Doctoral Thesis

Behavioural and cardiac reactions to acoustic stimuli in ruminants

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Publication Date:
2016

Permanent Link:
https://doi.org/10.3929/ethz-b-000168325

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Behavioural and cardiac reactions to acoustic stimuli in ruminants

A thesis submitted to attain the degree of
DOCTOR OF SCIENCES of ETH ZURICH
(Dr. sc. ETH Zurich)

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2016
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1 Summary

Farm animals are exposed to acoustic stimuli continually throughout their lives, such as ventilation, feed carts, barn cleaners, the vacuum machine in the milking parlour and the chime of a bell during pasturing seasons. However, depending on their characteristics and duration of exposure these acoustic stimuli may act as stressors. It is unknown if animals can adapt to such acoustic stimuli. Since cows and goats have a well-developed hearing capacity and an auditory perception that is comparable to the human perception, intense and/or long-lasting acoustic stimuli may act as stressors and result in behavioural and cardiac reactions that are indicative of a stress response. There is a lack of systematic research into the long-term exposure to acoustic stimuli in farm animals that may affect welfare. The aim of the present thesis was to investigate how short, medium and long-term exposure to acoustic stimuli affect behavioural and cardiac reactions of cows and goats and make inferences about their effects on the animals’ welfare.

The aim of the first study (chapter 4) was to test whether the short-term exposure to a non-uniform sound (chime of a bell) varying in amplitude and frequency and to a uniform sound (sinusoidal tone) with continuously increasing amplitude and constant frequency lead to stress responses in terms of behaviour (feeding and alertness duration), and heartbeat. Therefore, twenty-nine goats were tested individually in a test arena in two sessions (one for each sound), each lasting five consecutive days with one trial per day. A day before the first trial, reference values were collected without playback. During the first trial, the relative feeding duration was decreased and the relative alertness duration was increased during both stimuli, but more so when goats were exposed to the chime of a bell than the sinusoidal tone. For both stimuli, the relative feeding duration increased and the relative alertness duration decreased from the first to the fifth trial but returned to the levels of the reference values sooner when goats were exposed to the sinusoidal tone than the chime of a bell. Cardiac activity was not affected by the two stimuli. Taken together, the results showed that the short-term exposure to a chime of a bell led to higher behavioural arousal than the sinusoidal tone. In addition, with repeated exposure to the stimuli, goats habituated to both stimuli. However, habituation was faster to the sinusoidal tone than to the chime of a bell, indicating that goats perceived the chime stimulus as more aversive than the sinusoidal tone. Free-ranging animals in alpine regions are usually equipped with bells 24 h a day.
during summer season. Thus, the question arose whether the long-term exposure to the chime of a bell might affect animal welfare.

Because the goats habituated to the chime of the bell within 3 days when exposed to the sound for only 1 min daily (chapter 4), the study presented in chapter 5 investigated the medium-term impact of hearing and wearing a bell on behaviour (feeding, rumination and lying duration, number of chews per rumination per cud, leg movements as well as distance to the nearest neighbour) and heart rate variability in dairy cows. Nineteen non-lactating cows with bell experience were assigned to three different treatments. For 3 days each, cows were equipped with no bell (control), with a bell with inactivated clapper (silent bell) or with a functional bell (functional bell). The bells weighed 5.5 kg and had amplitudes between 90 and 113 dB at a distance of 20 cm. Data were collected on either the first and third or on all 3 days of each treatment. Duration of feeding and rumination was reduced with a functional bell and a silent bell compared with no bell. Head movements were reduced when wearing a silent bell compared with no bell and tended to be reduced when wearing a functional compared to no bell. With a functional bell, lying duration was reduced by almost 4 hours on the third day of treatment compared with the first day with a functional bell and compared with no bell or a silent bell. All additional behavioural measures (number of chews per rumination per cud, leg movements, and distance to the nearest neighbour) were consistent with the hypothesis of a restriction in the behaviour of the cows wearing bells, although this pattern did not reach significance. There was no treatment effect on heart rate variability, suggesting that the bells did not affect vago-sympathetic balance. An effect of experimental day was found for only 1 out of 10 behavioural parameters, as shown by a decrease in lying with a functional bell on day 3. The results showed behavioural changes in the cows wearing a bell over 3 days, without indication of habituation to the bell. Altogether, the behavioural changes suggested that the behaviour of the cows was disturbed by the medium-term exposure to the silent as well as the functional bell. If long-lasting, these effects may have implications for animal welfare.

Long-lasting exposure to sounds (e.g. a chime of a bell) may impair the cows’ hearing ability. Thus, the aim of the third study (chapter 6) was to assess the response of both, bell experienced and unexperienced cows to an acoustic stimulus. In addition, it was tested whether wearing earplugs, mimicking hearing damage, reduced the cows’ reactivity towards the sound. On 24 farms, half of them regularly using cow bells, 96 cows were tested individually in 2x2 factorial design (65/85 dB, without/with earplugs).
The effect of bell experience, amplitude and earplugs on the latency to the first reaction and avoidance of a five-second playback of pink noise was analysed, as well as cardiac reactivity. Cows reacted faster without earplugs and when they were exposed to 85 dB compared to 65 dB. The latency to the first behavioural reaction was not affected by bell experience. The proportion of leaving the feeding rack after onset of the playback was reduced by bell experience and earplugs, and was increased with 85 dB compared to 65 dB. Exposure without earplugs to 85 dB but not to 65 dB increased heart rate, indicating that cows were more alert when exposed to 85 dB. Heart rate as well as heart rate variability indicated increased sympathetic activation during the exposure to 85 dB compared with 65 dB and at the same time increased parasympathetic activity with earplugs than without earplugs. Heart rate and heart rate variability were not affected by bell experience. In general, no indication of complete hearing loss due to bells was found. The 85 dB stimulus increased arousal and avoidance compared with the 65 dB stimulus, with bell experience and earplugs leading to a general decrease in avoidance of the stimulus. This may reflect an altered emotional appraisal of the playback stimulus due to routine bell exposure.

Taken together, the results of this thesis show that the short-term exposure to a non-uniform sound (here: chime of a bell) can be more aversive than a uniform sound (here: sinusoidal tone). Behavioural changes suggest that the behaviour of cows is disturbed by the medium-term exposure to hearing and wearing a bell continuously over 3 days, without indication of habituation to the bell. In the long-term, bell exposure throughout summer season for several years did not lead to deafness in dairy cows. However, a 85 dB stimulus triggered increased arousal and avoidance compared to a 65 dB stimulus. Thus, exposing goats and cows to non-uniform sounds of more than 85 dB, e.g. bells should be avoided whenever possible in goat and cow husbandry.
2 Zusammenfassung


In der ersten Studie (Kapitel 4) wurde überprüft, wie sich die kurzfristige Exposition gegenüber einem Ton mit verschiedenen Frequenzzigenschaften und wechselnden Amplituden (Glockenton) und einem Ton mit gleichbleibender Frequenz und stetig ansteigender Amplitude (Sinuston) auswirkt und ob es zu stressanzeigenden Veränderungen im Verhalten (Fressdauer, Vigilanz), in der Herzfrequenz und Herzfrequenzvariabilität kommt. Dafür wurden 29 Ziegen einzeln in einer Test-Arena in 2 Versuchs durchgängen (eine für jeden Ton) an je 5 aufeinanderfolgenden Tagen getestet. Pro Tag fand ein trial statt. Der Tag vor dem jeweiligen ersten Versuchstag diente als Referenzwert (Test ohne Playback). Die entsprechende Fressdauer war am ersten Versuchstag bei beiden Tönen kürzer und die Dauer der Vigilanz länger. Dieser Effekt war insgesamt ausgeprägter beim Glockenton zu erkennen. Vom 1. bis zum 5. Versuchstag stiegen die entsprechenden Fressdauern sowohl beim Glockenton als auch beim Sinuston an, und die entsprechenden Dauern der Vigilanz nahmen ab. Insgesamt erreichten die Dauern von Fressen und Vigilanz das Niveau der Referenzwerte schneller beim Sinuston als beim Glockenton. Die Exposition hatte weder einen Einfluss auf die Herzfrequenz noch auf die Herzfrequenzvariabilität. Die Ergebnisse lassen darauf
schließen, dass die kurzfristige Exposition gegenüber einem Glockenton stärkere Verhaltensänderungen hervorrief als ein Sinuston. Im Verlauf der 5 Versuchstage war bei beiden Tönen ein Habitationseffekt erkennbar. Die Tiere gewöhnten sich jedoch schneller an den Sinuston als an den Glockenton, was darauf schließen lässt, dass sie den Glockenton als unangenehm empfanden als den Sinuston. Auf der Alp tragen freilaufende Tiere während der Sommermonate häufig für 24-Stunden am Tag eine Glocke. Somit stellt sich die Frage, ob das langfristige Tragen einer Glocke negative Auswirkungen auf das Wohlbefinden der Tiere haben könnte oder ob sich die Tiere an die Glocke gewöhnen.

Da die Ziegen sich in der ersten Studie innerhalb von 3 Tagen an den 1-minütigen Glockenton gewöhnten (Kapitel 4), verfolgte die zweite Studie (Kapitel 5) das Ziel, den mittelfristigen Einfluss einer Glocke auf Verhalten (Fress-, Wiederkau- und Liegedauer, Anzahl Kauschläge/Ruktus, Aktivität sowie Distanz zur nächsten Nachbarin), Herzfrequenz und Herzfrequenzvariabilität bei Milchkühen zu untersuchen. Für jeweils 3 Tage trugen 19 nicht-laktierende Kühe entweder keine Glocke (Kontrolle), eine Glocke mit inaktiviertem Klöppel (stille Glocke) oder eine funktionstüchtige Glocke (funktionstüchtige Glocke). Die Glocken wogen 5.5 kg und wiesen schwankende Amplituden zwischen 90-113 dB auf, was in einem Abstand von 20 cm gemessen wurde. Daten wurden entweder am 1. und 3. Versuchstag, oder an allen drei Versuchstagen erhoben.

es möglich ist, vermieden werden, dass Ziegen und Kühe Tönen mit verschiedenen Frequenzen und wechselnden Amplituden von mehr als 85 dB, zum Beispiel Glocken ausgesetzt werden.
3 General introduction

Farm animals are exposed to acoustic stimuli continually throughout their lives, such as ventilation, feed carts, barn cleaners, the vacuum machine in the milking parlour and the chime of a bell during pasturing seasons. However, depending on their characteristics and duration of exposure these acoustic stimuli may act as stressors. It is largely unknown if an animals can adapt to such acoustic stimuli.

In my dissertation, I want to investigate whether and how short, medium and long-term exposure to acoustic stimuli in general as well as hearing and wearing a bell particularly affect animal welfare. For this aim, four topics are addressed in more detail in the general introduction: First, the characteristics of acoustic stimuli and the hearing capacities of farm animals are described to understand how they might react to different acoustic stimuli. Then, the impact of acoustic stimuli that have already been tested in other animal species are reviewed. In the end, hearing loss caused by acoustic stimuli of high amplitudes will be described as it might occur as an ultimate damage.

3.1 Characteristics of acoustic stimuli

*Sound* is the result of pressure variations, or oscillations, in an elastic medium (e.g. air) generated by a vibrating surface (Hansen and Sehrndt 2001). The sound wave is characterised by the pitch (*frequency*) and the sound level (*amplitude*) (Maue 2009). The frequency is the number of pressure variation cycles in the medium per time unit, or, the number of cycles per second, and is expressed in Hertz (Hz) (Hansen and Sehrndt 2001). The amplitude refers to the pressure of a sound wave. It determines how loud a sound is perceived and is expressed in *decibel* (dB) (Maue 2009). Decibel is defined as the logarithm of the ratio of a quantity to a reference quantity of the same type (Casali 2012). Since power is directly proportional to the square of the pressure, the sound pressure level (SPL) is defined as

\[
\text{Sound pressure level (SPL; dB)} = 10 \log_{10} \frac{P_i^2}{P_r^2} = 20 \log_{10} \frac{P_i}{P_r}
\]

where \(P_i\) is the pressure level of the sound in micro-pascals (µPa) or other pressure unit and \(P_r\) is the pressure level of a reference sound in micro-pascals, usually taken to be the pressure at hearing threshold. Considering changes in sound level measured
in decibel, one relationship emanating from the decibel formula above are often helpful in practice. An increase (decrease) in SPL by 6 dB is equivalent to a doubling (halving) of the sound pressure. Similarly, on the power or intensity scales, an increase (decrease) of 3 dB is equivalent to a doubling (halving) of the sound power or intensity (Casali 2012).

From the acoustics point of view, sound and noise constitute the same phenomenon of atmospheric pressure fluctuations (Hansen and Sehrndt 2001). Noise is usually composed of many frequencies combined together (Hansen and Sehrndt 2001) and can be loosely defined as a subset of sound (Casali 2012); that is, noise is sound that is undesirable or offensive in some aspect. However, the distinction is largely situation- and listener-specific, as perhaps best stated in the old adage “one person’s music is another’s noise” (Casali 2012). Noise can be perceived as a stressor when a subject cannot escape from it and when it results in behavioural or physiological changes (Ames and Arehart 1972, Bowles et al 1990, Espmark et al 1974, Moberg 1987).

3.2 Hearing capacities of farm animals

Since cows and goats have a well-developed hearing capacity and an auditory perception comparable to humans, theirs will be exemplarily described in detail for farm animals. In the 1980s, investigations of auditory capacities of cows and goats were carried out, showing that cows are able to perceive sounds between 23 Hz and 35 kHz (Heffner and Heffner 1983). Turning to the midrange of the cows’ audiogram, it can be seen that the animals have a well-defined best frequency at 8 kHz. Goats can hear sounds between 78 Hz and 37 kHz, with the best sensitivity at 2 kHz. Cows and goats are quite sensitive, with a threshold of -11 dB (Heffner and Heffner 1990) and they have a similar but not exactly matched frequency sensitivity compared with humans. Hearing in humans ranged from 31 Hz to 17.6 kHz with the best sensitivity at 4 kHz (Heffner and Heffner 1998). By testing cows and goats in an operant conditioning paradigm, Heffner and Heffner (1983, 1990, and 1998) found the animals to be 1 dB more sensitive than humans and more sensitive than most other animals tested, e.g. pigs (42 Hz – 40.5 kHz, 9 dB), horses (55 Hz – 33.5 kHz, 7 dB) and sheep (125 Hz – 42 kHz, -6 dB). Altogether, it can be assumed that acoustic stimuli that are audible to humans can be well perceived by cows and goats.
3.3 Short, medium and long-term exposure to acoustic stimuli
Acoustic stimuli of high amplitudes have been found to act as stressors in several animals species (chickens: McAdie et al 1993; pigs: Talling et al 1996, 1998; cattle: Waynert et al 1999; sheep: Sevi et al 2001; rats: Masini et al 2008; koalas: Larsen et al 2014). Stress is a broad term that implies a threat to which the body needs to adjust. Stress may be classified as physical, psychological, or interceptive in nature, but usually contains components of all three classifications (von Borell 2000). The degree of stress can be measured using neuroendocrine (e.g. Smith and Vale 2006, Boissy and Le Neindre 1997), physiological (e.g. von Borell et al 2007, Aschwanden et al 2008, Gygax et al 2008), and behavioural (e.g. avoidance: Arnold et al 2008, Rushen 1996, Talling et al 1998; startle responses: Blaszczyk and Tajchert 1997, Lanier et al 2000, Clements and Kelly 1978; feeding, ruminating and lying behaviour: DeVries et al 2009, Bristow and Holmes 2007, Munksgaard and Simonsen 1996, Rushen et al 2012) indicators for an overall welfare assessment. To describe the impact of acoustic stimuli that might affect animals' welfare, short, medium and long-term effects will be distinguished in the following. For this thesis, short-term is defined as the exposure to acoustic stimuli for a maximum of 5 min either once only or for several consecutive repetitions during a short period, maximal one week. Medium-term is defined as the exposure to acoustic stimuli for more than 5 min until 24 hours a day either once or during several days and/or weeks. The exposure to acoustic stimuli for several hours a day until continuously 24 hours a day during a minimum of several month is defined as long-term.

3.3.1 Short-term
Several studies assessed the effect of short-term exposure of farm animals to acoustic stimuli. Horses (Christensen et al 2005) spent less time feeding and had an increased heart rate when exposed to ‘white noise’ of 60 dB for 2 min in a test arena. Pigs were repeatedly exposed to 5 min playbacks of a pig transporter, either intermittent with varying (59-86 dB) or constant (84 dB) amplitudes (Talling et al 1998). The intermittent stimulus appeared to be more aversive than the constant one as pigs avoided the former more than the latter. When exposed to a 2 min playback of background sound recorded in milking parlours and broadcast at 85 dB in a raceway 3 times daily, heifers showed an increased heart rate on day 1 and faster transit times on days 1-4, indicating an escape reaction, but habituated over a 5 day period with daily exposure (Arnold et al 2007). Heifers exposed to playbacks of people shouting (86 dB) and metal-on-metal
clanging (85 dB) had an increased heart rate and moved more often. However, they also showed signs of habituation to the sounds when exposed repeatedly over a 5 day period with daily exposure (Waynert et al 1999). Habituation is defined as the decline in response to a repeatedly presented stimulus (Mackintosh 1987). If an initial aversion to an acoustic stimulus is motivated mainly by neophobia, habituation would be expected after repeated exposure (Treisman 1984). Habituation to acoustic stimuli due to repeated exposure may result in a reduced reactivity towards unfamiliar sounds as can be seen in police dogs and horses after month and years of training (Polizeidirektion Hannover 2016). Nevertheless, this training is based on reward. However, systematic investigations of the reactions of goats to short-term exposure to acoustic stimuli that may affect welfare are not available.

### 3.3.2 Medium-term

During medium-term exposure, Talling et al (1996) found an increase in heart rate and locomotion in piglets that were exposed to various acoustic stimuli (farm, transport and abattoir recordings and ‘white noise’) at 80-97 dB for 15 min once per week over a total of 4 weeks, indicating increased arousal of the pigs. In another study, exposure to repeated sounds, either to broad-band sound (90 dB) during daily sessions of 2 hours or to the same sound three times a week over a period of 4 weeks caused changes in neuroendocrine regulations that reflected a state of chronic stress in growing pigs (Kanitz et al 2005). On the other hand, behavioural, immune and physiological responses as well as production performance were not affected when lambs were exposed 8 hours a day during 6 weeks to sounds of 2 kHz with amplitudes from 75 dB to 95 dB (Sevi et al 2001). In a follow-up study, contradicting results were found. Lambs exposed to recordings of high-speed motor vehicle traffic sound (75-95 dB) for 8 hours a day for 42 days reduced their feeding time, increased the time spent active, had reduced plasma cortisol concentrations, and a slower growth rate (Quaranta et al 2002). Caribou were found to increase time spent feeding and were lying down less in response to repeated exposure of low-flying jet aircraft overflights (in total 110 overflights, maximum amplitude: 130 dB) (Luick et al 1996). In mice, ABR (auditory brain-stem response) showed that a single medium-term exposure to noise of 100 dB for 2 hours induced temporary hearing loss (see chapter 3.3.3) (Chuang et al 2014), and a medium-term exposure to noise of 110 dB for 60 minutes even induced permanent hearing loss (Park et al 2013). Contrary, exposure to aircraft sounds (34-116 dB) one
to four times per day on 10 to 12 days in the waiting area just before milking did not lead to behavioural reactions in dairy cows (Head et al 1993), and Masini et al (2008) found that rats habituated to daily 30-min exposure to ‘white noise’ at 95 dB within 6 days. The difference in acoustic perception between species (Heffner and Heffner 1983, Fay 1988, Heffner and Heffner 1992, Flydal et al 2001) might explain to some extent why some studies found acoustic stimuli to elicit stress responses and other studies did not. Additionally, characteristics other than amplitude varied greatly between studies. Characteristics like frequency distribution and amplitude distribution were shown to affect responses to acoustic stimuli in humans and non-human animals (USEPA 1974, Molino 1979, Talling et al 1998, Sevi et al 2001, Quaranta et al 2002). Sounds with fluctuating frequencies and/or amplitudes led to stronger reactions in humans, pigs and sheep than sounds with constant frequency and/or amplitude (Molino 1979, Talling et al 1998, Sevi et al 2001, Quaranta et al 2002). All in all, a non-uniform sound that is intermittent, irregular, unpredictable may elicit a stronger stress response than a uniform, tonal and constant sound (chapter 4) because it is hardly possible to habituate to intermittent, unpredictable acoustic stimuli (Todt 1988, Moore 2012). Furthermore, both, the intensity and duration of the exposure might also affect the responses to acoustic stimuli. In humans, as intensity increases, the length of time for which the exposure does not affect their hearing capacity decreases (Fligor 2011). For example, being exposed to 85 dB for 8 hours may be equally at risk as being exposed to 110 dB for only a few minutes.

To my knowledge, the impact of medium-term exposure to acoustic stimuli 24 hours a day over several days has not been investigated in dairy cows. A stimulus, to which cows are exposed to under housing conditions in alpine regions are cowbells. Depending on the management, cows wear these bells during the day (medium-term) or during the whole pasturing season (long-term).

3.3.3 Long-term

In all studies mentioned above, the animals were exposed to the acoustic stimuli only during short- or medium-term intervals and not continuously over a longer period of time, i.e. several months. In humans, it has been shown in various studies that constant exposure to acoustic stimuli, can result in permanent physiological and psychological changes (e.g. Cohen et al 1970, Johnson 1991, Jansen 1992, Melamed et al 2001,
Noise-induced hearing loss is one of the most common consequences among the physiological ones (Miller and Schein 2008). Increased blood pressure and heart rate, appearance of muscle reflexes, and sleeping disorders may be considered as other physiological effects. The physiological effects of noise are more common compared to the psychological ones. Physiological ones can be seen in the forms of annoyance, stress, anger and concentration disorders as well as difficulties in resting and perception (Öhrström 1989, Finegold et al 1994, Cheung 2004, Atmaca et al 2005). The effects of noise described above, particularly noise-induced hearing loss, are well-known in human medicine (CDC National Institute for Occupational Safety and Health 2011, Dobie 1993). In humans, noise is considered to be hazardous if it exceed 85 dB over a typical 8-hour workday (Fligor 2011). It has been shown that constant exposure to such hazardous noise often results in irreversible hearing loss and even a single intense sound event can cause hearing loss and tinnitus (Holgers and Pettersson 2005, Daniel 2007).

So far, only very little research has been conducted investigating the long-term effect of noise on the hearing capacities of animals in general and of cows and goats in particular. In the following, according to humans, for animals noise is defined as sounds that exceed 85 dB. In the fruit fly *Drosophila melanogaster* it was found that the exposure to life-long music of 70 dB decreased their lifespan (Morales et al 2010). Contrary, rats exposed to life-long noise showed no difference in lifespan even with 100 dB (Borg and Järplid 2007), suggesting that insects and mammals may have different sensitivities to noise. Kenneled dogs that were constantly exposed to noise between 100 and 108 dB for 6 months developed hearing loss (Scheifele et al 2012).

Although anatomic differences among various animal species lead to differences in acoustic perception (Fay 1988), the basic physiologic processes underlying the detection and sensation of sound are homologous between humans, dogs, cattle and mice (Strain and Myers 2004, Fay and Popper 2000, Heffner and Heffner 1983 and 1990). Situations in which cows and goats are exposed to sounds of high amplitudes occur not only at the slaughterhouse or at the milking parlour, where exposure to the respective sounds lasts several minutes; in alpine region, cows and goats are often equipped with a bell throughout the summer season night and day. Since they have a well-developed hearing capacity, the long-term exposure to the chime of a bell might lead to behavioural and cardiac changes as well as negatively affect the cows’ hearing capacity.
3.4 Aim of this thesis, study design, and research questions

The aim of the present thesis was to investigate whether and how short, medium and long-term exposure to acoustic stimuli affects behaviour, heart rate parameters and hearing capacities in farm animals. Since under normal management conditions bells are used in goats and cows, I chose them as acoustic stimulus and model species’. Altogether, the results are supposed to establish a basis for the impact of acoustic stimuli on these species.

To investigate the short-term exposure to acoustic stimuli in goats, the first study (chapter 4) aimed at testing whether a non-uniform sound (chime of a bell) with varying frequency and increasing in amplitude and a uniform sound (sinusoidal tone) with continuously increasing amplitude and constant frequency lead to stress responses in terms of behaviour and heartbeat. Additionally, we tested for habituation effects by exposing goats to these stimuli repeatedly. Whereas the first study was conducted in a rather artificial situation, farm animals are confronted with acoustic stimuli throughout their lives. Thus, the second study (chapter 5) investigated the medium-term impact of hearing and wearing a bell on behaviour and heart rate variability in dairy cows in an applied situation. Besides, the question arises whether the long-term exposure to acoustic stimuli might have effects on cows’ responses to unfamiliar sounds as well as hearing capacity. The aim of the third study (chapter 6) was to test cows’ reactivity towards a sound of low (65 dB) and high (85 dB) amplitude and whether wearing ear-plugs, mimicking hearing damage, also reduces cows’ reactivity towards the sound.
Effects of sounds of different quality on the behaviour and heart beat parameters of goats

4 Effects of sounds of different quality on the behaviour and heart beat parameters of goats

Based on:
Julia Johns, Antonia Patt, Edna Hillmann (2015)
Applied Animal Behaviour Science 165, 72-80

Abstract
In alpine regions, bells are used to relocate free-ranging grazers like cows and goats. Considering that goats have a well-developed hearing capacity, sounds (e.g. a chime of a bell) may act as stressors depending on their characteristics. The aim of this study was to test whether a non-uniform sound (chime of a bell) varying in amplitude and frequency and a uniform sound (sinusoidal tone) with continuously increasing amplitude and constant frequency lead to stress responses in terms of behaviour and heart-beat. Twenty-nine goats were tested individually in a test arena in two sessions, each lasting five consecutive days with one trial per day. A day before the first trial, reference values were collected without playback. During the following five trials, playbacks were conducted. Differences in behaviour and heartbeat parameters between test and reference values were analysed by using generalised linear mixed-effects models. During the first trial, the relative feeding duration was decreased and the relative alertness duration was increased during both stimuli, but more when goats were exposed to the non-uniform than the uniform sound. For both stimuli, the relative feeding duration increased (trial × stimulus: $p = 0.05$) and the relative alertness duration decreased (trial × stimulus: $p = 0.004$) from the first to the fifth trial but returned to the levels of the reference values sooner when goats were exposed to the uniform than the non-uniform sound. Cardiac activity was not affected by the stimuli. Altogether, the chime of a bell led to higher behavioural arousal than the uniform sinusoidal tone, indicating a potential of the chime to being more aversive to goats than a uniform sound. With repeated exposure to the stimuli, goats habituated to both stimuli, but habituation was faster to the sinusoidal sound than to the chime of a bell. Free-ranging goats in alpine regions usually are equipped with bells 24 h a day during the summer season. Thus, the question arises whether the long-term exposure to the chime of a bell might have negative effects on animal welfare.
4.1 Introduction

In several animal species, bells are used either to relocate free-ranging animals (e.g. cows, goats, sheep, yak) or for ornamental or religious purpose (e.g. in elephants, cows, horses, falcons). Considering that goats have a well-developed hearing capacity (Tracey and Fleming 2007) and an auditory perception comparable to that of humans (Heffner and Heffner 1990), sounds may act as stressors, depending on their amplitude and/or frequency (McAdie et al 1993, Talling et al 1996, Talling et al 1998, Sevi et al 2001, Quaranta et al 2002, Moore 2012). Goats can hear sounds between 78 Hz and 37 kHz, with the best sensitivity at 2 kHz, and are capable to detect sounds at -11 dB, i.e. sounds the human ear cannot detect (Heffner and Heffner 1990).

Sounds of high amplitudes, i.e. ‘noise’, have been found to act as stressors in several species (chickens: McAdie et al 1993, pigs: Talling et al 1996, Talling et al 1998, cattle: Waynert et al 1999, sheep: Sevi et al 2001, rats: Masini et al 2008, koalas: Larsen et al 2014). As the hearing capacity differs between species (Heffner and Heffner 1983, Heffner and Heffner 1992), this might explain to some extent why some studies found sound to elicit stress responses in animals and other studies did not. Heffner and Heffner (1990) and Ames and Arehart (1972) showed that goats and sheep have a similar but not exactly matched frequency sensitivity compared with humans and pigs. By testing the animals in an operant conditioning paradigm, Heffner and Heffner (1990) found goats to be by 20 dB more sensitive than pigs and more sensitive than most other animals tested. Talling et al (1996) found that pigs had a higher heart rate and ambulation score when exposed to high compared with low amplitudes. Further, lambs that were exposed to sounds with amplitudes increasing from 45 dB to 95 dB showed increased plasma cortisol concentrations (Sevi et al 2001), and pregnant ewes exposed to the same increase in sound amplitudes reduced their feeding time, increased the time spent inactive, and had increased plasma cortisol concentrations (Quaranta et al 2002). Larsen et al (2014) showed that an increase from ‘little background noise without visitors’ to ‘loud visitor noise’ resulted in an increased time spent being vigilant in koalas. McAdie et al (1993) found chicken to show greater averseness to the sound of other chicken in a commercial poultry shed at 100 dB compared with a piece of music at 90 dB and 95 dB. Characteristics other than amplitude were shown to affect the effects of sound in different animal species additionally. In pigs, intermittent sounds with varying amplitudes appeared to be more aversive than a constant sound as pigs avoided the former more than the latter (Talling et al 1998). In general, a sound with
fluctuating frequencies and/or amplitudes led to a stronger reaction in humans than a sound with constant frequency and/or amplitude (Molino 1979). All in all, a non-uniform sound that is intermittent, irregular, unpredictable may elicit a stronger stress response than a uniform, tonal and constant sound because it is hardly possible to habituate to intermittent, unpredictable acoustic stimuli (Todt 1988, Moore 2012).

Additionally to the characteristics of the sound, duration of exposure affects the reaction to the sound. If an initial aversion to an acoustic stimulus is motivated mainly by neophobia, a reduction in arousal (i.e. habituation) would be expected after repeated exposure (Treisman 1984). It has been shown that the stronger the response to a sound, the longer it takes the animals to habituate (Koehler et al 1990, Voipio 1997, Biedenweg et al 2011).

Although much is known about the hearing capacity of goats in general and the impact of noise on other animal species, systematic investigations of the reactions of goats to acoustic stimuli that may affect welfare are not available. Our study thus aimed at investigating whether a uniform sound (i.e. sinusoidal tone) with continuously increasing amplitude and a non-uniform sound (i.e. chime of a bell) with varying frequencies and amplitudes differ in their effects on behaviour, heart rate and heart rate variability of goats. In addition, we tested for habituation effects by exposing goats to these stimuli repeatedly. We expected the behavioural and physiological responses to increase with increasing amplitude, and the non-uniform sound leading to increased arousal compared to a uniform sound, indicated by a stronger stress response and slower habituation.
4.2 Materials and methods

The experiment was approved by the ethical commission for animal experiments of the Thurgau Cantonal Veterinary Office, Switzerland (Approval No. 03/11).

4.2.1 Animals and housing

Experimental subjects were 29 horned non-lactating female goats of various Swiss milking breeds (Saanen, Toggenburger, Chamois Coloured, St. Gallen Booted, Grisons Striped, Peacock, Valais Blackneck and Nera Verzasca) or their crossbreeds. The goats were housed in eight identically equipped pens at the Agroscope Research Station (Tänikon, Switzerland). The total area of each pen was 15.3 m² (approx. 3 m × 5 m), consisting of a deep-bedded straw area of 11.7 m² (approx. 3 m × 4 m) and an elevated (0.5 m) feeding place (3.6 m²) divided by a wooden wall into two compartments of equal size (1.2 m × 1.5 m). The deep-bedded area was structured further by two wooden elements that provided climbing opportunities and protected lying areas. Hay and water were provided ad libitum.

4.2.2 Test arena and waiting area

The experiment was carried out in a test arena (10.5 m², Figure 1), which was acoustically separated from the goats’ home pens. The walls were constructed of wood and the floor was littered with long straw. A ladder for the experimenter to sit on was located opposite to the door (Figure 1). The room was lit with fluorescent light and was naturally ventilated, with an average temperature of 7.6 °C ± 4.4 °C throughout the experimental period. To record the behaviour of the animals, a video camera (SELVAG® OC-5 IR-Outdoor-Colour-Camera, Selvag c/o ELV, Leer, Germany) was mounted in one corner of the test arena (Figure 1). The acoustic stimuli were transmitted into the test arena via two loudspeakers (Edifier® S2000v, Edifier International, Hong Kong, China), which were attached to the ceiling (Figure 1). A microphone (Sennheiser® ME 62, adapter K6, Sennheiser electronic GmbH and CO KG, Wedemark, Germany) fixed to the ceiling in the middle of the test arena at a height of 2.5 m recorded the sound emitted by the speakers. The computers and all additional equipment needed to record the behavioural responses of the goats were located in an adjacent room. About 50 m away from the test arena, and thereby acoustically separated, an outdoor pen (18 m²) served as a waiting area for the goats shortly before they were used in the experiment.
4.2.3 Acoustic stimuli

The two acoustic stimuli (Figure 2) to which the goats were exposed were played for 1 min each. One stimulus was a uniform sound (sinusoidal tone) with a frequency of 2 kHz and a continuously increasing amplitude from 41 dB to 96 dB (A) with a rise time of 60 s. The other stimulus was a modified recording of a chime of a bell (non-uniform sound) with the first frequency band (i.e. lowest frequency of peak amplitude) measured on the spectrum at 1.9 kHz, and a maximum frequency of 17 kHz. This stimulus had an increasing and simultaneously varying amplitude between 41 dB and 96 dB (A) with a rise time of 0.9 ms. The rise time refers to the time required for the amplitude to change from the lowest value to the highest value. The amplitude was measured at
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approx. 1.5 m below the speakers (i.e. the estimated height of the goats' heads) by a precision noise level measuring instrument with integrated long-term storage (SoundTest-Master, Laserliner®, Umarex GmbH and Co. KG, Arnsberg, Germany). In the reference situation (no playback), the amplitude (i.e. background noise) was 42 dB (A-weighting scale (A)). The A-weighting scale assigns low weights to the low-frequency tones, to which the human ear and the ears of some animals are less sensitive, and high weights to the high-frequency tones, to which humans are more sensitive (Mancy et al 1988). Thus, the sounds were adjusted to match the goats’ (potential) hearing capacity. The rise time was measured on the oscillogram as the duration between the beginning of a bell sound and its peak amplitude. It was averaged over 128 peaks for which the beginning and peak amplitudes were clearly visible.

![Figure 2: Spectrogram (above) and oscillogram (below) of (a) the uniform sound and (b) the non-uniform sound.](image)

### 4.2.4 Experimental procedure

Before the start of the experiment, goats were habituated to the waiting area, the test arena, and the experimental procedure over a 10-day habituation period, and to thorax belts for measuring the cardiac activity during the last three days of this habituation period. The goats were trained daily to walk voluntarily into the test arena, to remain there for about 5 min without conspecifics and to feed hay and concentrate from a bucket within the test arena. During the first habituation trials, the experimenter was located in an adjacent room, observing the goats via video transmission. As most of
the goats still showed signs of high arousal in the test arena after the first habituation days, the experimenter stayed with them in the test arena during the habituation phase and later during the experiment. The experimenter sat on a ladder in a corner of the test arena (Figure 1) outside the goats’ immediate reach and did not interact with the goats (neither addressing them verbally nor touching them). At the end of the habituation period, the goats walked voluntarily into the test arena and remained calmly in the waiting area as well as in the test arena.

During the experiment, three goats that were housed together in the same home pen were always walked together to the waiting area. By this procedure, social separation during the experimental procedure was minimised. From the waiting area, one goat at a time was led to the test arena and fitted with a thorax belt to measure heartbeat parameters (see section 4.2.6 Heartbeat measurements). To enhance feeding motivation for the experiment, goats were offered only straw during the preceding feeding time. A bucket filled with hay and a handful of concentrate was placed in the centre of the test arena. After entering the test arena, the goat was allowed to feed freely from the bucket for 5 min. When a goat did not enter the test arena voluntarily, it was gently moved in. When the goat was feeding calmly (on average 1.37 min ± 1.05 min after entering the test arena), the playback was switched on for 1 min (= trial). Start and stop of the playback were controlled manually by the experimenter using an MP3 player (iPod nano 6th generation®, Apple Inc.). After the end of the playback, the thorax belt was removed and the goat was brought back to the waiting area; time needed to complete one trial was about 4 min. Subsequently, the second goat was led from the waiting area to the test arena and the next trial started. When a goat did not start feeding within 5 min, no data were collected for this trial and the goat was taken back to the waiting area (14 goats in 46 of 348 trials). The order in which the goats were tested was chosen randomly before the start of the experiment and remained constant over the whole experimental period. When all three goats had finished their trials on a given day, the group was brought back to the home pen; the total duration of absence from the home pen was approximately 30 min per experimental day.

Each goat was exposed to the acoustic stimuli in two sessions, each lasting six consecutive days with one trial per day (Figure 1). Half of the goats were exposed to the uniform sound first, the other half to the non-uniform sound. A day before the first trial of each session, reference values were recorded from each goat applying the experimental setting without playback for 5 min. The reference value was calculated as mean
duration or frequency per minute to reduce the variability within the course of the 5 min reference session. On the following 5 days, playbacks were conducted, each lasting for 1 min (29 goats × two sessions × 6 days = 348 trials in total). For all goats, there was a break of 2 days between the two test sessions (Figure 1).

4.2.5 Behavioural observations

The following behaviours were scored from video recordings by using INTERACT® (Mangold International GmbH, Arnstorf, Germany; version 9.0.7): duration of feeding and alertness, looking at the experimenter, standing in front of the door, latency to the first reaction, and ear postures (Table 1). Ear postures were analysed by using instantaneous recording (Martin and Bateson 2007) at 10-s intervals (seven sample points per trial at seconds 0, 10, 20, 30, 40, 50 and 60). According to the method described by Reefmann et al (2009a), the goats’ ear postures assessed were: axial ear, forward ear and backward ear (Table 1), which were recorded separately for the right and left ears. In addition, the total number of ear-posture changes (i.e. at least one ear is moved from forward, axial, or backward to another of these postures from one sampling point to the next) per trial and the proportion of asymmetric ears (i.e. right and left ears in a different posture) per sample point were calculated. The behavioural analysis was done by two different persons (one person analysed ear movements, and the other analysed all other behaviours) that had not participated in the conduction of the experiments. They did not know the goats and were not aware of the aim of the study. However, as she needed to record the behaviours related to the start of the playback, she was aware of the acoustic stimulus.
Effects of sounds of different quality on the behaviour and heart beat parameters of goats

Table 1: Ethogram of behaviours recorded during playback experiments

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Definition</th>
<th>Abbreviations used in the text</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding</td>
<td>Head in bucket or head raised with visible chewing movements.</td>
<td>Δ feeding duration</td>
</tr>
<tr>
<td>(Quaranta et al 2002)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding calmly</td>
<td>Head in bucket or head raised with regular chewing movements visible.</td>
<td>feeding calmly</td>
</tr>
<tr>
<td></td>
<td>No locomotion, alertness, fast ear or tail movements visible.</td>
<td></td>
</tr>
<tr>
<td>Alertness</td>
<td>Animals were standing still and raised their head with ears turned forward.</td>
<td>Δ alertness duration</td>
</tr>
<tr>
<td>(Tracey and Fleming 2007)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ear postures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Reefmann et al 2009a)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axial ear</td>
<td>Ear perpendicular to the head-rump axis.</td>
<td>Δ axial ears</td>
</tr>
<tr>
<td>Forward ear</td>
<td>Tip of the ear towards the front at an angle of more than 30° from the perpendicular.</td>
<td>Δ forward ears</td>
</tr>
<tr>
<td>Backward ear</td>
<td>Tip of the ear towards the back at more than 30° from the perpendicular.</td>
<td>Δ backward ears</td>
</tr>
<tr>
<td>Latency to the first reaction</td>
<td>Time [s] taken from first feeding until the goat raised its head or stopped feeding and left the bucket for the first time.</td>
<td>Δ latency to the first reaction</td>
</tr>
<tr>
<td>Looking at the experimenter</td>
<td>Goats were standing still in front of the ladder and looking in the direction of the experimenter sitting on the ladder.</td>
<td>None</td>
</tr>
<tr>
<td>Standing in front of the door</td>
<td>Goats were standing with the head in the direction of the door, distance to door &lt; 1 m.</td>
<td>None</td>
</tr>
</tbody>
</table>

*During negative situations, the number of ear-posture changes and the proportion of forward and asymmetric ear postures were high, whereas axial ear postures rarely occurred. By contrast, positive situations were characterised by few posture changes, a low proportion of asymmetric ear postures and high proportions of axial and backward ears (Reefmann et al 2009a).

4.2.6 Heartbeat measurements

Heart rate was recorded by using Polar® S810i (Polar Elektro Europe BV, Zug, Switzerland), allowing a non-invasive measurement of heartbeats (Langbein et al 2004, von Borell et al 2007). To increase the electrode-skin contact, the goats were shorn on a width of 15 cm from the left shoulder to the left armpit within the area of the infrasternal angle on the day before the first session. A thorax belt with two integrated electrodes
and a radio transmitter were fixed around the torso directly behind the forelegs. One electrode was positioned ventrally on the left side of the sternum and the other one at a given distance by the thorax belt on the left thoracic wall. Electrode gel (Anandic Medical Systems AG/SA, Feuerthalen, Switzerland) was used to improve conductivity. The thorax belt was protected additionally by an elastic belt of about 5 cm width. The data logger, shaped as a digital watch, was placed into a pocket on the elastic belt. As reference values for heartbeat parameters, an interval of 1 min of continuous feeding during the reference measurements was chosen. During the trials, heartbeat parameters were recorded during the 1-min playback. After the end of the playback, the data logger was detached and the data were downloaded onto a computer via an interface (Polar ProTrainer 5™ Equine Edition®, Polar Electro Oy, Kempele, Finland; version 5.35.161).

4.2.7 Statistical analyses
To reflect dependencies adequately in the experimental design (nesting, repeated measurements), generalised linear mixed-effects models were used to evaluate the outcome variables. Statistical analyses were performed in R 3.1.0 (R Development Core Team 2014) by using the lme and glmer methods from the nlme (Pinheiro et al 2014) and lme4 (Bates et al 2014) packages, respectively. Data of one animal could not be used for the analysis of the uniform sound because this goat never started feeding in these trials. Thus, data from 29 goats and 342 trials were available for behavioural analyses.

4.2.7.1 Behaviour
The reference values for all behaviours were calculated on the first day of each session from 5-min recordings without playback. The durations of feeding, alertness, looking at the experimenter and standing in front of the door were then divided by five, and a ‘reference-duration per minute’ was calculated. The reference values of ear postures were recorded while the goat was feeding continuously for 1 min during this 5-min period. Subsequently, the differences of the behavioural indicators (Table 1) to reference values were calculated and referred to as ‘Δ’. Separate models were set up for all outcome variables. No statistical analyses were possible for asymmetric ears, duration of looking at the experimenter and duration of standing in front of the door due to a rare occurrence of the respective behaviours.
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Fixed effects were ‘trial’ (factor with five levels: 1, 2, 3, 4, 5) and ‘stimulus’ (factor with two levels: uniform sound, non-uniform sound) and their two-way interaction. All models were reduced in a step-wise backwards procedure with a $P$-value of $> 0.05$ as criterion for exclusion of an interaction. Statistical assumptions (normal distribution, homoscedasticity) were checked by graphical analysis of residuals. To reflect the experimental design, the random effects ‘trial’ nested in ‘stimulus’ nested in ‘individual’ were included in all models.

### 4.2.7.2 Heart rate and heart rate variability

The number of R-R intervals and the root mean square of successive differences (RMSSD) were calculated by using the programs Polar ProTrainer 5<sup>™</sup> Equine Edition® and R 3.1.0. The playback was divided into three phases, namely first (0-20 s), middle (21-40 s) and last (41-60 s) 20 s, to reflect the increasing amplitude.

Automatic correction of the tachograms was carried out by using the correction routines included in the Polar software. Files with an error rate of more than 10% were excluded from the analysis analogous to the approach described by Langbein et al (2004). This criterion led to the exclusion of 39 trials. Twenty-four of these trials were of the same two goats, and these goats were completely excluded from the heart rate analysis. Thus, the number of subjects was $n = 27$ in a total number of 309 trials.

The reference values of R-R intervals and RMSSD were recorded on the first day of each session without playback while the goats were feeding continuously for 1 min. Subsequently, the difference in heart rate (beats/min) and the difference in RMSSD (ms) to reference values were calculated.

The statistical model included ‘trial’ (factor with five levels: 1, 2, 3, 4, 5), ‘stimulus’ (factor with two levels: uniform sound, non-uniform sound) and ‘phase’ (factor with three levels: first, middle, last 20 s of playback) as main effects, and all possible two-way interactions and their three-way interaction as fixed effects. The models for heart rate and RMSSD were reduced in a step-wise backwards procedure with a $P$-value of $> 0.05$ as criterion for exclusion of an interaction. For heart rate and RMSSD, random effects were ‘trial’ nested in ‘stimulus’ nested in ‘individual’. Statistical assumptions (normal distribution, homoscedasticity) were checked by graphical analysis of residuals.
4.3 Results

A positive difference ($\Delta$) reflected that the trial value was greater than the reference value, whereas a negative difference indicated a lower trial value compared with the reference value. In the figures, raw data and model estimations are shown; in the text, only model estimations (mean and confidence intervals [CI]) were used to interpret the results.
4.3.1 Behavioural observations

4.3.1.1 Feeding duration

During the first trial, Δ feeding duration was negative during both stimuli and more negative when goats were exposed to the non-uniform compared with the uniform sound (Figure 3). For both stimuli, Δ feeding duration increased from the second to the fourth trial, and it approached the level of the reference value by the third trial for the uniform sound and by the fifth trial for the non-uniform sound (trial × stimulus: $F_{4,171} = 2.4; p = 0.05$; Figure 3).

**Figure 3**: Difference in feeding duration [s] to the reference values depending on the stimulus (non-uniform sound/uniform sound) from the first to the fifth trial. Raw data are presented as box plots indicating observed median, first and third quartiles, and absolute range of data. The solid lines show the model estimation. A difference in feeding duration of ‘0’ indicates identical values during playback and reference situation. A difference of -60 means the goats did not feed during the playback exposure; a difference of +60 means the goats did not feed during the reference situation.
4.3.1.2 Alertness duration

During the first trial, $\Delta$ alertness duration was positive in both playback situations, and the difference was larger with the non-uniform sound than with the uniform sound. The $\Delta$ alertness duration decreased from the first to the fifth trial for both stimuli. Whereas almost no $\Delta$ alertness duration was detectable by the third trial when goats were exposed to the uniform sound, $\Delta$ alertness duration remained slightly positive until the fifth trial when goats were exposed to the non-uniform sound (trial $\times$ stimulus: $F_{4,171} = 9$; $p = 0.004$; Figure 4).

![Figure 4](image-url)

Figure 4: Difference in alertness duration [s] to the reference values depending on the stimulus (non-uniform sound/uniform sound) from the first to the fifth trial. Raw data are presented as box plots indicating observed median, first and third quartiles, and absolute range of data. The solid lines show the model estimation. A difference in alertness duration of ‘0’ indicates identical values during playback and reference situation. A difference of -60 means the goats did not show alertness behaviour during the playback exposure; a difference of +60 means the goats did not show alertness behaviour during the reference situation.
4.3.1.3 Latency to the first reaction

The Δ latency to the first reaction tended to be more negative when goats were exposed to the non-uniform sound (-13.4 s, CI [-20.5; -6.2]) compared with the uniform sound (-7.7 s, CI [-15.1; -0.2]) (stimulus: $F_{1,27} = 2.9; p = 0.1$). Thus, the goats showed the first reaction slightly earlier when being exposed to the non-uniform than to the uniform sound. The Δ latency increased from the first (-17.7 s, CI [-24.9; -10.4]) to the third trial (-4.4 s, CI [-11.8; 3.0]) and decreased again from the third to the fourth (-9.3 s, CI [-16.8; -1.9]) and the fifth trial (-10.3 s, CI [-17.4, -3.2]) (trial: $F_{4,173} = 5.1; p = 0.0006$).

4.3.1.4 Ear postures

The Δ forward ears tended to be more positive when goats were exposed to the non-uniform sound (20.5%, CI [12.2; 29.6]) compared with the uniform sound (13.5%, CI [4.4; 22.5]) (stimulus: $F_{1,27} = 3.08; p = 0.09$). The Δ forward ears decreased from the first (31.6%, CI [23.2; 39.9]) to the fourth trial (7.8%, CI [-1.0; 19.2]) and increased slightly during the fifth trial (9.5%, CI [-0.2; 19.2]) (trial: $F_{4,177} = 12.55; p < 0.0001$). In all five trials, Δ forward ears remained positive.

The Δ axial ears was more negative when goats were exposed to the non-uniform sound (-30.9%, CI [-41.0; -20.9]) than when goats were exposed to the uniform sound (-19.1%, CI [-29.6; -8.6]) (stimulus: $F_{1,27} = 4.29; p = 0.05$). It increased from the first (-41.6%, CI [-51.1; -32.1]) to the fourth trial (-14.8%, CI [-24.9; -4.7]) and decreased slightly during the fifth trial (-17.2%, CI [-28.2; -6.3]) (trial: $F_{4,177} = 12.35; p < 0.0001$). The Δ axial ears remained negative in all five trials.

The Δ ear posture changes was positive in all trials, tended to decrease from the first (2.4, CI [1.1; 3.6]) to the fourth trial (1.1, CI [-0.2; 2.4]) and increased slightly from the fourth to the fifth trial (1.6, CI [0.2; 3.4]) (trial: $F_{4,177} = 2.36; p = 0.06$). The Δ backward ears was not influenced by either stimulus (stimulus: $F_{1,27} = 0.1; p = 0.7$) or trial (trial: $F_{4,177} = 0.3; p = 0.8$).

4.3.2 Heart rate measurements

4.3.2.1 Heart rate

When goats were exposed to the non-uniform sound, Δ heart rate was at reference level during the playback; when goats were exposed to the uniform sound, Δ heart rate
was slightly decreased in the first (0-20 s) phase (phase × stimulus: $F_{2,575} = 3.5$; $p = 0.03$; Figure 5).

The $\Delta$ heart rate was positive during the first (2.0 beats/min, CI [-2.4; 6.4]) and second trials (2.7 beats/min, CI [-1.8; 7.1]). From the third to the fifth trial, $\Delta$ heart rate was negative and decreased from -5.6 (CI [-10.1; -1.1]) to -6.5 (CI [-11.1; -1.9]) to -8.1 (CI [-12.9; -3.3]) beats/min, independent of the stimulus (trial: $F_{4,575} = 19.63$; $p < 0.0001$; Figure 5). Thus, heart rate during the first and second trials was higher than during the reference situation whereas it was lower than reference values during the third, fourth and fifth trials.

**Figure 5:** (a) Difference in heart rate [beats/min] to the reference values depending on the stimulus (non-uniform sound/uniform sound) in the first (0-20 s), middle (21-40 s) and last (41-60 s) 20 s of the playback. (b) Difference in heart rate [beats/min] to the reference values depending on the trial (first to fifth). Raw data are presented as box plots indicating observed median, first and third quartiles, and absolute range of data. The solid lines show the model estimation. A difference in heart rate of ‘0’ indicates identical values during playback and reference situation.
4.3.2.2 RMSSD

When goats were exposed to the non-uniform sound, Δ RMSSD was positive during the first and fifth trials whereas there was almost no difference to reference values during the second, third and fourth trials (Figure 6). When goats were exposed to the uniform sound, there was almost no difference to reference values during the first and second trials; the Δ RMSSD increased slightly from the second to the fourth trial and decreased from the fourth to the fifth trial (trial × stimulus: $F_{4,573} = 4.91; p = 0.0007$; Figure 6).

The Δ RMSSD decreased slightly from 3.8 (CI [1.1; 6.5]) to 1.6 (CI [-1.1; 4.3]) to 0.9 (CI [-1.8; 3.5]) ms from the first (0-20 s) to the last (41-60 s) phase, independent of the stimulus (phase: $F_{2,573} = 6.7; p = 0.0013$).

![Figure 6](image-url)

**Figure 6**: Difference in heart rate variability RMSSD [ms] to the reference values depending on the stimulus (non-uniform sound/uniform sound) from the first to the fifth trial. Raw data are presented as box plots indicating observed median, first and third quartiles, and absolute range of data. The solid lines show the model estimation. A difference in heart rate variability of ‘0’ indicates identical values during playback and reference situation.
4.4 Discussion

4.4.1 Uniform sinusoidal tone vs. non-uniform chime of a bell

Overall, the behaviour of goats in this study suggested a stronger response to a chime of a bell – a non-uniform sound with varying frequencies and amplitudes as well as a short rise time – than to a uniform sinusoidal tone consisting of only one frequency and continuously increasing amplitude with a long rise time. When goats were exposed to the chime of a bell, the feeding duration was shorter, and the alertness duration was longer, and the proportion of axial ears was lower than when they were exposed to the uniform sound. These findings indicate that the chime of a bell led to an increased arousal compared to the constant sound, despite the identical maximum amplitude of 96 dB which correlates to the maximum amplitude of traditional goat bells. Cardiac activity was not affected by the stimulus, suggesting that the playback situation did not lead to a physiological stress reaction.

Our results correspond to previous studies that found sounds with varying frequencies and amplitudes as well as short rise times being more aversive to humans and non-human mammals than sounds with uniform amplitudes, one frequency and long rise times (Molino 1979, Talling et al 1996, Talling et al 1998). The differing intensity of responses to the two acoustic stimuli might be explained by the chime of a bell having acoustic characteristics similar to those of certain animal calls, such as alarm calls: both sounds are characterised by rapid rise times, high amplitudes and varying frequencies (Fischer 1998). The auditory system might be designed to react to such sounds as they may contain biologically relevant information. The importance of the amplitude and frequency characteristics for potential aversive effects of a sound is underlined by the results of Moore (2012), who showed that humans adapted to a sound only if it was below 30 dB and adapted more to sinusoidal tones than to noisy sounds.

If the acoustic stimuli used in this study not only led to an increased arousal but were aversive to the goats, activation in form of increased cardiac and ear activity would have been expected. Reefmann et al (2009b) found that in sheep emotionally negative situations were characterised by a high number of ear-posture changes and a high proportion of forward ear postures, whereas axial ear postures occurred rarely. By contrast, during positive situations, ear-posture changes were few and the proportion of axial ear postures was high. In this study, only the proportion of axial ears was lower when goats were exposed to the chime of a bell indicating that the goats might have perceived the chime of a bell as more negative than the sinusoidal tone. As we did not
find consistent differences in other ear postures, the ear activity has to be considered carefully in this study.

Both heart rate and RMSSD were only weakly affected by the stimulus, and thus we can read little into the physiological stress caused by the acoustic stimuli.

Overall, the feeding duration was shorter, the alertness duration longer, and the proportion of axial ears lower when goats were exposed to the chime of a bell compared with the sinusoidal tone, suggesting that the sound characteristics of the chime stimulus caused an increased behavioural arousal compared to the sinusoidal tone.

4.4.2 Habituation

During the first trial, regardless of the type of acoustic stimulus, the feeding duration was substantially shorter and the alertness duration longer than during the subsequent four trials, suggesting that the first exposure to an acoustic stimulus of high amplitude was distracting the animals. Similar to our results, Harbers et al (1975) found that feed intake of sheep was decreased when they were subjected to 75 dB or 100 dB compared to 45 dB. Furthermore, Australian fur seals were more alert when they heard boat noise of 75-85 dB compared with boat noise of 64-70 dB (Tripovich et al 2012). Over the course of five trials in our study, the initial reactions in feeding and alertness diminished, indicating a habituation to both stimuli after a few trials. Apparently, habituation to the sinusoidal tone was faster than to the chime of a bell as feeding duration increased and alertness decreased sooner with the sinusoidal sound. Previous studies have shown that sounds that provoke the strongest response are expected to take longer to habituate to compared with sounds that initiate a weaker response (Voipio 1997).

The observations from this study are similar to those from experiments done with pigs (Talling et al 1998), heifers (Waynert et al 1999) and rats (Masini et al 2008). Pigs habituated to loud, uniform sounds after 40 consecutive 5-min tests but did not habituate to loud, intermittent sounds (Talling et al 1998). Heifers showed signs of habituation to handling noises composed of humans shouting and metal clanging during a 1-min exposure over 5-day trials (Waynert et al 1999). Masini et al (2008) found that rats habituated to daily 30-min exposures to white noise at 95 dB within 6 days. Contrary, the results of Kanitz et al (2005) indicated that exposure of domestic pigs to repeated noise levels of 90 dB during daily sessions of 2 h over a period of 28 days resulted in a state of chronic stress which may have negative implications on animal welfare. In
our study, we used playbacks of 1 min over only five consecutive days, but a longer exposure of the goats to the playbacks might have led to different effects. Free-ranging goats in alpine regions are equipped with bells 24 h a day during the summer season on the alp. Thus, the question arises whether the long-term exposure to the chime of the bell might lead not only to increased arousal but might negatively affect welfare.

4.5 Conclusion
Despite the same maximum amplitude, the irregularly intermittent chime of a bell led to an increased behavioural arousal reaction compared to the uniform sinusoidal tone. With repeated exposure to the stimuli, goats seemed to habituate to both stimuli, but habituation was faster to the sinusoidal sound than to the chime of a bell. Free-ranging goats in alpine regions usually are equipped with bells 24 h a day during the summer season. Thus, the question arises whether the long-term exposure to the chime of a bell might have negative effects on animal welfare.
5 Do bells affect behaviour and heart rate variability in grazing dairy cows?

Based on:
Julia Johns, Antonia Patt, Edna Hillmann (2015)

Abstract
In alpine regions cows are often equipped with bells. The present study investigated the impact of wearing a bell on behaviour and heart rate variability in dairy cows. Nineteen non-lactating Brown-Swiss cows with bell experience were assigned to three different treatments. For 3 days each, cows were equipped with no bell (control), with a bell with inactivated clapper (silent bell) or with a functional bell (functional bell). The bells weighed 5.5 kg and had frequencies between 532 Hz and 2.8 kHz and amplitudes between 90 and 113 dB at a distance of 20 cm. Data were collected on either the first and third or on all 3 days of each treatment. Whereas duration of rumination was reduced with a functional bell and a silent bell compared with no bell, feeding duration was reduced with a silent bell and was intermediate with a functional bell. Head movements were reduced when wearing a silent bell compared with no bell and tended to be reduced when wearing a functional compared to no bell. With a functional bell, lying duration was reduced by almost 4 hours on the third day of treatment compared with the first day with a functional bell and compared with no bell or a silent bell. All additional behavioural measures are consistent with the hypothesis of a restriction in the behaviour of the cows wearing bells, although this pattern did not reach significance. There was no treatment effect on heart rate variability, suggesting that the bells did not affect vago-sympathetic balance. An effect of experimental day was found for only 1 out of 10 behavioural parameters, as shown by a decrease in lying with a functional bell on day 3. The results indicate behavioural changes in the cows wearing a bell over 3 days, without indication of habituation to the bell. Altogether, the behavioural changes suggest that the behaviour of the cows was disturbed by wearing a bell. If long-lasting, these effects may have implications for animal welfare.
5.1 Introduction
Noise is described as an acute, chronic or intermittent sound (Head et al 1993), which can act as a potential stressor in farmed species such as pigs (Talling et al 1996, Otten et al 2004), horses (Christensen et al 2005), goats (Johns et al 2015 (chapter 4), Liu et al 2012) and cattle (Head et al 1993, Waynert et al 1999, Arnold et al 2007, Arnold et al 2008). In piglets, Talling et al (1996) found that, within 15 min of acute exposures to various noise stimuli at 80–97 dB once per week over a total of 4 weeks, initial increase in heart rate and locomotion indicated an activation of the pigs’ defence mechanisms, followed by habituation. In another study, exposure to repeated noise during daily sessions of 2 hours over a period of 4 weeks caused changes in neuroendocrine regulations that reflected a state of chronic stress in growing pigs (Kanitz et al 2005). Horses (Christensen et al 2005) and goats (Johns et al 2015 (chapter 4)) spent less time feeding when exposed to acute noise for 1–2 min in a test arena. The goats habituated to the noise (chime of bell and sinusoidal sound with maximum amplitude of 96 dB) within 3 days, i.e. from the third day of playbacks there was no difference between baseline and playback values in feeding and vigilance behaviour (Johns et al 2015 (chapter 4)). Whereas these studies were conducted in rather artificial situations, farm animals are confronted with several acoustic stimuli throughout their lives, including ventilation, feed carts, barn cleaners or the vacuum machine in the milking parlour. The noise of the latter is considered to be stressful for dairy cows as it resulted in both fearful reactions and avoidance in a y-maze choice task (Arnold et al 2008). When exposed to playback of noise recorded in milking parlours and played at 85 dB in a raceway 3 times daily, heifers showed an increased heart rate on day 1 and faster transit times on days 1–4, indicating an escape reaction, but habituated over a 5 day period with daily exposure (Arnold et al 2007). A reduction in noise and vibration in the milking parlour for 3 months improved udder health, which was, however, mediated mainly by the reduction in vibration rather than noise (Gygax and Nosal 2006). Furthermore, exposure to aircraft noise in the waiting area just before milking did not lead to behavioural reactions in dairy cows (Head et al 1993). When examining the effect of noise on cow behaviour, noise exposure was conducted either in a raceway or in the waiting area just before or during milking, i.e. at times when cows were already aroused, and the effects of noise during resting times were not taken into account (Head et al 1993, Arnold et al 2007, Arnold et al 2008, Gygax and Nosal 2006).
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In alpine regions cows are often equipped with a bell throughout the summer season, i.e. for several months, to ensure that farmers can locate their animals on the wide alpine pastures, many areas of which are obstructed from the view. Various types of bells are available, ranging from small ones for goats and calves to large and heavy ones that are used for traditional purposes like seasonal cattle drives (‘Alpabzug’) or exhibitions. Whereas cows are equipped with the larger and heavier bells during these traditional purposes, the smaller bells are used on pasture. However, although the bells used on pasture are smaller than the ones used during traditional purposes, the animals are exposed to them continuously throughout the day for several months. Consequently, cows are also exposed to the bells during resting periods. The chime of a bell is characterised by high and varying amplitudes, varying frequencies and sounds arising intermittently, depending on the movement of the bell. These characteristics have been shown to be more aversive than uniform sounds of similar amplitudes in pigs (Talling et al 1996, Talling et al 1998), goats (Johns et al 2015 (chapter 4)) and lambs (Ames and Arehart 1972). As cows have a well-developed hearing capacity (Heffner and Heffner 1983), a bell may thus lead to deviations in behaviour, indicating aversiveness, and may lead to reduced welfare in the long term if the animals do not habituate to the sound of the bell.

In ruminants, feeding and rumination are indispensable activities and provide useful in-formation regarding health and welfare (DeVries et al 2009, Bristow and Holmes 2007). Rumination in dairy cattle is associated with saliva production, which helps buffer the acidic conditions in the rumen and prevent rumen acidosis (Owens et al 1998, Beauchemin 1991). A reduction in rumination time might result in a reduction of saliva production and eventually challenge health through an increased risk of rumen acidosis.

Although rumination can take place during standing, it mainly takes place while cows are lying (Beauchemin 1991). As feeding and lying are mutually exclusive behaviours, there is direct competition for the time allocated to each of them. The time cows spend lying down is an important measure of cow comfort, and cattle welfare standards are increasingly addressing the issue of lying times in dairy cattle (Charlton et al 2014, EFSA 2009, Pelzer et al 2015, Rushen et al 2012). The need for lying is described as relatively constant (13h per day, Jensen et al 2005) and can dominate other basic needs after only a few hours of forced standing (Metz 1985, Munksgaard and Simonson 1996). A lying duration of 11-13h per day is widely recommended as best practice,
and reduced lying times are a risk for lameness, especially in cows kept indoors (Cook et al 2004, Bell et al 2009, Chapinel et al 2009, Proudfoot et al 2010). In addition to behaviour, changes in the vago-sympathetic balance (reflected by heart rate variability parameters) have been used as an animal welfare indicator which allows comparing different management procedures, technologies and handling methods in terms of animal welfare (Gygax et al 2008, Steward et al 2008, von Borell et al 2007).

To our knowledge, the impact of wearing a bell on welfare-related indicators has not been investigated in dairy cows. We examined whether and how wearing a functional bell (exposure to weight and chime) and a bell that does not produce any noise (exposure to weight only) for 3 days affects behaviour as well as vagal activity of cows compared with not wearing a bell. Bells become audible when the animals move their heads. Thus, we were interested in whether the cows would move less to quieten the bell or to possibly reduce the burden of its weight. As a consequence, we expected feeding and ruminating durations to decrease because both behaviours include head movements. Moreover, we expected lying duration to increase and locomotion activity as well as head movements to decrease as an adaptation to wearing a functional bell.

In addition, herd members may increase the distance to a cow that is wearing a functional bell to avoid the sound. Furthermore, wearing a bell may lead to a change in vago-sympathetic balance resulting in reduced heart rate variability. All these changes were expected to be more pronounced when cows were exposed to both the chime and the weight of the bell compared with only the weight. According to the finding that goats were habituating to once daily minute-long playbacks of bell sounds within 3 days (Johns et al 2015 (chapter 4)), we expected cows to habituate to the bells within the 3 day observation time and, thus, measurements to return to baseline values by the third day.
5.2 Animals, materials and methods

To test our hypotheses, dairy cows were equipped with no bell (control), with a bell with inactivated clapper (silent bell) or with a functional bell (functional bell) for 3 days each in a balanced order. During the treatment period, lying, feeding and ruminating behaviours, leg and head movements, nearest neighbour distances and heart rate variability were recorded. The study was performed between June and November 2012 on a working farm near Zurich, Switzerland (47°31′05.48″ N; 8°50′13.15″ E; 429 m.a.s.l.). The owner of the land gave permission to conduct the study on this site. Procedures used on the animals included fixing a belt for heart rate measurements, a halter and a pedometer. All cows used in this study had previously been habituated to wearing these devices and were used to human contact. Hence, no negative effect of either the equipment or the handling on the cows’ behaviour was expected. The study did not involve endangered or protected species. Ethical approval to conduct the study was obtained from the Zurich Cantonal Veterinary Office, Switzerland (Approval No. 77/2012). Animal care and all experimental procedures were in accordance with the ARRIVE guidelines for animal research (Kilkenny et al 2009).

5.2.1 Animals, housing and management

In this study, we used 19 dry, multiparous Brown-Swiss cows with an average milk yield of 8,000 kg per 305 days of standard lactation, between 3 and 10 years of age. All cows had previous experience with wearing a bell, as all of them had been equipped with a bell on an Alp for 4–5 months when they were 1 year old. Because the experiment had to be conducted on pasture, its duration was limited to the grazing period (end of May – mid-November). During the experiment, the cows were managed under standard summer grazing conditions with 24 hours access to two flat lowland pastures of approximately 3.5 ha in total. Trees provided protection against rain, wind and sun. Hay was provided ad libitum in a round rack with headlocks, and the cows had access to a water trough 24 hours a day. The herd of dry cows was managed in that cows were added to the herd approximately 5 weeks before calving and were removed from the herd 3–5 days before the estimated calving date. Thus, the herd varied in size and consisted of both focal and non-focal cows (Table 1). During the experiment, the average temperature and precipitation were 15 °C (min/max: 0/23.5 °C) and 13 mm (min/max: 0/485 mm), respectively.
5.2.2 Data collection

The experiment was conducted during the grazing season (May to November) in six consecutive batches using one unique group of focal cows per batch. Each group of focal cows consisted of two to five individuals with groups being tested one after the other (Table 1). The number of animals per group and batch depended on the number of dry cows available. Each focal cow was exposed to three different treatments: control treatment (‘no bell’), a bell with inactivated clapper (‘silent bell’) or a functional bell (‘functional bell’). For each batch, the order of treatments was balanced over groups and each treatment lasted for three consecutive days. We chose this duration for two reasons: first, farmers said that cows would get used to bells very quickly (within hours). Second, we found in a previous experiment that goats habituated to a playback of a bell within 3 days when exposed to the sound for only 1 min daily (Johns et al 2015 (chapter 4)). We thus assumed that cows would begin to habituate to a bell they wear 24 hours daily within 3 days, eliminating the need for measurements beyond 3 days. To minimise the risk of carry-over effects, we allowed a break of 3–4 days between two treatments. Due to the memory capacity of the data loggers, data were collected on the first and third experimental day of each treatment for lying behaviour and activity (hind leg acceleration). Nearest neighbour distance and heart rate variability were also recorded on days 1 and 3, whereas feeding and rumination behaviours as well as head movements were recorded on all 3 days of each treatment. This resulted in 19 cows tested in 3 treatments, each treatment lasting 3 days with a break of 3–4 days between. Depending on the variable, measurements were taken on either 2 or 3 days. In the morning (between 8:00 am and 9:00 am) of the day before the start of the first treatment, cows were fitted with the measuring equipment (halter, logger with 3D-accelerometer and thorax belt, Figure 1) for habituation (i.e. a habituation period of 24 hours before measurements started) and were then wearing the devices continuously until the first treatment was finished.
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Figure 1: Experimental cow wearing a bell and measuring equipment. A thorax belt for measuring heart rate variability, a halter for recording feeding, ruminating, and head movements, and a 3D accelerometer at the hind leg for recording activity and lying were used for data recording after 24 hours of habituation.

In the morning (between 8:00 am and 9:00 am) of the first day of the second and third treatments, the focal cows were again fitted with a halter, a 3D-accelerometer logger on the hind leg and a thorax belt. At this time on the first day of each bell treatment, cows were also fitted with a silent or a functioning bell (Figure 1). During the fitting procedure, cows were restrained in the headlocks of the hayrack on pasture. Then, the measuring devices were attached in the following order: 1. halter, 2. accelerometer on the leg, 3. belt for heart rate variability measurements, 4. bell. Attaching the devices took approx. 5 min per cow, and the cows usually did not show signs of disturbance except one cow that tried to get rid of the bell by vigorously shaking its head, jumping and running. We had to exclude this cow from the experiment to avoid severe stress and/or injuries to the animal. All handling procedures and observations were conducted by the same person (Julia Johns). Data collection started when all focal cows were fitted with the measuring equipment and bells and released from the hayrack. After the first 24 hours of data collection (first experimental day), the loggers on the leg and the thorax belts were removed and data were downloaded. In the morning of the third day (third experimental day), cows were again equipped with the loggers on the leg and the thorax belts and data collection started for another 24 hours. The halters were not
removed and data collection lasted continuously over the 3 days of each treatment. The data from the loggers of the halters were downloaded after the third experimental day. Based on this experimental design, only the focal cows were observed, except for recording the distance to the nearest neighbour, for which all cows on pasture were included. The cows of the same experimental group (six groups) were observed on the same calendar dates. Cows that had finished the treatments stayed in the herd until calving but were not observed anymore. Thus, herd size varied in the course of the experiment (Table 1).

Table 1: Experimental design. Calendar date, number of animals and order of treatments per batch are shown as well as total number of animals present on pasture during each experimental period.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Number of focal cows</th>
<th>Treatment order</th>
<th>Total number of cows on pasture</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 20 to July 5</td>
<td>5</td>
<td>No bell, Functional bell, Silent bell</td>
<td>7–9</td>
</tr>
<tr>
<td>July 11 to July 26</td>
<td>3</td>
<td>Functional bell, Silent bell, No bell</td>
<td>5–7</td>
</tr>
<tr>
<td>July 31 to August 15</td>
<td>3</td>
<td>No bell, Silent bell, Functional bell</td>
<td>4–6</td>
</tr>
<tr>
<td>August 29 to September 14</td>
<td>2</td>
<td>Functional bell, No bell, Silent bell</td>
<td>4–6</td>
</tr>
<tr>
<td>September 25 to October 10</td>
<td>3</td>
<td>Silent bell, No bell, Functional bell</td>
<td>6–9</td>
</tr>
<tr>
<td>October 14 to November 8</td>
<td>3</td>
<td>Silent bell, Functional bell, No bell</td>
<td>9–10</td>
</tr>
</tbody>
</table>

5.2.3 Bells

The casted cow bells that were used in this study were bells made from a bronze containing about 23% tin known as bell metal, and all were similar in size and identical in weight (5.5 kg, approx. 1% of cow body weight) including the strap used to attach the bell to the neck of the cows (Figure 1). The bells were part of a traditional set of bells for cows that would produce a harmonised sound when being worn by cows. The sound of the bells was measured at a distance of 20 cm, which corresponds to the estimated distance between the bell and the cow’s ears. It was characterised by a first
frequency band of 532–875 Hz (the lowest visible band on the spectrogram) and a peak frequency of 1.2–2.8 kHz (frequency of highest amplitude, as measured on a spectrum; details are shown in Table 2). In addition, the amplitudes of the bells were measured at distances up to 80 m to see the distance at which the amplitude of the bells decreases substantially (Figure 2). The measurements were conducted by two persons, one moving the bell and the other recording sound and amplitudes. The bells were moved in a standardized way, aimed at mimicking a walking cow.

Table 2: Frequency characteristics and amplitudes of the bells used in the experiment recorded at a distance of approx. 20 cm while manually moving the bells in a standardized way for 1 min each. Sound analysis was done using PRAAT software; amplitude analysis was done using SoundTest-Master, Laserliner, Umarex GmbH and Co. KG, Arnsberg, Germany.

<table>
<thead>
<tr>
<th>Bell</th>
<th>First frequency band (Hz)</th>
<th>Peak frequency (kHz)</th>
<th>Mean amplitude (dB[A]) and range (min–max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>609</td>
<td>2.6</td>
<td>104.7 (98–113)</td>
</tr>
<tr>
<td>2</td>
<td>581</td>
<td>2.8</td>
<td>99.6 (93–109)</td>
</tr>
<tr>
<td>3</td>
<td>581</td>
<td>1.7</td>
<td>96.7 (90–104)</td>
</tr>
<tr>
<td>4</td>
<td>875</td>
<td>1.2</td>
<td>106.4 (101–111)</td>
</tr>
<tr>
<td>5</td>
<td>532</td>
<td>1.3</td>
<td>97.6 (93–103)</td>
</tr>
</tbody>
</table>

Amplitudes were measured in dB (A) by using a precision noise level measuring instrument with integrated long-term storage (SoundTest-Master, Laserliner, Umarex GmbH and Co. KG, Arnsberg, Germany). The A-weighting scale assigns low weights to the low-frequency tones, to which the human ear and the ears of some animals are less sensitive, and high weights to the typically more audible high-frequency tones (Manci et al 1988). Thus, the tones were adjusted to match the cows’ (potential) hearing capacity. For the sound analysis, PRAAT v.5.3.41 DSP Package was used.
Figure 2: Amplitudes of the bells at distances up to 80 m [dB (A)]. Solid line: model estimate, dashed lines: 95% intervals of confidence based on a model predicting amplitude by the square root of the distance, controlling for bell identity and multiple measurements per distance.

5.2.4 Behavioural observations

The experimental design resulted in a sample size of 38 cow-days (i.e. 19 cows × 2 days) per treatment for lying behaviour, leg accelerations and nearest neighbour distance, and 57 cow-days (i.e. 19 cows × 3 days) per treatment for feeding and rumination behaviours and acceleration of head movements. Due to technical problems, data collected on five animals of the treatment ‘functional bell’ and on one animal of the treatment ‘silent bell’ could not be used for the analysis of lying behaviour and activity, and data collected on six animals of the treatment ‘functional bell’ and on five animals of the treatments ‘no bell’ and ‘silent bell’ could not be used for feeding and rumination analysis. One cow approached calving and had to be excluded from the treatment ‘functional bell’, and data collected on another cow had to be excluded from the treatment ‘silent bell’ because the strap of the bell was broken. Concerning lying behaviour and leg acceleration, this resulted in a remaining sample size of 34 cow-days for the treatment ‘functional bell’, 38 cow-days for ‘silent bell’ and 38 cow-days for ‘no bell’.
For feeding and rumination behaviours, we had a remaining sample size of 46 cow-days for ‘functional bell’, 47 cow-days for ‘silent bell’ and 52 cow-days for ‘no bell’.

5.2.4.1 Lying behaviour and leg movements
According to the method described by Helmreich et al (2009) as well as Patt et al (2012, 2013), lying behaviour and activity during locomotion were recorded using a commercial 3D-acceleration logger attached to the left hind leg of each cow (MSR145, MSR Electronics GmbH, Seuzach, Switzerland; 18 × 14 × 62 mm, 33 g). Acceleration in the direction of the y-axis, parallel to the longitudinal axis of the cow’s hind leg, was recorded. Sampling rate was set at 10 Hz (10 measurements/s) and sensitivity of the sensor at ± 10 g. The accelerometer was powered by a 260 mAh lithium-polymer battery, rechargeable via USB connection, which enabled measurements for several days. Data were recorded on an integrated memory chip with a capacity of 2 million data points, transferred to a computer via USB connection and stored in a Microsoft CSV-file. MSR PC-Software was used for data transfer and analysis as well as settings of the data loggers (MSR Electronics GmbH 2011). Due to the way the logger was attached to the cow’s left hind leg, acceleration values observed while the cow was lying (quietly) and standing equalled 0 g and −1 g, respectively. When the leg was moving, acceleration values reached values both higher and lower than −1 g. The different positions of the cows’ hind legs while lying down (almost horizontal) as opposed to standing and walking (almost vertical) meant that the lying duration per cow could be calculated per 24-hour period. Leg movements were calculated during the periods in which the cows were standing or walking, i.e. not lying, and the sum of acceleration changes per 24 hours was calculated in g.

5.2.4.2 Feeding and rumination behaviours, and head movements
According to the method described by Nydegger et al (2011) as well as Braun et al (2013), feeding and rumination behaviours of the focal cows were recorded using a halter (500 g) that included a commercial logger (MSR145, MSR Electronics GmbH; 31 × 31 × 72 mm, 33 g) fitted with a pressure-sensitive sensor combined with an oil-filled silicon tube (40 g). This tube was attached to the halter above the cow’s nose. Opening of the mouth caused bending of the tube and increased pressure inside the tube. These pressure changes were trans-mitted through the oil-filled tube and regis-tered by the sensor. The signal was saved at a rate of 10 Hz. Data were stored in a
data logger, which was attached to the side of the halter in a leather pouch. The data logger additionally recorded the 3D acceleration of head movements. Acceleration was measureable on x-, y- and z-axes. Sampling rate was set at 10 Hz (10 measurements/s) and sensitivity of the sensor at ± 10 g. The data logger was powered by a 260 mAh lithium-polymer battery, rechargeable via USB connection, and had an SD card, which enabled measurements over several days. It was possible to differentiate between feeding and ruminating owing to the differences in the characteristics of the pressure pattern generated by each (Braun et al 2013, Scheidegger 2008). MSR PC-Software (MSR Electronics GmbH 2011) was used for data analysis, and the amount of time spent feeding and ruminating was calculated per 24-hour period for each cow. Head movement was assessed by summing up acceleration value changes of all three dimensions over 24 hours in each treatment, as well as calculating the variance in acceleration value changes, which served as indicator of sudden movements (e.g. displacing flies, self-grooming).

5.2.4.3 Nearest neighbour distance
Using the scan sampling method (Martin and Bateson 2007), the distance between each focal cow and the nearest (focal or non-focal) cow was recorded on the first and third experimental day outside the common resting time. Due to the size and structure of the pasture, focal cows could not all be observed at the same time but needed to be observed one after another. The observer did not interact with the cows and slowly followed the focal cow at a constant distance of 10-20 m. Each focal cow was directly observed at two times per day for 30 min between 10:00 am and noon and between 13:30 pm and 15:30 pm. The distance to the nearest neighbour was recorded every 5 min.

5.2.5 Heart rate variability
Heart rate variability was recorded using Polar Equine (Polar Elektro Europe BV, Zug, Switzerland, 120 g), allowing a non-invasive measurement of heartbeats (von Borell 2007). To improve conductivity between electrode and skin contact, electrode gel (Anandic Medical Systems AG/SA, Feuerthalen, Switzerland) was applied. A thorax belt with two integrated electrodes was fixed around the torso directly behind the forelegs. One electrode was positioned ventrally on the left side of the sternum and the other one in a distance given by the thorax belt on the left thoracic wall. A receiver for
recording the data was placed between the two electrodes. The thorax belt was additionally protected by an elastic belt of about 5 cm width, and withers were additionally protected by foam material. Data were downloaded to a computer via a base station using Blue-tooth (Polar Team2 Pro, version 1.3.0.3, Polar Electro Oy, Kempele, Finland).

All calculations were carried out using the programs Polar ProTrainer 5 Equine Edition (version 5.35.161, Polar Elektro Europe BV, Zug, Switzerland) and R version 3.1.2 (R Core Team 2015). The root mean square of successive differences of heartbeats (RMSSD, ms), and the ratio between RMSSD and the standard deviation of all heartbeats (SDNN, ms), RMSSD/SDNN, were calculated for 5 × 5 min time windows during 24 hours (3 × 5 min during the day [6:00 am – 10:59 am, 11:00 am – 15:59 pm, 16:00 pm – 20:59 pm], 2 × 5 min during the night [21:00 pm – 1:59 am, 2:00 am – 5:59 am]). To minimize influences on heartbeats caused by different levels of physical activity and to study long-term effects on heart rate variability related to the different treatments, we only considered parts of the tachogram when the animals were lying (Langbein et al 2004). The RMSSD reflects alternations in the vago-sympathetic balance that are vagally mediated. The SDNN is a more complex parameter reflecting vagal as well as sympathetic activation. The ratio between RMSSD and SDNN is a global indicator for general changes of the vago-sympathetic balance in the organism.

Automatic correction of the tachograms was carried out using the correction routines included in the Polar Software (Polar ProTrainer 5 Equine Edition, version 5.35.161, Polar Elektro Europe BV). Data with an error rate of more than 5% were excluded from the analysis according to von Borell et al (2007). The experimental design resulted in a sample size of 38 cow-days (i.e. 19 cows × 2 days) per treatment. One cow approached calving and had to be excluded from the treatment ‘functional bell’, and data collected on another cow had to be excluded from the treatment ‘silent bell’ because the strap of the bell was broken. This resulted in a remaining sample size of 37 cow-days for the treatment ‘functional bell’, 37 cow-days for ‘silent bell’ and 38 cow-days for ‘no bell’.
5.2.6 Statistical analyses

Linear mixed-effects models were performed for the different outcome variables defined above using the lmer method from the lme4 package (Bates et al 2014) in R (version 3.1.2, R Core Team 2015). Statistical assumptions (normal distribution, homoscedasticity) were checked by graphical analysis of residuals. To satisfy these assumptions, the sum and variance of acceleration changes in head movements and the summed acceleration changes of leg movements needed to be log transformed.

To adequately reflect dependencies in the experimental design, the effect of observation day was nested in individual identity nested in batch. For heart rate variability, time period was additionally nested within a given observation day. In addition, a crossed random effect of calendar date was included to all models to reflect day-to-day variation that was, for example, caused by differing weather conditions and potentially affected all the simultaneously observed cows in a similar manner. The data missing due to technical problems were distributed equally over treatments and animals, and the respective sample size for each outcome variable was accounted for by the random effects.

For each outcome variable, we set up a maximum model including the fixed effects of treatment (factor with three levels: no bell, silent bell, functional bell), experimental day (factor with two levels: first and third day for lying, leg movements and nearest neighbour distance; or three levels: first, second and third day for feeding and ruminating behaviours and head movements), and their interaction. The minimal model considered was the model including treatment only. Maximum models were reduced in a step-wise backward procedure using $p > 0.05$ as the exclusion criterion. The $p$-value for the treatment is reported independently of whether or not it reaches significance. The $p$-values for the step-wise backward model selection and for treatment were calculated using a parametric bootstrap approach with 1,000 bootstrap samples as implemented in package pbkrtest; this test is more adequate than the raw likelihood-ratio test because it does not rely on large-sample asymptotic analysis and correctly takes the random-effects structure into account (Halekoh and Hojsgaard 2014). Post-hoc pairwise comparisons with Tukey correction were conducted using glht from the multcomp.
5.3 Results

The feeding duration with a silent bell was reduced by 115 min compared with the no bell treatment \( (z = -3.0, p = 0.008; \text{Figure 3A}) \), and by 40 min in the functional bell treatment with no significant difference to the no bell or silent bell treatment (functional bell vs. no bell: \( z = -1.0, p = 0.55 \); functional bell vs. silent bell: \( z = 1.9, p = 0.14 \)). The duration of rumination was reduced by 131 min in the functional bell treatment compared with the no bell treatment \( (z = -3.1, p = 0.005) \) and by 160 min in the silent bell treatment compared with the no bell treatment \( (z = -3.8, p < 0.001; \text{Figure 3B}) \). There was no difference between the silent bell and the functional bell treatments \( (z = 0.7, p = 0.76) \). The number of chews per cud numerically decreased from the no bell to the silent bell and the functional bell treatments, but this pattern did not reach significance (Figure 3C). There was no effect of the experimental day on any of the feed intake behaviours.

The summed changes in acceleration of head movements were larger with no bell compared with the silent bell \( (z = -2.68, p = 0.02; \text{Figure 3D}) \) and tended to be larger with no bell compared with the functional bell \( (z = -2.1, p = 0.09) \). There was no difference in the summed changes in head acceleration between the silent bell and the functional bell \( (z = 0.5, p = 0.8) \). The variability of head accelerations showed a similar pattern in that there was a reduction in variability with the silent bell \( (z = -2.4, p = 0.04; \text{Figure 3E}) \) and a tendency for a reduction with the functional bell \( (z = -2.3, p = 0.06) \) compared with no bell. Leg acceleration during standing bouts was slightly but not significantly reduced with the silent bell and the functional bell (Figure 3F). There was no effect of experimental day on leg movements.

The lying duration with the functional bell was reduced by 231 min on the third day of treatment compared with no bell on the first day (significant treatment x day interaction followed by post-hoc pairwise comparisons; day 3 with functional bell vs. all other day-treatment combinations: \( z \leq -4.3, p \leq 0.01 \); all other comparisons: \( z \geq -1.5, p \geq 0.6 \); Figure 2G). With the functional bell, lying duration was reduced by 65 min on the third day compared with the first day \( (z \leq -3.0, p = 0.04) \). The proportion of scans with another cow in a distance of up to 2 m to the focal animal slightly decreased from the no bell to the silent bell and the functional bell treatments, but this pattern did not reach significance (Figure 3H). There was no significant effect of treatment or experimental day on RMSSD (all \( p \geq 0.6 \)) or RMSSD/SDNN (all \( p > 0.5 \)).
Do bells affect behaviour and heart rate variability in grazing dairy cows?

Figure 3: Effects of bells on the behaviour of dairy cows on pasture. A. Feeding duration per 24 hours [min], B. Duration of rumination depending on the treatment [min], C. Number of chews per rumination cud, D. Summed changes in head acceleration per 24 hours [g], E. Variance in head acceleration changes per 24 hours [g], F. Changes in leg acceleration during standing bouts [g] depending on the treatment, G. Lying duration per 24 hours [min] depending on the treatment and the day of observation, H. Proportion of scans with the distance to the nearest neighbour < 2 m depending on the treatment. Raw data are presented as box plots indicating observed median, first and third quartiles and absolute range of data. The solid lines show the model estimation, the dashed lines the 95% intervals of confidence. Different letters reflect significant differences (p ≤ 0.05) and letters in parentheses p < 0.1 from the Tukey corrected post-hoc comparisons.
5.4 Discussion

Compared with the no bell treatment, feeding duration was reduced with a silent bell. With a functional bell, however, feeding duration was intermediate between the no bell and the silent bell treatments. Compared with the no bell treatment, rumination duration was reduced with both a silent bell and a functional bell. Lying duration clearly decreased with a functional bell on the third treatment day but did not do so with a silent bell or with no bell. All other behavioural measures showed the expected tendencies, i.e. largest effect of functional bells and less effect of silent bells compared to no bell, but these effects did not reach significance. Heart rate variability was not affected by the applied treatments. There was no effect of experimental day on any of the variables except lying duration, which was reduced on the third day compared with the first day with a functional bell.

There are contradictory findings regarding the effect of noise on feeding behaviour in ungulates (Broucek 2014). Caribou were found to increase time spent feeding in response to increased noise exposure (Luick et al 1996). By contrast, feed intake in horses (Christensen et al 2005) and goats (Johns et al 2015 (chapter 4)) decreased when animals were exposed to acute noise. In dairy cows, immediate exposure to high-intensity noise at 105 dB, which is comparable to the bells tested in the current study, reduced feed intake and milk yield (Kovalcik and Sottnik 1971). However, in the studies on the domestic species, the animals were exposed to noise only during short intervals. In the current study, cows were exposed to the sound of the bells continuously. Compared with cows wearing no bell, cows wearing a silent bell reduced their feeding duration by almost 2 hours per day whereas cows wearing a functional bell reduced their feeding duration by only 40 min. Thus, the weight of the bell seemed to affect feeding behaviour more than the sound of the bell, although it is hard to explain why feeding duration was less affected by the functional bell, i.e. weight and sound, compared with the silent bell, i.e. only weight. Longer observation periods and/or a larger sample size are needed to clarify this finding.

The rumination duration, on the other hand, was clearly reduced in both bell treatments, indicating that rumination behaviour was restricted by the weight of the bell. Reduced rumination was previously observed in cows at risk of rumen acidosis (Bristow and Holmes 2007) and in cows after regrouping, which is widely accepted as a stressful event (Schirmann et al 2011). Although rumination can take place con-currently with standing, most rumination takes place while cows are lying (Schirmann et
Do bells affect behaviour and heart rate variability in grazing dairy cows? al 2012, Cooper et al 2007). Thus, the reduction in lying duration might to a certain extent explain the reduction in duration of rumination. Following the argumentation of Owens et al (1998) and of Munksgaard and Simonsen (1996) that reduced rumination is an indicator of stress in cattle, the weight of the bell may have reduced the duration of rumination by triggering some kind of stress or discomfort. Consequently, a prolonged reduction in feeding and rumination might eventually challenge the animals’ health and welfare (Stöber 2006, Bailey and Balch 1961).

Both summed head acceleration changes and variability in head acceleration changes were reduced when the cows were wearing a bell, indicating that the cows avoided intense movements like head shaking when wearing a bell. This explanation would be supported by the correlation between head acceleration changes and amplitude that was strongest with the functional bell (the recorded amplitude was also increased in the “silent” treatments to a certain extent, due to the sensitivity of the dosimeter that recorded rubbing movements between halter and skin). Interestingly, there was no additional reduction by the sound of the bell although this was expected when the cows had learned to avoid the chiming by reducing head movements. Cows are assumed to be capable to learn noise avoidance as shown in dairy heifers that learned to avoid tape recorded milking facility noise (Arnold et al 2008). It thus seems that the cows did not learn how to avoid the chiming, were not able to further reduce head movements or were not affected by the chime of the bell more than by the weight of the bell.

Surprisingly, cows were lying for shorter durations when wearing a functional bell on the third day of observation compared with wearing a silent bell or no bell. The lying duration was expected to increase with functional bells compared with the other treatments, because a reduced movement may quieten the bell. In agreement with our study, the lying duration in dairy cows was found to decrease when cows were exposed to 90 dB several times per day over 3 weeks (Kovalcik and Sottnik 1971). Similarly, caribou exposed to military jet aircraft noise were lying down less in response to increased noise exposure (Luick et al 1996). Lying down is widely accepted to be a basic requirement for the well-being of dairy cows. Heifers were found to have an inelastic demand for rest of about 12 hours per day (Jensen et al 2005), and after 3 hours of lying and feed deprivation, cows compensated for lying but not for feeding (Metz 1985). The reduced lying duration (by almost 4 hours per day on the third day of observation) in combination with the reduced feeding and rumination durations as well as the reduced head movements and the numerically reduced leg acceleration changes as
found in the current study indicate that the cows spent more time standing idle. Haley et al (2000) concluded from their study on behaviour of dairy cows in two types of housing a decrease in lying duration combined with an increase in standing idle to be probable indicators of cow discomfort.

The focal cows in this study spent the same proportion of time in close distance (< 2 m) to other cows independent of wearing a bell or not, although the chime of the bells was reduced to levels below 80 dB only at distances > 10 m. We thus assume that the need for close spatial contact was stronger than the avoidance of the chime of the bell. Nevertheless, although these effects were not significant, the mean values for the time spent in close distance to another cow, leg acceleration changes and the number of rumination chews per cud tended to decrease when cows were wearing a bell, suggesting that the behaviour of the cows may have been restricted in this situation. By contrast, the physiological measure heart rate variability did not differ between any of the treatments. Although some studies found an increase in heart rate during noise exposure in pigs and cattle (Talling et al 1996, Arnold et al 2007), we focussed on heart rate variability during lying periods. Heart rate variability during periods of rest is assumed to be suitable to reflect long-term effects better than heart rate (Frondelius et al 2015). However, no effect of either of the bell treatments was found, indicating no impact of wearing a bell on vago-sympathetic balance within the duration of the study. Finally, none of the eight behavioural measures indicated habituation by the cows although all cows had had some experience wearing a bell during one summer season as heifers. This finding contradicts those from studies on goats (Johns et al 2015 (chapter 4)), pigs (Talling et al 1996) and cattle (Arnold et al 2007) that used short-term noise exposures. We thus assume that the continuous exposure to sound and weight prevented habituation within the 3 days of observation. A follow-up study with a longer observation period would be necessary to find out the latency to habituate to wearing a bell. Such a study could also inform us about whether the effects represent short-term behavioural adaptions to wearing a bell or long-term changes that might challenge animal welfare.

Added effects by the functional bell in comparison with the silent bell may help distinguish the effects of weight alone from further effects of the sound of the bell. The functional bell reduced lying duration on the third day of treatment more than the silent bell, indicating that the cows may have felt more uncomfortable through the sound than the weight alone. The reduced duration of rumination with a bell, independent of sound
production, may be explained by the head position of the animal during rumination. Cows usually ruminate with an upright position of the head, often while lying. The weight of the bell may result in discomfort when the cows are holding the head in this position, and thus the animals reduced the duration of rumination accordingly. By contrast, the head is always lowered for feeding, i.e. grazing, probably allowing the weight of the bell to be less burdensome. Cows do not have upper incisors, and thus jerky head movements, which make the bell chime, are required to pick a cluster of grass with the tongue. Nevertheless, feeding duration was reduced by almost 2 hours with a silent bell and 40 min with a functional bell. More research is needed to completely disentangle the effects of weight and sound.

A bell usually combines weight and sound, with heavy bells being louder than light bells. Bells need to produce sound of certain (high) amplitudes because otherwise they are use-less. Thus, a cow has to cope with the combined effects of weight and sound on-farm.

5.5 Conclusion

Wearing a bell for 3 days interfered with feeding, ruminating and lying behaviours as well as head movements of cows compared with not wearing a bell, but it did not affect heart rate variability. Cows did not habituate to the bells over the 3 days of observation. The observed behavioural changes might challenge welfare if they lasted for an extended time period, but long-term observations are necessary to quantify the effects of bells on welfare.
6 Effects of cowbells on acoustic perception of dairy cows

Based on:
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Submitted to Frontiers in Veterinary Science

Abstract
In alpine regions, cows are often equipped with bells during pasture season to ensure that farmers can locate them. Considering that cows have a well-developed hearing capacity, constant exposure to the chime of a bell may affect cows' hearing. The aim of the present study was to test the behavioural and cardiac reactivity of cows that were either used to wearing a bell or not towards a playback of low and high amplitude. Additionally, we tested whether wearing earplugs, mimicking hearing damage, reduced the cows' reactivity towards the playback. On 24 farms, half of them routinely using cowbells, 96 Brown Swiss cows were tested in a 2×2 factorial cross-over design (65 or 85 dB, without or with earplugs) in a balanced order. The effect of bell experience, amplitude and earplugs on the latency to the first behavioural and to the cardiac response to a 5-second playback was analysed using linear mixed effects models, considering dependencies within the dataset. Cows reacted faster without earplugs and when they were exposed to 85 dB compared with 65 dB. The latency to the first behavioural reaction was not affected by bell experience. The proportion of cows leaving the feeding rack after onset of the playback was reduced by bell experience and earplugs, and was increased with 85 dB compared to 65 dB. Exposure without earplugs to 85 dB but not to 65 dB increased heart rate, indicating that cows were more alert when exposed to 85 dB. Heart rate as well as heart rate variability indicated increased sympathetic activation during the exposure to 85 dB compared with 65 dB and at the same time increased parasympathetic activity with earplugs than without earplugs. Heart rate and heart rate variability were not affected by bell experience. In general, no indication of complete hearing loss due to bells was found. The 85 dB stimulus increased arousal and avoidance compared with the 65 dB stimulus, with bell experience and earplugs leading to a general decrease in avoidance of the stimulus. This may reflect an altered emotional appraisal of the playback stimulus due to routine bell exposure.
6.1 Introduction

In alpine regions, cows are often equipped with a bell throughout the summer season to ensure that farmers can locate their animals on the wide alpine pastures, many areas of which are obstructed from view. The chime of these cowbells is characterised by high and varying amplitudes from 90 dB to 113 dB at a distance of 20 cm, the approximated distance between the bell and the cows’ ears (Johns et al 2015 (chapter 5)). Goats have been found to show a reduced feeding duration and increased alertness when being exposed to a playback of a bell. This playback led to higher behaviour arousal than a playback of a uniform sinusoidal sound, indicating a potential of the bell sound to be more aversive to goats than a uniform sound. With repeated exposure, goats habituated to both stimuli (Johns et al 2015 (chapter 4)).

Different from the findings obtained in the goat experiment (Johns et al 2015), noise exposure is often accompanied by an increase of heart rate in humans (Holand et al 1999, Carter et al 2002). Lee et al (2010) evaluated instant responses of the cardiac autonomic nervous system to short-duration noises by using heart rate variability analysis. The results indicated that, compared with background noise of 38 dB, exposure to noise between 50 dB and 80 dB increased sympathetic activity. For humans, “hazardous” noise is defined as sounds that exceed 85 dB over a typical 8-hour workday (Holgers and Pettersson 2005, Daniel 2007). It has been shown that constant exposure to such hazardous noise can result in irreversible hearing loss and even a single intense sound event can cause hearing loss and tinnitus (Holgers and Pettersson 2005, Daniel 2007).

So far, little research has been conducted investigating the effect of noise on the hearing capacities of animals. Kennelled dogs that were constantly exposed to noise between 100 dB and 108 dB for 6 months developed hearing loss by measuring the auditory brainstem response (ABR) (Scheifele et al 2012). In mice, ABR recordings showed that a single exposure to noise of 100 dB for 2 hours induced temporary hearing loss (Chuang et al 2014), and an exposure to noise of 110 dB for 60 minutes even induced permanent hearing loss (Park et al 2013).

Noise-induced hearing loss is one of the most common causes of exogenously acquired sensorineural hearing loss in adult humans (Miller and Schein 2008). Although anatomic differences among mammal species lead to differences in hearing capacities (Fay 1988), the basic physiologic processes underlying the detection and sensation of sound are essentially identical between humans, dogs, cattle and mice (Strain and
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Myers 2004, Fay 2000, Heffner and Heffner 1983, Heffner and Heffner 1990). Considering that cows can hear sounds between 23 Hz and 35 kHz, with the best sensitivity at 8 kHz, and are able to detect sounds at −11 dB, i.e., amplitudes the human ear cannot detect (Heffner and Heffner 1990), the continuous exposure to bells during pasturing season might impair the cows' hearing capacity.

A non-invasive but indirect test of hearing capacity is the acoustic startle response. This test has been used in a variety of species, such as rats (Blaszczyk and Tajchert 1997), cows (Lanier et al 2000), cats (Clements and Kelly 1978) and humans (Braff et al 1978). A startle response can be any reaction of any part of the body, such as body movements, movements of limbs and facial movements, or any behavioural reaction to sound stimulation (Ehret 1983). In rodents, the acoustic startle response is elicited by stimuli with an amplitude of more than 80 dB (Pilz et al 1987, Valsamis and Schmid 2011), and the latency of the acoustic startle response is very short (5–10 ms; Cassella et al 1986, Pilz et al 1988, Koch and Schnitzler 1997). The degree of the startle response increases with increasing amplitude and reaches a maximum at 100–110 dB in rodents (Valsamis and Schmid 2011). Accordingly, a short latency of the acoustic startle response may be interpreted as increased arousal. Although arousal itself does not necessarily imply averseness of a stimulus, avoidance reactions in response to acoustic stimuli indicate that stimuli are perceived as aversive by the animals (Rushen 1996, Talling et al 1998, Arnold et al 2008).

A widespread solution used to protect humans and horses from noise exposure consists in using hearing protection devices such as earplugs (Viallet et al 2015, MacFarlane et al 2010). Commercially available earplugs for horses are made of memory foam (MacFarlane et al 2010). In cattle, acoustic earphones were inserted into the ear canal and were held in position with either silicone earplugs or earplugs of compressible foam while measuring brainstem auditory evoked potentials (BAEP) (Strain et al 1989, Arai and Matsui 2008, Arai et al 2009). Such earplugs occlude the ear canal and attenuate background noise.

Although some studies on the general hearing capacity of cows are available, to our knowledge no literature exist on hearing capacities of cows that have been exposed to the chime of bells for several pasturing seasons. In this study, we thus examined the reactivity towards sounds of low (65 dB) and high (85 dB) amplitudes in bell-experienced and bell-unexperienced cows on 24 Swiss dairy farms. We additionally tested whether mimicking hearing loss with earplugs would reduce the reactivity to the
sounds. We hypothesized that cows that had been exposed regularly to a bell on alpine pastures (bell-experienced cows) would show reduced reactivity towards these sounds compared with cows that were only equipped with a bell as heifers or never before (bell-unexperienced cows). We expected avoidance and heart rate to be increased, and the latency of the first behavioural reaction and heart rate variability to be reduced in bell-unexperienced cows, in response to a stimulus of high amplitude, and in cows without earplugs. Contrary, we expected avoidance and heart rate to be lower, and the latency of the first behavioural reaction and heart rate variability to be increased in bell-experienced cows, in response to a stimulus of low amplitude, and in cows with earplugs. Altogether, if earplugs do not diminish the reaction of a given cow, this might be an indicator of either a low-reactive animal or hearing impairment.
6.2 Animals, material and methods

Ethical approval to conduct the study was obtained from the Zurich Cantonal Veterinary Office, Switzerland (Approval No. 77/2012).

6.2.1 Animals, housing and management

The study was performed between September and November 2013 on 24 Swiss farms, with 96 multiparous Brown-Swiss cows that were between 3 years and 10 years of age. The owners of the cows gave permission to conduct the study on their farms. The size of the farms varied between 8 and 100 animals. On each farm, four experimental cows were selected randomly. On 12 farms, cows were used to wearing a bell either every year for 4–5 months during the summer season or all year round (48 cows on 12 farms, “experienced”). On the other 12 farms, cows had no or very little experience with wearing a bell, having either never worn a bell before or only once for 4–5 months when they were 1 year old (48 cows on 12 farms, “unexperienced”). On all 24 farms, cows were kept in cubicle housing systems with a feeding rack and headlocks. They were fed with hay, fresh grass or a mixed ration of hay, corn and grass silage. Feed and water were provided ad libitum. The cows were milked twice a day.

6.2.2 Test area within barn

The experiment was carried out at the feeding rack of each farm during the course of 1 day. A 5-m section of the feeding rack served as test area for the experiment. During the experiment, only the four experimental cows had access to this area, 1 cow at a time. To record the animals’ behaviour, a video camera with an integrated microphone (Canon® Legria FS 200 digital camera) was mounted on a tripod and positioned in front of the separated feeding rack (Figure 1). The acoustic stimulus was transmitted via two loudspeakers (Edifier® S2000v, Edifier International, Hong Kong, China) that were positioned on the floor next to the camera. The loudspeakers, the tripod and cables were hidden behind a visual cover of white fabric (Figure 1) and placed on a board (approx. 1.5 m × 1.5 m).
6.2.3 Acoustic stimuli

Cows were exposed to a pink noise stimulus broadcast 4 times for 5 seconds each in a 2×2 factorial design: cows were tested with and without earplugs at 65 dB (A-weighting scale, A) and 85 dB (A), (Figure 2; each amplitude with 2 phases: without and with earplugs, see below). The A-weighting scale assigns low weights to the low frequency tones, to which the human ear and the ears of some animals are less sensitive, and high weights to the high frequency tones, to which humans are more sensitive (Manci et al 1988).

Pink noise is characterized by uniformly distributed energy throughout the range of human hearing, approx. 20 Hz to 20 kHz. Most people perceive pink noise as having uniform spectral power density—i.e., the same loudness at all frequencies (Zhou et al 2012). The noise stimulus was automatically generated by a Tone Generator Pro v1.0.8 (Performance Audio®) for iPhone®. We chose the pink noise for two reasons: first, it has no biologic relevance for the cows and therefore the reactions to the acoustic stimuli were mostly likely limited to the perception of the acoustic stimuli per se. Second, it contains all frequencies from 20 Hz to 20 kHz at the same amplitude and should thus match the cows’ (potential) hearing capacity. The volume of the playback was set at a level that ensured an amplitude of either 65 dB or 85 dB (A) at the feeding rack at approx. 60 cm above the floor (i.e., the estimated position of the cows’ heads.
when feeding). The front edge of the board with the loudspeakers was placed in the feeding alley at a distance of 2.15 m from the feeding rack to ensure that the intended amplitude reached the cows’ ears. The amplitude was measured with a precision noise level-measuring instrument with integrated long-term storage (SoundTest-Master, Laserliner®, Umarex GmbH and Co.KG, Arnsberg, Germany). The background noises at the farms measured before the start of the experiments ranged from 40 dB to 60 dB (A).

### 6.2.4 Experimental procedure

In the morning around 8 o’clock, the visual cover of white fabric with all technical equipment was set up. Before the start of the experiment, the four experimental cows were habituated to the technical equipment (Figure 1), the thorax belt to measure heartbeat parameters (see section 2.6 Heartbeat measurements) and the earplugs (Figure 2). Each experimental cow was exposed to the pink noise stimulus in four trials (Figure 3). The order in which the cows were tested was chosen randomly before the start of the experiment. To reduce handling of the cows, the two playbacks during which cows were equipped with earplugs were tested in consecutive trials (2 phases: with and without earplugs). Thus, each cow was equipped with the earplugs only once during the experiment with the order of phases and amplitudes chosen randomly for each animal.

*Figure 2: Earplugs.* Cow with earplug (70 mm × 40 mm × 40 mm) made of memory foam (left) in the ear canal (right).
On each farm, the four experimental cows were led to the separated 5-m section of the feeding rack, restrained in the headlocks (for feeding), and fitted with a thorax belt. Then, they were habituated to the earplugs. The earplugs (70 mm × 40 mm × 40 mm; Figure 2) were made of memory foam (polyurethane with additional non-toxic chemicals increasing its viscosity and density, Vibraplast AG®, Aadorf, Switzerland). Earplugs were compressed and placed into the ear canal during feeding, where they expanded and plugged the ear canal. To avoid experimental cows to be irritated by a sudden loss of hearing, a first earplug was positioned into the left ear for 15 minutes before the second earplug was placed into the right ear. Experimental cows were left undisturbed for 30 minutes with both thorax belt and earplugs. During these 30 minutes, the two earplugs remained in position for at least 15 minutes. If the animal shook its head resulting in the loss of an earplug, the earplug was repositioned. During the first 15 minutes, the first earplug had to be repositioned 1.3 times (min 1, max 6 times), and during the following 30 minutes, an earplug had to be repositioned 1.9 times (min 1, max 7 times). At the end of the habituation phase, all experimental cows accepted the earplugs and continued to feed calmly. After the habituation phase, the earplugs were removed and the experimental cows were allowed to re-join the herd for 1 hour. To ensure that experimental cows were motivated to feed, cows had no access to feed while re-joining the herd.

After the 1-hour break, experimental cows were individually led into the separated area of the feeding rack, restrained in the headlocks and fitted with the earplugs depending on stimulus order (Figure 3). To avoid exposure to the acoustic stimuli prior to testing, the other experimental cows were either separated in an outdoor paddock (n = 11 farms) or fitted with earplugs and led to the farthest part of the stable (n = 13 farms). A handful of concentrate was then sprinkled on the usual feed to enhance feeding motivation in the experimental cow. As soon as the experimental cow was feeding calmly for at least 1 minute, the second experimenter carefully opened the headlocks, and the playback was switched on for 5 seconds (20 ± 3 seconds after opening the headlocks). Start and stop of the playback was controlled manually by the experimenter using a mobile phone (iPhone 4s®, Apple Inc.). After the playback ended, behavioural observations and heartbeat measurements continued for two more minutes (playback + 2 minutes = trial). Each experimental cow was tested in all four trials in 1 session. When the session was finished, the thorax belt (and earplugs, depending on phase order) was removed and the cow was allowed to re-join the herd. If a cow did
not start feeding within 1 minute after offering the concentrate, no playback was conducted and another experimental cow was tested.

**Figure 3: Experimental procedure.** The 2 playbacks (65 and 85dB) with earplugs were tested in consecutive trials; the order of amplitude within a phase (without or with earplugs) and between phases was chosen randomly for each cow.

### 6.2.5 Behaviour

Behavioural analyses were conducted by two different blinded persons (one person analysed the latency to the first reaction, and the other analysed avoidance) that had not participated in the conduction of the experiments. They did not know the farms and were not aware of the aim of the study. However, as they needed to record the behaviours related to the start of the playback, they were aware of the acoustic stimuli but not of the bell-experience of the animals, the amplitude or if the animals were equipped with earplugs.

In addition, for testing the intra-observer reliability for the latency to the first reaction, person one assessed 96 trials twice.

#### 6.2.5.1 Latency to the first reaction

The latency to the first reaction was described as time [seconds] it took the experimental cows from the onset of the playback to react with either a clearly visible change of the ear posture, pausing (cow stopped her current behaviour and froze shortly) or a sudden head movement (cow stopped feeding and raised its head quickly). The latency to the first reaction was analysed in slow motion (0.5x real time) from video using
VLC media player® (version 2.2.4 Weatherwax, VideoLAN, Paris, France) and a stopwatch for Smartphone Shift5.1® (Android Version 5.1, SHIFT GmbH, Falkenberg, Germany) for 15 s after onset of the playback.

6.2.5.2 Avoidance
Avoidance reaction to the playback was recorded whenever a cow left the feeding rack (completely withdrawing the head from the feeding rack) within 60 seconds after onset of the playback using INTERACT® (Mangold International GmbH, version 9.0.7).

6.2.6 Heartbeat measurements
Heartbeat measurements were recorded using Polar® Equine (Polar® Elektro Europe BV, Zug, Switzerland), allowing a non-invasive measurement of heartbeats (Langbein et al 2004, von Borell et al 2007). To increase the electrode–skin contact, electrode gel (Anandic Medical Systems AG/SA, Feuerthalen, Switzerland) was used. A thorax belt with two integrated electrodes was fixed around the torso directly behind the forelegs. One electrode was positioned ventrally on the left side of the sternum and the other one at a given distance by the thorax belt on the left thoracic wall. A receiver for recording the data was placed between the 2 electrodes. An elastic belt of about five cm width additionally protected the thorax belt. The heartbeat was recorded for 1 minute of continuous feeding before the playback started, during the playback (5 seconds), and during the 2 minutes following the playback. Data were downloaded onto a computer via a base station using Bluetooth (Polar® Team2 Pro, version 1.3.0.3).
All calculations were carried out using the programs Polar® ProTrainer 5 Equine Edition (version 5.35.161, © Polar Electro Europe AG, Zug, Switzerland) and R 3.2.3 (R Core Team 2016). Root mean square of successive differences of heartbeats (RMSSD) reflects changes in the vago-sympathetic balance that are vagally mediated (von Borell et al 2007) and represents parasympathetic activity, whereas standard deviation of heartbeats (SDNN) is a more complex parameter reflecting vagal as well as sympathetic activation (von Borell et al 2007). The ratio between RMSSD and SDNN is thus a global indicator of general changes of the vago-sympathetic balance in the organism (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996). An increased ratio between RMSSD and SDNN reflects a high heart rate variability and is thus interpreted as an expression of increased capacity in coping with stress.
Automatic correction of the tachograms was carried out using the correction routines included in the Polar® software (Polar® ProTrainer 5 Equine Edition, version 5.35.161). Data with an error rate of more than 10% were excluded from the analysis according to Langbein et al. (2004). In addition, data of one cow were excluded from analysis due to extremely high heart rate regardless of experimental treatment. This led to the exclusion of 272 trials, and a remaining sample size of 112 trials.

The number of R-R intervals (heart rate in beats per minute, bpm), the RMSSD (ms) and the ratio between RMSSD and SDNN (RMSSD/SDNN) were calculated. The 1-minute preceding the playback and the first minute of the trial were both divided into first (0–20 seconds), middle (21–40 seconds) and last (41–60 seconds) 20 seconds. The middle 20 seconds of the minute preceding the playback was chosen as reference value for heartbeat parameters. This reference time window was compared with the first time window (0–20 seconds) after start of the playback (trial value) by calculating the ratios of heart rate (bpm), RMSSD (ms) and RMSSD/SDNN between reference and trial value.

6.2.7 Data analysis

The experimental design resulted in a sample size of 384 trials (i.e., 24 farms × 4 experimental cows × 4 trials). However, five cows older than 10 years were excluded from data analysis to avoid age-dependent hearing impairment (presbyacusis) interference with experimental treatments. One cow had to be excluded from data analysis due to technical problems, and another cow due to not feeding at all. In eight trials (five cows on five farms), cows did not start feeding again after a playback exposure. In these cases, we had to quit the session. All these cows had no or very little experience with wearing a bell. Technical problems with video recording occurred in another seven trials. Thus, the total sample size was 89 cows on 24 farms in 341 trials.

Statistical analyses were performed in R 3.3.1 and 3.3.2 (R Core Team 2016). We used the agreement package (Yue and Lin 2015) to check the intra-observer agreement concerning the latency to the first reaction. To adequately reflect dependencies in the experimental design (nesting, repeated measurements) linear mixed-effects models were used to evaluate the latency to the first reaction and heartbeat measurements with the lmer methods from ‘lme4‘ and ‘lmerTest‘ packages, respectively (Bates
et al 2015). The occurrence of leaving the feeding rack was analysed using a generalized linear mixed-effects model (glmer method from package ‘lme4’, Christensen 2015).

Full models consisted of the fixed effects “bell experience” (factor with two levels: experienced, unexperienced), “earplugs” (factor with two levels: with, without) and “amplitude” (factor with two levels: 65 dB, 85 dB) and all possible interactions. Models were reduced in a stepwise backwards procedure. A P-value of >0.05 was used as criterion for exclusion of non-significant interactions. P-values were calculated based on likelihood ratio tests. Trial nested in individual identity nested in farm served as random effects. To satisfy model assumptions, heartbeat parameters were log transformed.
6.3 Results

In the figures, raw data as well as model estimations are shown; in the text, model estimations were used to interpret the results.

6.3.1 Latency to the first reaction

The intra-observer agreement for the assessment of the latency to the first reaction was good, with a concordance coefficient (CCC) of 0.91.

As expected, cows reacted faster when exposed to the 85 dB stimulus compared with the 65 dB stimulus ($F_{1,212.1} = 56.65, p < 0.001$), and slower when equipped with earplugs ($F_{1,214.6} = 65.05, p < 0.001$). Bell experience did not affect the latency to the first reaction ($F_{1,24.5} = 0.5, p = 0.4863$, Figure 4).

![Figure 4: Latency to the first reaction 15 s after onset of the playback stimulus depending on bell experience (U = unexperienced, E = experienced), earplugs (without, with) and amplitude (65 dB, 85 dB). Raw data are presented as box plots indicating observed median, first and third quartiles, and absolute range of data. Solid lines show the model estimation, dotted lines the lower and upper 95% confidence intervals.](image-url)
6.3.2 Avoidance

The probability for a cow to leave the feeding rack within 60 seconds after exposure to the sound stimulus was strongly increased when cows were exposed to 85 dB compared with 65 dB (odds ratio = 3.20; \( \chi^2 = 17.29; p < 0.001 \); Figure 5). Similarly, the probability to leave the feeding rack was reduced when wearing earplugs (odds ratio = 0.30; \( \chi^2 = 17.63; p < 0.001 \); Figure 5) and by bell experience (odds ratio = 0.33; \( \chi^2 = 4.92; p = 0.0265 \); Figure 5).

![Figure 5: Avoidance. Proportion of cows leaving the feeding rack within 60 seconds after playback depending on bell experience (U = unexperienced, E = experienced), earplugs (without, with) and amplitude (65 dB, 85 dB). Solid lines show the model estimation, dotted lines the lower and upper 95% confidence intervals.](image)
6.3.3 Heartbeat measurements

In the description of the results of heartbeat measurements, a ratio >1 indicates that the trial value was greater than the reference value, and vice versa for a ratio <1. The mean absolute heart rate was 80.2 bpm (min: 54 bpm, max: 188 bpm) in the minute before, and 79.6 bpm (min: 54 bpm, max: 180 bpm) in the minute after playback exposure and showed a large inter-individual variability. When cows were exposed to 85 dB without ear-plugs, heart rate during the first 20 seconds after onset of the playback was increased compared with the baseline heart rate (amplitude × earplugs: $F_{1,114.8} = 4.41; p = 0.0380$; Figure 6A). We found no effect of bell experience ($F_{1,22.3} = 0.22; p = 0.6399$) on heart rate response.

The RMSSD ratio following the playback at 85 dB was increased compared with the RMSSD ratio following the playback at 65 dB ($F_{1,106.5} = 7.64; p = 0.0067$). Further, RMSSD ratio was lower when wearing earplugs than when not wearing earplugs ($F_{1,53.9} = 5.95; p = 0.0181$). We found no effect of bell experience ($F_{1,60.1} = 0.0004; p = 0.7462$) on RMSSD response (Figure 6B).

The RMSSD/SDNN ratio was reduced when cows were exposed to 85 dB compared with 65 dB ($F_{1,118.8} = 12.71; p = 0.0005$), and it was slightly increased by earplugs ($F_{1,60.1} = 2.88; p = 0.0951$; Figure 6C). Again, no effect of bell experience was detectable ($F_{1,66.4} = 0.001; p = 0.9724$).
Figure 6: Heart rate, RMSSD and RMSSD/SDNN. Ratio of (A) heart rate, (B) RMSSD and (C) RMSSD/SDNN between playback situation and reference depending on bell experience (U = unexperienced, E = experienced), earplugs (without, with) and amplitude (65 dB, 85 dB). Raw data are presented as box plots indicating observed median, first and third quartiles, and absolute range of data. Solid lines show the model estimation, dotted lines the lower and upper 95% confidence intervals. The green dotted line represents the ratio value in case of identical values during trial and reference.
6.4 Discussion

The experimental setting in this study allowed an assessment of the cows’ reactions to acoustic stimuli as both amplitude and earplugs affected all outcome variables in a meaningful way. Overall, the cows responded to the 85 dB stimulus stronger than to the 65 dB stimulus. Bell experience as well as earplugs reduced avoidance of the sound stimulus. The high-amplitude stimulus triggered a stronger startle response, as seen in a shorter latency to the first reaction, increased heart rate and avoidance, than the low-amplitude stimulus. With earplugs, the latency to the first reaction was longer, and the cows showed less avoidance of the sound stimulus. However, bell experience had no effect on the latency to the first reaction.

Similar to our results, Tripovich et al (2012) found that Australian fur seals were more alert when they heard boat noise of 75–85 dB compared with boat noise of 64–70 dB. Furthermore, Talling et al (1996) found that pigs had a higher heart rate and ambulation score when exposed to very loud (97 dB) compared with loud (85 dB) stimuli. In addition, a sudden loud sound (110 dB, 1–20 kHz, 0.15 seconds) evoked an immediate increase of heart rate in humans (Holand et al 1999). In our study, changes in heart rate, RMSSD and in the ratio between RMSSD and SDNN were rather small (i.e. ratios varied little and were close to 1), thus, the cardiac reaction caused by the playback has to be interpreted with caution. However, Désiré et al (2004, 2006) showed that, in lambs, the sudden appearance of an object elicited a startle response and an increase in heart rate most likely due to enhanced sympathetic activity, i.e., increased arousal. Additionally, the exposure to a novel object elicited an orientation response and an increase in RMSSD indicating an increased parasympathetic activity. In the current study, the pink noise stimulus was both sudden and novel. The increased heart rate during the 85 dB stimulus without earplugs can be explained by the suddenness of the high-amplitude stimulus. Accordingly, the decrease in RMSSD/SDNN indicated increased sympathetic activation during the exposure to the high-amplitude stimulus compared with the low-amplitude stimulus. The increased RMSSD reflected an additional increase in parasympathetic activity during the exposure to the high-amplitude stimulus, which might be a result of the novelty of the stimulus (Lee et al 2010, Hainsworth 1995).

Cows avoided the playback stimulus to a lesser extent when wearing earplugs, or when their farm of origin used bells during the pasture season, which may reflect an altered
emotional appraisal of the playback stimulus due to routine bell exposure. Furthermore, only cows from farms that did not use bells for their cows did not start feeding again after being exposed to the playback. This may indicate that the playback was perceived as aversive by these cows. Although the experimental setting of our study did not allow us to conclusively assess the hearing capacity of the cows, hearing damage cannot be excluded. Studies indeed have shown that hearing loss in humans, dogs and mice occurred after they were exposed to noise with amplitudes similar to those of cowbells. Further, exposure time was similar to the time cows are exposed to bells while on pasture (Daniel 2007, Scheifele et al 2012, Chuang et al 2014, Park et al 2013). However, given the effect of the earplugs and the fact that all cows in our study showed a reaction to the playback in at least one trial, none of them was completely deaf to the used stimuli.

Considering the experimental setting in retrospect, the pink noise stimulus might not have been optimal to assess the full hearing capacity of cows. Given that cows can hear sounds between 23 Hz and 35 kHz (Heffner and Heffner 1983), a high-pass filter that blocks low frequencies and passes high frequencies (Johnson 2012), or a stimulus that contains only higher frequencies might be better suitable to test cows’ hearing capacity in further research. In addition, the 65 dB and 85 dB as used in our study might have been too loud to detect subtle differences in hearing capacities. On the other hand, the amplitude used to elicit an acoustic startle reflex was more than 80 dB in other studies (Holand et al 1999, Lee et al 2010, Blaszczyk and Tajchert 1997, Pilz et al 1987, Valsamis and Schmid 2011). Thus, inferring information about hearing capacity from a startle response might partly be misleading when using a low-amplitude stimulus. Furthermore, the studies mentioned above (Daniel 2007, Scheifele et al 2012, Chuang et al 2014, Park et al 2013) used standardized clinical hearing tests (BAEP, ABR). BAEP are bioelectric waves that can be recorded within 10 ms after an auditory stimulus, and are used to assess auditory function (Chiappa 1997). Due to the influence of excessive muscle movements on the measurement, it is necessary for the subject to be motionless during the procedure (Arai and Matsui 2008). Clinically, sedation is therefore needed when measuring BAEP in young children or animals. Accordingly, it was not possible to use the BAEP procedure in this study since it should have been conducted in a veterinary hospital rather than on-farm to be able to monitor the animals more closely.
Altogether, we cannot clinically assess the hearing capacity of bell-experienced cows. Nevertheless, our results show that long-term bell experience led to a mitigation of the behavioural response to novel acoustic stimuli.

6.5 Conclusion
Our results demonstrated that acute exposure to a 85 dB pink noise stimulus triggered increased arousal and avoidance compared with a 65 dB stimulus, with bell experience and ear-plugs leading to a general decrease in avoidance of the stimulus. This may reflect an altered emotional appraisal of the playback stimulus due to noise habituation or impaired hearing capacity with routine bell exposure.
7 General Discussion

The aim of this thesis was to examine whether and how short, medium and long-term exposure to acoustic stimuli in general as well as with bell exposure, as management tool regularly applied on-farm, affects behavioural and cardiac responses, and hearing capacities in cows and goats. In chapter 7.1, it is discussed if habituation to the playback stimuli and to wearing a bell occurred. The next chapter (7.2) contains recommendations for farming practice based on the outcomes of the project. The last part of the discussion offers ideas for future research to answer remaining open questions (chapter 7.3).

7.1 Habituation to acoustic stimuli and to hearing and wearing a bell

The results of this thesis addressed aspects associated with the habituation to acoustic stimuli and to hearing and wearing a bell. Short-term exposure to a 1-min playback of a non-uniform sound (chime of a bell) and a uniform sound (sinusoidal tone) over the course of five trials indicated habituation to both stimuli in goats (chapter 4). Apparently, habituation to the sinusoidal tone was faster than to the chime of a bell as feeding duration increased and alertness duration decreased sooner with the sinusoidal tone. Contrary, in the second study, none of eight behavioural measures (feeding, ruminating and lying behaviours as well as head movements) indicated habituation to the medium-term exposure to hearing and wearing a bell 24 hours within 3 days of observation in cows (chapter 5). Regarding habituation to acoustic stimuli, there are ambiguous results in literature. Previous studies have shown that pigs (Talling et al 1996, Talling et al 1998, Kanitz et al 2005), heifers (Waynert et al 1999) and rats (Masini et al 2008) reacted different to acoustic stimuli regarding habituation. Pigs (Talling et al 1998) did not habituate to the short-term exposure to loud, intermittent acoustic stimuli after 40 consecutive 5 min tests. The results of Kanitz et al (2005) indicated that exposing domestic pigs to repeated acoustic stimuli of 90 dB during a period of 28 days for two hours daily in the medium-term caused changes in neuroendocrine regulations reflecting a state of chronic stress. Contrary, Talling et al (1996) found that, within 15 min of acute medium-term exposure to various acoustic stimuli at 80 - 97 dB once per week over a total of 4 weeks, initial increase in heart rate and locomotion was followed by habituation. Heifers showed signs of habituation to handling acoustic stimuli composed of humans shouting and metal clanging during a 1 min short-term exposure over 5-day
trials (Waynert et al 1999). Masini et al (2008) found that rats habituated to daily 30-min medium-term exposures of 95 dB white noise within 6 days as plasma corticosterone and ACTH responses, heart rate and core body temperature decreased. As the hearing capacity differs between species (Heffner and Heffner 1983, Heffner and Heffner 1992), this might explain to some extent why some studies found stress responses in animals and other studies did not. All studies mentioned above, except the study described in chapter 5, used acoustic stimuli of limited durations and longer exposure might have led to different effects. In the second study (chapter 5), the cows were exposed to the bells 24 hours during three consecutive days. Contrary to my hypothesis, the continuous exposure to the bell did not lead to habituation within 3 days of observation. If the initial behavioural changes of hearing and wearing a bell were motivated mainly by neophobia, habituation would be expected after 24 hours during 3 days of exposure. A longer observation period would be necessary to find out if habituation would occur or whether the long-term hearing and wearing of a bell might negatively affect animal welfare.

7.2 Recommendations for practice based on the outcomes of the project

Farm animals (e.g. cows, goats, pigs) are exposed to acoustic stimuli continually throughout their lives such as ventilation, feed carts, barn cleaners, the vacuum machine in the milking parlour and the chime of a bell (Gygax and Nosal 2006, Johns et al 2015 (chapter 5)). Bsi-Schwarzenbek (2013) recommends an optimal average noise level of less than 75 dB for cattle and pigs at the slaughterhouse and if it cannot be avoided exposing animals to noise of more than 5 minutes, the noise level should not exceed 80 dB. The Swiss Federal Food Safety and Veterinary Office generally recommends that it’s not allowed exposing animals to excessive noise during longer periods. Noise is defined as ‘excessive’ if it evokes escape-, avoidance- or aggressive behaviour as well as freezing and if an animal cannot escape from the source of noise (FSVO 2008).

Beside noises at the slaughterhouse or in the barn that have already been partly researched, there are still other noises, e.g. bells. In several animal species, bells are used either to relocate free-ranging animals (e.g. cows, goats, sheep, yak) or for ornamental or religious purpose (e.g. in elephants, cows, horses, falcons, as well as cows during “Alpabzug”). The chime of a bell is characterised by high and varying amplitudes (90 – 113 dB), varying frequencies and sounds arising intermittently, depending on the
movement of the bell (chapter 5). The results of the first and third study (chapters 4 and 6) showed that the short-term exposure to a chime of a bell led to more arousal than a uniform sound and a 85 dB stimulus led to more arousal and avoidance than a 65 dB stimulus. Furthermore, the medium-term exposure to hearing and wearing a bell (chapter 5) resulted in a reduction of rumination, feeding and lying as well as reduced head movements. Cows that were exposed to a chime of a bell in the long-term were avoiding a pink noise stimulus less compared to cows that were equipped with a bell only as heifers for 1 summer or never before (chapter 6). Thus, the question arises if it is indispensable exposing cows and goats to bells for several month considering costs and benefits. Here, bell use on the Alps and on fenced pastures must be considered separately. Alpine grazing systems can improve product quality of milk and cheese (Leiber et al 2005), help to maintain landscape conservation and can have economic advantages for farmers (WallisDeVries et al 2002). Another important issue in relation with alpine grazing systems is that cowbells are an essential part of Swiss tradition, not only for farmers but also for tourism (“Soundtrack der Alpen”, Theile 2015). Traditionally, grazing goats and cows on alpine pasture only was possible when animals were equipped with bells. There are some aspects associated with welfare and health that it would be better when cows and goats being grazed on the Alps with bells instead of staying in the stable without bells. Pasture can provide certain welfare benefits: cows have access to a more natural environment, they can perform behaviours that may be important to them such as grazing (Krohn 1994), and cows on pasture sometimes experience a lower incidence of diseases such as mastitis (Washburn et al 2002) and lameness (Hernandez-Mendo et al 2007). However, alternatives to cowbells are available in the meantime. The concept of virtual fencing is increasingly considered for managing free-ranging livestock (Umstatter 2011) and was especially interesting because of its potential to initially enhance ecological management as well as reducing workload of livestock managers. All of these would have the potential to reduce costs. Moreover, it opened up the possibility of managing regions that are not manageable at the moment. In her review, Umstatter (2011) described different concepts of virtual fencing. The first approach was to keep animals in or out of a defined area using devices that were animal-borne such as GPS receivers as a tracking facility (Marsh 1999, Talamas and Bianco 2006). The next category contained the development of a virtual fence where no device was mounted onto the animal (Mikuski et al 2007, Brown 2009). The last category dealt with moving fence lines (Butler et al 2004,
General discussion

Anderson and Rus (2009). Most of the virtual fence inventions worked on the basis of audio warning sounds and electric stimulation and could have animal welfare implications. The use of electric shock was not necessarily a problem if the animal had a chance to learn the link between an audio cue and the electric stimulus (Tibbs et al. 1995, Thiedemann et al. 1999, Lee et al. 2008, reviewed in Umstatter, 2011). Still, more research is needed to develop the different systems. However, as it is the advantage of cowbells to relocate the animals on the wide alpine pastures and not to keep the animals in or out of a defined area, using GPS as a tracking facility might be one of the best approaches. Furthermore, recording the precise position and behavioural data of the animals could be the basis for developing control strategies to reduce environmental damage from grazing animals and to facilitate pasture management for the farmer in mountainous regions (Braunreiter et al. 2007). Turner et al. (2000) described the development of lightweight GPS collar receivers for cows suitable for monitoring animal position at 5-min intervals. During the 1990s, GPS tracking was already used in wildlife research, e.g. in caribous (Bradshaw et al. 1995) or camels (Grigg et al. 1995), but also in cattle (Harbin 1995) and sheep (Rutter et al. 1997). In 2014, the project “GPS-Weidemanagementsystem” of the Institute for Agricultural Engineering and Animal Husbandry in Freising, Germany, was awarded from an initiative called “Deutschland – Land der Ideen”. The “GPS-Weidemanagementsystem” for Smartphones was developed to facilitate work of the farmers (Maxa et al. 2014). With this system, searching for the animals, is not longer necessary. Besides this reduction of workload, the GPS system is able to locate an animal even when it does not move, e.g. due to injury. As soon as a cow would not move, its bell would not chime, making locating of this animal difficult. In such a case, it would be an asset being equipped with a GPS-receiver. In Switzerland, there are some farmers who use a GPS-receiver for their cows at the Alps (SRF Schweiz aktuell 2015).

All in all, the results of this study showed, that the short-term exposure to loud acoustic stimuli resulted in increased stress reactivity and avoidance. In the medium-term, animals did not always habituate to the acoustic stimuli combined with the weight of a bell. However, in the long-term, the acoustic perception seemed to change. Against this background, the question arises if equipping cows with bells should be reduced to minimum regarding animal welfare. Considering that alternatives to bells exists, the necessity of cowbells on fenced pasture in the valley has to be questioned. Exposing
cows and goats to loud, non-uniform acoustic stimuli should be avoided whenever possible in practice if it is not indispensable.

7.3 Prospects for future research

The present thesis is, to my knowledge, the first that highlights short-, medium-, and long-term effects of bell exposure in cows and goats. However, from what is discussed above and to answer the remaining open questions, three approaches for further research are presented in the following. The first one takes into account a more applied investigation on the behavioural changes in cows hearing and wearing a bell more than 3 days. The second approach focuses on using BAEP (brainstem auditory evoked potentials) as a clinical test in cows and goats to clinically assess their hearing capacity. The third approach is a more general one and suggests measuring multiple physiological systems to investigate the impact of acoustic stimuli on animals.

7.3.1 Behavioural changes in cows wearing a bell more than 3 days

Behavioural changes suggested that the behaviour of cows was disturbed by hearing and wearing a bell over 3 days without indication of habituation. Bell exposure for several weeks should therefore be further investigated to find out if and when habituation would occur. Lying duration, duration of rumination and feeding duration were all reduced when the cows were equipped with a bell compared to no bell (chapter 5). As increased lying, ruminating and feeding durations are associated with longevity, reduced health costs, increased productivity and improved cow welfare (Botheras 2007), one of the primary objectives of dairy producers should be to promote these behaviours. Therefore, a basic interest not only for scientist, but also for farmers should be a further investigation if wearing a bell may have implications for the reasons given above.

Although cows have to cope with the combined effects of weight and sound of bells on-farm, still no consistent results were found for the functional bell (weight and sound) in comparison with the silent bell (only weight) in the second study (chapter 5). Therefore, more research is needed to completely disentangle these effects. For example, by using a lightweight loudspeaker that is mounted on the neckband of cows to produce sound without weight burden.

In Switzerland, almost all cows are exposed to a bell regularly every year for 4-5 months during the summer season, either all year round, or on the Alp for 4-5 months.
when they are 1 year old. Keuroghlian and Knudsen (2007) described in their review that the exposure of animals (e.g. rat, cat, barn owl, bat, guinea pig etc.) to atypical auditory environments could result in large functional changes in certain auditory circuits. Thus, cows that had been regularly exposed to a bell on alpine pastures might have perceived the sound differently based on divergent development of the auditory system compared to unexperienced cows. Therefore, I suggest including animals without any experience with bells for further investigations.

7.3.2 Measuring BAEP to assess the hearing capacity of cows and goats

The aim of the third study (chapter 6) was to test if long-term bell exposure impairs cows’ hearing capacity by testing their reactivity towards a sound. Interestingly, cows that had regularly been exposed to a bell seemed to react faster to playbacks without earplugs but slower with earplugs than cows that were equipped with a bell only as heifers for one summer or never before. The exposure of animals to atypical auditory environments could result in large functional changes in certain auditory circuits (Keuroghlian and Knudsen 2007, see above). Thus, bell-experienced cows might have perceived the playback differently based on divergent development of the auditory system compared to unexperienced cows. However, hearing and wearing a bell did not lead to deafness of the cows, since all cows were reacting to a playback stimulus at least once. To assess the exactly hearing capacity of cows and goats, it seems necessary to test their ability to hear by measuring BAEP in a clinical test. The electrocochleogram consists of electrical responses of the cochlea and the auditory nerve to acoustic stimulation. These include the cochlear microphonics, the summating potential and the auditory nerve compound action potential (American Clinical Neurophysiology Society 2008). The BAEP consists of a sequence of peaks in the electric potential recorded from scalp electrodes within the first 10 ms after an auditory stimulus presentation (Strain et al 1989). Each electric potential is generated by an activation of a subcortical component of the acoustic pathway (Arai and Matsui 2008). It is recommended that “broad-band” clicks, the acoustic energy which is spread over a wide range of audio frequencies, is used for the neurologic applications of auditory evoked potentials (American Clinical Neurophysiology Society 2008). In cows, the BAEP has four peaks (Arai et al 2009). Clinically, it is useful to use a sedative when measuring BAEP in cattle to control excessive movement of the cattle without influencing the peak latencies (Arai and Matsui 2008).
A conclusive assessment of the impact of long-term exposure to a chime of a bell on cows’ hearing capacity might be based on clinical evidence by measuring BAEP.

7.3.3 Measuring physiological stress caused by acoustic stimuli

In this thesis, the potential physiological stress that might be caused by acoustic stimuli was assessed on the basis of a single physiological alteration by measuring heart rate and heart rate variability. Various studies that investigated the physiological stress among others caused by acoustic stimuli on farm animals were either based on the HPA axis (Kanitz et al 2005, Quaranta et al 2002) or on the ANS (Christensen et al 2005, Arnold et al 2007, Waynert et al 1999, Talling et al 1996) without considering that multiple physiological systems are altered during stress (von Borell 2000). So far, this kind of research has received only little attention in animal welfare research. However, for example, Masini et al (2008) investigated the physiological stress caused by acoustic stimuli on rats by measuring both, HPA axis and ANS. Furthermore, Patt et al (2016) found that a selection of variables, that was, for example, only behavioural variables or only variables of the HPA axis or heart rate variability, would have led to different interpretations of the effects of two regrouping experiments in goats on animal welfare. Therefore, to get a better understanding of the complexity of the physiological stress response caused by acoustic stimuli, comparisons between the effects of the ANS and the HPA-axis might be useful and should be considered in further investigations.
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Acknowledgements

The project was funded by budget funds of ETH Zurich and Felsentorstiftung.

I would like to thank:

Dr. Edna Hillmann for dedicated and proficient supervision, helpful and constructive comments on the thesis, many enjoyable and very constructive discussion, and constant statistical and social support throughout all parts of my PhD.

Prof. Dr. Michael Kreuzer for taking over the responsibility as my doctoral advisor at ETH Zurich.

Dr. Lars Schrader and Prof. Dr. Josef Troxler for agreeing to be co-examiner.

Dr. Antonia Patt for all her support in so many things (experimental design, data collection, statistical analysis, interpretation of results, comments on manuscripts, etc.), discussions, support, sharing numberless laughs and good times, and for her friendship.

PD Dr. Lorenz Gygax for constant and profound statistical support as well as helpful and constructive comments on experimental design.

Dr. Verena Lietze for professional proofreading.

Marc Wymann, Gallus Jöhl and Vid Vidovic for committedly and reliably caring for the goats.

Dr. Peter-Christian Schön and Dr. Elodie Briefer for analysing the acoustic stimuli.

Christina Rufener, Karoline Margreitter, Karin Scheuss and Sarina Fetscher for analysing the video recordings as well as Simon Peter Luzi and Sophie Masneuf for their assistance during data collection.

The Team of the Ethology and Animal Welfare Unit, especially Joan Burla, Anic Ostertag, Heike Schulze Westerath, Cornelia Buchli and Elodie Mandel-Briefer for discussions, support, sharing laughs and good times.

Tasja Kälber, Yvonne Lötscher, Janina Meier and Helen Willems, and Patrick Flütsch and Michi Egli from the workshop for sharing laughs and many memorable lunch and coffee breaks.
The Team of the Centre for Proper Housing of Ruminants and Pigs of Prof. Dr. Beat Wechsler for providing the goats and premises, and for their valuable comments to experimental designs and presentations during doctoral colloquiums.

All farmers for their participation in the studies, their openness and trust towards us, many informative discussions, and often caring hospitality.

All my dear friends, especially Sabrina for always being there for me and having faith in me during times of crisis.

The “Brunch-Truppe”, especially Karin and Nici for sharing numberless laughs and events (Fasnacht, Chilbi, matches, “Männerabende”, etc.) and always being there for me. Life in Switzerland wouldn’t have been what it was without you!

Benni for your patient mental support during the last weeks and months and for pushing me while completing the final document. Thanks a lot for stepping in and doing all the daily tasks for me while I was sitting at the desk!

My dear parents for their endless love, support and faith in me. I wouldn’t be where I am today without you! There are no words for how grateful I am.
10 Curriculum vitae

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Publications

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Hat das Saugen bei der Mutter im Vergleich zum Saugen am Tränkeautomaten für Kälber eine Entspannungswirkung?
43. Internationale Arbeitstagung Angewandte Ethologie bei Nutztieren der Deutschen Veterinärmedizinischen Gesellschaft e.V. (DVG), Freiburg im Breisgau, KTBL 489, 88-97

Unrestricted contact of dairy calves to their mothers: behavior and weight-gain.
ISAE Regional Meeting – Maternal behavior and survival of the offspring, Kostelec nad cernymi Lesy, Czech Republic