (0.2) in 0.5 lx compared to 5 lx (0.1) and all observations came from different individuals. Two of the calves came from the same group (Group A).

The mean number of vocalizations observed was higher in 225 lx (2.4), compared to 5 lx (0.3) and 0.5 lx white light (0.5). No vocalization at all was observed in 0.5 lx red light.

The mean number of retreats increased in 0.5 lx (1.1 for white light and 2.0 for red) compared to 225 lx (0.6) and 5 lx (0.3). A large proportion of calves (9 out of 12) performed this behaviour at least once in one of the intensities.

Table 4. *The table presents least square means ± standard error of means and p>F values in recordings of statistical relevance. TOCT= Time in the obstacle course, SOCT= number of steps in the obstacle course, COCT= number of contacts in the obstacle course. LSB=licking, smelling or biting the Novel object (NO), Moving = Number of times moving the NO, NcNOT= number of contacts with the NO, FcNOT= Time of first contact with the NO, LcNOT= mean length of contact with the NO, Uncont= the number of unintended contacts with the NO. Significant differences between treatments or groups are marked with different superscripts in capital letters ABCD Tendencies of differences is marked in lower case letters ab. Significant levels SOCT: A-B = p< 0. 05. Significant levels Moving: AC-B = p<0. 01, AC-BD = p<0. 05 and B-CD = p<0. 05.*

	Least square means (LSMEAN) ± Standard error of means (SEM)								Type 3 fixed effects		
	Treatment				Group				P > F		
Rec.	225 lx	5 lx	0,5lx w	0,5lx r	A	B	C	D	Treatm.	Group	Order
TOCT	51.9 ± 22.2	65.3 ± 24.0	48.9 ± 21.6	79.7 ± 20.2	90.2 ± 27.6	34.0 ± 36.9	65.9 ± 27.0	55.7 ± 27.0	0.6808	0.5583	0.4977
SOCT	32.3 ± 4.2	28.9^{A} ± 4.6	39.6 ± 4.0	39.9^{B} ± 3.7	35.4 ± 4.6	26.6 ± 4.4	39.7 ± 4.4	39.0 ± 4.4	0.0297	0.2171	0.2253
COCT	1.7 ± 0.7	3.1 ± 0.7	1.9 ± 0.6	3.4 ± 0.6	2.1 ± 0.6	1.5 ± 0.6	3.6 ± 0.6	2.9 ± 0.6	0.3460	0.1407	0.9556
LSB	17.3 ± 2.7	5.7 ± 3.2	13.3 ± 2.6	10.5 ± 2.3	10.0 ± 2.5	13.6 ± 2.4	13.2 ± 2.3	10.0 ± 2.4	0.1511	0.6107	0.0947
Moving	13.5^{AC} ± 2.6	- 2.3^{B} ± 3.5	9.6^{CD} ± 2.5	4.0^{BD} ± 2.2	2.8 ± 2.6	7.8 ± 2.3	10.3 ± 2.5	3.8 ± 2.8	0.0395	0.2122	0.0582
NcNOT	18.4 ± 2.5	8.0 ± 3.0	13.8 ± 2.2	10.0 ± 2.1	10.4 ± 2.2	10.1 ± 2.1	17.7 ± 2.1	11.9 ± 2.2	0.1099	0.1025	0.2184
FcNOT	31.0 ± 38.3	50.6 ± 45.0	14.9 ± 32.5	50.3 ± 30.8	13.5 ± 27.8	5.7 ± 26.4	20.6 ± 26.4	107.1 ± 28.0	0.8994	0.1009	0.8953
LcNOT	9.9 ± 3.1	8.3 ± 3.6	13.6 ± 2.7	8.9 ± 2.5	10.0 ± 2.4	12.2 ± 2.3	10.3 ± 2.3	8.2 ± 2.4	0.5622	0.6922	0.4250
Uncont	3.6 ± 1.4	6.2 ± 1.9	2.7 ± 1.2	2.2 ± 1.3	3.2 ± 1.2	1.1^{a} ± 1.2	5.8^{b} ± 1.0	4.6 ± 1.2	0.3196	0.0786	0.5696

5 Discussion

Overall the results show very little difference between treatments. However, this was a small study that only included a homogeneous group of calves of one gender, a specific age group and of milk breed. This was made on purpose to minimize the variance to be able to draw statistically relevant conclusions with a small amount of animals. Consequently, this study would have to include more animals in order to draw relevant conclusions for calves in different systems and of different breeds and gender.

The experiment design used in this study did not follow a Latin square, in which case the groups would have had to be evaluated in all intensities following a random test order. Since some of the groups in some cases went from the same specific intensity to the same other specific intensity, this might have given deceptive results on the measured parameters that may have been related to the order of testing rather than the actual intensity. For example if the calves went more times from light to dark intensities or the other way around. In some of the parameters tested there were big differences between groups, the reason for this is probably that the groups contained only three calves and that the individual differences therefore was more prominent than it would have been if the groups consisted of more animals.

Another factor that could have affected the results was the fact that this study measured a lot of different parameters; this increases the risk to get significance by coincidence (Type 1 errors) wich may lead to false conclusions regarding the results. One way to minimize this risk would have been to raise the level of significance to $p<0.01$, in wich case all of the significant differences found in this study would no longer be true.

5.1 Obstacle course test

The obstacle course was deliberately designed in a way that gave the calves two opportunities, either to walk the course avoiding the obstacles or to jump over the obstacles. This was mainly because the calves tended to walk or gallop in a straight line and did not know how to continue if they had walked into a “dead end” between the wall and the obstacle. This was discovered during the test runs where the obstacles initially were higher and the angles between the wall and the obstacle were sharper. According to Moran and Doyle, (2015), cattle can react this way if they feel pressured in to doing something they don’t want to do. The less sharp angles and lower obstacles offered an easier solution and seemed to reduce the confusion and disorientation from the calves. The obstacles were also easy to knock down, and therefore prevented the calves from tripping if they tried to jump over them but did not lift their legs up properly. Additionally, the white colour is reflective, which made the obstacles easy to detect even in dim light.

The results from the obstacle course showed no significant difference in time or number of contacts between the intensities, however, there was a significant increase in number of steps in the lowest intensity (0.5 lx) red light compared to 5 lx. This is similar to the results by Philips et al. (2000), where it was shown that the stepping rate increased in dark pathways compared to lit pathways (259 lx), and that the step length was shortened in the dark pathway. However, no difference in speed was shown. Although step length was not measured in this study, the time from start to finish can be seen as a measure of speed. Since there were no differences in time between the intensities, and the calves took more steps in 0.5 red light compared to 5 lx, it is likely that the step length was shorter in 0.5 lx compared to 5 lx.

Since the number of steps was manually counted once per calf, they cannot be regarded as exact values. A use of for example video recordings as done in Philips et al. (2000) to monitor the position and angle of the cow’s feet would have given the opportunity to recheck all results if necessary, but would not affect the counting as such, since it is affected by the definition of what counts as a step, which was sometimes hard to determine since the calves did not always show clean steps.

The use of the feed concentrate bucket as a motivator was very efficient. The reason for this was probably because it stimulated the calf’s natural instinct to eat feed and their sensory behaviour of sound and smell (Moran and Doyle, 2015). On the down side, some of the calves were highly feed motivated and quickly learned to follow the bucket. Because of this, it is possible that a lack of vision or possible fear responses may have been suppressed by their motivation to eat or that they learned to blindly follow the smell or the sound of the bucket to get feed. Furthermore, there were a few of the younger calves that did not want to eat concentrate. These calves were harder to entice, and were instead offered gentle patting (positive reinforcement) and an empty milking bottle that they had before weaning to suckle on. The calves, however, quickly lost interest in the milking bottles, making patting the best motivation for these calves. However it was not as successful as the concentrate bucket. This suggests that enticing the calves with feed overall is better than enticing with gently patting or suckling on milk bottles.

Another enticing strategy used was to design the OCT so that the calves were moving towards the boxes where the other calves were held, taking advantage of their need of social contact (Moran and Doyle, 2015). However it is unclear if the calves were aware of their presence in the darkest intensity, in which they might not have been able to spot them. Furthermore, it is hard to conclude if their motivation of social contact were higher or lower than the willingness to proceed in the OCT, since this was used additionally to feed motivation or gentle patting.

5.2 Novel object study

The choice of placing the novel object arena in the aisle next to the OCT passage was made mainly for practical reasons but also to make sure that the novel object arena was familiar, minimising the risk that the calves show fearfulness towards the environment rather than to the darkness and the object. All calves were tested for 15 minutes each in the novel object test so that the results could be comparable and to make space for fearful calves to approach the novel object. Some novel objects seemed to have aroused more attentiveness than others, for example the inflatable beach toys (ball and frog) and the pinwheel was pushed and moved frequently, perhaps because they were more colourful or easily moved. The plastic cat was the least noticed, probably for the opposite reasons. The novel objects used in the experiment was mainly chosen to make sure that none of the objects were similar to things that the calves might have seen in the stable prior to the experiment. One example of a novel object used in another study was an iron- truncated pyramid painted with green and white stripes, similar to the traffic cone used in this study. The pyramid was used in Boissy and Bouissou (1995) in a NOT to evaluate and correlate fear behaviours in heifers.

There were significantly more moving of the novel object in full intensity compared to 5 lx and 0.5 lx red light, and more in 0.5 lx white light compared to 5 lx. The fact that the calves moved the object more in full intensity may have been because they had a better visual capacity (Philips, 2010) and that they therefore showed more investigatory behaviour. Another explanation may be that the investigatory behaviour was suppressed by fear in similarity to the findings in Boissy and Bouissou (1995), where latency to approach the novel object and time spent away from the object was found to be indications of fear. The results from the present study also show a tendency that the calves moved the object more when they were in their third or fourth treatment, which suggest that they probably got more accustomed to the situation after a few trials. This is consistent with normal cattle behaviour as they have good memories and can learn to accept an unfamiliar situation if they are exposed to it routinely (Moran and Doyle, 2015).

5.3 Other results

The other results from the novel object study that was not observed enough number of times to be statistically relevant was number of lie downs, vocals and retreats. Although only performed a few times, they gave some information about the calves' behaviour in the novel object study. For example, the calves chose to lie down in the test arena only in 5 lx and in 0.5 lx red light, twice in 0.5 lx red light and once in 5 lx. This is especially interesting since Muthuramalingam et al. (2006) found that intensities under 10 lx could be used at night without affecting the night melatonin levels. Since melatonin is responsible for sleep regulation (Sjaastad et al., 2010) this might be one explanation to why the resting behaviour was only observed in 5 lx and 0.5 lx. However, it is questionable if melatonin could have this effect in such a short time. Furthermore, all the calves that showed the lie down behaviour were in their fourth treatment and were tested in 5 lx or 0.5 lx in their previous light intensity, suggesting that previous light intensity in combination with habituation may be a more likely reason for this behaviour.

The number of retreats increased in 0.5 lx compared to 5 lx and full intensity and was highest in 0.5 lx red light. This suggests that the calves may have had difficulties discriminating the objects and that they were more hesitant to approach it in low light intensities. This is perhaps not surprising since the other results from the NOT showed that the calves tended to move the novel object more in the higher light intensities and less in the lower intensities, which could be translated to a certain level of insecurity in the low light intensities, as seen in the higher number of retreats. Since the retreating behaviour measured in this study is similar to the latency to approach the novel object described in Boissy and Bouissou (1995), it is possible that the retreating behaviour also indicates fear. Another fact supporting this is that the behaviour was noticed in a high number of individuals compared to the resting and vocalizing behaviour.

The number of vocalizations was highest in full intensity and lowest in 0.5 lx red light where no vocalization at all was observed. Since vocalization can be interpreted as a sign of fear (Moran and Doyle, 2015), this contradicts the other findings in this study, where the calves have shown more fearful behaviour in the lower intensities. However, it is consistent with the findings in Boissy and Bouissou (1995) that found that vocalizations and sniffing on the novel object was not indicators of fear. Perhaps the calves reacted this way because it was more apparent in full intensity that they were isolated from their herd, or that they were more motivated to perform behaviours that could not be done in the novel object arena, for example eating or playing. Another explanation to this may be that that all groups had the full intensity light in the first or second trial, when they were more new to the procedure.

5.4 Behaviour in red light

None of the parameters tested showed any significant difference between white and red coloured light in 0.5 lx. Although there were no significant differences, some of the trials showed more pronounced results in 0.5 lx red light, for example number of steps, how often the calves lied down and the number of vocalizations. This partly contradicts the theory that cattle should be more active in red light (Philips, 2010; Philips and Lomas, 2001), at low light intensities, since these findings indicate that there is less vocalization and more resting behaviour. Another possible explanation to these results is that red light may be perceived as darker than white light, since it is suggested that cattle perceive the red colour as shades of grey due to their dichromatic colour vision (Hörndahl et al, 2013).

5.5 Conclusion

In conclusion, only few significant effects were found between the treatments. This suggests that calves can cope with intensities down to 0.5 lx without large significant effects on their behaviour or motion. There were no significant differences in willingness to pass the course or on the behaviour in the NOT between white light and red coloured light in 0.5 lx.

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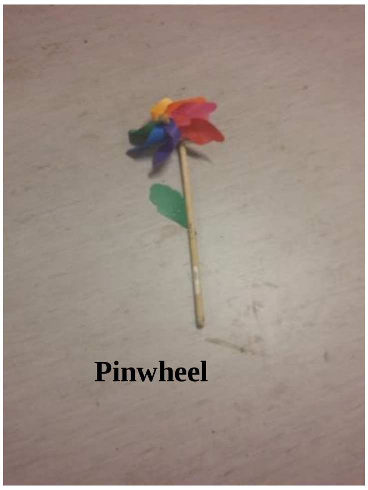
Appendix: Pictures of the Novel objects



Pinwheel

Pink box

Inflatable beach ball

Inflatable beach frog


Traffic cone


Plastic cat

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