TECHNISCHE UNIVERSITÄT MÜNCHEN

Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt

Lehrstuhl für Tierökologie

Phenotyping personality of young cattle (*Bos taurus*) – a multidimensional approach

Katharina Luise Graunke

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften

genehmigten Dissertation.

Vorsitzender: Univ.-Prof. Dr. H.-R. Fries

Prüfer der Dissertation:

1. apl. Prof. Dr. R. Gerstmeier
2. Univ.-Prof. Dr. M. Pfaffl
3. Univ.-Prof. Dr. B. Puppe, Universität Rostock

Die Dissertation wurde am 18.12.2013 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 10.03.2014 angenommen.

In loving memory of Nico.

You were grand, noble, and kind-hearted. Always.

CONTENTS

Summary

Inter-individually different but intra-individually consistent behaviour in similar situations cannot always be explained by physiological states or environmental factors; hence, these differences may reveal the strategy an individual employs to act with and react to environmental stimuli. This is described as personality and is thought to be innate and consistent over time and across situations.

The aim of this thesis was to assess the personality of young cattle (*Bos taurus*) to gain more knowledge on the phenotype of this economically important livestock species by combining ethological observations, physiological measures, and objectively measured applied physical force. The thesis was divided into three studies.

In Study I, an appliance was to be established that allows to objectively measuring escape behaviour and reluctance in young cattle restrained by being tethered on a halter, independently of the impact and rating of a more or less subjective observer or handler. While restrained, a tractive force-time diagram was recorded and later analysed. To evaluate, 24 three-month-old calves were restrained by being tethered for 30 min on a halter connected to a force transducer. Most of the calves showed a similar behaviour pattern with a slight decrease in effort across the 30 min test. Still, several animals showed clearly higher values in the parameters. Thus, two clusters of calves were found, which did not differ in weight of the animals.

To phenotype personality, behaviour and simultaneously measured heart rate variability (HRV) during a novel-object test, which is a so far unique methodology in personality research in animals, was analysed in Study II. We tested 400 calves at 90 days *post natum* (dpn). The test was conducted a second time four months later on 39 of these calves to test for stability over time. Four distinct personality types could be described by combining the ethological and physiological measurements: “neophobic/fearful – alert”, “interested – stressed”, “subdued/uninterested – calm”, and “neophilic/outgoing – alert”. In the evaluation of the stability of the personality types over time, more than 40 % of the calves showed a similar behaviour pattern.

In Study III, data of 356 calves tested at 90 and 91 dpn, respectively, from the developed restraint test of Study I was correlated with the multidimensional depiction of cattle personality established in Study II. By linking automatically measured data to ethological and physiological data, we aimed to test whether the automated restraint test is a practicable way to test large numbers of individuals for their personality with maintained level of measure reliability. Tractive force was found to be influenced by the activation of the autonomous nervous system during the novel-object test. Although the character of this connection was surprising, this can be seen as a candidate measure that provides a basis for further developments.

To conclude, it was possible to describe four distinct personality types using a multidimensional analysis of behaviour recorded during a standard behaviour test and simultaneous measurements of the HRV. Correlating the types with data of the developed restraint test revealed one candidate measure, which appeared promising for further developments of this test.

Zusammenfassung

Inter-individuell unterschiedliches, jedoch intra-individuell konsistentes Verhalten in vergleichbaren Situationen kann nicht immer durch physiologische Faktoren oder Umweltfaktoren erklärt werden. Die daraus folgenden Unterschiede lassen die Strategie erkennen, die ein Individuum anwendet, um mit der Umwelt zu agieren und auf sie zu reagieren. Diese Strategie wird Personalität genannt und als angeboren und konstant über Zeit und Situationen angesehen.

Ziel dieser Arbeit war die Erfassung von Personalität bei Jungrindern (*Bos taurus*) durch die Kombination von ethologischen Beobachtungen, physiologischen Messungen und objektiv gemessener, aufgewandter physikalischer Kraft. Damit sollten mehr Kenntnisse über den Phänotyp dieser ökonomisch wichtigen Nutztierart gewonnen werden. Die Arbeit besteht aus drei Studien.

In Studie I sollte ein Apparat entwickelt und eingeführt werden, der unabhängig vom Einfluss und von der Bewertung eines mehr oder weniger subjektiven Beobachters oder Tierpflegers das Fluchtverhalten und den Widerwillen eines mit einem Halfter angebundenen Jungrindes misst. Während der Anbindung wurde ein Zugkraft-Diagramm aufgenommen und später analysiert. Zur Evaluation wurden 24 Kälber im Alter von 91 Lebenstagen für 30 min mit einem Halfter angebunden, der mit einer Kraftmessdose verbunden war. Die meisten Kälber zeigten ein ähnliches Verhaltensmuster mit einem leichten Rückgang der Zugkraft im Laufe des 30-minütigen Tests. Einige erreichten jedoch deutlich höhere Werte in den Parametern, sodass zwei Cluster gefunden wurden, deren Kälber sich aber nicht in ihrem Gewicht unterschieden.

Um Personalität zu phänotypisieren, wurden in Studie II während eines Novel-Object-Tests das Verhalten und die gleichzeitig gemessene Herzfrequenzvariabilität (HRV) ausgewertet. Mit dieser in der Personalitätsforschung bei Tieren bisher einmaligen Methodik wurden 400 Kälber mit 90 Lebenstagen getestet. Bei 39 dieser Kälber wurde der Test vier Monate später ein zweites Mal durchgeführt, um die Stabilität über die Zeit zu prüfen. Mithilfe der kombinierten Analyse der ethologischen und physiologischen Daten konnten vier besonders stark ausgeprägte Personalitätstypen beschrieben werden: “neophob/ängstlich – aufmerksam”, “interessiert – gestresst”, “gehemmt/desinteressiert – ruhig” und “neophil/kontaktfreudig – aufmerksam”. Beim zweiten Testalter zeigten mehr als 40 % der Kälber ein mit ihrem ersten Test übereinstimmendes Verhaltensmuster.

In Studie III sollte untersucht werden, ob ein Zusammenhang zwischen den Daten von 356 Kälbern aus dem in Studie I entwickelten Anbindetest und mit der in Studie II etablierten, multidimensionalen Darstellung ihrer Personalität hergestellt werden kann. So sollte ein praktikabler Test entstehen, mit dem eine große Zahl von Individuen – bei gleichzeitigem

Erhalt der Messverlässlichkeit – auf ihre Personalität getestet werden kann. Die Zugkraft hing mit der Aktivität des autonomen Nervensystems während des Novel-Object-Tests zusammen. Obwohl die Art dieses Zusammenhangs überraschend war, kann die Zugkraft als vielversprechender Parameter für die Weiterentwicklung des Anbindetests gesehen werden.

Abschließend sei bemerkt, dass es gelungen ist, vier besonders stark ausgeprägte Personalitätstypen mithilfe einer multidimensionalen Analyse eines Standardverhaltenstests und einer gleichzeitigen HRV-Messung zu beschreiben. Bei der Korrelation der Personalitätstypen mit Daten aus dem entwickelten Anbindetest zeigte sich ein Parameter vielversprechend für die Weiterentwicklung dieses Tests.

Abbreviations

d	Day
D	Duration
dpn	Days *post natum*
F	Frequency
HR	Heart rate
kNs	Kilo-Newton-second
L	Latency
LSM	Least square mean
MSA	Measure of sampling adequacy
NN	Beat to beat interval in heart rate
PC	Principal Component
PCA	Principal Component Analysis
PNS	Parasympathetic nervous system
RMSSD	Root mean square of successive differences
RR	Beat to beat interval in heart rate
SC	Score class
SD	Standard deviation
SDNN	Standard deviation of all R-R-intervals
SNS	Sympathetic nervous system

List of papers

Study I – Pulling test methodology

Graunke KL, Langbein J, Repsilber D, Schön P-C, 2013. Objectively measuring behaviour traits in an automated restraint-test for ungulates: towards making temperament measurable. The Journal of Agricultural Science 151, 141-149.

doi:10.1017/S0021859612000408

Study II – Multidimensional personality depiction

Graunke KL, Nürnberg G, Repsilber D, Puppe B, Langbein J, 2013. Describing temperament in an ungulate: A multidimensional approach. PLoS ONE 8, e74579.

doi:10.1371/journal.pone.0074579

Study III – Towards an easy applicable personality test

Graunke KL, Nürnberg G, Langbein J, Repsilber D, Schön P-C, 2013. Connecting data of an automated restraint test and a standard behaviour test: An attempt in developing an easy applicable personality test for cattle. (manuscript)

The main aims and results of these papers are summarised in 4 Summary of the studies, p. 25ff. The papers and license agreements are included at the end of this thesis.

Contributions

Katharina L Graunke – KG	Gerd Nürnberg – GN	Dirk Repsilber – DR
Jan Langbein – JL	Birger Puppe – BP	Peter-Christian Schön – PS

Study I – Pulling test methodology

KG wrote the following parts of the article: Introduction, Results, Discussion, and Animals and housing of Material and Methods; KG translated the parts "Measuring appliance and experimental outlay" and "Recording and parameters of tractive force" from German to English; KG executed the test on several animals; KG analysed the results; KG took part in the statistical analysis

JL designed the study; JL took part in analysing the results; JL took part in the statistical analysis; JL commented on the manuscript

DR designed the statistical analysis; DR wrote the part "Statistical analysis"; DR commented on the manuscript

PS designed the measuring appliance; PS programmed software to record the pulling effort of the test subjects and programmed software to analyse the force-time diagrams; PS wrote the following parts of the article in German: "Measuring appliance and experimental outlay" and "Recording and parameters of tractive force"; PS took part in the statistical analysis; PS commented on the manuscript

Study II – Multidimensional personality depiction

KG wrote the article with the exception of the part on HRV in the Introduction and most of the "Statistical analysis" of Material and Methods; KG executed the test on numerous animals; KG analysed the results; KG initiated the statistical analysis with a PCA; KG statistically analysed the data

GN designed the statistical analysis; GN wrote most of the part "Statistical analysis"; GN commented on the analysis of the results; GN commented on the manuscript

DR took part in designing the statistical analysis; DR commented on the analysis of the results; DR commented on the manuscript

BP designed the study; BP took part in analysing the results; BP commented on the manuscript

JL designed the study; JL wrote the part on HRV in the Introduction; JL took part in the statistical analysis; JL took part in analysing the results; JL commented on the manuscript; JL executed the test on some animals

Study III – Towards an easy applicable personality test

KG wrote the article with the exception of the part “Measuring appliance and experimental outlay” and most of the “Statistical analysis” of Material and Methods; KG translated the part “Measuring appliance and experimental outlay” from German to English; KG executed the tests on numerous animals; KG designed the statistical analysis apart from the PCA; KG statistically analysed the data; KG analysed the results

JL designed the study; JL took part in analysing the results; JL took part in the statistical analysis; JL commented on the manuscript; JL executed the test on some animals

GN designed the PCA of the statistical analysis; GN wrote most of the part “Statistical analysis”; GN commented on the manuscript

DR took part in designing the PCA of the statistical analysis; DR commented on the manuscript

PS designed the measuring appliance; PS programmed software to record the pulling effort of the test subjects and programmed software to analyse the force-time diagrams; PS wrote the following part of the article in German: “Measuring appliance and experimental outlay”; PS commented on the manuscript

1 General introduction

For a long time in ethological studies, it was tried to generalise behaviour over a group, a treatment unit, a population, even a species, etc., often rather desperately as differences between individuals occurred more often than not and with larger variances than expected and desired (Hawley 2011). These differences were long called “noise” that were to be avoided and minimised by choosing the “right” individuals (Hawley 2011), e.g., from inbred animal strains, and by reducing environmental influences as much as possible. The results of these studies, though, have in fact been argued to have little external generalisation value as they might detect effects with little or no external validity, because of the fact that they “reduce individual differences within study populations” (Würbel 2000). However, intra-individually consistent behaviour in similar situations began to assert itself with time, which meant that even the inter-individual differences turned out to be consistent in similar situations. These differences in behaviour between individuals can be caused by a range of environmental and state-dependent factors such as sex, age, reproductive status, or environment. However, not all of these differences can be explained by physiological states or environmental factors; these differences reveal the strategy an individual employs to act with and react to environmental stimuli (Manteca & Deag 1993, Locurto 2007, Wolf & Weissing 2010). This strategy is described as temperament and is thought to be innate and consistent over time and across different situations (Grandin 1993, Grignard *et al.* 2001, Range *et al.* 2006, Réale *et al.* 2007, Forkman *et al.* 2007, Dingemanse & Wolf 2010).

1.1 Concepts, definitions, and usage of terms

Besides temperament there are other terms used in this context, e.g., personality, individuality, or coping style (Forkman *et al.* 1995, Réale *et al.* 2007, Bell & Sih 2007, Boersma *et al.* 2009). However, there are no generally accepted definitions of these terms (Mills *et al.* 2010). Until recently, temperament was defined as mentioned above as relatively stable behaviour traits, while personality was supposedly a more flexible set of traits; therefore, there was to be a difference between the two terms (Mills *et al.* 2010). Gagne (2013) states that “temperament is an early developing set of characteristics related to later personality”. However, more recent research shows that both temperament and personality are influenced by similar factors such as genetics, hormones, etc. in a likewise manner and amount (Mills *et al.* 2010).

Due to the lack of a general definition authors have to define what they understand as temperament or personality and whether there is a difference between the terms. This leads to almost every publication using its very own definition. Gosling (2001) explains that personality is a consistent pattern of behaviour, while temperament is a set of early shown tendencies as foundation of personality. Sullivan *et al.* (2011) say temperament is a behavioural response to environment stable across time and situations, while Lowe & Bradshaw (2001) define the same as personality. Réale *et al.* (2007) provide a summary of the different terms and their definitions depending on the author(s), which clearly shows how arbitrary the distinctions between the different terms are. They conclude that the two most common terms, temperament and personality, are often artificially distinguished. Therefore, they understand these two terms as synonyms. I am in accordance with this understanding and use the terms synonymously throughout this thesis. Although being consistent over time and situations, personality should not be imagined as a fixed and completely inflexible construct, but rather as an adjustable tool for adaptation to exterior circumstances during individual ontogeny (Stamps & Groothuis 2010, Trillmich & Hudson 2011). Very early in life, personality seems to be rather flexible (Sulloway 2011, Hudson *et al.* 2011), while later on it is maintained more and more rigidly (Trillmich & Hudson 2011). However, depending on genetics and epigenetics, the starting point is different in each individual. Therefore, I understand personality as the strategy an individual employs to act with and react to environmental stimuli, which starting point is innate and which is relatively consistent over time.

Very often the usage of the terms depends on the field of science the author originates from. Scientists approaching animal personality from a psychological or developmental psychobiological point of view usually use the term “personality” (Trillmich & Hudson 2011, Gracceva *et al.* 2011); those with neurophysiological background use “coping style” (Koolhaas *et al.* 1999, Coppens *et al.* 2010) or “behavioural syndrome” (Sih *et al.* 2004, Herczeg & Garamszegi 2012) and others speak of “individuality” (Le Neindre *et al.* 1995, Coleman *et al.* 2005, Kilgour *et al.* 2006). “Temperament” is mainly used by scientists working with livestock (e.g., Dickson *et al.* 1970, Gauly *et al.* 2001, Benhajali *et al.* 2010). It also depends on the examined species which term is used. Interestingly, in fish and birds the term personality is frequently used (Bell & Sih 2007, Korsten *et al.* 2010, David *et al.* 2011, Meager *et al.* 2012), while there seemed and to some extend still seems to be a great fear in scientists working with livestock to grant these species personality (pers. comm.). Only in horses, the species of livestock many people have emotional relationships with, few authors speak of personality (Visser *et al.* 2003).

Although apes are the closest relatives to human kind and although there have been numerous descriptions of individual differences in their behaviour since the 1950s, partly pointing directly at the existence of personality in these non-human animals (Nissen 1956, Goodall 1964, Kutsukake *et al.* 2012, Rosati & Hare 2013), only about a decade ago research focussing on personality in apes started to become a subject (Koski 2011, Herrelko *et al.* 2012, Weiss *et al.* 2012, Kramer & Ward 2012). Most studies focussed and still focus on vertebrate model animal species like mice, rats or sticklebacks (Benus *et al.* 1989, Bell & Sih 2007, Boersma *et al.* 2009), and on species with rather close relationships to humans. These include dogs, horses, cattle, and sheep (Svartberg & Forkman 2002, Lansade *et al.* 2008b, Pajor *et al.* 2010, Hoppe *et al.* 2010). The continuingly increasing acceptance of animals as sentient beings with feelings and emotions (Toates 1998, Boissy *et al.* 2007, Reefmann *et al.* 2009, Veissier *et al.* 2012) and the inclusion of personality with their different perceptions of the environment and hence differing feelings and emotions into the concept of gradual evolution after Charles Darwin (Réale *et al.* 2007, Wolf *et al.* 2007, Dingemanse *et al.* 2009, Buss & Hawley 2011, Klefoth *et al.* 2012), however, will probably expand the attention to other species sooner or later. In fact, the European Union makes no difference between various animal classes, let alone species, and has already included not only agriculture (working with mammals and birds), but also fisheries (working with fish, cephalopods, bivalves, and some classes of crustaceans) into the branches of economy needing to implement animal welfare as they are dealing with sentient beings: "Article 13. In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions and customs of the Member States relating in particular to religious rites, cultural traditions and regional heritage." (Official Journal of the European Union 2008). This also has consequences for research in, e.g., conservation biology: Any interference with nature or renaturation plans should take into account what actions favour which personality types. The awareness of sentience and personality in various animal species and the consequences for research and action plans in, e.g., ecology and conservation biology raise the relevance of any information on that topic. The evolutionary benefits of specific personality types and the consequences of their existence for populations as a whole have to be taken into account within these fields. Also for animals living under human care, may it be livestock, pets, or zoo, circus, or laboratory animals, the consequences of the existence of personality for the more or less artificially mixed groups and the individuals' perception of their own welfare must become relevant.

1.2 Personality in evolution

Researchers in personality of all fields have slowly become aware that the concept of personality cannot have suddenly popped up in humans when it is such a complex construct (Gosling & John 1999, Stamps & Groothuis 2010, Trillmich & Hudson 2011); instead, like physiology, neurology, or genetics personality is consequently to be included into the concept of gradual evolution after Charles Darwin (Réale *et al.* 2007, Wolf *et al.* 2007, Dingemanse *et al.* 2009, Buss & Hawley 2011, Klefoth *et al.* 2012). As a result, there is more and more research done in developmental psychology, ecology and evolution, and ethology addressing questions like what the advantage of certain personality types is in an evolutionary and ecological context (Dingemanse *et al.* 2009, Hudson *et al.* 2011, David *et al.* 2011, Carter *et al.* 2012), when and if in the individual ontogeny personality is stable (Rödel & Meyer 2011, Eccard & Rödel 2011), what role siblings play (Hudson *et al.* 2011, Eccard & Rödel 2011), or how genetically fixated personality is (Korsten *et al.* 2010, Tschirren & Bensch 2010). E.g., male European rabbits from small litter sizes have been found to be more likely to show escalated offensive behaviour (= chasing) as juveniles (Hudson *et al.* 2011). While this could be related to larger body mass (Hudson *et al.* 2011), the same individuals were also more offensive and successful during fights as adults independent of their current weight (Rödel & von Holst 2009). Rhesus monkeys were found to be bolder towards humans when they were quickly approaching food in a novel-food test (Coleman *et al.* 2005) and zebra finches spent more time feeding when they were more exploratory, active, and faster to resume feeding after a startle (David *et al.* 2011). These results indicate that personality undoubtedly plays an important role in evolution. Bolder, more exploratory individuals certainly have an advantage over shyer, less exploratory animals during times of food shortage; however, they also take a higher predation risk. This risk or cost, respectively, probably explains why it is evolutionary useful to not eradicate the shy individuals: they might have disadvantages during food shortage, but their more cautious personality provides great advantages regarding predation risk, which during times of food prosperity is lifesaving. Still, the above statements, as logically as they may seem, are yet to be scientifically proven.

It is now no longer challenged whether non-human animals have temperament or personality or not, but rather how complex their personality is, when in the process of evolution it occurred and started to become relevant for survival, and in which species there is personality (Stamps & Groothuis 2010). The study of animal personality has also become more and more important to understanding the ontogenetic development of personality and to determine influential factors, because one can do studies that can be more controlled than studies with humans (Hudson *et al.* 2011). However, the knowledge about personality in context of evolution is still very little and the research on this topic has only just begun; an appeal to researchers of ecology and evolution to take notice of personality in animals and start researching on it from their perspective has been given by Réale *et al.* (2007).

Interesting also in context of evolution is the study of personality in domestic animals. Domestication is the “process by which a population of animals becomes adapted to man and to the captive environment by some combination of genetic changes occurring over generations and environmentally induced developmental events reoccurring during each generation” (Price 1984). This adaptation more than likely includes changes in behaviour and personality. I consider it probable that extremely shy, aggressive, and fearful personality types were greatly diminished and that the distribution of the existing personality types shifted towards more docile, bolder, and curious types. This, however, is yet to be scientifically investigated. Potentially, personality types have evolved that are not found in the wild counterparts as domesticated animals have also evolved abilities other than those they were explicitly bred for. E.g., dogs are able to understand what humans see (Kaminski *et al.* 2013), even if this is different to what they see (Kaminski *et al.* 2009) and they are further able to understand human gaze, pointing direction, and other given physical cues (Marshall-Pescini *et al.* 2012), which seems to be innate (Hare *et al.* 2002). Their wild counterparts, wolves, on the other hand do not have these abilities, even when kept and raised like dogs from day 4 (Miklósi *et al.* 2003). Still, wolves and dogs are equal to one another in non-social memory tasks (Hare *et al.* 2002). There are first hints that domesticated pigs are receptive to human gaze as well (Nawroth *et al.* 2013). If wild boars show the same ability is – to the best of my knowledge – unknown to this day. It is conceivable, if not probable, that other domesticated animals have developed similar abilities. Still, research needs to go a long way until these questions and others regarding new personality types in domesticated animals can be answered sufficiently.

1.3 Human vs. non-human animal personality

In human psychology, personality is described by the so-called “big five” factors (de Raad 1998). These are the five dimensions extraversion, agreeableness, conscientiousness, emotional stability (or neuroticism), and openness. Each dimension consists of six underlying aspects and is explained as follows (taken from Bagby *et al.* 1996):

- Extraversion describes the “quantity and intensity of interpersonal interaction” and the “capacity for joy”; includes i. a. activity, excitement-seeking, and assertiveness;
- Agreeableness describes the “interpersonal orientation along a continuum from compassion to antagonism”; includes i. a. trust, compliance, and tender-mindedness;
- Conscientiousness describes the “degree of organisation, persistence, and motivation in goal directed behaviour”; includes i. a. dutifulness, achievement striving, and self-discipline;
- Neuroticism describes “adjustment vs. emotional stability”; includes i. a. anxiety, angry hostility, and impulsiveness;
- Openness describes the “appreciation of experience for its own sake” and the “tolerance for and exploration of the unfamiliar”; includes i. a. curiosity, need for variety, and non-dogmatic attitudes.

Interestingly, when comparing the descriptions of the big five personality dimensions in human psychology with the five categories of temperament in animals described in the context of ecology and evolution by Réale *et al.* (2007) we find three of the categories overlapping with three of the big five factors, and a fourth being rather similar in its meaning. Their five categories are described as follows (Réale *et al.* 2007):

- Exploration – avoidance: reaction to new situations including new food, habitat, or novel objects; situations can be perceived as or actually be risky; overlaps with big five dimension openness;
- Aggressiveness: “agonistic reaction towards conspecifics”; overlaps with big five dimension neuroticism/emotional stability;
- Sociability: reaction to the presence or absence of conspecifics (excluding antagonistic behaviour); seeking or avoiding conspecifics; overlaps with big five dimension agreeableness;
- Shyness – boldness: reaction to a risky, but not new situation; this includes the reaction towards humans (potential predators) often described as docility, tameness, or fearfulness; similar to big five dimension extraversion;
- Activity: refers to the “general level of activity of an individual”; “can interfere with the measurement of exploration or of boldness”.

The personality trait conscientiousness of human psychology does obviously not appear in the description of Réale *et al.* (2007) and seems to be a very anthropomorphic trait. However, professional working, guide, and assistance dog trainers often characterise successful dogs as persistent, highly motivated to fulfil a given task, and with a strong will to please (pers. comm.). In other words, they could be described as determined, achievement striving, and dutiful, which is very close to the conscientiousness personality factor. Svartberg (2002) concluded that to succeed in known tasks under distraction, prolonged training but not more repetitions is the key for dogs scoring low in boldness. I.e. they are more likely to succeed because of advanced maturity and not because of more repetitions of the tasks, but the author failed to explain why that is. It could be argued that a low boldness score inhibits the striving to fulfil given tasks, thus inhibits a conscientiousness-like personality trait. Dogs with a low boldness score might need prolonged training and further developed maturity to overcome their low boldness score and to be able to develop and show their actual conscientiousness-like trait, which is described as “trainability” in another work (Svartberg 2005). These findings might possibly display a conscientiousness-like personality trait, although this has not yet been described as such.

1.4 Assessing animal personality

According to Manteca & Deag (1993) there are three ways of assessing personality-relevant behaviours: 1) standard behavioural observation; 2) observers’ rating with the help of predefined categories; 3) behavioural tests. A non-exclusive list of tests, a short description, the studied species, and the belonging publications can be found in Table 1. Common in many of the tests is to confront a test animal with an unknown and/or unpleasant stimulus, which can, e.g., be an object, food, a human, a conspecific, or an electric shock.

Table 1: Non-exclusive list of behaviour tests, their short description, species the test is used for, and references

Test	Description	Species	References
Elevated maze	Elevated platform with usually two narrow arms with or without boundaries at the sides, number of quadrants counted the animal enters, behaviour recording, is thought to measure anxiety	Rats, mice	(Weiss *et al.* 1998, Leshem 2011)
Open field	Behaviour recording in larger but closed area either known or unknown to the animal prior testing, field usually unfurnished but sometimes furnished depending on the species, is thought to measure boldness	Fish, grey mouse lemurs, cattle, rhesus macaques, pigs, sheep, chickens, great tits	(Fraser 1974, Gallup & Suarez 1980, Moberg & Wood 1982, Boissy & Bouissou 1988, Verbeek *et al.* 1994, Coleman *et al.* 2005, Yayou *et al.* 2010, Klefoth *et al.* 2012, Dammhahn 2012)
Novel stimuli	Unknown stimulus is presented to an animal, most often stimulus is an object, other unfamiliar stimuli are a human, food, etc., is thought to measure openness towards novelty	Cattle, sheep, dogs, fallow deer, zebra finches, rhesus macaques, pigs, horses, great tits	(Goddard & Beilharz 1986, Forkman *et al.* 1995, Plusquellec *et al.* 2001, Visser *et al.* 2003, Kilgour *et al.* 2006, Sibbald *et al.* 2009, David *et al.* 2011, Bergvall *et al.* 2011)
Back test	Test animal laid on its back and held in position, struggles against the position recorded, is thought to measure coping strategy	Pigs	(Forkman *et al.* 1995, Bolhuis *et al.* 2004)
Unknown intruder	An unfamiliar conspecific is let into the home pen/tank of the test animal, is thought to measure aggression	Fish, mice	(Huntingford 1976, Sluyter *et al.* 1995, Bell & Sih 2007)
Defensive burying	Prod giving an electric shock is presented to the test animal, animals react either with avoidance or with actively burying the prod with nesting material, is thought to measure proactive or reactive coping style	Rats, mice	(Sluyter *et al.* 1996, Boersma *et al.* 2009)
Human approach	Distance when animal steps back or flees while a human approaches it, is thought to measure fear of humans	Cattle	(Murphey *et al.* 1980, Boissy & Bouissou 1988, Kilgour *et al.* 2006)
Crush test	Temperament scoring while the animal is confined in a crush or scale, is thought to measure ease of handling and docility	Cattle, sheep	(Grandin 1993, Kilgour *et al.* 2006, Behrends *et al.* 2009, Pajor *et al.* 2010)
Docility test	Animal is kept in a corner by a human handler while being separated from its pen mates, is thought to measure docility	Cattle	(Boivin *et al.* 1992, Plusquellec *et al.* 2001)
Flight time	Time it takes an animal to leave after confinement (e.g., in a crush or scale) through an alleyway, is thought to measure ease of handling and docility	Cattle	(Müller & von Keyserlingk 2006, Kilgour *et al.* 2006, Behrends *et al.* 2009, Gibbons *et al.* 2011)

In many of these tests, human interaction with the tested animals is inevitable or part of the tests themselves (for detailed descriptions of common methods of personality trait and temperament measurements, see, e.g., Manteca & Deag 1993, Burrow 1997, Lanier *et al.* 2000, Waiblinger *et al.* 2006, Forkman *et al.* 2007). Some methods are therefore at least partly subjective and judgemental, especially when categories are used. According to the mood-biased judgement hypothesis human judgements will be positive during a good mood and negative during a bad mood (Mayer 1986). Thus, when measuring personality traits, the results of judgemental methods are always influenced by the mood of the observer during the rating and by what the observer thinks is a positive or a negative judgement of the animals. Therefore, the inter- and even intra-observer reliability are difficult to obtain (Welfare Quality® 2009b); however, especially for practical purposes, e.g., when animals are selected for breeding, an objective, reliable, and observer- and mood-independent method of measuring personality traits is required.

In the literature, original research on non-human animals often describes personality on a one-dimensional scale using expressions such as “proactive – reactive”, “aggressive – non-aggressive”, “bold – shy”, etc. (e.g., Benus *et al.* 1989, Koolhaas *et al.* 1999, Boersma *et al.* 2009). As mentioned above, human psychology and thereon based recent theoretical framework on personality in non-human animals, however, mostly argue for two or more dimensions using terms such as “valence”, “arousal”, or “activity” to describe the different dimensions (Koolhaas *et al.* 2007, Réale *et al.* 2007, Koolhaas *et al.* 2010, Mendl *et al.* 2010). Following their arguments, different personality types can either be located in a circumplex model as a linear combination of these dimensions like Mendl *et al.* (2010) and Koolhaas *et al.* (2010) do, and which has first been suggested for humans by Eysenck (e.g., Eysenck 1991, Figure 1). As mentioned above, Réale *et al.* (2007) even defines five categories of temperament in animals. Therefore, two or more dimensions are more likely to reflect the entire nature of temperament or personality in non-human animals than one dimension. Especially, the valence or perception dimension of a situation is highly individual and very difficult to measure, yet most important to an animal’s welfare (Veissier *et al.* 2012).

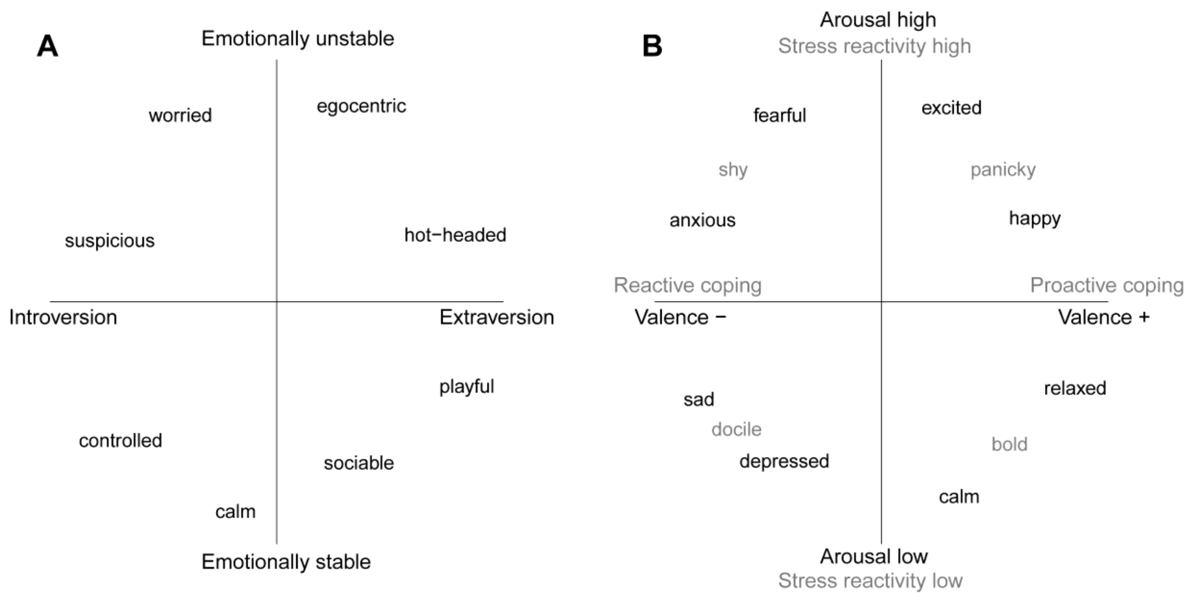


Figure 1: Circumplex models of (A) human personality (after Eysenck 1991) and (B) non-human personality (taken from Koolhaas *et al.* 2010, grey font) and core affect (taken from Mendl *et al.* 2010, black font).

1.5 Measuring perception

In psychological research, not only behaviour tests and observation can be conducted but also questionnaires about the inner emotional world of the test persons can be answered (Gomez & Gomez 2002). Naturally, questionnaires on the perception of different situations that are used in personality research in human psychology are impossible in non-human animals; therefore, one must include measures of physiological or neurophysiological activation revealing information about the probable perception and processing of a test situation by an individual. The analysis of cardio-vascular measurements has been found to be a suitable approach for determining the activity of the autonomous nervous system (Beauchaine 2001, van Reenen *et al.* 2005, Santucci *et al.* 2008). The length between heart beats differs with each beat as long as the individual is in good health. This is called the heart rate variability (HRV). Depending on which branch of the autonomous nervous system – the parasympathetic or the sympathetic branch – is more activated, the beat to beat changes in heart rate (R-R/N-N interval, R-R in ms) vary differently (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996, von Borell *et al.* 2007, Figure 2). Changes in heart rate occur within 5 s after the activation of the parasympathetic branch while after activation of the sympathetic branch an up to 5 s response delay arises followed by a gradual increase and maximum response after 30 s (von Borell *et al.* 2007). Changes in HRV, therefore, depict a rather clear and immediate image of the individual inner perception of a situation and have been suggested as a psychophysiological marker of internal regulation and of certain aspects of psychological adjustment in humans and animals (Calkins & Keane 2004, Boissy *et al.* 2007). The parasympathetic branch (vagus nerve) regulates the inner organs and the blood circuit and induces regeneration of the body, digestion, and rest (Eckert *et al.* 2000). An individual with an active parasympathetic branch is therefore likely to perceive the current situation as calming, not threatening, and in extreme activation as subduing. The sympathetic branch increases the body's performance ability by circulating blood to the muscular blood vessels, increasing blood pressure, and prepares it for stressful events (Eckert *et al.* 2000). Individuals with an active sympathetic branch are likely to perceive the current situation as stressful, exciting, threatening, and in extreme activation as ready for "fight-or-flight".

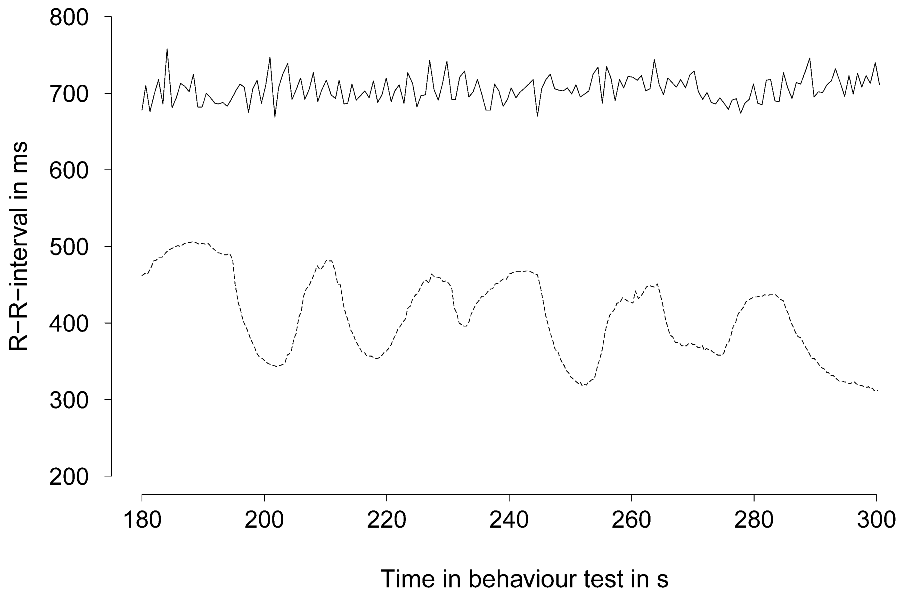


Figure 2: R-R-interval curves of a highly parasympathetically activated individual (solid line) and a highly sympathetically activated individual (dashed line) during minute 3 to 5 of the behaviour test applied in this thesis.

Common variables of the HRV measures in the time domain are (1) the heart rate in beats per minute (HR in bpm), (2) the root mean square of successive differences (RMSSD in ms), (3) the standard deviation of all R-R-intervals (SDNN in ms), and the ratio of RMSSD and SDNN (RMSSD/SDNN). Table 2 provides a description of the effects of the autonomous nervous system on these measures.

$$HR = \frac{60000}{\overline{NN}} \tag{1}$$

$$RMSSD = \sqrt{\frac{1}{1-n} \sum_{i=1}^{n-1} (NN_i - NN_{i+1})^2} \tag{2}$$

$$SDNN = \sqrt{\frac{1}{1-n} \sum_{i=1}^{n} (NN_i - \overline{NN})^2} \tag{3}$$

Table 2: Measures of heart rate variability (HRV), branches of the autonomous nervous system that influence the HRV measures, and their consequences on the respective measure (after von Borell *et al.* 2007); PNS = parasympathetic nervous system, SNS = sympathetic nervous system

HRV measures	Influence of the autonomous nervous system	Consequences on HRV measure
HR	Additive and non-additive effects of PNS and SNS	HR decreases, when PNS activity increases and/or SNS activity decreases
RMSSD	Only influenced by PNS	RMSSD increases, when PNS activity increases
SDNN	PNS and SNS act synergetically	SDNN increases mainly, when SNS activity increases, but is also influenced by PNS activity
RMSSD/SDNN	PNS and SNS affect measure antagonistically	RMSSD/SDNN increases, when PNS activity increases and/or SNS activity decreases

1.6 Consequences for animal welfare and animal husbandry

Since the welfare of an animal depends on its perception of and its ability to cope with its environment (Broom 1988), personality is an important factor in animal welfare and in breeding. For a long time, animal welfare focussed on avoiding the presence of negative stimuli. In 1965, the Brambell Report was the first agreement in written form on what welfare in animals meant (Brambell 1965). The postulations were reformulated in 1979 to the much referred Five Freedoms of Animal Welfare, which are (taken from Farm Animal Welfare Council 1979):

- "Freedom from hunger and thirst – by ready access to fresh water and a diet to maintain full health and vigour";
- "Freedom from discomfort – by providing an appropriate environment including shelter and a comfortable resting area";

- "Freedom from pain, injury or disease – by prevention or rapid diagnosis and treatment";
- "Freedom to express normal behaviour – by providing sufficient space, proper facilities and company of the animal's own kind";
- "Freedom from fear and distress – by ensuring conditions and treatment which avoid mental suffering".

These freedoms have been developed further to criteria for welfare quality by the EU-financed project Welfare Quality®, which combine both the Five Freedoms of Animal Welfare and explicitly the presence of "positive emotions such as security and contentment" (Welfare Quality® 2009a) to 12 standards for welfare. Although in modern intensive housing systems at least the freedom to express normal behaviour is still all too seldom reality, the written agreement on the importance of the presence of positive emotions is a big step towards providing good animal welfare for livestock. However, the challenge remains how to provide opportunity for positive emotions and an overall pleasant perception and valence of an individual's situation, when these are, especially in comparison to the other criteria, very individual (Veissier *et al.* 2012). Veissier *et al.* (2012) point out that although exterior conditions like housing system, quality of diet, etc. loose no importance to animal welfare, one mandatorily needs to take into account the valence of the animals themselves, when seriously trying to evaluate an animal's welfare. The valence of a situation differs vastly between individuals and depends greatly on their personality, former experiences, and to some extent also to i. a. health and reproductive status. Therefore, research on personality and their differing valences of identical situations is mandatory to reach the goal of providing good animal welfare. When choosing the "right" animals for a particular barn not only the individual valence of, e.g., the housing system is to be taken into account, but also the personality type when attempting to compose a harmonic group of individually different animals. After all, a herd of only fearful cows could cause severe problems and time losses in handling (Burrow 1998) as well as a decrease in productivity (Fordyce *et al.* 1988b, Voisinet *et al.* 1997a, Voisinet *et al.* 1997b, Fell *et al.* 1999). There might also be personality types that are hazardous in domesticated animals due to the danger they might cause for the handlers. Also, it is conceivable that certain personality types are more adapted to outdoor housing conditions providing more space but also presence of predators, while others might be better able to adapt to indoor housing conditions with limited space and crowded circumstances.

1.7 Cattle and cattle personality in research and practice

The ungulates cattle are ruminants and have been domesticated since approximately 10,000 years by man. They stem from the aurochs and are divided into *Bos taurus* and *Bos indicus*, both of which have their main occurrence at different geographical areas around the world. Both were domesticated in areas of southeast Europe and southeast Turkey (Sambraus 2006). While *Bos taurus* has a straight back, is best adapted to colder environments, and is now mostly distributed over the colder parts of the northern hemisphere, *Bos indicus* has a humped back consisting of six muscles, is much smaller and lighter than *Bos taurus*, has less coat, more surface compared to its volume, and is therefore better adapted to hotter climates and survives much higher temperatures (Sambraus 2006). Therefore, it is now mostly found in hot climates and in the southern hemisphere, where it can also often been found in crossbreeds of *Bos indicus* and *Bos taurus*. Cattle played a crucial role during the settlement of humans and helped making it possible to survive harsh winters in Europe by providing milk and lasting milk products. Nowadays, there are three different types of breeds besides landraces and working breeds: dairy breeds, which are kept solely for milk production and provide meat only as a by-product through male calves and old cows, examples are Holstein Friesian, Jersey, Brown Swiss; beef breeds, which are only kept for beef production, their milk is directly nursed to their own calves, examples are Charolais, Aberdeen Angus, Limousin; and dual-purpose breeds, which are kept for both milk and beef production, these breeds produce neither most milk nor most beef in comparison to the specialised breeds, but are above average in both areas, examples are Fleckvieh, Vorderwald Cattle, Tyrolese Grey Cattle (Sambraus 1991). In Europe and North America dairy breeds are kept mostly indoors in same sex groups, same age or same production status groups, respectively. Most often the females are artificially inseminated, only seldom and often as a last resort natural insemination is applied (pers. comm.). Calves are taken from their mothers usually within the first 24 hours and raised apart from their mothers in calves groups. Average lifespan of a dairy cow in Germany was 5.3 years in 2012 (Vereinigte Informationssysteme Tierhaltung w.V. VIT 2012). Beef cattle are often kept outdoors at least during summers. Calves are raised by the mothers and usually weaned at 6-8 months. They are then assigned to either single sex fattening groups or recruitment. Bulls are kept with the female herds for about 8 weeks each year to reproduce; artificial insemination is used seldom and usually only for pure-breeding purposes. Cows are often kept as long as they birth calves annually without assistance and as long the calves gain good weight until weaning or slaughter. They can then reach 20 years of age; usually though, they are slaughtered between 8-10 years of age (pers. comm.). When fattened, beef cattle are slaughtered at either a certain predefined weight or a predefined age, mostly between 15 and 18 months.

In cattle, personality traits are associated with the ease of handling (Burrow 1998), live weight gains in feedlots (Voisinet *et al.* 1997b, Fell *et al.* 1999) and on pasture (Fordyce *et al.* 1985, Fordyce *et al.* 1988a), carcass damage (Fordyce *et al.* 1988b), and meat quality (Fordyce *et al.* 1988b, Voisinet *et al.* 1997a). Some official beef breeders have already added certain personality traits to their breeding goals (e.g., Zuchtbetrieb Zachert 2013, Traditional Herefords 2013). They are usually told to use the flight speed/time test, crush test, or docility test to determine an individual's personality (Gaden *et al.* 2004, International Beef Recording Scheme 2012). Common for these behavioural tests is the measurement or rating of the motivation of an animal to elude an unpleasant or frightening situation either while restrained (flight speed test and crush test) or while moving freely (docility test). The behaviour shown in these situations is either fear-related escape behaviour or the expression of the animal's resentment of the situation which is an important part of personality (Kilgour *et al.* 2006, Benhajali *et al.* 2010). Still, the often used crush-test measures the behaviour indirectly by having observers judge the strength, frequency, and duration of escape attempts and rating the animals according to a predefined category scale (Grandin 1993, Kilgour *et al.* 2006, Benhajali *et al.* 2010, Gibbons *et al.* 2011). The flight speed test simply measures the time it takes an animal to leave the crush and pass the first few meters after restraint (Müller & von Keyserlingk 2006, Kilgour *et al.* 2006, Behrends *et al.* 2009, Gibbons *et al.* 2011), while other developed tests such as the docility test can be time consuming and rather complicated to execute (Le Neindre *et al.* 1995, Boivin *et al.* 2009). Human interaction with the animals (Boissy & Bouissou 1988) and observer-dependent rating are fundamental parts of the tests (Grandin 1993). Eventually, all previously named tests measure docility, which is an important part of animal personality, especially for livestock species, but not even close to a more complex depiction of it. Docility can further not be found within the theoretical frameworks of Réale *et al.* (2007), Mendl *et al.* (2010), and Koolhaas *et al.* (2010). Whether it does exist as a single personality trait or is the result of the combination of the other traits, remains to be studied. One therefore needs an easy applicable test revealing other parts of personality than docility, which can be used by anyone anywhere in the world, trained or untrained, educated or not, in any housing system and will yield objective comparable results. To know whether such an easy applicable test actually does detect different personality types it has to be correlated with a more complex depiction of cattle personality.

2 Aims

The aim of this thesis was the assessment of personality in young cattle (*Bos taurus*) to gain more knowledge on the phenotype of this economically important livestock species. By combining ethological observations, physiological measures, and objectively measured applied physical force, it was thought to provide a broad phenotypical basis for further analyses on metabolome, proteome, and transcriptome level.

At first, an appliance was to be established that allows to objectively measuring escape behaviour and reluctance in young cattle restrained by being tethered on a halter, independently of the impact and rating of a more or less subjective observer or handler. (Study I – Pulling test methodology)

By analysing the behaviour and simultaneously measured heart rate variability during a standard behaviour test in calves a depiction of personality was to be developed and tested for stability over time. Taking this combined multidimensional approach based on experimental original data, I intended to provide foundational support for the theoretical framework suggesting two or more dimensions in animal personality. (Study II – Multidimensional personality depiction)

To take a first step in developing an easy applicable test for personality in cattle, data from an automated restraint test was to be correlated with a multidimensional depiction of cattle personality resulting from a standard behaviour test. By linking automatically measured data to ethological and physiological data, I aimed to test whether the automated restraint test is a practicable way to test large numbers of individuals for their personality with maintained level of measure reliability. (Study III – Towards an easy applicable personality test)

I hypothesised that the animals would have different strategies in their reaction to being tethered (Study I), that personality could be depicted with at least two dimensions from conducting a standard behaviour test (Study II), and that the behaviour patterns during the restraint would be linked to some parameters of the personality depiction (Study III).

3 Animals, materials, and methods

3.1 Animals and housing

In all studies, animals of the F_2-generation of a running breeding project (Holstein Friesian × Charolais cross breeding) were tested with 90 dpn (± 3 dpn, days *post natum*). The parental generation consisted of five Charolais bulls and 26 Holstein Friesian cows. Of each bull one male offspring was selected to be paired with the female offspring of another bull. Thereof descending offspring were part of the studies presented in this thesis. For more information about the breeding scheme and the relations between the F_2-generation see Appendix 1. All calves were bred via embryo transfer into unrelated Holstein Friesian heifers as recipient mothers and were born and tested between 2004 and 2010. All tests started at noon. The calves were kept in various small groups of up to nine animals of similar age, apart from their recipient mothers from day one. Pens had a size of 6 m × 7 m and were covered with deep litter. Calves were fed colostrum and first milk from the recipient mothers four times per day until 6 dpn with 1.5-2.0 l per meal. From 7-107 dpn they had access to milk substitute (SALVANA M-15/SW, concentration 125 g/l) from an automatic calf feeder with up to 8 l/d until weaning started at 71 dpn when the daily meal size gradually decreased to 1 l/d at 100 dpn. Simultaneously, the concentrate meal size increased from 0.1 kg/d to 2.6 kg/d while having access to hay *ad libitum*. Until 90 dpn, the calves were not subject to any other experiment, and handling did not exceed routine handling by the animal keepers except in the case of animals requiring treatment for sickness.

After weaning, the animals were weighed at 111 dpn (± 3 dpn). The weight at the day of the experiment was calculated with the help of the average daily weight gain from birth to weaning and the exact age at the experiment.

We further tested each 20 calves (10 male, 10 female) of the founder breeds Holstein Friesian and Charolais at 91 dpn (± 3 dpn) and a second time at 197 dpn (± 12 dpn). The calves were purchased from breeders and arrived at our facilities two days after birth at the latest. They were housed in the same barn and received the same amount of feed as the crossbreeds and male and female calves were housed together until the second test had been conducted. After weaning, calves were moved to a larger pen covered with deep litter and received about 15 g/kg liveweight of concentrate on top of *ad libitum* access to silage and hay. These calves were born and tested between 2008 and 2012. All tests started at noon.

All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Ministry of Agriculture, the Environment and Consumer Protection of the federal state Mecklenburg-Vorpommern, Germany.

3.1.1 Study I – Pulling test methodology

Twenty-four calves (12 male, 12 female) of the F_2-generation were tested with 91 dpn (± 3 dpn) in a restraint situation (further called pulling test). Sixteen of them (8 male, 8 female) were not subject to any other experiment presented in this thesis. On average the calves weighed 115 kg (range: 89-144 kg, SD ± 13 kg) at the day of the experiment.

3.1.2 Study II – Multidimensional personality depiction

We tested 361 calves (175 male, 186 female) of the F_2-generation with 90 dpn (± 3 dpn) in a novel-object test, eight (4 male, 4 female) of which were also subjects in Study I. On average, the calves weighed 118 kg (range: 74-159 kg, SD ± 14 kg) at the day of the experiment. Each 20 calves (10 male, 10 female) of the founder breeds Holstein Friesian and Charolais were tested at 91 dpn (± 3 dpn) and a second time at 197 dpn (± 12 dpn) to evaluate stability of personality over time and to detect possible breed differences. Due to the early death of one male Charolais calf, there were 19 Charolais calves tested at 91 dpn. At the second test age, one male Charolais calf became extremely distressed during testing and risked serious injury. The test was terminated; thus there is data of 18 (8 male, 10 female) Charolais calves at 197 dpn.

3.1.3 Study III – Towards an easy applicable personality test

The same individuals as in Study II were additionally tested in the pulling test with 91 dpn (± 3 dpn). On average, the calves weighed 119 kg (range: 74-160 kg, SD ± 14 kg) at the test day. Each 20 calves (10 male, 10 female) of the founder breeds Holstein Friesian and Charolais were additionally tested at 92 dpn (± 3 dpn) and a second time at 198 dpn (± 12 dpn). Due to the reasons mentioned above, there were 19 Charolais calves tested at the first test age and there is data of this test of 18 (8 male, 10 female) Charolais calves at 197 dpn.

3.2 Experimental procedures

3.2.1 Study I – Pulling test methodology

Measuring appliance and experimental outlay

To automatically measure escape behaviour and reluctance in calves, we recorded the animals' expended power during the pulling test with the force transducer Megatron KT1400 (Megatron Elektronik AG & Co., Putzbrunn, Germany). The force transducer was built onto a metal bar of the home pen of the animals so that the cable elongated the force direction of the transducer, which was essential to avoid resultant force, and was connected with an analogue/digital interface (NI PCI-6503, National Instruments Germany GmbH, Munich, Germany) to a PC.

During the pulling test the tested calf stayed in one half of its home pen, while the pen mates were confined to the other half of the pen using metal bars to avoid falsifying the measurement. Visual, acoustic, and olfactory contact between the test calf and its pen mates therefore persisted in the hope of minimising effects and influences of isolation. The test calves were captured, put on a halter, and tied to the cable leading to the force transducer (see Fig. 1 in the publication of Study I for pictures of the technical equipment and the experimental set-up with a test animal). The software recording of the tractive force developed for this study was then initiated and all human handlers left the barn. After a 30 min recording period, a handler entered the barn, released the test calf, and reunited it with its pen mates, which were allowed back into the experimental part of the pen.

Recording and parameters of tractive force

To save the tractive force-time diagram and to calculate the parameters from this diagram, a software program using LabView 6.1 (National Instruments GmbH Germany, Munich, Germany) was developed.

In this experiment, the 30 min recording period was divided into six 5 min-intervals, and for each interval, the following parameters were calculated:

- Total force: an integral of the tractive force diagram in kNs consisting of:
    - Tractive force: an integral of positive values of the derivation of the tractive force diagram (meaning only the upward tractive phases) in kNs (force multiplied by time that a calf pulls on the cable with elevating effort)
    - Holding force: an integral of zero values of the derivation of the tractive force diagram (meaning only the holding tractive phases) in kNs (force multiplied by time that a calf pulls on the cable with stable effort; i.e. “hanging” in the cable without moving)
    - Dwindling force: an integral of negative values of the derivation of the tractive force diagram (meaning only the downward tractive phases) in kNs (force multiplied by time that a calf pulls on the cable with declining effort; i.e. stop pulling on the cable)
- Number of pulls: all local maxima of the tractive force diagram above a threshold level of 60 N (empirical value)

– Maximal tractive force: the highest amplitude of the tractive force diagram (global maximum) in N

3.2.2 Study II – Multidimensional personality depiction

Novel-object test

The behaviour test was performed in an open field of 9.6 m × 4.0 m in size, which was unknown to the calves prior to testing. Each calf was tested on its own without visual and olfactory contact to its pen mates. Acoustic contact, however, was possible through the closed door between the home pen and the open field. The field was divided into four segments of 2.4 m × 4.0 m each. Two sides (one short and one long side) of the open field consisted of solid brickwork, while the other two sides consisted of chip boards of 1.4-2.0 m in height. These sides were consolidated on the outside with metal bars and several wooden constructions to prevent the chip board walls from tilting. The observer stood on an elevated desk in the middle of the longer side of the chip board wall with a laptop for live-recording. The floor of the open field consisted of concrete. After allowing the test animal to acclimatise to the open field for 10 min, a novel-object test was conducted with an orange traffic pylon of 0.5 m height, a diameter of 0.3 m at the bottom, and two white stripes of 0.1 m width as novel object. It was let down into the outer segment, which was the farthest from where the calf stood. We chose this test as it is known to provoke behaviour, which correlates with behaviour during other tests (Lansade *et al.* 2008a, David *et al.* 2011) or with social cues (David *et al.* 2011). Most importantly, though, it triggers exploration behaviour, which is an important parameter in describing personality in non-human animals (Verbeek *et al.* 1994, Budaev 1997, Korsten *et al.* 2010). The novel-object test lasted for 10 min. During the test, behaviour was live-recorded using the observation software tool The Observer 5.0 (Noldus, The Netherlands). Of in total 438 behaviour test sessions, 428 were conducted by three experienced observers whose observation highly correlated during a 90 min-test session (Pearson's Rho 0.973, $p < 0.001$). The remaining 10 sessions were conducted by three other experienced observers, who did not take part in the test session. Recorded behaviours with their definitions and type of recording are listed in Table 3. For analysis, the latency of the behaviours an individual did not show during the 10 min behaviour test was set to the maximum time of 600 s (10 min).

Table 3: Definition and type of recording of the behaviours live-recorded during the novel-object test; D = duration (total time in s), F = frequency, L = latency (time in s until behaviour was first shown)

Behaviour	Type of recording	Definition
Contact with novel object (contact)	D, F, L	Physical contact with any part of the body with the novel object or sniffing the novel object while being closer than 0.1 m to it
Inactivity	D	At least three legs touch the ground, no forward movement
Exploration	D, L	Sniffing or licking the wall or floor of the open field
Grooming	D	Calf licking or scratching itself with one hind leg
Activity	D, L	Max. 3 legs touch the ground, forward movement
Running	D	Max. 2 legs touch the ground, fast forward movement
Vocalisation	F	Any kind of sound the calf makes
Change of segment	F	Leaving one segment and entering another with at least the forelegs
Habitation in segment where the novel object is placed (object segment)	D, L	With at least the forelegs in the segment in which the novel object is placed
Habitation in segment next to segment where the novel object is placed (object neighbouring segment)	L	With at least the forelegs in the segment next to the segment in which the novel object is placed

Heart rate variability (HRV)

To measure the heart beat activity during the test, we applied a heart monitor system (Polar S810i, Polar Electro, Oy, Finland). Prior to the beginning of the experiment, calves were fitted with flexible belts with two integrated electrodes and a transmitter for wireless transmission of the R-R-interval data series to a separate storage device and were then left alone with their pen mates in their home pen to gain base measurements. After 30 min, they were led into the open field for acclimatisation and testing. Later on, the R-R data series were transferred to a computer and corrected when necessary using Polar Precision Performance SW version 4.03 (Polar Electro, Oy, Finland) with the standard set-up. The curves were divided into 5-min intervals and an error correction of up to 10 % per interval was accepted. In further processing of the data, neither differences between two R-R-intervals larger than 150 ms nor identical values of five or more consecutive R-R-intervals were accepted. A program developed with LabView 2009 version 9.0 (National Instruments Germany GmbH, Munich, Germany) detected complete 1-min intervals in the base measurements (starting 5 min after the experimenters left the barn) and the test, and calculated HR, RMSSD, SDNN, and RMSSD/SDNN for each complete 1-min interval (see General introduction 1.5 equations (1)-(3) for the exact calculation and Table 2 for an explanation of the variables). When there were at least seven complete 1-min intervals per base measurement and per test, the program further determined the mean of the first seven values of HR, RMSSD, SDNN, and RMSSD/SDNN. This was the case in 272 crossbreed calves (134 male, 138 female), 17 Holstein Friesian calves at 90 dpn (8 male, 9 female), all 20 Holstein Friesian calves at 197 dpn, all 19 Charolais calves at 90 dpn, and 17 Charolais calves at 197 dpn (8 male, 9 female). The differences between test and base measurements of HR, RMSSD, and SDNN and the ratio of test and base measurement of RMSSD/SDNN were used for further analyses. In later analysis, it turned out to be completely independent of the personality, which calves did and which did not have complete HRV measures (Appendix 2). Therefore, there is no further discussion of this fact.

3.2.3 Study III – Towards an easy applicable personality test

In this study, we combined data from the automated pulling test and the more elaborate novel-object test to take a first step towards an easy applicable personality test for cattle. Of the pulling test, the parameters total force consisting of tractive force, holding force, and dwindling force, number of pulls and maximal tractive force were calculated for the complete test period of 30 min. Of the novel-object test, the results of the behaviour analysis were used and the ratio of RMSSD/SDNN of test and base measurement as it provides the most reliable information about the balance of the autonomous nervous system’s activity. Due to technical problems in the pulling test, data from one male and four female crossbreed calves was lost. Combined with the losses in the HRV measurements during the novel-object test, there was therefore complete data of behaviour and HRV measures during the novel-object test and data from the pulling test in 268 crossbreed calves (133 male, 135 female), 17 Holstein Friesian calves at 90/91 dpn (8 male, 9 female), all 20 Holstein Friesian calves at 197/198 dpn, all 19 Charolais calves at 90/91 dpn, and 17 Charolais calves at 197/198 dpn (8 male, 9 female).

3.3 Statistical analysis

3.3.1 Study I – Pulling test methodology

The parameters tractive force, maximal tractive force, and number of pulls were used in this exemplary analysis. For matter of clearness, the other two parameters were left aside and a parameter vector with a dimension of 18 (each three parameters in six 5 min-intervals) was therefore used. Since the measured parameters of the vectors were multivariate, we conducted multivariate analyses with the statistics program R version 2.12.1 (The R Foundation for Statistical Computing, Austria). With these analyses, we tried to classify the different reactions of the tested animals on the basis of the parameter vectors without a priori knowledge. The k-means-algorithm ("kcca") was used to perform a k-centroids clustering on the data matrix (Leisch 2006). Since the number of clusters k is unknown per default, the algorithm was conducted iteratively from k = 2 to k = 20. The algorithm was conducted 20 times with randomly differing initial conditions for each number of clusters k. By using the score of the "silhouette" function, the optimal number of clusters was determined. A hierarchical cluster analysis (Euclidean distance, complete linkage) was performed with the function "hclust", which uses a set of dissimilarities for the clustered objects. At first, each object is allocated to its own cluster and then the algorithm proceeds iteratively by stepwise combining the two most similar clusters and continuing until there is only one single cluster. The animals' weights were tested for differences between the clusters with Welch's Two Sample t-test. See Appendix 3 for the R-script used in this analysis.

3.3.2 Study II – Multidimensional personality depiction

All statistical analyses were performed using SAS 9.3, SAS Institute Inc., USA. In a preliminary analysis, we checked for the influence of sex and weight on all behaviours and HRV measures using a one-way analysis of covariance model (ANCOVA, The GLM Procedure) with the fixed factor sex and the co-variable weight.

As main analysis, we performed a Principal Component Analysis (PCA), which described the relationship between new (latent) factors and our 15 behaviours. A PCA is used to condense several correlated measures into a smaller number of principal components (PC). The loadings of each measure on a PC represent the correlation between the component and this measure. I.e., the loadings reflect the importance of each measure for the component. A measure for sampling adequacy (MSA) of the correlation matrix is the Kaiser-Meyer-Olkin criterion (KMO). We decided to use a PCA instead of a Factor Analysis, because many of our behaviour measurements were non-normally distributed (Budaev 2010), and because no a-priori theory or model exists (Gorsuch 1983). The PCA was conducted with The FACTOR Procedure with the parameter settings: method=PRIN, prior=ONE, rotation=VARIMAX. As input data set, we used a correlation matrix of all pairwise correlations of our 15 behavioural measures applying the non-parametric Spearman's rank correlation test (using The CORR Procedure), because some of the behaviours were not continuous and/or normally distributed. One crucial point when using a PCA is the choice of the final number of extracted principal components (Budaev 2010). Several methods are available for this decision. We performed four methods: Kaiser's number of eigenvalues > 1 (Kaiser 1960), Cattell's scree-test (Cattell 1966), Horn's Parallel test (Horn 1965), and Velicer's Minimum Average Partial (MAP) test (Velicer 1976). For the Parallel test and MAP test, we applied the SAS syntaxes provided by O'Connor (2000). We decided for two PCs in the final PCA calculation, since three of these methods led to a two PC solution (except number of eigenvalues > 1). Corresponding PC scores for each calf were finally calculated with The SCORE Procedure. These scores were further used to determine score classes and to identify the calves with differing behaviour. See Appendix 4 for the complete SAS-script used for this analysis.

The influence of these score classes and sex on the HRV measures was tested by a two-way analysis of variance model (ANOVA, The MIXED Procedure) with the fixed factors score class, sex, and their interaction. Posthoc tests were performed with a Tukey-Kramer correction for multiple testing (Appendix 5).

The PC scores of the calves of the founder breeds (Holstein Friesian, Charolais) were calculated with The SCORE Procedure using the resulting loadings from the crossbreeds as it is no use to perform a PCA on such a low number of animals (Comrey & Lee 1992). The influence of breed and sex on the score class was calculated with a two-way analysis of variance model (ANOVA, The MIXED Procedure) with the fixed factors breed, sex, and their interaction. To analyse the stability of the scores over time, we applied Spearman's rank correlation test on scores of the two test ages (The CORR Procedure).

3.3.3 Study III – Towards an easy applicable personality test

All statistical analyses were performed using SAS 9.3, SAS Institute Inc., USA. In a preliminary analysis, we checked for the influence of sex and weight on the parameters of the pulling test using a one-way analysis of covariance model (ANCOVA, The GLM Procedure) with the fixed factor sex and the co-variable weight, and further the influence of breed, sex, and their interaction on the parameters of the pulling test (Appendix 6). To connect the two tests, the influence of the RMSSD/SDNN-ratio, the PC scores, and the score class of the novel-object test on parameters measured during the pulling test was tested by an analysis of variance model (ANOVA, The MIXED Procedure) with the fixed factors RMSSD/SDNN-ratio, PC 1, PC 2, and score class. For the variables which were influenced by weight, sex or both, the random effects of weight at day of experiment, sex, or both were included. Posthoc tests were performed with a Tukey-Kramer correction for multiple testing (Appendix 7).

4 Summary of the studies

4.1 Study I – Pulling test methodology

In this study, an appliance was to be established that allows to objectively measuring escape behaviour and reluctance in young cattle restrained by being tethered on a halter, independently of the impact and rating of a more or less subjective observer or handler. While restrained, a tractive force-time diagram describing escape behaviour was recorded and later analysed with specifically developed software. To evaluate, 24 three-month-old calves were restrained by being tethered for 30 min on a halter that was connected to a force transducer. Tractive force, maximal tractive force, and the number of pulls, that the calves performed during 5 min-intervals, were calculated from the tractive force-time diagram. The multivariate results were analysed with a k-means-algorithm (function “kcca”) and a hierarchical clustering (function “hclust”) included in R version 2.12.1. The parameters tractive force, maximal tractive force, and number of pulls during 5-min intervals described the escape behaviour and reluctance of the calves. Especially in tractive force and number of pulls, most of the calves showed a similar behaviour pattern with a slight decrease in effort across the 30 min test. Still, several animals showed clearly higher values in these parameters. Both cluster analyses found two clusters, which allocated the same individuals into the two clusters. The clusters were balanced for sex (cluster 1: 4 male, 5 female calves; cluster 2: 8 male, 7 female calves) and did not differ significantly in weight of the animals ($t = 0.77$; $p = 0.452$). For the three measured parameters the mean values ± SD over time were distinctively different between the two clusters. Cluster 1 showed a clearly higher curve in all parameters than cluster 2. Both curves had the highest values at the beginning of the test, decreased over time, and showed a slight increase in the middle of the pulling test before decreasing even further towards the end. However, cluster 1 showed clearly higher values than cluster 2. Also, the variability in tractive force and number of pulls was smaller in cluster 2, although it consisted of more animals. It further displayed a more stable reaction level throughout the 30-min test period in spite of slight increases and decreases. With this newly developed method it was possible to detect differences in the animals’ escape behaviour patterns and reluctance with the measured parameters.

Katharina L Graunke (KG) wrote the following parts of the article: Introduction, Results, Discussion, and Animals and housing of Material and Methods; KG translated the parts “Measuring appliance and experimental outlay” and “Recording and parameters of tractive force” from German to English; KG executed the test on several animals; KG analysed the results; KG took part in the statistical analysis.

4.2 Study II – Multidimensional personality depiction

A depiction of personality was developed by analysing the behaviour and simultaneously measured heart rate variability during a novel-object test in calves. The test was conducted at two ages on an additional, small sample size to test for stability over time. Taking this combined multidimensional approach based on experimental original data, it was intended to provide foundational support for the theoretical framework suggesting two or more dimensions in animal personality. We tested 361 calves at 90 days *post natum* (dpn) in a novel-object test. We condensed numerous behaviours into fewer variables to describe temperament using a principal component analysis (PCA), and correlated these variables with simultaneously measured heart rate variability (HRV) data. The 15 observed behaviours were combined with the PCA to two principal components (PC), of which PC 1 represented behaviours in context with the novel object and PC 2 exploration of the open field (but not the novel object) and general activity. The PCs explained 46.8 % and 11.2 %, respectively, of the variation of the data. Each individual animal received a score in each PC calculated from their original behavioural data and the respective PC loading. After classifying the calves according to their scores into nine score classes (SC), we analysed the influence of SC on HRV. The variable giving most reliable information on the activity of the autonomous nervous system, the RMSSD/SDNN-ratio, was significantly different between the SCs and shifted towards parasympathetic activation compared to the base measurement in one SC and towards sympathetic activation in two other SCs. We could therefore describe the most distinct personality types by combining the ethological and physiological measurements: “neophobic/fearful – alert”, “interested – stressed”, “subdued/uninterested – calm”, and “neophilic/outgoing – alert”. To evaluate the stability of the scores, one needs to take all individuals into account as each individual – intermediate or distinct – has its own specific personality. The additionally tested 19 Charolais and 20 Holstein Friesian calves were evenly distributed in the scores plot and revealed no breed differences. During the repetition of the novel-object test 4 months after the first test, 42.1 % of the calves scored within 1 SD around their first score, 44.7 % scored within 1-2 SD, of which 88.2 % showed the greater change in only one dimension but not the other, and 13.2 % scored farther than 2 SD away from their first score. Correlation between the test ages was higher in PC 1 ($r = 0.36$. $p = 0.028$) than PC 2 ($r = 0.29$. $p = 0.079$). No animal changed from one extreme SC to the opposite SC. To conclude, distinct temperament types in calves could be described based on behavioural and physiological measures emphasising the benefits of a multidimensional approach.

Katharina L Graunke (KG) wrote the article with the exception of the part on HRV in the Introduction and most of the “Statistical analysis” of Material and Methods; KG executed the test on numerous animals; KG analysed the results; KG initiated the statistical analysis with a PCA; KG statistically analysed the data.

4.3 Study III – Towards an easy applicable personality test

In this study, data from an automated restraint test was correlated with a multidimensional depiction of cattle personality resulting from a standard behaviour test to take a first step in developing an easy applicable test for personality in cattle. By linking automatically measured data to ethological and physiological data, it was aimed to test whether the automated restraint test is a practicable way to test large numbers of individuals for their personality with maintained level of measure reliability. Data of 356 crossbreed calves tested at 90 and 91 dpn, respectively, in the in Study I developed pulling test was correlated with multidimensional personality types retrieved from a novel-object test (NO) including physiological measures of heart rate variability (HRV) in Study II. These and the HRV-measure RMSSD/SDNN-ratio were correlated with a generalised linear mixed model (The MIXED Procedure, SAS 9.3, SAS Institute Inc., USA) to measures of the pulling test. Tractive force, holding force, dwindling force, and total force were not influenced by weight and sex, whereas number of pulls was influenced by weight ($F = 4.27$, $p = 0.040$) and maximal tractive force was influenced by weight ($F = 48.6$, $p < 0.001$) and sex ($F = 4.02$, $p = 0.046$) with heavier calves pulling more often and with more maximal tractive force and with female calves pulling with greater force. The additionally tested breeds Charolais and Holstein Friesian did not differ in their pulling behaviour except for the holding force with the 19 Charolais calves holding stronger than the 20 Holstein Friesian calves ($F = 12.77$, $p < 0.001$). Number of pulls and maximal tractive force tended to correlate between the test ages ($r = 0.31$, $p = 0.056$; $r = 0.30$, $p = 0.067$). The behaviour parameters were combined to two PCs with a PCA, which explained 46.8 % and 11.2 %, respectively, of the variation in the data. We divided the calves into nine score classes, which significantly differed in the RMSSD/SDNN-ratio ($F = 5.04$; $p < 0.001$), hence in the activity of the autonomous nervous system. Tractive force was significantly influenced by the RMSSD/SDNN-ratio between NO and base measurement ($F = 4.23$, $p = 0.041$) with calves with a lower RMSSD/SDNN-ratio pulled with less force than calves with a higher ratio. While neither PC 1 nor PC 2 influenced any parameter, SC tended to influence the parameters of the pulling test except for holding force and maximal tractive force (tractive force: $F = 1.84$, $p = 0.070$; dwindling force: $F = 1.81$, $p = 0.075$; total force: $F = 1.84$, $p = 0.070$; number of pulls: $F = 1.74$, $p = 0.090$). We found the candidate measure tractive force for further investigation in developing an easy applicable automated test for measuring cattle's personality on large practice scale.

Katharina L Graunke (KG) wrote the article with the exception of the part "Measuring appliance and experimental outlay" and most of the "Statistical analysis" of Material and Methods; KG translated the part "Measuring appliance and experimental outlay" from German to English; KG executed the tests on numerous animals; KG designed the statistical analysis apart from the PCA; KG statistically analysed the data; KG analysed the results.

5 General discussion

Research with original data on animal personality has so far used one dimension to describe different personalities (Benus *et al.* 1989, Koolhaas *et al.* 1999, Boersma *et al.* 2009) whereas theoretical framework has suggested two or more dimensions to sufficiently describe them (Koolhaas *et al.* 2007, Réale *et al.* 2007, Koolhaas *et al.* 2010, Mendl *et al.* 2010, Coppens *et al.* 2010). In the past few years, studies have started to display behavioural data condensed into two to five dimensions, although except for one study in 2012 on fish (Meager *et al.* 2012), without characterising the individuals' personality (Müller & Schrader 2005, Bergvall *et al.* 2011, Koski 2011, Tanaka *et al.* 2012). To my knowledge, this thesis is therefore the first scientific study examining this topic so elaborately and the first to support the claims of the theoretical framework with original data of mammalian non-hominidae. As agricultural practice has become aware of the importance of animal personality for them, the need of an easy applicable more practicable behaviour test to predict an animal's personality has been addressed by developing an automated restraint test. The combination of behavioural observations and simultaneously recorded physiological data with the automated measurement of applied tractive force during restraint made it possible to take a first step towards a practicable and easy applicable personality test with the potential for a wide use in breeding stations as well as on farm level.

The methodology of the developed pulling test was established in Study I. This form of restraint test made it possible to physically measure the exact tractive force an individual applied over time in an easy applicable way. It can therefore be seen as the advancement of the widely practiced crush test, where an observer judges the test animal's reaction to the restraint using a predefined category scale. Although the crush test is said to work well in practice (pers. comm.), it is questionable which animals really are selected and which really are sorted out and what consequences this might entail for the future population as the one-dimensionality of this test again prohibits a differentiated analysis of an animal's personality. Further, the lacking objectivity and comparability between different farms can cause severe problems for its usage in breeding indices or for the comparison of animals of different origins. Therefore, a differentiated test excluding human-animal interaction like the developed pulling test is needed for practical purposes and for research on the topic of cattle personality.

In Study II, it was possible to retrieve two behavioural dimensions of personality with the conducted novel-object test; one that consisted of behaviours in context with the novel object (PC 1) and one that summarised exploration of the open field (but not the novel object) and activity behaviours (PC 2). These two behaviour dimensions were complemented by a third physiological dimension that gave additional information on the probable individual perception of the test situation from the HRV, especially from the RMSSD/SDNN-ratio. Only with this third physiological dimension it was possible to describe all distinct personality types with certainty, since behaviour alone could, e.g., not explain whether calves showing no interest in either novel object or open field were truly uninterested or possibly petrified. It further added valuable information on perception and the inner emotional world of the tested individual that often lacks in animal studies while it is a fundamental part in research on human personality. The perception dimension, however, is also crucial to animal welfare as it is indispensable to take into account when trying to provide good animal welfare.

In Study III, a fourth dimension, the reaction to being restraint, was added and was tried to be put in relation to the other three already measured dimensions. The perception dimension of the novel-object test was correlated with tractive force, opening up the possibility to predict some of an animal’s more complex personality traits by measurements during a completely different personality test. The character of this connection might be surprising; however, one should keep in mind that in the NO the test subjects could move freely. The restraint though forced them to stay at the spot and tied up. This lack of controllability of the situation might have triggered a panic reaction in individuals parasympathetically activated during the NO, while animals sympathetically activated in the NO did clearly not react in the same way to the restraint. The combined two behavioural dimensions in the form of the score classes gave hints for some relation between them and the measurements of the pulling test; however, this was a tendency and not significant. Therefore, more research needs to be done, possibly by applying more complex statistical analyses like the partial least squares projections to latent structures on data of the pulling test or by slight changes in its experimental set-up. Possibly though, the pulling test is not correlated with any other personality traits depicted in the novel-object test than the perception dimension.

The dimensions measured in the present thesis can be recognised in Réale *et al.*’s (2007) five categories “exploration – avoidance” (PC 1), “activity” (PC 2, tractive force, number of pulls), “shyness – boldness” (PC 2), or “sociability” (PC 2, tractive force, number of pulls) and in Koolhaas *et al.*’s (2010) and Mendl *et al.*’s (2010) arousal/stress reactivity dimension (RMSSD/SDNN-ratio). Although the descriptive evaluation of the different types of Koolhaas *et al.* (2010) and Mendl *et al.* (2010) fits well with our depiction of the found distinct temperament types, it cannot be concluded with certainty that their valence (Mendl *et al.* 2010) and coping strategy (Koolhaas *et al.* 2010) dimensions were indirectly measured in this study.

5.1 Critical review of the study design

The study design including recorded behaviours, live recording, the age of the test calves, a genetically limited sample (361 animals descending from 5 bulls and 26 cows), the sample size of repeatedly tested animals, and the lack of alternating common behaviour tests like the crush test are owed to this study being included in an already 5-year running breeding project at more or less no costs. The project demanded a strict protocol of treatment of the test animals, which naturally could not be altered 5 years into the project. Therefore, the latest possible time both male and female animals could be tested without the influence of a changed environment, of different housing systems, and different diets for males and females was before weaning and separating the sexes at about 100 dpn. Due to the part of research addressing nutritional and metabolic questions, it was further not possible to start testing either all individuals or at least one sex repeatedly after the project had been running for several years. If this project were designed to serve as research study on personality only, one should certainly have included one or several test repetitions on all individuals, record a few more detailed behaviours like head, ear, and tail posture, and could have freely chosen reasonable test ages, e.g., after developmental stages. Including more detailed behaviours into analysis (also at a later stage) would have only been possible by making videos of the tests. However, these limitations in design should not be understood as diminishing the results found in this study. One could rather argue that they were found despite the limitations which then would in fact strengthen the findings. Indeed, it has been postulated that external validity of results “will not be affected by standardization“ (Würbel 2000). This means, if the here reported results can be widely generalised, increased standardisation like testing in a shorter time period, during one season or month only, more detailed behaviour recording, etc. should have no impact on the overall results.

The comparison of behaviour tests repeatedly conducted at different reproduction states and therefore differing states of metabolic rates could have been enlightening as it has been found in coral reef fish that temperature increases, which influence metabolic rates, alter their personality (Biro *et al.* 2010). Of course, fish are ectotherms, but the correlation of metabolic rates and personality traits could have an effect in endotherms like mammals as well. In fact, it has been found that progress of day and temperature, respectively, (morning vs. afternoon) changed boldness in marmots (Petelle *et al.* 2013) and activity in cattle (Graunke *et al.* 2011). Although not reported, in this study we did not find any influence of season or year on the occurrence of personality types found in the novel-object test. It is imaginable that in endotherms the metabolic rate is more influenced by short term changes, but adapts to long term changes such as seasons rather well and is therefore less influenced by long term changes. One further has to be aware that temperatures throughout the years at the study site neither fell below the lower critical temperature (-31 °C for growing cattle, Christopherson

1984) nor exceeded heat stress temperature (24 °C for heifers, Carroll *et al.* 2013) for longer than a few days of the year, if at all when taking the overall higher thermo neutral temperatures for young into account (Christopherson 1984).

5.2 Plasticity in personality and its role for evolutionary success

Personality traits have been found to correlate with learning abilities in wild cavies and also their reversal learning skills; these, however, are conversely correlated (Guenther *et al.* 2013). The bolder and the more aggressive the cavy, the faster it learnt; vice versa the bolder and the more aggressive the animal, the slower it was in reversal learning (Guenther *et al.* 2013). Similar observations have also been reported in great tits, ravens, pigs, and rhesus macaques (Bolhuis *et al.* 2004, Coleman *et al.* 2005, Range *et al.* 2006, Titulaer *et al.* 2012). In an evolutionary context, it is advantageous to conserve contrary learning types as both have their advantages in different environments, especially when the environment the animals live in is very diverse and offers many changes throughout the years regarding food and predation density. The bolder and more explorative an individual, the faster it will find new food resources or mates, which is advantageous in a scarce environment and increases survival rate. At the same time, it also entails a higher predation risk, which decreases survival rate. Especially in a rich and prosperous environment, it can be disadvantageous when the higher risk of predation does not outweigh the increased survival rate by gathering more food. On population level, it is crucial to conserve contrary personality types to secure the overall survival despite various differing environmental preconditions. It is yet unknown, whether personality traits and learning ability are dependent on one another, because they are influenced by the same genes or genes with nearby location in the genome, or if learning abilities are the result of personality traits. However, although in pigs this correlation was found (Bolhuis *et al.* 2004), in a study on dwarf goats, another domesticated livestock species, this correlation could not be found (Langbein, unpublished data). The lacking correlation here, however, could have been lost due to the relaxed selection process during domestication (Price 1984).

In a very recent study, it was found that different personality traits were stable and plastic, respectively, during different periods of the ontogenesis (Petelle *et al.* 2013). While boldness was only stable during puberty in marmots, docility was stable at all ages and could even be predicted for adulthood from their juvenile docility (Petelle *et al.* 2013). It seems logic and useful for survival that some personality traits are stable during certain periods of the ontogenesis and plastic during the rest of the time while others become more and more stable, the older and more experienced an individual becomes. The lack of further knowledge on this topic should be kept in mind when interpreting the results on stability over time of this thesis. Plasticity leaves room for adaptation to differing environments occurring during the life time of an individual, while stable traits could be very beneficial for social behaviour making individuals predictable for others in their group. Factors such as high selection pressure may also favour animals with stable traits like, e.g., rigid maternal behaviour in wild species (Price 1984). This, however, complicates the research on personality in general, while at the same time it takes the pressure from results being only accepted as relevant for personality when they show at least moderate stability at different random times of measurement. A lack of consistency should not automatically be considered a mistake in measurement or lack of relevance for personality (although this could well be the case), but should be further investigated whether there could be ontogenetic reasons for its plasticity. In the future, the varying plasticity of different traits should also be taken into account in definitions of personality and temperament.

5.3 Domesticating personality

It is highly interesting to try investigating the role of domestication for personality types, the range of now existing personality types, and their occurrence. Logically, certain personality types were ought to be favoured during the domestication process as they were better accustomed to the new environment around humans. In pigs, e.g., it was found that active coping types occurred less often than reactive coping types (Zebunke *et al.* 2013). Especially docility towards humans was the first and foremost trait that was selected for. Whether these favoured types were actively supported by humans, more successful in the new surroundings, or both can most probably never be found out; however, this is in my opinion the less relevant question. Much more important is to find out, how the presumed shift of personality type occurrence came about, what this means in terms of allele frequency change, allele loss and acquisition, what mechanisms are behind these changes, and how they can be induced or avoided. The difficulty remains of how to investigate the past anagenesis of a species by domestication. It is probably safe to say that of most, if not all domesticated species, there exists no original, “undomesticated”, unchanged version of them; meaning the original genetic material of thousands of years back does no longer exist. For one thing, it is questionable whether one can know what the gene pool of the undomesticated species looked like and whether the original population that was domesticated represented the total wild population. But even if the domesticated population represented the total wild population, it would be ignoring environmental changes, (genetic) adaptations, and such simple genetics like crossing-overs and mutations occurring over thousands of years that both domesticated and undomesticated strains underwent. Additionally, the ancestral undomesticated species of some of our domesticated animals (aurochs, Przewalski's horse, wild boar, wolf, mouflon; Sambraus 1991) have been (almost) extinct. The now living individuals originate from very few individuals, which have been protected from extinction. In Przewalksi's horses, e.g., the now existing population originates from about 50 individuals captured in the late 1800s and early 1900s and is therefore affected by inbreeding, besides the fact that it is unknown whether the captured horses had not been mixed with domesticated horses (Volf 1996). Hence, their gene pool has hardly anything to do with the original one several thousand years ago. Also, experiments with individuals from breeding back or dedomestication (Heck cattle, Tarpans, Mustang horses, dingoes, etc.) that sometimes are considered the original, undomesticated versions of domesticated animals will and cannot help shedding light on these questions, because of the aforementioned genetic changes and limitations.

One possibility to study influences of domestication on personality is to start artificially domesticating a wild species first and foremost for tameness as has been done since 1959 with silver foxes originally kept for fur production (Trut 1999). Within only 40 generations of selecting for non-aggressive behaviour towards humans, there have been found not only behaviour alterations (Kukekova *et al.* 2008), but also brain gene expression changes (Lindberg *et al.* 2005) as has also been reported in the comparison of dogs and wolves (Saetre *et al.* 2004). This, however, may carry certain difficulties with it such as a limited and pre-selected gene pool, especially in the wild individuals, and very artificial circumstances for domestication. Another way could be comparative genetic and behavioural studies of species that include individuals living within or close to human settlements as well as individuals living with rare contact to humans. Examples for such species are foxes, wild boars, sparrows, or badgers. Individuals living within or close to human settlements either chose themselves or some ancestors of them chose to live in this environment while those without or rare contact to humans did the opposite. These animals could be seen as an early stage domesticated subpopulation of which the original wild version without human contact should still have the original genetic and phenotypic variety. A third thinkable method to investigate the domestication process could be the comparison of specific traits that individuals of the same domesticated species were selected for differently. For example, the comparison of dairy cattle breeds with beef cattle breeds regarding maternal behaviour could offer tremendous information on the influence of relaxed vs. high selection pressure on certain traits. Dairy cows are extremely pampered and cared for regarding maternal behaviour allowing individuals with completely lacking maternal behaviour reproduce successfully many times as humans take care of their offspring immediately after birth. In fact, individuals with stronger maternal instincts are more or less consciously sorted out either because they become dangerous for the handlers when protecting their calf or because they silently suffer from their loss and produce less milk or become ill. Beef cows on the other hand receive probably almost as high a selection pressure as their wild ancestors regarding maternal behaviour, because they are rigidly selected for easy, unsupported births and good maternal characteristics like sufficient milk production and protection of their young. However, as it was reported in fish that presence of predators influenced the personality trait boldness (Brown *et al.* 2005), one needs to be careful when designing such experiments with cattle as the environment might play a result changing role. Thus, jumping to a conclusion could be avoided.

Overall, I cannot imagine that any method will enlighten the process of domestication fully and the role it plays for personality, genetics, or anything else. The mechanisms behind domestication, however, could be understood, even though different disciplines of nature and life sciences will have to work together as they did and do for research on evolution. After all, domestication is nothing else than a small and tiny part of a special evolution.

5.4 Perception and valence

As has been shown in this thesis, including and implementing the physiological dimension of heart rate variability (HRV) into measurements of personality is crucial for the understanding of animal personality. It is surprising that HRV can hardly been found in research on animal personality so far. The simultaneous measurement of HRV and behaviour has not been done in personality research until now, probably as a lot of studies on animal personality have been carried out on classic model animal species like mice, rats, fish, or birds. All of them do not fit for non-invasive HRV measurements simply due to their size. Hence, one needs to revert to well-studied species, which are large enough for those measurements, used to handling by humans to accept wearing HRV equipment, and which can be kept on a larger scale under similar conditions. The larger mammalian livestock species (cattle, sheep, pigs, goats) seem to be the perfect species that come in mind for those studies. The here presented study can therefore serve as a model for future studies on multidimensional animal personality including the perception dimension. The perception or valence of a situation has not been measured directly in this study, though, which should be kept in mind. Only humans can formulate their current perception of a situation with words. Correlating such formulations with simultaneously measured HRV could shed tremendous light on how HRV data can be interpreted more than, e.g., a high RMSSD showing high activity of the parasympathetic branch of the autonomous nervous system, which in turn is known to regulate digestion, sleep, blood pressure, etc. (Eckert *et al.* 2000) and then drawing conclusions from that. After all, there could be a vast difference in perception between a slight increase in RMSSD (comforting, sleepy) compared to an extreme increase (numbing, unable to move) as between a slight increase in SDNN (exciting, thrilling) vs. an extreme increase (panicking, strong urge to move). This should even be kept in mind when interpreting the results of this thesis. Calves with slight increases might experience the situation as positive, while those with high increases might experience them as negative stress, regardless of the RMSSD or the SDNN increasing. This, however, cannot be appreciated without humans formulating their perception of such differences in activation and conclude in analogy of that for other species.

Questionable is also the plasticity of HRV within the same situation over different life stages and whether it should be included as an own personality trait instead of a helping tool to understand perception in animals. In humans, HRV has been found to be very stable, however, measured over 24 hours and not in specific situations (Kleiger *et al.* 1991). In another study, most HRV parameters have been found to be stable in specific situations, except for SDNN, which was in fact very unstable; this, however, on patients with stable coronary artery disease (Tarkiainen *et al.* 2005). Respiratory sinus arrhythmia, a component of HRV, is used in research on infant temperament development (Richards & Cameron 1989, Beauchaine 2001), mother-infant-bond (Propper & Moore 2006), or behaviour disorders (Porges *et al.* 1996). In adult personality research, it is also widely used (Sher 2005, Craig *et al.* 2009). Maybe it would be possible to do studies on infants with behaviour tests more similar to those used in animals to be able to compare the two. That could result in interesting comparisons between human infant personality and animal personality and could shed light on how similar or different human and non-human animal personality really is.

5.5 Consequences for animal welfare

The Five Freedoms of Animal Welfare from 1979 include freedom from (1) hunger and thirst, (2) discomfort, (3) pain, injury, or disease, (4) fear and distress, and (5) freedom to express normal behaviour (Farm Animal Welfare Council 1979). Additionally, the 12 standards for welfare explicitly include the presence of “positive emotions such as security and contentment” (Welfare Quality® 2009a). The latter is only possible to take into consideration when adding the animals’ perspective, their perception, of their situation to the calculation of welfare. Still, also the freedom to express normal behaviour “by providing sufficient space, proper facilities and company of the animal’s own kind” (Farm Animal Welfare Council 1979) must include the animals’ perspective when taking animal welfare seriously. Otherwise humans decide what is “proper” and “sufficient” for the animals. The problem with this approach is obvious as it is very sensitive to mistakes, misinterpretation, abuse, anthropomorphism, amongst others. Even the “company of the animal’s own kind” is valued very differently by different individuals (cp. Réale *et al.* 2007: category (5) sociability). Thus, the essentiality of the individual perception cannot be emphasised enough.

Also, when composing a group one should acknowledge personality types to avoid putting together a group of, e.g., aggressive individuals, which will not stop fighting with each other, or of very shy animals, which will be stressed by the slightest change in the environment or handling and reassure each other in their fear. Ultimately, a well composed group should cause least stress, injury, but also least danger for the handlers; it should create a calm atmosphere of little stress, which in turn saves individuals from being slaughtered due to severe injuries from fights, their danger for handlers, etc. I would recommend a balanced mix of all personality types and try to avoid extreme types of any kind. According to one's own taste and needs, the "average" personality type of the group can lean towards the type one prefers. In other words, if I want to keep my animals in a similar environment or housing system with regular contact to humans, but want to move them regularly through the facilities, I would prefer a more active type as average that is more easily scared (cp. paper of Study II: SC I, II, IV). If I want to keep my animals in varying environments with little contact to humans and also want to move them once in a while, I would recommend a less active but curious type that keeps calm but does not stop moving (cp. paper of Study II: SC VIII, IX). The first named suggestion would be for classic dairy cattle herds and the second for classic beef cattle herds. To successfully handle the two types and herds, one necessarily needs to alter one's actions, meaning the distance to the herd while moving them, and expectations, meaning the expected speed of the herds' movements.

Anyone who takes animal welfare seriously will have to include the animals' personality into his or her calculation of welfare. Individual perception is either part of personality or at least influenced by personality, depending on future research results and definitions of personality. Independent of these results, both personality and perception play an equally central role in animal welfare as nutrition and health. Similar amounts of knowledge as on these topics need to be available on perception and personality.

5.6 Cattle personality in practice

In agricultural practice, traits influencing productivity were and are most important for breeding, recruitment, and finishing. So far (with some exceptions, cp. International Beef Recording Scheme 2012), these traits only included physiological characteristics like milk yield, average daily weight gain, weaning weight of the young, meat quality, etc. The fact that these traits are or may be influenced by or correlated with certain personality traits slowly reaches agricultural practice; further, the awareness of certain personality types being less dangerous for handlers and being less stressed by handling routines, environmental changes, etc. than other types slowly arises. However, for the latter it is still feared that the inclusion or choice of certain preferable personality types might have a negative effect on productivity and thus on the economic value of the farm or breed. This is a legitimate objection that researchers cannot ignore. Therefore, future research on possible limitations in productivity of certain personality types must be conducted.

As has been mentioned in the General introduction, some personality traits have been included into breeding goals of official beef cattle breeders (Zuchtbetrieb Zachert 2013, Traditional Herefords 2013) and the personality characteristic docility has even been implemented into beef cattle breeding plans in Australia (International Beef Recording Scheme 2012). Nowadays, the crush test and flight speed test are advised to be used for selecting beef animals (Gaden *et al.* 2004, International Beef Recording Scheme 2012) and could have been implemented in the test design relatively easily. However, although the crush test has first been reported in 1993 (Grandin 1993), in practice it has been suggested only in 2004 together with the flight speed test (Gaden *et al.* 2004) and has not been applied on a wider scale until the recent years (International Beef Recording Scheme 2012), i.e. until after most test animals of this study had been tested. The willingness of practice to work and acknowledge personality traits in cattle now demands tests giving more detailed information about personality than a crush test or flight speed test can offer. In order to being applicable in practice, these tests need to be simple to execute, quickly done, and easily integrated into routine handling. Only in that way, they have a realistic chance to being widely employed. The here implemented novel-object test is a very widely used behaviour test. While experimental set-ups are not entirely different, there still are differences in length, recorded behaviours, open-field size and shape, etc. These differences along with the difficulties of a behaviour test of that kind (such as the need for a trained observer, intra- and inter-observer-reliability (Welfare Quality® 2009b), etc.) make this test understandably unpractical and unrealistic for practice. Therefore, we need to further develop an easy applicable personality test for practice such as the here developed restraint test.

6 Conclusion

This thesis was designed to phenotype personality traits in cattle, to develop an easy applicable automated behaviour test, and to make a first attempt in correlating the phenotyped personality of the test animals with data of the automated behaviour test that could be used in practice. After developing a behaviour test, the pulling test, that can be applied by anyone in the world without further knowledge and training in behaviour observation and analysis, the test calves were successfully phenotyped in their personality using a standard behaviour test, the novel-object test, and simultaneous measurements of their heart rate variability. This so far unique methodology in personality research in animals made it possible to effectively describe four distinct personality types using a multidimensional analysis of the observed behaviours and the activity of the autonomous nervous system, indirectly measured by heart rate variability. Merging data from the pulling test with data of the novel-object test, tractive force applied in the pulling test was found to correlate with the activation of the autonomous nervous system during the novel-object test. Although the character of this connection was surprising at first, tractive force can be seen as a candidate measure that appeared promising for further development of the automated pulling test.

Only broad, profound, and deep knowledge on every aspect of an animal, including personality and its complexity lets us make correct and knowledgeable choices concerning the handling and management of animals, whether that would be animals living under human care, or animals living in the wild. The impact of humans, human settlements, environmental changes, etc. probably affects different personality types differently. One should at least be aware of this effect, if not trying to countervail it for animals living in the wild and to select individuals that are most capable to live in the different conditions under human care. The consequences of this knowledge are to be included into all aspects of animal husbandry, research, experiments, welfare, handling, game management, ecological action plans, and renaturation plans.

References

Bagby RM, Schuller DR, Levitt AJ, Joffe RT, Harkness KL, 1996. Seasonal and non-seasonal depression and the five-factor model of personality. *J Affect Disord* **38,** 89-95.

Beauchaine T, 2001. Vagal tone, development, and Gray's motivational theory: Toward an integrated model of autonomic nervous system functioning in psychopathology. *Dev Psychopathol* **30,** 183-214.

Behrends S, Miller R, Rouquette F, Randel R, Warrington B, Forbes T, Welsh T, Lippke H, Behrends J, Carstens G, Holloway J, 2009. Relationship of temperament, growth, carcass characteristics and tenderness in beef steers. *Meat Sci* **81,** 433-438.

Bell AM, Sih A, 2007. Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). *Ecol Lett* **10,** 828-834.

Benhajali H, Boivin X, Sapa J, Pellegrini P, Boulesteix P, Lajudie P, Phocas F, 2010. Assessment of different on-farm measures of beef cattle temperament for use in genetic evaluation. *J Anim Sci* **88,** 3529-3537.

Benus RF, Bohus B, Koolhaas JM, van Oortmerssen GA, 1989. Behavioral Strategies of Aggressive and Nonaggressive Male-Mice in Active Shock Avoidance. *Behav Processes* **20,** 1-12.

Bergvall UA, Schapers A, Kjellander P, Weiss A, 2011. Personality and foraging decisions in fallow deer, *Dama dama*. *Anim Behav* **81,** 101-112.

Biro PA, Beckmann C, Stamps JA, 2010. Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proc R Soc B* **277,** 71-77.

Boersma G, Scheurink A, Wielinga P, Steimer T, Benthem L, 2009. The passive coping Roman Low Avoidance rat, a non-obese rat model for insulin resistance. *Physiol Behav* **97,** 353-358.

Boissy A, Bouissou MF, 1988. Effects of Early Handling on Heifers Subsequent Reactivity to Humans and to Unfamiliar Situations. *Appl Anim Behav Sci* **20,** 259-273.

Boissy A, Manteuffel G, Jensen MB, Moe RO, Spruijt B, Keeling LJ, Winckler C, Forkman B, Dimitrov I, Langbein J, Bakken M, Veissier I, Aubert A, 2007. Assessment of positive emotions in animals to improve their welfare. *Physiol Behav* **92,** 375-397.

Boivin X, Gilard F, Egal D, 2009. The effect of early human contact and the separation method from the dam on responses of beef calves to humans. *Appl Anim Behav Sci* **120,** 132-139.

Boivin X, Le Neindre P, Chupin JM, Garel JP, Trillat G, 1992. Influence of Breed and Early Management on Ease of Handling and Open-Field Behavior of Cattle. *Appl Anim Behav Sci* **32,** 313-323.

Bolhuis JE, Schouten WGP, de Leeuw JA, Schrama JW, Wiegant VA, 2004. Individual coping characteristics, rearing conditions and behavioural flexibility in pigs. *Behav Brain Res* **152,** 351-360.

Brambell FWR, 1965. *Report of the Technical Committee to enquire into the welfare of animals kept under intensive livestock husbandry systems*. London, UK: H.M.S.O.

Broom DM, 1988. The scientific assessment of animal welfare. *Appl Anim Behav Sci* **20,** 5-19.

Brown C, Jones F, Braithwaite V, 2005. In situ examination of boldness-shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. *Anim Behav* **70,** 1003-1009.

Budaev SV, 1997. "Personality" in the guppy (*Poecilia reticulata*): A correlational study of exploratory behavior and social tendency. *J Comp Psychol* **111,** 399-411.

Budaev SV, 2010. Using Principal Components and Factor Analysis in Animal Behaviour Research: Caveats and Guidelines. *Ethology* **116,** 472-480.

Burrow H, 1997. Measurements of temperament and their relationships with performance traits of beef cattle. *Anim Breed Abstr* **65,** 477-495.

Burrow HM, 1998. The effects of inbreeding on productive and adaptive traits and temperament of tropical beef cattle. *Livest Prod Sci* **55,** 227-243.

Buss D, Hawley PH, 2011. *The Evolution of Personality and Individual Differences*. Oxford: Oxford University Press.

Calkins SD, Keane SP, 2004. Cardiac Vagal Regulation across the Preschool Period: Stability, Continuity, and Implications for Childhood Adjustment. *Dev Psychobiol* **45,** 101-112.

Carroll JA, Burdick Sanchez NC, Chaffin R, Chase J, Coleman SW, Spiers DE, 2013. Heat-tolerant versus heat-sensitive *Bos taurus* cattle: influence of air temperature and breed on the acute phase response to a provocative immune challenge. *Domest Anim Endocrin* **45,** 163-169.

Carter AJ, Marshall HH, Heinsohn R, Cowlishaw G, 2012. How not to measure boldness: novel object and antipredator responses are not the same in wild baboons. *Anim Behav* **84,** 603-609.

Cattell RB, 1966. The Scree Test For The Number Of Factors. *Multivar Behav Res* **1,** 245-276.

Christopherson RJ, 1984. Management and housing of animals in cold environments. In: *Stress physiology in livestock* (ed. Yousef MK), pp. 175-194. Boca Raton, Florida: CRC Press.Inc.

Coleman K, Tully LA, McMillan JL, 2005. Temperament correlates with training success in adult rhesus macaques. *Am J Primatol* **65,** 63-71.

Comrey AL, Lee HB, 1992. *A First Course in Factor Analysis*, Second edn. New York, USA: Psychology Press.

Coppens C, de Boer SF, Koolhaas JM, 2010. Coping styles and behavioural flexibility: towards underlying mechanisms. *Philos Trans R Soc B* **365,** 4021-4028.

Craig A, Tran Y, Hermens G, Williams LM, Kemp A, Morris C, Gordon E, 2009. Psychological and neural correlates of emotional intelligence in a large sample of adult males and females. *Person Indivual Diff* **46,** 111-115.

Dammhahn M, 2012. Are personality differences in a small iteroparous mammal maintained by a life-history trade-off? *Proc R Soc B* **279,** 2645-2651.

David M, Auclair Y, Cezilly F, 2011. Personality predicts social dominance in female zebra finches, *Taeniopygia guttata*, in a feeding context. *Anim Behav* **81,** 219-224.

de Raad B, 1998. Five big, big five issues: Rationale, content, structure, status, and crosscultural assessment. *Europ Psychol* **3,** 113-124.

Dickson DP, Barr GR, Johnson LP, Wieckert DA, 1970. Social Dominance and Temperament of Holstein Cows. *J Dairy Sci* **53,** 904-907.

Dingemanse NJ, van der Plas F, Wright J, Réale D, Schrama M, Roff DA, van der Zee E, Barber I, 2009. Individual experience and evolutionary history of predation affect expression of heritable variation in fish personality and morphology. *Proc R Soc B* **276,** 1285-1293.

Dingemanse NJ, Wolf M, 2010. Recent models for adaptive personality differences: a review. *Philos Trans R Soc B* **365,** 3947-3958.

Eccard JA, Rödel HG, 2011. Optimizing temperament through litter size in short-lived, iteroparous mammals in seasonal environments. *Dev Psychobiol* **53,** 585-591.

Eckert R, Burggren W, French K, Randall D, 2000. *Tierphysiologie*, 3rd edn. Stuttgart: Georg Thieme Verlag.

Eysenck HJ, 1991. Dimensions of personality: 16, 5 or 3?-Criteria for a taxonomic paradigm. *Person Indivual Diff* **12,** 773-790.

Farm Animal Welfare Council, 1979. *Press Statement December 5, 1979.* http://www.fawc.org.uk/pdf/fivefreedoms1979.pdf.

Fell LR, Colditz IG, Walker KH, Watson DL, 1999. Associations between temperament, performance and immune function in cattle entering a commercial feedlot. *Aust J Exp Agric* **39,** 795-802.

Fordyce G, Dodt RM, Wythes JR, 1988a. Cattle temperaments in extensive beef herds in northern Queensland. 1. Factors affecting temperament. *Aust J Exp Agric* **28,** 683-687.

Fordyce G, Goddard ME, Tyler R, Williams G, Toleman MA, 1985. Temperament and bruising of *Bos indicus* cross cattle. *Aust J Exp Agric* **25,** 283-288.

Fordyce G, Wythes JR, Shorthose WR, Underwood DW, Shepherd RK, 1988b. Cattle temperaments in extensive beef herds in northern Queensland. 2. Effect of temperament on carcass and meat quality. *Aust J Exp Agric* **28,** 689-693.

Forkman B, Boissy A, Meunier-Salauen M, Canali E, Jones R, 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiol Behav* **92,** 340-374.

Forkman B, Furuhaug IL, Jensen P, 1995. Personality, Coping Patterns and Aggression in Piglets. *Appl Anim Behav Sci* **45,** 31-42.

Fraser D, 1974. The vocalizations and other behaviour of growing pigs in an "open field" test. *Appl Anim Ethol* **1,** 3-16.

Gaden B, Burrow H, Pettiford S, 2004. Flight time - a practical way to improve feedlot performance, temperament and tenderness. *Producing Quality Beef* 26-28.

Gagne JR, 2013. Personality and Temperament. In: *eLS*. Chichester, UK: John Wiley & Sons, Ltd.

Gallup GG, Suarez SD, 1980. An Ethological Analysis of Open-Field Behaviour in Chickens. *Anim Behav* **28,** 368-378.

Gauly M, Mathiak H, Hoffmann K, Kraus M, Erhardt G, 2001. Estimating genetic variability in temperamental traits in German Angus and Simmental cattle. *Appl Anim Behav Sci* **74,** 109-119.

Gibbons JM, Lawrence AB, Haskell MJ, 2011. Consistency of flight speed and response to restraint in a crush in dairy cattle. *Appl Anim Behav Sci* **131,** 15-20.

Goddard ME, Beilharz RG, 1986. Early Prediction of Adult Behavior in Potential Guide Dogs. *Appl Anim Behav Sci* **15,** 247-260.

Gomez A, Gomez R, 2002. Personality traits of the behavioural approach and inhibition systems: associations with processing of emotional stimuli. *Person Indivual Diff* **32,** 1299-1316.

Goodall J, 1964. Tool-Using and Aimed Throwing in a Community of Free-Living Chimpanzees. *Nature* **201,** 1264-1266.

Gorsuch RL, 1983. *Factor Analysis*, 2nd edn. Hillsdale, New Jersey: Erlbaum.

Gosling SD, 2001. From mice to men: What can we learn about personality from animal research? *Psychol Bull* **127,** 45-86.

Gosling SD, John OP, 1999. Personality Dimensions in Nonhuman Animals: A Cross-Species Review. *Curr Directions Psychol Sci* **8,** 69-75.

Gracceva G, Koolhaas JM, Groothuis TG, 2011. Does the Early Social Environment Affect Structure and Consistency of Personality in Wild-Type Male Rats? *Dev Psychobiol* **53,** 614-623.

Grandin T, 1993. Behavioral Agitation During Handling of Cattle Is Persistent Over Time. *Appl Anim Behav Sci* **36,** 1-9.

Graunke KL, Schuster T, Lidfors LM, 2011. Influence of weather on the behaviour of outdoor-wintered beef cattle in Scandinavia. *Livest Sci* **136,** 247-255.

Grignard L, Boivin X, Boissy A, Le Neindre P, 2001. Do beef cattle react consistently to different handling situations? *Appl Anim Behav Sci* **71,** 263-276.

Guenther A, Brust V, Dersen M, Trillmich F, 2013. Learning and Personality Types Are Related in Cavies (*Cavia aperea*). *J Comp Psychol* No Pagination Specified.

Hare B, Brown M, Williamson C, Tomasello M, 2002. The domestication of social cognition in dogs. *Science* **298,** 1634-1636.

Hawley PH, 2011. The Role of Competition and Cooperation in Shaping Personality: An Evolutionary Perspective on Social Dominance, Machiavellianism, and Childern's Social Development. In: *The Evolution of Personality and Individual Differences* (eds. Buss D, Hawley PH), pp. 61-85. Oxford: Oxford University Press.

Herczeg G, Garamszegi LZ, 2012. Individual deviation from behavioural correlations: a simple approach to study the evolution of behavioural syndromes. *Behav Ecol Sociobiol* **66,** 161-169.

Herrelko ES, Vick SJ, Buchanan-Smith HM, 2012. Cognitive Research in Zoo-Housed Chimpanzees: Influence of Personality and Impact on Welfare. *Am J Primatol* **74,** 828-840.

Hoppe S, Brandt HR, König S, Erhardt G, Gauly M, 2010. Temperament traits of beef calves measured under field conditions and their relationships to performance. *J Anim Sci* **88,** 1982-1989.

Horn JL, 1965. A rationale and test for the number of factors in factor analysis. *Psychometrika* **30,** 179-185.

Hudson R, Bautista A, Reyes-Meza V, Montor JM, Rödel HG, 2011. The effect of siblings on early development: A potential contributor to personality differences in mammals. *Dev Psychobiol* **53,** 564-574.

Huntingford FA, 1976. Relationship Between Anti-Predator Behavior and Aggression Among Conspecifics in 3-Spined Stickleback, *Gasterosteus aculeatus*. *Anim Behav* **24,** 245-260.

International Beef Recording Scheme, 2012. *A Basic Guide to Breedplan EBVs*. Armidale, Australia: BREEDPLAN, University of New England.

Kaiser HF, 1960. The Application of Electronic Computers to Factor Analysis. *Educ Psychol Meas* **20,** 141-151.

Kaminski J, Bräuer J, Call J, Tomasello M, 2009. Domestic dogs are sensitive to a human's perspective. *Behaviour* **146,** 979-998.

Kaminski J, Pitsch A, Tomasello M, 2013. Dogs steal in the dark. *Anim Cogn* **16,** 385-394.

Kilgour RJ, Melville GJ, Greenwood PL, 2006. Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity of humans, restraint and novelty. *Appl Anim Behav Sci* **99,** 21-40.

Klefoth T, Skov C, Krause J, Arlinghaus R, 2012. The role of ecological context and predation risk-stimuli in revealing the true picture about the genetic basis of boldness evolution in fish. *Behav Ecol Sociobiol* **66,** 547-559.

Kleiger RE, Bigger JT, Bosner MS, Chung MK, Cook JR, Rolnitzky LM, Steinman R, Fleiss JL, 1991. Stability over time of variables measuring heart rate variability in normal subjects. *Am J Cardiol* **68,** 626-630.

Koolhaas JM, de Boer SF, Buwalda B, van Reenen K, 2007. Individual variation in coping with stress: A multidimensional approach of ultimate and proximate mechanisms. *Brain Behav Evol* **70,** 218-226.

Koolhaas JM, de Boer SF, Coppens C, Buwalda B, 2010. Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Front Neuroendocrinol* **31,** 307-321.

Koolhaas JM, Korte SM, de Boer SF, van der Vegt BJ, van Reenen CG, Hopster H, de Jong IC, Ruis MAW, Blokhuis HJ, 1999. Coping styles in animals: current status in behavior and stress-physiology. *Neurosci Biobehav Rev* **23,** 925-935.

Korsten P, Mueller JC, Hermannstadter C, Bouwman KM, Dingemanse NJ, Drent PJ, Liedvogel M, Matthysen E, van Oers K, van Overveld T, Patrick SC, Quinn JL, Sheldon BC, Tinbergen JM, Kempenaers B, 2010. Association between DRD4 gene polymorphism and personality variation in great tits: a test across four wild populations. *Mol Ecol* **19,** 832-843.

Koski SE, 2011. Social personality traits in chimpanzees: temporal stability and structure of behaviourally assessed personality traits in three captive populations. *Behav Ecol Sociobiol* **65,** 2161-2174.

Kramer RSS, Ward R, 2012. Cues to Personality and Health in the Facial Appearance of Chimpanzees (*Pan troglodytes*). *Evol Psychol* **10,** 320-337.

Kukekova AV, Trut LN, Chase K, Shepeleva DV, Vladimirova AV, Kharlamova AV, Oskina IN, Stepika A, Klebanov S, Erb HN, Acland GM, 2008. Measurement of Segregating Behaviors in Experimental Silver Fox Pedigrees. *Behav Genet* **38,** 185-194.

Kutsukake N, Teramoto M, Homma S, Mori Y, Matsudaira K, Kobayashi H, Ishida T, Okanoya K, Hasegawa T, 2012. Individual Variation in Behavioural Reactions to Unfamiliar Conspecific Vocalisation and Hormonal Underpinnings in Male Chimpanzees. *Ethology* **118,** 269-280.

Lanier JL, Grandin T, Green RD, Avery D, McGee K, 2000. The relationship between reaction to sudden, intermittent movements and sounds and temperament. *J Anim Sci* **78,** 1467-1474.

Lansade L, Bouissou MF, Erhard HW, 2008a. Fearfulness in horses: A temperament trait stable across time and situations. *Appl Anim Behav Sci* **115,** 182-200.

Lansade L, Bouissou MF, Erhard HW, 2008b. Reactivity to isolation and association with conspecifics: A temperament trait stable across time and situations. *Appl Anim Behav Sci* **109,** 355-373.

Le Neindre P, Trillat G, Sapa J, Menissier F, Bonnet JN, Chupin JM, 1995. Individual-Differences in Docility in Limousin Cattle. *J Anim Sci* **73,** 2249-2253.

Leisch F, 2006. A toolbox for K-centroids cluster analysis. *Comput Stat Data An* **51,** 526-544.

Leshem M, 2011. Low dietary sodium is anxiogenic in rats. *Physiol Behav* **103,** 453-458.

Lindberg J, Björnerfeldt S, Saetre P, Svartberg K, Seehuus B, Bakken M, Vilà C, Jazin E, 2005. Selection for tameness has changed brain gene expression in silver foxes. *Curr Biol* **15,** R915-R916.

Locurto C, 2007. Individual differences and animal personality. *Comp Cogn Behav Rev* **2,** 67-78.

Lowe SE, Bradshaw JWS, 2001. Ontogeny of individuality in the domestic cat in the home environment. *Anim Behav* **61,** 231-237.

Manteca X, Deag JM, 1993. Individual differences in temperament of domestic animals: a review of methodolgy. *Anim Welf* **2,** 247-268.

Marshall-Pescini S, Passalacqua C, Miletto Petrazzini ME, Valsecchi P, Prato-Previde E, 2012. Do Dogs (*Canis lupus familiaris*) Make Counterproductive Choices Because They Are Sensitive to Human Ostensive Cues? *Plos One* **7,** e35437.

Mayer JD, 1986. How mood influences cognition. In: *Advances in Cognitive Science* (ed. Sharkey NE), pp. 290-314. Chichester: Ellis Horwood Limited.

Meager JJ, Ferno A, Skjæraasen JE, Jarvi T, Rodewald P, Sverdrup G, Winberg S, Mayer I, 2012. Multidimensionality of behavioural phenotypes in Atlantic cod, *Gadus morhua*. *Physiol Behav* **106,** 462-470.

Mendl M, Burman OH, Paul ES, 2010. An integrative and functional framework for the study of animal emotion and mood. *Proc R Soc B* **277,** 2895-2904.

Miklósi Á, Kubinyi E, Topál J, Gácsi M, Virányi Z, Csányi V, 2003. A simple reason for a big difference: Wolves do not look back at humans, but dogs do. *Curr Biol* **13,** 763-766.

Mills DS, Marchant-Forde JN, McGreevy PD, Morton DB, Nicol CJ, Phillips CJC, Sandøe P, Swaisgood RR, 2010. *The Encyclopedia of Applied Animal Behaviour & Welfare*. Wallingford, UK: CAB International.

Moberg GP, Wood VA, 1982. Effect of Differential Rearing on the Behavioral and Adrenocortical-Response of Lambs to A Novel Environment. *Appl Anim Ethol* **8,** 269-279.

Müller R, Schrader L, 2005. Behavioural consistency during social separation and personality in dairy cows. *Behaviour* **142,** 1289-1306.

Müller R, von Keyserlingk MAG, 2006. Consistency of flight speed and its correlation to productivity and to personality in *Bos taurus* beef cattle. *Appl Anim Behav Sci* **99,** 193-204.

Murphey RM, Duarte FAM, Penedo MCT, 1980. Approachability of Bovine Cattle in Pastures - Breed Comparisons and A Breed × Treatment Analysis. *Behav Genet* **10,** 171-181.

Nawroth C, Ebersbach M, von Borell E, 2013. Are juvenile domestic pigs (*Sus scrota domestica*) sensitive to the attentive states of humans?-The impact of impulsivity on choice behaviour. *Behav Processes* **96,** 53-58.

Nissen H, 1956. Individuality in the behavior of chimpanzees. *Amer Anthropol* **58,** 407-413.

O'Connor BP, 2000. SPSS and SAS programs for determining the number of components using parallel analysis and Velicer's MAP test. *Behav Res Methods Instrum Comput* **32,** 396-402.

Official Journal of the European Union, 2008. Consolidated versions of the Treaty on European Union and the Treaty on the Functioning of the European Union. **C 115,** 47-199.

Pajor F, Muranyi A, Szentleleki A, Tozser J, Poti P, 2010. Effect of temperament of ewes on their maternal ability and their lambs' postweaning traits in Tsigai breed. *Arch Tierz - Arch Anim Breed* **53,** 465-474.

Petelle MB, McCoy DE, Alejandro V, Martin JGA, Blumstein DT, 2013. Development of boldness and docility in yellow-bellied marmots. *Anim Behav* **86,** 1147-1154.

Plusquellec P, Bouissou MF, Le Pape G, 2001. Early predictors of dominance ability in heifers (*Bos taurus*, L.) of the Hérens breed. *Behaviour* **138,** 1009-1031.

Porges SW, Doussard-Roosevelt JA, Portales AL, Greenspan SI, 1996. Infant regulation of the vagal "brake" predicts child behavior problems: A psychobiological model of social behavior. *Dev Psychobiol* **29,** 697-712.

Price EO, 1984. Behavioral Aspects of Animal Domestication. *Q Rev Biol* **59,** 1-32.

Propper C, Moore GA, 2006. The influence of parenting on infant emotionality: A multi-level psychobiological perspective. *Dev Rev* **26,** 427-460.

Range F, Bugnyar T, Schloegl C, Kotrschal K, 2006. Individual and sex differences in learning abilities of ravens. *Behav Processes* **73,** 100-106.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ, 2007. Integrating animal temperament within ecology and evolution. *Biol Rev* **82,** 291-318.

Reefmann N, Kaszas FB, Wechsler B, Gygax L, 2009. Physiological expression of emotional reactions in sheep. *Physiol Behav* **98,** 235-241.

Richards JE, Cameron D, 1989. Infant heart-rate variability and behavioral developmental status. *Infant Behav Dev* **12,** 45-58.

Rödel HG, Meyer S, 2011. Early development influences ontogeny of personality types in young laboratory rats. *Dev Psychobiol* **53,** 601-613.

Rödel HG, von Holst D, 2009. Features of the early juvenile development predict competitive performance in male European rabbits. *Physiol Behav* **97,** 495-502.

Rosati AG, Hare B, 2013. Chimpanzees and Bonobos Exhibit Emotional Responses to Decision Outcomes. *Plos One* **8,** e63058.

Saetre P, Lindberg J, Leonard JA, Olsson K, Pettersson U, Ellegren H, Bergström TF, Vilà C, Jazin E, 2004. From wild wolf to domestic dog: gene expression changes in the brain. *Mol Brain Res* **126,** 198-206.

Sambraus HH, 1991. *Nutztierkunde*. Stuttgart: Ulmer.

Sambraus HH, 2006. *Exotische Rinder*. Stuttgart: Ulmer.

Santucci AK, Silk JS, Shaw DS, Gentzler A, Fox NA, Kovacs M, 2008. Vagal tone and temperament as predictors of emotion regulation strategies in young children. *Dev Psychobiol* **50,** 205-216.

Sher L, 2005. Type D personality: the heart, stress, and cortisol. *QJM* **98,** 323-329.

Sibbald AM, Erhard HW, McLeod JE, Hooper RJ, 2009. Individual personality and the spatial distribution of groups of grazing animals: An example with sheep. *Behav Processes* **82,** 319-326.

Sih A, Bell AM, Johnson JC, Ziemba RE, 2004. Behavioral syndromes: An integrative overview. *Q Rev Biol* **79,** 241-277.

Sluyter F, Bult A, Lynch CB, van Oortmerssen GA, Koolhaas JM, 1995. A Comparison Between House Mouse Lines Selected for Attack Latency Or Nest-Building - Evidence for A Genetic-Basis of Alternative Behavioral Strategies. *Behav Genet* **25,** 247-252.

Sluyter F, Korte SM, Bohus B, van Oortmerssen GA, 1996. Behavioral stress response of genetically selected aggressive and nonaggressive wild house mice in the shock-probe/defensive burying test. *Pharmacol Biochem Behav* **54,** 113-116.

Stamps JA, Groothuis TGG, 2010. Developmental perspectives on personality: implications for ecological and evolutionary studies of individual differences. *Philos Trans R Soc B* **365,** 4029-4041.

Sullivan EC, Mendoza SP, Capitanio JP, 2011. Similarity in Temperament Between Mother and Offspring Rhesus Monkeys: Sex Differences and the Role of Monoamine Oxidase-A and Serotonin Transporter Promoter Polymorphism Genotypes. *Dev Psychobiol* **53,** 549-563.

Sulloway FJ, 2011. Why Siblings Are Like Darwin's Finches: Birth Order, Sibling Competition, and Adaptive Ddivergence within the Family. In: *The Evolution of Personality and Individual Differences* (eds. Buss D, Hawley PH), pp. 86-119. Oxford: Oxford University Press.

Svartberg K, Forkman B, 2002. Personality traits in the domestic dog (Canis familiaris). *Appl Anim Behav Sci* **79,** 133-155.

Svartberg K, 2002. Shyness-boldness predicts performance in working dogs. *Appl Anim Behav Sci* **79,** 157-174.

Svartberg K, 2005. A comparison of behaviour in test and in everyday life: evidence of three consistent boldness-related personality traits in dogs. *Appl Anim Behav Sci* **91,** 103-128.

Tanaka S, Young JW, Halberstadt AL, Masten VL, Geyer MA, 2012. Four factors underlying mouse behavior in an open field. *Behav Brain Res* **233,** 55-61.

Tarkiainen TH, Timonen KL, Tiittanen P, Hartikainen JEK, Pekkanen J, Hoek G, Ibald-Mulli A, Vanninen EJ, 2005. Stability over time of short-term heart rate variability. *Clin Auton Res* **15,** 394-399.

Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996. Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *Eur Heart J* **17,** 354-381.

Titulaer M, van Oers K, Naguib M, 2012. Personality affects learning performance in difficult tasks in a sex-dependent way. *Anim Behav* **83,** 723-730.

Toates F, 1998. The interaction of cognitive and stimulus-response processes in the control of behaviour. *Neurosci Biobehav Rev* **22,** 59-83.

Traditional Herefords, 2013. *Traditional Hereford Breeders Club - The original efficient beef breed.* http://www.traditionalherefords.org/#/the-breed/4565836802.

Trillmich F, Hudson R, 2011. The Emergence of Personality in Animals: The Need for a Developmental Approach. *Dev Psychobiol* **53,** 505-509.

Trut LN, 1999. Early canid domestication: The farm-fox experiment. *American Scientist* **87,** 160-169.

Tschirren B, Bensch S, 2010. Genetics of personalities: no simple answers for complex traits. *Mol Ecol* **19,** 624-626.

van Reenen CG, O'Connell NE, van der Werf JTN, Korte SM, Hopster H, Jones RB, Blokhuis HJ, 2005. Responses of calves to acute stress: Individual consistency and relations between behavioral and physiological measures. *Physiol Behav* **85,** 557-570.

Veissier I, Aubert A, Boissy A, 2012. Animal welfare: a result of animal background and perception of its environment. *Anim Front* **2,** 7-15.

Velicer WF, 1976. Determining Number of Components from Matrix of Partial Correlations. *Psychometrika* **41,** 321-327.

Verbeek MEM, Drent PJ, Wiepkema PR, 1994. Consistent Individual-Differences in Early Exploratory-Behavior of Male Great Tits. *Anim Behav* **48,** 1113-1121.

Vereinigte Informationssysteme Tierhaltung w.V. VIT, 2012. *Trends Fakten Zahlen 2012.* http://www.vit.de/fileadmin/user_upload/wirsindvit/jahresberichte/vit-JB2012-gesamt_140dpi.pdf.

Visser EK, van Reenen CG, Engel B, Schilder MBH, Barnveld A, Blokhuis HJ, 2003. The association between performance in show-jumping and personality traits earlier in life. *Appl Anim Behav Sci* **82,** 279-295.

Voisinet BD, Grandin T, O'Connor SF, Tatum JD, Deesing MJ, 1997a. *Bos indicus*-cross feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. *Meat Sci* **46,** 367-377.

Voisinet BD, Grandin T, Tatum JD, OConnor SF, Struthers JJ, 1997b. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. *J Anim Sci* **75,** 892-896.

Volf J, 1996. *Das Urwildpferd*, 4th edn. Heidelberg: Spektrum Akademischer Verlag.

von Borell E, Langbein J, Despres G, Hansen S, Leterrier C, Marchant-Forde J, Marchant-Forde R, Minero M, Mohr E, Prunier A, Valance D, Veissier I, 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals - A review. *Physiol Behav* **92,** 293-316.

Waiblinger S, Boivin X, Pedersen V, Tosi MV, Janczak AM, Visser EK, Jones RB, 2006. Assessing the human-animal relationship in farmed species: A critical review. *Appl Anim Behav Sci* **101,** 185-242.

Weiss A, Inoue-Murayama M, King JE, Adams MJ, Matsuzawa T, 2012. All too human? Chimpanzee and orang-utan personalities are not anthropomorphic projections. *Anim Behav* **83,** 1355-1365.

Weiss SM, Wadsworth G, Fletcher A, Dourish CT, 1998. Utility of ethological analysis to overcome locomotor confounds in elevated maze models of anxiety. *Neurosci Biobehav Rev* **23,** 265-271.

Welfare Quality®, 2009a. *Principles and criteria of good Animal Welfare, Welfare Quality® Science and society improving animal welfare.* http://www.welfarequality.net/downloadattachment/41858/19874/WQ%20_%20Factsheet_10_07_eng2.pdf.

Welfare Quality®, 2009b. *Training in the Welfare Quality® assessment protocols, Welfare Quality® Science and society improving animal welfare.* http://www.welfarequality.net/downloadattachment/43299/20259/2009-Nov_adapted_leaflet WQ Training Assessment Protocols.pdf.

Wolf M, van Doorn GS, Leimar O, Weissing FJ, 2007. Life-history trade-offs favour the evolution of animal personalities. *Nature* **447,** 581-584.

Wolf M, Weissing FJ, 2010. An explanatory framework for adaptive personality differences. *Philos Trans R Soc B* **365,** 3959-3968.

Würbel H, 2000. Behaviour and the standardization fallacy. *Nature Genetics* **26,** 263.

Yayou K, Ito S, Yamamoto N, Kitagawa S, Okamura H, 2010. Relationships of stress responses with plasma oxytocin and prolactin in heifer calves. *Physiol Behav* **99,** 362-369.

Zebunke M, Nürnberg G, Repsilber D, Puppe B, 2013. Der Backtest beim Schwein - Individuelle Variation im Verhalten oder Verhaltensstrategie? *KTBL Schrift* **503,** 188-197.

Zuchtbetrieb Zachert, 2013. *Charolais + Blonde aus dem Havelland.* http://www.zachert-zucht.de/zuchtziel.php?id=3.

Acknowledgements

First of all, I would like to thank all the involuntary study subjects, the “SEGFAM-calves” and the “pure-breds”. The range of their behaviour in the exact same situation is astonishing.

I thank Dr Jan Langbein and Prof Dr Birger Puppe for giving me the opportunity and their confidence to work on this controversially discussed and highly complicated topic of animal personality with the mountains of data that emerged from the different tests. They always had an open ear for my suggestions and guided me through all shallows of this thesis with many helpful comments on analyses, interpretation, and manuscripts.

I am deeply grateful to Prof Dr Roland Gerstmeier for accepting me as doctoral candidate on short notice and for the smooth handling of the administrative work.

There are many colleagues without whom this thesis would not have been possible: Katrin Siebert and Ursula Engel tested numerous calves already long before my graduation, Dr Peter-Christian Schön programmed several crucial and time-saving software applications for measurements, analyses, and data processing, Dieter Sehland and Kurt Wendland constructed the measuring appliance of the pulling test and tested plenty of calves, Heinz Deike constructed and built the open field, the animal caretakers helped capturing the calves. I thank you all.

Dr Gerd Nürnberg and Dr Dirk Repsilber are entitled to many thanks for their statistical support and for their commitment to making the analyses the best possible.

My office “inmates” Dr Lisette Leliveld and Jenny Stracke are gratefully appreciated for the friendly atmosphere, Dr Sandra Düpjan and Dr Elisa Wirthgen for many inspiring conversations about our research, research in general, and lots of other important topics, and the extended coffee gang for chatting, moaning, and laughing with me over a Milchschäumchen.

Most importantly, I thank my family and friends. I do not know what I would do without them:

My parents Gabriele and Michael and my sister Theresa for their never ending support: I have no doubt we will always be able to tell us the plain truth and trust each other;

my dear friends Martin Kunze for being such a good team player especially during our time as representatives of the doctoral candidates and for stoically bearing my southern temperament, Dr Michael Oster for many cheerful, inspiring, meaningful, and philosophical conversations, Annelie Braune for the many hours of joy, laugh, and sorrow we share, Carolin Werner for being my constant for almost 30 years now, and Tatjana Schweizer, my southern companion in “mis”fortune, for her sympathies during our PhD time.

They have an open ear for me at all times and help me solicitously through rough times.

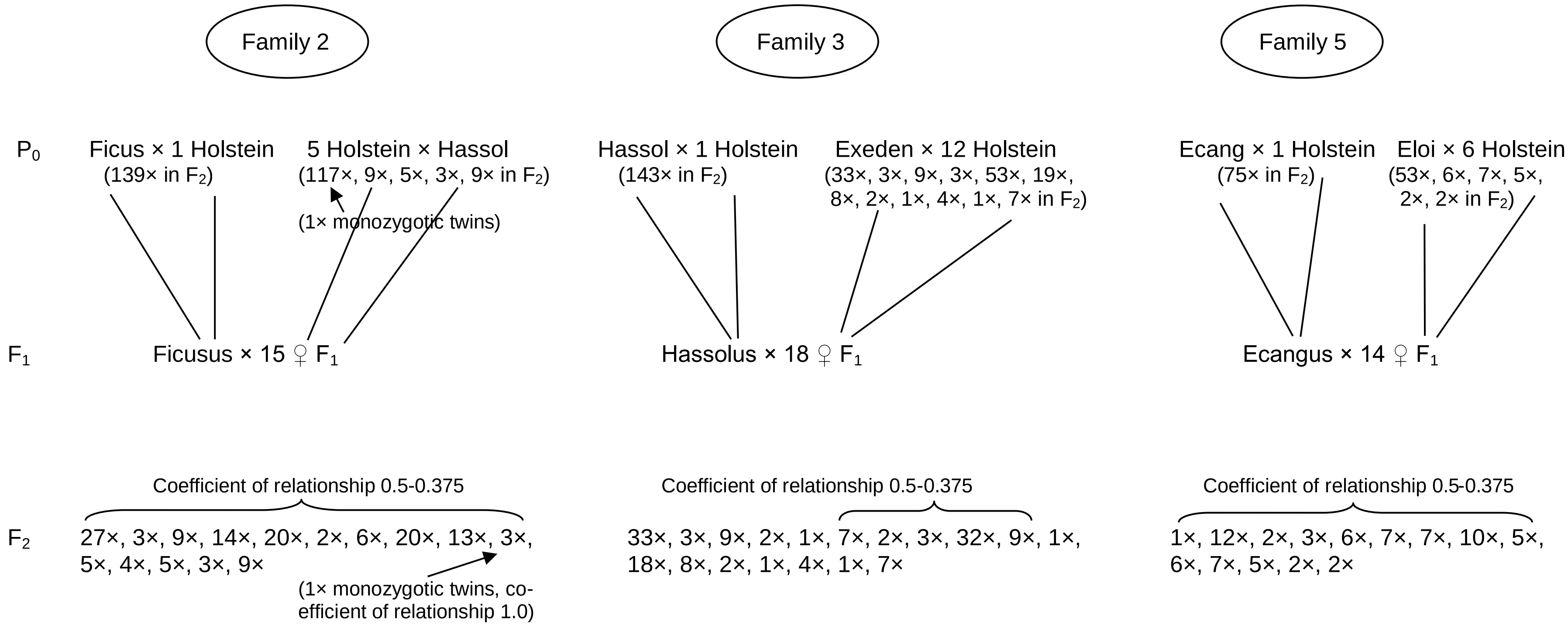
There are three other personalities I would like to acknowledge: Charlie with his endless dedication, his work attitude is adorable; Fritzi, my brave little fighter, I cannot imagine how frightening the world may seem to him; still, he overcomes all his fears.

Nico, I am so lucky he was part of my life. Together we have grown. He is sadly missed.

My time as a doctoral candidate was financed by the German agriculture network PHENOMICS, grant no. 0315536G, supported by the Federal Ministry of Education and Research, Germany.

Appendix

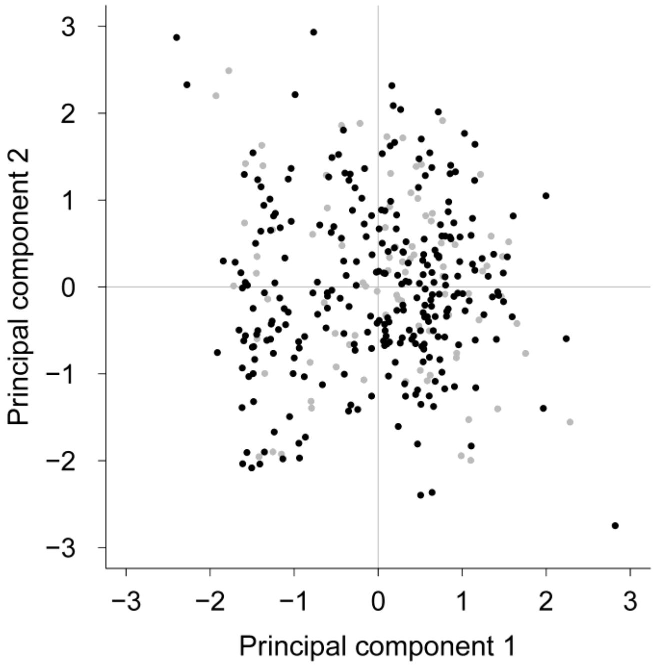
Appendix 1: Genealogy of the breeding project SEGFAM, which was designed to segregate genes and chromosomes from one another from the beef breed Charolais and the dairy breed Holstein Friesian; names in the parental generation (P_0) refer to the Charolais bulls and names in the F_1-generation are their sons used for breeding the F_2-generation, numbers in parenthesis declare how often the females (separated with commas) above or in the generation before are represented in the F_2-generation.



Coefficient of relationship $r = \sum (0.5)^L$; L = number of generations involved

		F_1 paternal		F_1 maternal	
Full siblings:		$r = 1 * 0.5^2$	+	$1 * 0.5^2$	= 0.5
4 identical P_0-animals, but 2 different F_1-mothers, mothers full siblings:		$r = 1 * 0.5^2$	+	$½ * 0.5^2$	= 0.375
3 identical P_0-animals, but 2 different F_1-mothers, mothers half siblings:		$r = 1 * 0.5^2$	+	$¼ * 0.5^2$	= 0.3125
Grandfather Hassol identical, F_1-mothers Family 2 and Hassolus half siblings:	Family 2:	$r = 0 * 0.5^2$	+	$¼ * 0.5^2$	= 0.0625
	Family 3:	$r = ¼ * 0.5^2$	+	$0 * 0.5^2$	= 0.0625

Appendix 2: Distribution of 272 calves with heart rate variability data (black dots) and 89 calves without heart rate variability data (grey dots) in the scores plot of the novel-object test.



Appendix 3: R-script of the “kcca” and “hclust” functions used for analysis in Study I.

```
X <- dat

## Clustering-kmeans and evaluating cluster silhouttes:
########################################################
library(flexclust) ## provides clustering with arbitrary distance functions
library(cluster)   ## provides the Silhouette function
library(e1071)     ## provides the corrected Rand index
X.scaled <- scale(X,scale = TRUE, center = TRUE)
distMat <- dist(X.scaled)
set.seed(1)
n.trial <- 20
res <- matrix(nrow=n.trial,ncol=19)
cll <- matrix(nrow=24,ncol=n.trial)
par(mfrow=c(4,5))
for(trial in 1:n.trial){
  cl <- sapply(2:20, function(i) kcca(X.scaled, k=i)@cluster)
  cll[,trial] <- cl[,1]
  res[trial,] <- apply(cl, 2, function(i) summary(silhouette(i,
distMat))$avg.width)
  barplot(res[trial,], ylab = "Average Silhouette width", xlab = "K",
names.arg = 2:20)
}

write.table (cll, file="D:\\Temp\\zugehoerigkeit.dat", sep="\t")

par(mfrow=c(1,1))
sums <- apply(res,2,sum)
barplot(sums,names.arg=2:20)
write.table (sums, file="D:\\Temp\\Test.dat", sep="\t")
postscript(file="D:\\Temp\\Clust_KMeans.eps",paper="special",horizontal=FAL
SE,
           width=5,height=5)
image(abs(barplot(sums,names.arg=2:20)))
dev.off()

print(paste("best number of clusters:",which(sums==max(sums))+1))

## Clustering-hclust:
########################################################
plot(hclust(distMat,method="average"))
plot (hclust (distMat,method="complete"))
dendo <- hclust(distMat,method="complete")
postscript(file="D:\\Temp\\Cluster.eps",paper="special",horizontal=FALSE,
           width=5,height=5)
plot(dendo)
dev.off()
```

Appendix 4: SAS-script used for the principal component analysis in Study II.

```
data test2;
set sasuser.SEGtest2;
run;

title 'Correlations Test2';
proc corr data=test2 outs=CorrOutsTest2;
      var d_kontakt--d_segObj_seg1;
   run;

proc iml;
reset noname;

/*  Specify the data for the analyses: */
USE work.CorrOutsTest2;  read all var _num_ into whole;  cr =
whole[4:nrow(whole),];

/* computes the correlation matrix, if necessary. */
if (nrow(raw) > 1) then do;
ncases = nrow(raw);
nvars  = ncol(raw);
ones = j(ncases,1,1);
xi1 = ( 1 / ncases) * t(ones);
nm1 = 1 / (ncases-1);
vcv = nm1 * (t(raw)*raw - ((t(raw[+,])*raw[+,])/ncases));
d = inv(diag(sqrt(vecdiag(vcv))));
cr = (d * vcv * d);
end;

/* MAP test computations */
call eigen (eigval,eigvect,cr);
loadings = eigvect * sqrt(diag(eigval));
nvars  = ncol(cr);
fm = j(nvars,2,-9999);
fm[1,2] = (ssq(cr) - nvars)/(nvars*(nvars-1));
fm4 = fm;
fm4[1,2] = (sum(cr##4)-nvars)/(nvars*(nvars-1));
do m = 1 to nvars - 1;
a = loadings[,1:m];
partcov = cr - (a * t(a));
d = diag( 1 / (sqrt(vecdiag(partcov))) );
pr = d * partcov * d;
fm[m+1,2] = (ssq(pr)-nvars) / (nvars*(nvars-1));
fm4[m+1,2] = (sum(pr##4)-nvars)/(nvars*(nvars-1));
end;

/* identifying the smallest fm value & its location (= the of factors) */
minfm = fm[1,2];
nfacts = 0;
minfm4 = fm4[1,2];
nfacts4 = 0;
do s = 1 to nrow(fm);
fm[s,1] = s - 1;
if ( fm[s,2] < minfm ) then do;
minfm = fm[s,2];
nfacts = s - 1;
end;
if ( fm4[s,2] < minfm4 ) then do;
minfm4 = fm4[s,2];
nfacts4 = s - 1;
end;end;
```

```
print, "Velicer's Minimum Average Partial (MAP) Test:";
print, "Eigenvalues", eigval[format=12.4];
labels = ("  "||"squared"||"power4");
print,, "Average Partial Correlations", (fm || fm4[,2]) [colname=labels
format=f12.4];
print, "The smallest average squared partial correlation is",
minfm[format=f12.4];
print, "The smallest average 4rth power partial correlation is",
minfm4[format=f12.4];
print, "The Number of Components According to the Original (1976) MAP Test
is", nfacts[format=f12.4];
print, "The Number of Components According to the Revised (2000) MAP Test
is", nfacts4[format=f12.4];

quit;



proc factor data= CorrOutsTest2  method=PRIN  n=2  priors=one scree score
outstat=score2
           rotate=varimax  MSA  ;
var d_kontakt--d_segObj_seg1 ;     /*alle Merkmale (15) */
run;quit;

proc score data= test2 score=score2 out=tierscore2;
var d_kontakt--d_segObj_seg1 ;     /*alle Merkmale (15) */
id Kalb_Nr;
run;
```

Appendix 5: SAS-script used for the two-way analysis of variance in Study II; example with the parameter heart rate (HR).

```
data SEGTempTypHRV;
set sasuser.SEGTempTypHRV;
run;


proc mixed data=SEGTempTypHRV method=reml;
class Sex TempTyp9T2;
model T2D_HR=Sex TempTyp9T2 TempTyp9T2*Sex /ddfm=kr;
lsmeans Sex TempTyp9T2 TempTyp9T2*Sex / pdiff cl adjust=tukey;

ods output      lsmeans=SASUSER.LSM
                Diffs=SASUSER.Diffs
                fitStatistics=SASUSER.fitstat
                Tests3=SASUSER.Type3_Tests;
run;
```

Appendix 6: SAS-script used for the analysis of the pulling data in Study III.

```
data zug;
set sasuser.Zugtest;
run;

title 'Einfluss Gewicht Geschlecht auf ZugTot';
proc glm data=zug;
        class sex;
        model pFtot--maxFtot= Gewicht_Zugtest_kg sex/ss3 ;
        lsmeans sex / stderr pdiff adjust=Tukey;

ods output      ModelANOVA=SASUSER.ANOVA
                lsmeans=SASUSER.LSM
                Diff=SASUSER.Diff;
run;
```

Appendix 7: SAS-script used to combine pulling data and ethological and physiological data from the novel-object test in Study III; example tractive force was similarly used for holding force, dwindling force, and total force alike.

```
title'Tractive force';
proc mixed data=zug method=reml;
class TempTyp9;
model pFtot=Ratio_RMSSD_SDNN Factor1 Factor2 TempTyp9 /ddfm=kr;
lsmeans TempTyp9 / pdiff cl adjust=tukey;

ods output      lsmeans=SASUSER.LSM
                Diffs=SASUSER.Diffs
                fitStatistics=SASUSER.fitstat
                Tests3=SASUSER.Type3_Tests;
run;

title'Number of pulls';
proc mixed data=zug method=reml;
class TempTyp9;
model Nr_Pulltot= Ratio_RMSSD_SDNN Factor1 Factor2 TempTyp9 /ddfm=kr;
random Gewicht_Zugtest_kg / type=AR(1);
lsmeans TempTyp9 / pdiff cl adjust=tukey;

ods output      lsmeans=SASUSER.LSM
                Diffs=SASUSER.Diffs
                fitStatistics=SASUSER.fitstat
                Tests3=SASUSER.Type3_Tests;
run;

title'Maximal tractive force';
proc mixed data=zug method=reml;
class TempTyp9 sex;
model maxFtot=Ratio_RMSSD_SDNN Factor1 Factor2 TempTyp9 /ddfm=kr;
random Gewicht_Zugtest_kg / type=AR(1);
random sex / type=CS;
lsmeans TempTyp9 / pdiff cl adjust=tukey;

ods output      lsmeans=SASUSER.LSM
                Diffs=SASUSER.Diffs
                fitStatistics=SASUSER.fitstat
                Tests3=SASUSER.Type3_Tests;
run;
```

Study I

Pulling test methodology

I

Journal of Agricultural Science (2013), **151**, 141–149.
doi:10.1017/S0021859612000408

ANIMAL RESEARCH PAPER

Objectively measuring behaviour traits in an automated restraint-test for ungulates: towards making temperament measurable

K. L. GRAUNKE[1], J. LANGBEIN[1], D. REPSILBER[2] AND P-C. SCHÖN[1]*

[1] *Leibniz Institute for Farm Animal Biology (FBN), Research Unit Behavioural Physiology, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany*
[2] *Leibniz Institute for Farm Animal Biology (FBN), Research Unit Genetics and Biometry, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany*

(*Received 8 February 2012; accepted 19 March 2012; first published online 27 April 2012*)

SUMMARY

The personality of an animal is described by traits that cause consistent actions and reactions to environmental stimuli. An important part of personality is the reaction to unpleasant or uncontrollable situations. Methods described in the literature to measure personality in animals are often based on measuring or rating escape behaviour in these situations. In the methods described, human handlers are frequently part of the experiment or the animals' personalities are scored by humans. Thus, these methods are at least partly subjective.

In the current study, an appliance to measure objectively the escape behaviour of ungulates and their reluctance during an uncontrollable situation (restraint) with a rather simple and comprehensible methodology is presented using a force transducer with adequate peripheral equipment. While the animals were restrained, a tractive force-time diagram describing escape behaviour was recorded and later analysed with software developed specifically.

To evaluate this newly developed technical method, 24 three-month-old calves were restrained by being tethered for 30 min on a halter that was connected to the force transducer. From the tractive force-time diagram, tractive force, maximal tractive force and the number of pulls that the calves performed during 5-min intervals were calculated. The multivariate results were analysed with a *k*-means-algorithm (function 'kcca') and a hierarchical clustering (function 'hclust') included in R version 2.12.1.

Both analyses revealed two clearly separated clusters including the same individuals in each analysis. The animals of cluster 1 showed a continuously higher reaction level than those of cluster 2 with a strong reaction in the beginning, a short decrease before increasing during the middle of the experiment and a final decrease at the end of the test. The animals of cluster 2 had a lower and quite steady reaction level throughout the experiment, although even here a slight increase during the middle of the experiment could be detected before a final decrease towards the end of the test was shown. There was no significant difference in weight between the two clusters.

The results showed that this newly developed method was able to detect differences in the animals' escape behaviour patterns and reluctance with the measured parameters.

INTRODUCTION

The general strategy an individual employs to act with and react to environmental stimuli describes his or her personality and is thought to be innate and consistent over time (Grandin 1993; Grignard *et al.* 2001) and in different situations (Forkman *et al.* 2007). Individual differences in behaviour between animals can not only be caused by sex, age, reproductive status and environment; hence, they reveal the personality of an individual (Manteca & Deag 1993; Locurto 2007). In cattle, personality traits influence the ease of handling (Matthews *et al.* 1997; Burrow 1998), live weight gains in feedlots (Voisinet *et al.* 1997*a*; Fell *et al.* 1999) and on pasture (Fordyce *et al.* 1985, 1988*a*), carcass damage (Fordyce *et al.* 1988*b*) and meat quality

* To whom all correspondence should be addressed. Email: Schoen@fbn-dummerstorf.de

(Voisinet *et al.* 1997*b*; Fordyce *et al.* 1988*b*). Since the welfare of an animal depends on its ability to cope with its environment (Broom 1988), personality is an important factor in animal welfare and in breeding. Some official breeders have already added certain personality traits to their breeding goals (e.g. Fleischrind Stölln GmbH 2007; Traditional Herefords 2008).

According to Manteca & Deag (1993) there are three ways of assessing personality-relevant behaviours: (i) standard behavioural observation; (ii) observers' rating with the help of predefined categories; and (iii) behavioural tests. In many of these tests, human interaction with the tested animals is inevitable or part of the tests themselves (for descriptions of common methods of personality trait and temperament measurements, see, e.g. Manteca & Deag 1993; Burrow 1997; Lanier *et al.* 2000; Waiblinger *et al.* 2006; Forkman *et al.* 2007). Some methods are therefore at least partly subjective and judgemental, especially when categories are used. According to the mood-biased judgement hypothesis, human judgements will be positive during a good mood and negative during a bad mood (Mayer 1986). Thus, when measuring personality traits, the results of judgemental methods are always influenced by the mood of the observer during the rating and by what the observer thinks is a positive or a negative judgement of the animals. Thus, inter- and even intra-observer reliability are difficult to obtain (Welfare Quality® 2009); however, an objective, reliable and observer- and mood-independent method of measuring personality traits is required, especially for practical purposes such as when animals are selected for breeding.

The measurement or rating of the motivation of an animal to elude an unpleasant or frightening situation either while restrained or while moving freely is common for many behavioural tests, such as the crush-test or the human-approach-test. The behaviour shown in these situations is either fear-related escape behaviour or an expression of the animal's dislike of the situation. It describes an important part of personality (Kilgour *et al.* 2006; Benhajali *et al.* 2010) and with regard to restraint situations, it is characterized by three main features: the number of escape attempts per time unit, the maximal force and the force per time unit. However, the often-used crush-test measures the behaviour indirectly by having observers judge the strength, frequency and duration of escape attempts and rating the animals according to a predefined category scale (Grandin 1993; Kilgour *et al.* 2006; Benhajali *et al.* 2010; Gibbons *et al.* 2011). Other tests developed, such as the docility test, can be time-consuming and rather complicated to execute (Le Neindre *et al.* 1995; Boivin *et al.* 2009), and human interaction with the animals (Boissy & Bouissou 1988) and observer-dependent rating are fundamental parts of the tests (Grandin 1993).

In the current study, an appliance is introduced that allows objective measurement of the escape behaviour and reluctance in young cattle restrained by being tethered on a halter, independently of the impact and rating of a more or less subjective observer or handler.

MATERIALS AND METHODS

Animals and housing

Animals in an existing breeding experiment were used to test this new measuring appliance. Twenty-four calves (12 male, 12 female) of the F_2 generation of a cross-breeding of Holstein–Friesian and Charolais were tested at 90 days (±3 days) of age. All calves were bred via embryo transfer into unrelated Holstein Friesian heifers as recipient mothers and born and tested between 2004 and 2010.

The calves were kept in various small groups of up to nine animals of similar age separate from their recipient mothers from day one. Pens were 6 × 7 m and were covered with deep litter. Each pen contained two hay racks, one milk feeder, one concentrate feeder and one drinking trough for water supply. Until 90 days of age, the calves were not subject to any other experiment, and handling did not exceed routine handling by the animal keepers except in the case of animals requiring treatment for sickness.

After weaning, the animals were weighed at an age of 111 days (±3 days). The weight at the day of the experiment was calculated with the help of the average daily weight gain from birth to weaning and the exact age at the experiment. On average the calves weighed 115 kg (range: 89–144 kg, s.d. ±13 kg) at the day of the experiment.

Measuring appliance and experimental outlay

To measure escape behaviour and reluctance in calves, the animals' expended power was recorded during a restraint situation (further called pulling-test) with the force transducer Megatron KT1400 (Megatron Elektronik AG & Co., Putzbrunn, Germany; Fig. 1). Its measuring principle is a DMS Wheatstone bridge.

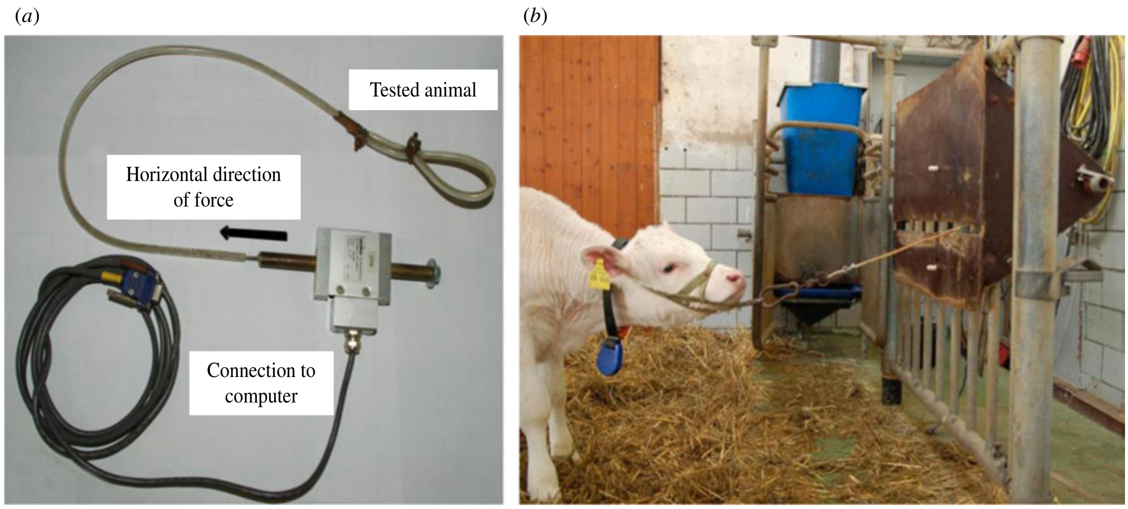


Fig. 1. (*a*) Force transducer Megatron KT1400 with the cable leading to the tested animal and the computer-interface connection; (*b*) a calf restrained during the pulling-test, put on a halter and tied with the cable to the force transducer hidden behind the wooden shield to the right.

With a signal of 0–5 V, it is calibrated from 0 to 1 kN, and it is useful for applications with traction force because of its S-beam shape. An M8 internal screw thread is used for force application.

The force transducer was connected with an analogue/digital interface (NI PCI-6503, National Instruments Germany GmbH, Munich, Germany) to a PC (Fig. 1*a*) and was built onto a metal bar of the home pen of the animals so that the cable elongated the force direction of the transducer (see pulling calf in Fig. 1*b*), which was essential to avoid resultant force.

During the pulling-test the tested calf stayed in one-half of its home pen, while the pen mates were confined to the other half of the pen using metal bars to avoid falsifying the measurement. Visual, acoustic and olfactory contact between the test calf and its pen mates therefore persisted, in the hope of minimizing the effects and influences of isolation. The test calves were captured, put on a halter and tied to the cable leading to the force transducer (Fig. 1*b*). The software recording the tractive force developed for the current study was then initiated and all human handlers left the barn. After a 30 min recording period, a handler entered the barn, released the test calf and reunited it with its pen mates, which were allowed back into the experimental part of the pen.

All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Ministry of Agriculture, the Environment and Consumer Protection of the federal state Mecklenburg-Vorpommern, Germany.

Recording and parameters of tractive force

Data measured by the force transducer were digitized with a sampling rate of 20 Hz and were transferred to a computer. A software program using LabView 6.1 (National Instruments GmbH Germany, Munich, Germany) was developed to save the tractive force-time diagram and to calculate the parameters from this diagram. The software included two applications: a recording program, which saved the force-time diagram with a time stamp during the complete recording period (Fig. 2), and an analysing program, which analysed the force-time diagrams in the lab. With the recording program one could freely choose sampling rate and the start time and duration of the recording period. The analysing program made it possible to choose interval length for analysis.

In the experiment, the 30-min recording period was divided into six 5-min intervals, and for each interval, the following parameters were calculated:

- Total force: an integral of the tractive force diagram in kNs consisting of:
  - Tractive force: an integral of positive values of the derivation of the tractive force diagram (meaning only the upward tractive phases) in kNs (force multiplied by time that a calf pulls upwards on the cable).
  - Holding force: an integral of zero values of the derivation of the tractive force diagram (meaning only the holding tractive phases) in kNs (force multiplied by time that a calf pulls on the cable

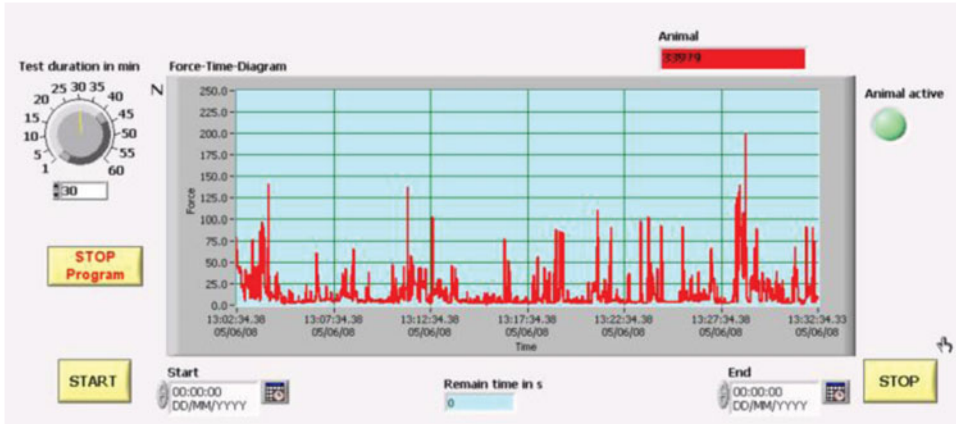


Fig. 2. Screenshot of a force-time diagram from the recording program in the barn.

with stable effort; i.e. 'hanging' in the cable without moving).

- Dwindling force: an integral of negative values of the derivation of the tractive force diagram (meaning only the downward tractive phases) in kNs (force multiplied by time that a calf pulls on the cable with downward effort; i.e. stops pulling on the cable).

– Number of pulls: a local maxima of the tractive force diagram above a threshold level of 60 N (empirical value).

– Maximal tractive force: the highest amplitude of the tractive force diagram (global maximum) in N.

To eliminate high-frequency perturbations, the recorded diagram was smoothed with a moving average ($n = 10$).

The procedures 'integral' and 'derivation' used to calculate the parameters were part of the program library of LabView. The numerical integration was performed with the trapezoidal rule, which calculates the integral with consecutive applications of the basic formula. The basic formula to calculate the partial sum with the trapezoidal rule is:

$$\text{partial sum} = \frac{1}{2}(x_i + x_{i+1}) \times \mathrm{d}t \tag{1}$$

for $i = 0, 1, 2, 3, 4, \ldots,$ whole-number part of (N–1), where N is the number of points of the whole plot and x is the initial array.

The result was the sum of these consecutive partial calculations:

$$\text{result} = \int_{t_0}^{t_1} f(t)\mathrm{d}t = \sum_j \text{partial sum} \tag{2}$$

where j is the range depending on the number of measuring points.

The discrete differentiation was carried out with a second-order differentiation method. The differentiation $f(t)$ of the function $F(t)$ is defined as follows:

$$f(t) = \frac{\mathrm{d}}{\mathrm{d}t}F(t) \tag{3}$$

Y is given as sampled start sequence dX/dt. When using the second-order method, Y is calculated with the following equation:

$$Y_i = \frac{1}{2\mathrm{d}t}(x_{i+1} - x_{i-1}) \tag{4}$$

for $i = 0, 1, 2, \ldots, n$–1, where n is the number of values in $x(t)$, x_{-1} is the first value in the start constraint and x_n is the first value of the final constraint.

The local and global maxima of the tractive force-time diagram were calculated with the subprogram 'peak detection' from the program library of LabView, which works with an algorithm fitting a quadratic polynomial to moving data blocks. The width of the quadratic polynomial determines the number of measuring points; in these analyses, a width of 5 was convenient (empirical value).

Statistical analysis

The parameters tractive force, maximal tractive force and number of pulls were used in the current exemplary analysis. The other two parameters were left aside to improve clarity in the results; therefore a parameter vector with a dimension of 18 (three parameters at six 5-min intervals) was used. Since the measured parameters of the vectors were multivariate, multivariate analyses with the statistics program R version 2.12.1 (R Development Core Team 2008) were conducted. The aim was to classify the different reactions of the tested animals on the basis of the parameter vectors without *a priori* knowledge. The *k*-means-algorithm ('kcca') was used to perform a *k*-centroids clustering on the data matrix (Leisch 2006). The algorithm forms a predetermined number of *k* differing clusters with similar subjects from the data volume. Since the number of clusters *k* is unknown per default, the algorithm was conducted iterative from $k=2$ to $k=20$. The *k*-means-algorithm is dependent on the initial conditions and can yield different results. Thus, the algorithm was conducted 20 times with randomly differing initial conditions for each number of clusters *k*. By using the score of the 'silhouette' function, the optimal number of clusters was determined. For each observation *i*, the silhouette score S_i is defined as follows:

$$S_i = \frac{b_i - a_i}{\max(a_i - b_i)} \quad (5)$$

where a_i is the average dissimilarity between *i* and all other points of the cluster to which *i* belongs, and b_i is the minimum of all average dissimilarity of *i* to all observations and can be seen as the dissimilarity between *i* and its neighbouring cluster, i.e. the nearest one to which it does not belong.

A hierarchical cluster analysis (Euclidean distance, complete linkage) was performed with the function 'hclust', which uses a set of dissimilarities for the clustered objects. At first, each object is allocated to its own cluster and then the algorithm proceeds iteratively by stepwise combining of the two most similar clusters and continuing until there is only one single cluster. At each stage, metric Euclidean distances between clusters are recomputed by the Lance–Williams dissimilarity. The result of this clustering can be shown graphically in a dendrogram. The animals' weights were tested for differences between the clusters with Welch's two-sample *t*-test.

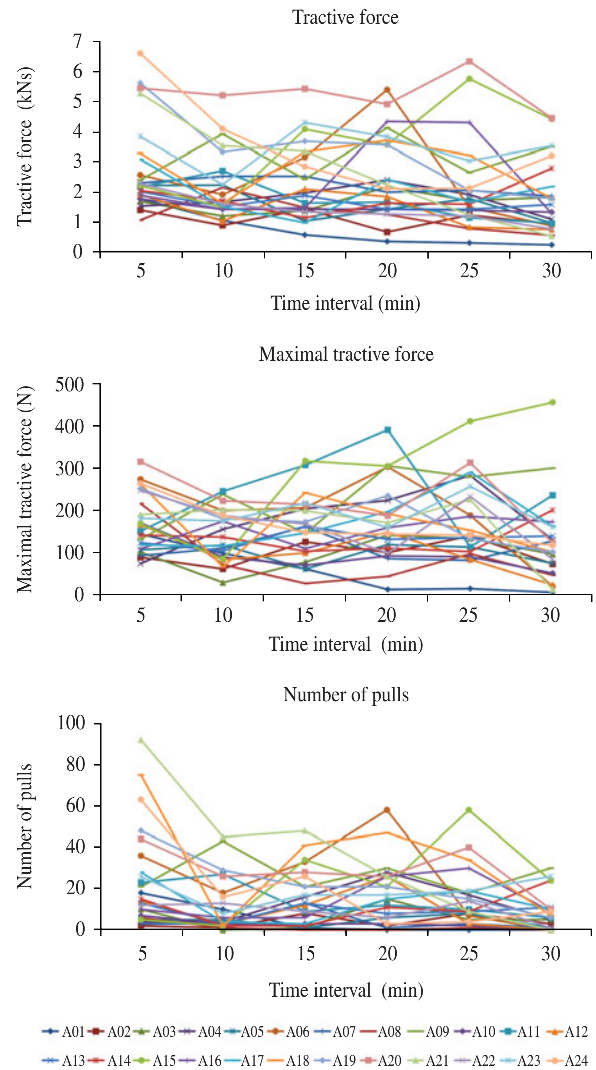


Fig. 3. Tractive force in kNs, maximal tractive force in N and number of pulls of calves tied to a force transducer for 30 min summarized in 5-min intervals.

RESULTS

Raw data

The escape behaviour and reluctance of the calves were described by tractive force, maximal tractive force and the number of pulls during 5-min intervals (Fig. 3). The majority of the calves showed a very similar behavioural pattern, especially with regard to the tractive force and number of pulls. The tractive force decreased from about 2 kNs at the beginning to 1 kNs at the end of the restraint situation. The number of pulls ranged from 5 to 10 for most calves and showed only a slight decrease across the 30 min. Several animals, however, showed higher values in both these parameters (Fig. 3).

Clustering

The silhouette function commended a cluster number of two when clustering with the *k*-means-algorithm 'kcca' without *a priori* knowledge and also when using the function 'hclust'. The latter function enabled the data structure and clustering to be displayed graphically via a dendrogram (Fig. 4). Both clustering

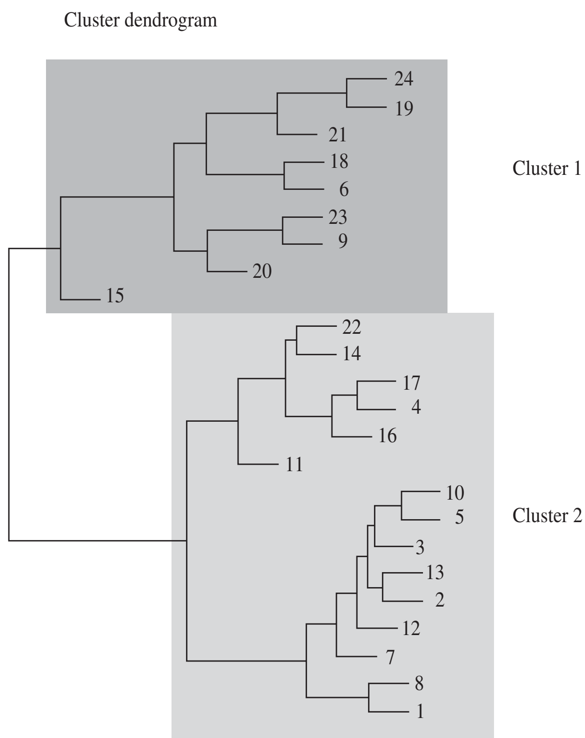


Fig. 4. Graphical display of the data structure of the 24 calves during the pulling-test shown as a cluster dendrogram.

methods allocated the same individuals into two clusters (Table 1). The first sub-structure to be found was named cluster 1 (four male and five female calves), and the next was named cluster 2 (eight male and seven female calves). Table 1 lists each individual in the clusters, its weight and sex. In cluster 2, two sub-clusters could be determined. The animal A15 could clearly be determined as an outlier in all three parameters and A06 showed a strong increase in reaction until the 20-min interval in all parameters (Fig. 3). A20 showed a continuously high tractive force level and A11 increased its maximal tractive force until the 20-min interval (Fig. 3). The mean weight of the animals in the two clusters was 117 kg (s.d. ±11 kg, range: 101–135 kg) for cluster 1 and 114 kg (s.d. ±14 kg, range: 89–144 kg) for cluster 2, and did not significantly differ from each other ($t=0.77$; $P=0.452$).

The mean values over time (± s.d.) in each cluster are shown in Fig. 5 for the three measured parameters showing a distinct differentiation. In all parameters, the curve for cluster 1 is clearly above that for cluster 2. The curves of both clusters showed similarities with the highest values in the beginning of the pulling test, a decrease over time and a slight increase in the middle of the test before decreasing even further towards the end. However, this pattern was far more distinctive in cluster 1 than in cluster 2. Furthermore, the variability in the tractive force and number of pulls was larger in cluster 1 than in cluster 2, although the latter consisted of more animals. Despite slight decreases and increases, the calves in cluster 2 displayed a relatively stable reaction level throughout the recording period.

Table 1. *Animal, sex and weight (kg) of the calves to cluster 1 and 2 analysed with the functions 'kcca' and 'hclust' in R; the number of an animal indicates the number in the cluster dendrogram (Fig. 4); m = male, f = female*

Animal	Sex	Weight (kg)	Clustering method: *k*-means	Clustering method: hclust
A01	f	106	2	2
A02	m	116	2	2
A03	f	118	2	2
A04	m	112	2	2
A05	f	101	2	2
A06	f	103	1	1
A07	m	98	2	2
A08	m	89	2	2
A09	m	135	1	1
A10	m	122	2	2
A11	f	112	2	2
A12	f	108	2	2
A13	m	108	2	2
A14	m	125	2	2
A15	f	110	1	1
A16	f	131	2	2
A17	m	144	2	2
A18	m	117	1	1
A19	m	124	1	1
A20	m	126	1	1
A21	f	123	1	1
A22	f	114	2	2
A23	f	117	1	1
A24	f	101	1	1

DISCUSSION

The current paper presents a technical method to record objectively the individual escape behaviour and reluctance in calves during restraint. The appliance can be used by anyone anywhere in the world, trained or untrained, educated or not, in any housing system (except for old-fashioned tied-barns) and will yield objective comparable results. Escape behaviour in a restraint situation is an important element of

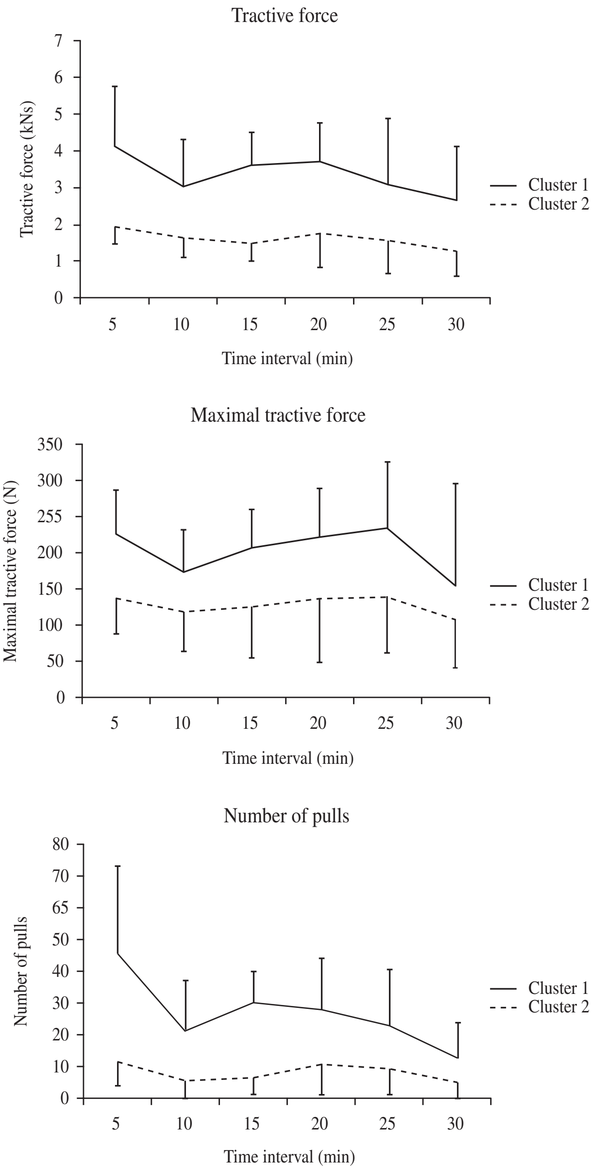


Fig. 5. Diagrams of the mean values over time (±S.D.) of the three measured parameters tractive force in kNs, maximal tractive force in N and number of pulls for cluster 1 and 2.

personality in prey animals (Boissy 1995; Koolhaas *et al.* 2010). One can expect that animals with different personality traits display differing behaviour patterns that can be classified according to their main features. Therefore, its standardized measurement has the potential to become an element of an easily applicable test in future breeding programme when phenotyping animals' personalities (Benhajali *et al.* 2010). So far, beef producers willing to include personality to the breed value of their cattle are instructed to measure flight time when leaving a crush once and use this simple measure to describe an animal's temperament (Gaden *et al.* 2004). To our knowledge, the behaviour of cattle during a restraint or tethered situation has either been described with temperament scores (e.g. Grandin 1993; Lanier *et al.* 2000) or has been observed and measured behaviourally (Boissy & Bouissou 1988), but until now this behaviour has only rarely been measured physically (Veissier *et al.* 1989).

With the animals tested in the current experiment, two different behaviour patterns revealing two intensities in the reaction were found. It is likely that more behaviour patterns would be found when testing more animals, e.g. one that shows an increase towards the end of the recording period or one with a constantly high reaction level. The first decrease in the reaction of the calves of cluster 1 could reflect exhaustion but could also be due to an initial evaluation of other possibilities for escaping from the restraint, since pulling has not worked so far. When no possibility was found and the calves had rested briefly, a new increase in reaction level could be detected. Calves of cluster 2 consistently showed a low level of reaction. Coleman *et al.* (2005) found that rhesus macaque monkeys that showed a quick reaction to an unknown stimulus were faster in learning a task by operant conditioning than individuals showing a slower or no reaction to an unknown stimulus; thus the animals of cluster 1 in the current study might be disadvantaged in other, different, situations. However, to evaluate this statement, further research needs to be conducted.

In the current study, data from one single measurement per animal at 90 days of age are presented. The young age of the calves tested made it possible to ensure similar housing conditions for both male and female animals. However, the repeatability and hence the reliability of the assessed behaviour pattern of each calf needs to be investigated. Grandin (1993) suggested three repetitions of temperament-scoring in her study on slightly older male cattle, to gain reliable results.

The reaction to the restraint is clearly not the only parameter defining the personality of cattle. Reactions toward humans may be similarly important. However, these reactions depend on the experience an individual has had with humans and are therefore not only dependent on personality traits but also highly influenced by the age of the animals, previous experience and the human himself (de Passillé *et al.* 1996). The behavioural reaction towards a restraint situation where the animals have the possibility to accept the restraint or not, as in the docility test

(Le Neindre *et al.* 1995; Boivin *et al.* 2009), are especially important for agricultural practice but are not entirely objective since humans are a fundamental part of the test (see intra- and inter-observer reliability in the introduction of the current paper). Moreover, their results are also dependent on previous experience of the test animal with humans (de Passillé *et al.* 1996). Reactions to an unknown object, as in the classic novel-object-test, give further important information on an animal's personality, which are not primarily covered with the method presented in the current paper. Possible correlations between the pulling-test and other tests named above need further research.

As a result of the current investigation, it is concluded that animals with different behaviour patterns can be successfully detected on the basis of their pulling behaviour during a 30-min period of restraint. The newly developed method, including the suggested statistical procedures, can be applied easily in research and in practice. It is suitable for phenotyping in breeding programmes, because it offers a standardized objective procedure independent of human observers and handlers. However, more research on the long-term reliability of the results needs to be conducted. Other aspects of personality also need to be accounted for in addition to those measured with the pulling-test. Therefore, the pulling-test should be seen as an objective and practicable basis-test.

The authors would like to thank Dieter Sehland and Kurt Wendland for their help in constructing the appliance and for testing the animals. Furthermore, they thank the animal keepers for helping to capture the calves. The first author Katharina L. Graunke was financed by the German agriculture network PHENOMCIS, grant no. 0315536G, supported by the Federal Ministry of Education and Research, Germany.

REFERENCES

Benhajali, H., Boivin, X., Sapa, J., Pellegrini, P., Boulesteix, P., Lajudie, P. & Phocas, F. (2010). Assessment of different on-farm measures of beef cattle temperament for use in genetic evaluation. *Journal of Animal Science* **88**, 3529–3537.

Boissy, A. (1995). Fear and fearfulness in animals. *Quarterly Review of Biology* **70**, 165–191.

Boissy, A. & Bouissou, M.-F. (1988). Effects of early handling on heifers' subsequent reactivity to humans and to unfamiliar situations. *Applied Animal Behaviour Science* **20**, 259–273.

Boivin, X., Gilard, F. & Egal, D. (2009). The effect of early human contact and the separation method from the dam on responses of beef calves to humans. *Applied Animal Behaviour Science* **120**, 132–139.

Broom, D. M. (1988). The scientific assessment of animal welfare. *Applied Animal Behaviour Science* **20**, 5–19.

Burrow, H. M. (1997). Measurements of temperament and their relationship with performance traits of beef cattle. *Animal Breeding Abstracts* **65**, 477–495.

Burrow, H. M. (1998). The effects of inbreeding on productive and adaptive traits and temperament of tropical beef cattle. *Livestock Production Science* **55**, 227–243.

Coleman, K., Tully, L. A. & McMillan, J. L. (2005). Temperament correlates with training success in adult rhesus macaques. *American Journal of Primatology* **65**, 63–71.

de Passillé, A. M., Rushen, J., Ladewig, J. & Petherick, C. (1996). Dairy calves' discrimination of people based on previous handling. *Journal of Animal Science* **74**, 969–974.

Fell, L. R., Colditz, I. G., Walker, K. H. & Watson, D. L. (1999). Associations between temperament, performance and immune function in cattle entering a commercial feedlot. *Australian Journal of Experimental Agriculture* **39**, 795–802.

Fleischrind Stölln GmbH (2007). *Charolais-Rinder aus dem Havelland*. Wilfried Zachert. Available online at: http://www.charolais-stoelln.de/zuchtziel.php?id=3 (verified 7 March 2012).

Fordyce, G., Dodt, R. M. & Wythes, J. R. (1988*a*). Cattle temperaments in extensive beef herds in Northern Queensland. 1. Factors affecting temperament. *Australian Journal of Experimental Agriculture* **28**, 683–687.

Fordyce, G., Goddard, M. E., Tyler, R., Williams, G. & Toleman, M. (1985). Temperament and bruising of *Bos indicus* cattle. *Australian Journal of Experimental Agriculture* **25**, 283–288.

Fordyce, G., Wythes, J. R., Shorthose, W. R., Underwood, D. W. & Shepherd, R. K. (1988*b*). Cattle temperaments in extensive beef herds in Northern Queensland. 2. Effect of temperament on carcass and meat quality. *Australian Journal of Experimental Agriculture* **28**, 689–693.

Forkman, B., Boissy, A., Meunier-Salaün, M.-C., Canali, E. & Jones, R. B. (2007). A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology and Behaviour* **92**, 340–374.

Gaden, B., Burrow, H. & Pettiford, S. (2004). Flight time – a practical way to improve feedlot performance, temperament and tenderness. In *Producing Quality Beef – Opportunities for Beef Producers from the CRC for Cattle and Beef Quality* (Ed B. Gaden), pp. 26–28. Armidale, NSW, Australia: CRC for Cattle and Beef Quality.

Gibbons, J. M., Lawrence, A. B. & Haskell, M. J. (2011). Consistency of flight speed and response to restraint in a crush in dairy cattle. *Applied Animal Behaviour Science* **131**, 15–20.

Grandin, T. (1993). Behavioral agitation during handling of cattle is persistent over time. *Applied Animal Behaviour Science* **36**, 1–9.

Grignard, L., Boivin, X., Boissy, A. & Le Neindre, P. (2001). Do beef cattle react consistently to different handling situations? *Applied Animal Behaviour Science* **71**, 263–276.

Kilgour, R. J., Melville, G. J. & Greenwood, P. L. (2006). Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity of humans, restraint and novelty. *Applied Animal Behaviour Science* **99**, 21–40.

Koolhaas, J. M., De Boer, S. F., Coppens, C. M. & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology* **31**, 307–321.

Lanier, J. L., Grandin, T., Green, R. D., Avery, D. & McGee, K. (2000). The relationship between reaction to sudden, intermittent movements and sounds and temperament. *Journal of Animal Science* **78**, 1467–1474.

Le Neindre, P., Trillat, G., Sapa, J., Ménissier, F., Bonnet, J. N. & Chupin, J. M. (1995). Individual differences in docility in Limousin cattle. *Journal of Animal Science* **73**, 2249–2253.

Leisch, F. (2006). A toolbox for *k*-centroids cluster analysis. *Computational Statistics and Data Analysis* **51**, 526–544.

Locurto, C. (2007). Individual differences and animal personality. *Comparative Cognition and Behaviour Reviews* **2**, 67–78.

Manteca, X. & Deag, J. M. (1993). Individual differences in temperament of domestic animals: a review of methodology. *Animal Welfare* **2**, 247–268.

Matthews, L. R., Carragher, J. F. & Slater, J. L. (1997). Effects of flightiness, sociability and previous handling experience on the behaviour of cattle in yards (abstract). In *Proceedings of the 31st International Conference of the ISAE, 13–16 August 1997* (Eds M. Špinka, G. Illmann, J. Maletînská, Z. Štìtková & L. Bartoš), p. 94. Prague, Czech Republic: International Society of Applied Ethology.

Mayer, J. D. (1986). How mood influences cognition. In *Advances in Cognitive Science* (Ed. N. E. Sharkey), pp. 290–314. Chichester, West Sussex, UK: Ellis Horwood Limited.

R Development Core Team (2008). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Traditional Herefords (2008). *The Official Traditional Hereford Web Site*. Available online at http://www.traditionalherefords.org/ (verified 7 March 2012).

Veissier, I., Le Neindre, P. & Trillat, G. (1989). Adaptability of calves during weaning. *Biology of Behaviour* **14**, 66–87.

Voisinet, B. D., Grandin, T., O'Connor, S. F., Tatum, J. D. & Deesing, M. J. (1997b). *Bos indicus* cross feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. *Meat Science* **46**, 367–377.

Voisinet, B. D., Grandin, T., Tatum, J. D., O'Connor, S. F. & Struthers, J. J. (1997*a*). Feedlot cattle with calm temperaments have higher average daily weight gains than cattle with excitable temperaments. *Journal of Animal Science* **75**, 892–896.

Waiblinger, S., Boivin, X., Pedersen, V., Tosi, M.-V., Janczak, A. M., Visser, E. K. & Jones, R. B. (2006). Assessing the human-animal relationship in farmed species: a critical review. *Applied Animal Behaviour Science* **101**, 185–242.

Welfare Quality® (2009). *Training in the Welfare Quality® Assessment Protocols*. Lelystad, The Netherlands: Welfare Quality®. Available online at: http://www.welfarequality.net/downloadattachment/43299/20259/2009-Nov_adapted_leafletWQTrainingAssessmentProtocols.pdf (verified 3 March 2012).

CAMBRIDGE UNIVERSITY PRESS LICENSE
TERMS AND CONDITIONS

Aug 30, 2013

This is a License Agreement between Katharina L Graunke ("You") and Cambridge University Press ("Cambridge University Press") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Cambridge University Press, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number	3218750514812
License date	Aug 30, 2013
Licensed content publisher	Cambridge University Press
Licensed content publication	The Journal of Agricultural Science
Licensed content title	Objectively measuring behaviour traits in an automated restraint-test for ungulates: towards making temperament measurable
Licensed content author	
Licensed content date	Dec 31, 1969
Volume number	151
Issue number	01
Start page	141
End page	149
Type of Use	Dissertation/Thesis
Requestor type	Author
Portion	Full article
Author of this Cambridge University Press article	Yes
Author / editor of the new work	Yes
Order reference number	
Territory for reuse	World
Title of your thesis / dissertation	Characterisation of temperament associated behaviour traits in cattle (Bos taurus)
Expected completion date	Nov 2013
Estimated size(pages)	80
Billing Type	Invoice
Billing address	Wilhelm-Stahl-Allee 2
	Dummerstorf, 18196
	Germany
Total	0.00 EUR
Terms and Conditions	

Terms and Conditions are not available at this time.

If you would like to pay for this license now, please remit this license along with your payment made payable to "COPYRIGHT CLEARANCE CENTER" otherwise you will be invoiced within 48 hours of the license date. Payment should be in the form of a check or money order referencing your account number and this invoice number RLNK501101802.
Once you receive your invoice for this order, you may pay your invoice by credit card. Please follow instructions provided at that time.

Make Payment To:
Copyright Clearance Center
Dept 001
P.O. Box 843006
Boston, MA 02284-3006

For suggestions or comments regarding this order, contact RightsLink Customer Support: customercare@copyright.com or +1-877-622-5543 (toll free in the US) or +1-978-646-2777.

Gratis licenses (referencing $0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.

Study II

Multidimensional personality depiction

II

Describing Temperament in an Ungulate: A Multidimensional Approach

Katharina L. Graunke[1,3], Gerd Nürnberg[2], Dirk Repsilber[2], Birger Puppe[1,4], Jan Langbein[1*]

1 Ethology Unit, Institute of Behavioural Physiology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, **2** Institute of Genetics and Biometry, Bioinformatics and Biomathematics Unit, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, **3** Faculty of Agricultural and Environmental Sciences (AUF), PHENOMICS office, University of Rostock, Rostock, Germany, **4** Faculty of Agricultural and Environmental Sciences (AUF), Behavioural Sciences, University of Rostock, Rostock, Germany

Abstract

Studies on animal temperament have often described temperament using a one-dimensional scale, whereas theoretical framework has recently suggested two or more dimensions using terms like “valence” or “arousal” to describe these dimensions. Yet, the valence or assessment of a situation is highly individual. The aim of this study was to provide support for the multidimensional framework with experimental data originating from an economically important species (*Bos taurus*). We tested 361 calves at 90 days *post natum* (dpn) in a novel-object test. Using a principal component analysis (PCA), we condensed numerous behaviours into fewer variables to describe temperament and correlated these variables with simultaneously measured heart rate variability (HRV) data. The PCA resulted in two behavioural dimensions (principal components, PC): novel-object-related (PC 1) and exploration-activity-related (PC 2). These PCs explained 58% of the variability in our data. The animals were distributed evenly within the two behavioural dimensions independent of their sex. Calves with different scores in these PCs differed significantly in HRV, and thus in the autonomous nervous system’s activity. Based on these combined behavioural and physiological data we described four distinct temperament types resulting from two behavioural dimensions: “neophobic/fearful – alert”, “interested – stressed”, “subdued/uninterested – calm”, and “neoophilic/outgoing – alert”. Additionally, 38 calves were tested at 90 and 197 dpn. Using the same PCA-model, they correlated significantly in PC 1 and tended to correlate in PC 2 between the two test ages. Of these calves, 42% expressed a similar behaviour pattern in both dimensions and 47% in one. No differences in temperament scores were found between sexes or breeds. In conclusion, we described distinct temperament types in calves based on behavioural and physiological measures emphasising the benefits of a multidimensional approach.

Citation: Graunke KL, Nürnberg G, Repsilber D, Puppe B, Langbein J (2013) Describing Temperament in an Ungulate: A Multidimensional Approach. PLoS ONE 8(9): e74579. doi:10.1371/journal.pone.0074579

Editor: Cédric Sueur, Institut Pluridisciplinaire Hubert Curien, France

Received February 12, 2013; **Accepted** August 6, 2013; **Published** September 10, 2013

Funding: The first author Katharina L. Graunke was financed by the German agriculture network PHENOMCIS, grant no. 0315536G, supported by the Federal Ministry of Education and Research, Germany (http://www.phaenomics.auf.uni-rostock.de/en-home.html). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

* E-mail: langbein@fbn-dummerstorf.de

Introduction

Differences in behaviour between animals can be caused by a range of environmental and state-dependent factors, e.g., sex, age, reproductive status, or environment. However, not all differences can be explained by physiological states or environmental factors; the remaining differences may therefore reveal the strategy an individual employs to act with and react to environmental stimuli [1–3]. This individual strategy is described as temperament and is thought to be innate and consistent over time and in different situations [4–9]. Besides temperament there are other terms used in this context, e.g., personality, individuality or coping style [6,10–12]. Réale et al. [6] provide a summary of the different terms and their definitions depending on the author(s), which clearly shows how arbitrary the distinctions between the different terms are. They conclude that the two most common terms, temperament and personality, are often artificially distinguished. Therefore, they understand these two terms as synonyms, which we are in accordance with. Although being consistent over time and situations, temperament should not be imagined as a fixed and completely inflexible construct, but rather as an adjustable tool for adaptation to exterior circumstances during individual ontogeny [13]. Very early in life, temperament seems to be rather flexible [14,15], while later on it is maintained more and more rigidly [13]. However, depending on genetics and epigenetics, the starting point is different for each individual.

In the literature, original research on non-human animals often describes temperament on a one-dimensional scale using expressions such as “proactive – reactive”, “aggressive – non-aggressive”, “bold – shy”, etc. (e.g., [11,16,17]). Human psychology and thereon based recent theoretical framework on temperament in non-human animals, however, mostly argue for two or more dimensions using terms such as “valence”, “arousal” or “activity” to describe the different dimensions [6,18–22]. Following their arguments, different temperament types can be located in a circumplex model as a linear combination of these dimensions. Therefore, two or more dimensions are more likely to reflect the entire nature of temperament or personality in non-human animals than one dimension. Especially, the valence or perception of a situation is highly individual, yet most important to an animal’s welfare [23]. Veissier et al. [23] point out that while exterior conditions like housing system, quality of diet, etc. loose no importance to animal welfare, one mandatorily needs to take into account the valence of the animals themselves, when seriously trying to evaluate an animal’s welfare.

Naturally, questionnaires on the perception of different situations that can be answered in personality research in human psychology are impossible in non-human animals; therefore, one must include measures of physiological or neurophysiological activation revealing information about the probable perception and processing of a test situation by an individual. The analysis of cardio-vascular measurements has been found to be a suitable approach for determining the activity of the autonomous nervous system in the study of temperament [24–26]. The cardiac vagal tone represents parasympathetic nervous activity at the level of the heart and derives from heart rate variability (HRV). HRV measures can be calculated from beat to beat changes in heart rate (R-R interval) in the electrocardiogram. The cardiac vagal tone has been suggested as a psychophysiological marker of internal regulation and of certain aspects of psychological adjustment in humans and animals [27,28]. Changes in the length of consecutive R-R-intervals reflect differential activation of the two branches of the autonomous nervous system [29,30] and can therefore give an understanding of a test subject’s perception or its valence of a situation. Common variables of HRV measures in the time domain are the heart rate in beats per minute (HR in bpm), the root mean square of successive differences (RMSSD in ms), the standard deviation of all R-R-intervals (SDNN in ms) and the ratio of RMSSD and SDNN (RMSSD/SDNN). Table 1 provides a description of the effects of the autonomous nervous system on these measures.

The aim of this study was to develop a description of temperament in young cattle (*Bos taurus*) by analysing their behaviour and simultaneously measured heart rate variability during a standard behaviour test. To test for stability over time, we conducted the test at two ages on an additional, small sample size. Taking this combined multidimensional approach based on experimental original data, we intended to provide foundational support for the theoretical framework suggesting two or more dimensions in animal temperament.

Table 1. Influence of the autonomous nervous system on measures of heart rate variability.

HRV measures	Influence of the autonomous nervous system	Consequences on HRV measure
HR	Additive and non-additive effects of PNS and SNS	HR decreases, when PNS activity increases and/or SNS activity decreases
RMSSD	Only influenced by PNS	RMSSD increases, when PNS activity increases
SDNN	PNS and SNS act synergetically	SDNN increases mainly, when SNS activity increases, but is also influenced by PNS activity
RMSSD/ SDNN	PNS and SNS affect measure antagonistically	RMSSD/SDNN increases, when PNS activity increases and/or SNS activity decreases

Measures of heart rate variability (HRV), branches of the autonomous nervous system that influence the HRV measures, and their consequences on the respective measure (after [29]); PNS = parasympathetic nervous system, SNS = sympathetic nervous system

doi: 10.1371/journal.pone.0074579.t001

Animals, Materials and Methods

2.1: Animals and housing

We tested 361 calves (175 male, 186 female) of the F_2-generation of a running breeding project (Holstein Friesian × Charolais cross breeding) with 90 dpn (± 3 dpn, days *post natum*). All calves were bred via embryo transfer into unrelated Holstein Friesian heifers as recipient mothers and were born and tested between 2004 and 2010. The calves were kept in various small groups of up to nine animals of similar age, apart from their recipient mothers from day one. Pens had a size of 6 x 7 m and were covered with deep litter. Until 90 dpn, the calves were not subject to any other experiment, and handling did not exceed routine handling by the animal keepers except in the case of animals requiring treatment for sickness.

After weaning, the animals were weighed at 111 dpn (± 3 dpn). The weight at the day of the experiment was calculated with the help of the average daily weight gain from birth to weaning and the exact age at the experiment. On average, the calves weighed 118 kg (range: 74-159 kg, SD ± 14 kg) at the day of the experiment.

We further tested each 20 calves (10 male, 10 female) of the founder breeds Holstein Friesian and Charolais at 91 dpn (± 3 dpn) and a second time at 197 dpn (± 12 dpn) to evaluate stability of temperament over time and to detect possible breed differences. The calves were purchased from breeders and arrived at our facilities two days after birth at the latest. They were housed in the same barn as the crossbreeds and male and female calves were housed together until the second test had been conducted. These calves were born and tested between 2008 and 2012. Due to the early death of one male Charolais calf, there were 19 Charolais calves tested at 91 dpn. At the second test age, one male Charolais calf became extremely distressed during testing and risked serious injury.

The test was terminated; thus there is data of 18 (8 male, 10 female) Charolais calves at 197 dpn.

2.2: Experimental procedure

The behaviour test was performed in an open field of 9.6 × 4.0 m in size, which was unknown to the calves prior to testing. It was divided into four segments of 2.4 × 4.0 m each. After allowing the test animal to acclimatise to the open field for 10 min, a novel-object test was conducted with a traffic pylon of 0.5 m height as novel object. It was let down into the outer segment, which was the farthest from where the calf stood (Figure S1). We chose this test as it is known to provoke behaviour, which correlates with behaviour during other tests [31,32] or with social cues [31]. The novel-object test lasted for 10 min. During the test, behaviour was live-recorded using the observation software tool The Observer 5.0 (Noldus, The Netherlands). Of in total 438 behaviour test sessions, 428 were conducted by three experienced observers whose observation highly correlated during a 90 min-test session (Pearson's Rho 0.973, $p < 0.001$). The residual 10 behaviour test sessions were conducted by three other experienced observers. Recorded behaviours with their definitions and type of recording are listed in Table 2. For further analysis, the latency of the behaviours an individual did not show during the 10 min behaviour test was set to the maximum time of 600 s (10 min).

2.3: Heart rate variability (HRV)

To measure the heart beat activity during the test, we applied a heart monitor system (Polar S810i, Polar Electro, Oy, Finland). The calves were fitted with flexible belts with two integrated electrodes and a transmitter for wireless transmission of the R-R-interval data series to a separate storage device. The two electrodes were placed on the left side of the most cranial part of the chest behind the forelegs: one next to the sternum, and the other behind the scapula. The coat under the electrodes was shaved and a conductive gel was used for better electrical conductivity. Prior to the beginning of the experiment, calves were fitted the belts and were then left alone with their pen mates in their home pen to gain base measurements. After 30 min, they were led into the open field for acclimatisation and testing. Later on, the R–R data series were transferred to a computer and corrected when necessary using Polar Precision Performance SW version 4.03 (Polar Electro, Oy, Finland) with the standard set-up. The curves were divided into 5-min intervals and an error correction of up to 10% per interval was accepted. In further processing of the data, neither differences between two R-R-intervals larger than 150 ms nor identical values of five or more consecutive R-R-intervals were accepted. A program developed with LabView 2009 version 9.0 (National Instruments Germany GmbH, Munich, Germany) detected complete 1-min intervals in the base measurements (starting 5 min after the experimenters left the barn) and the test, and calculated HR, RMSSD, SDNN, and RMSSD/SDNN for each complete 1-min interval (see [30] for the exact calculation of the variables, see Introduction for an explanation of the variables). When there were at least seven complete 1-min intervals per base measurement and per test (134 male, 138 female), the program further determined the mean of the first seven values of HR, RMSSD, SDNN and RMSSD/SDNN. The differences between test and base measurements of HR, RMSSD and SDNN and the ratio of test and base measurement of RMSSD/SDNN were used for further analyses. Later analysis showed that it was completely coincidental and independent of the animals' behaviour during the novel-object test (e.g., running or activity duration), which calves did and which did not have complete HRV measures. Therefore, there is no further discussion of this fact.

All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Ministry of Agriculture, the Environment and Consumer Protection of the federal state Mecklenburg-Vorpommern, Germany.

Table 2. Definition of live-recorded behaviours.

Behaviour	Type of recording	Definition
Contact with novel object (contact)	D, F, L	Physical contact with any part of the body with the novel object or sniffing the novel object while being closer than 0.1 m to it
Inactivity	D	At least three legs touch the ground, no forward movement
Exploration	D, L	Sniffing or licking the wall or floor of the open field
Grooming	D	Calf licking or scratching itself with one hind leg
Activity	D, L	Max. 3 legs touch the ground, forward movement
Running	D	Max. 2 legs touch the ground, fast forward movement
Vocalisation	F	Any kind of sound the calf makes
Change of segment	F	Leaving one segment and entering another with at least the forelegs
Habitation in segment where the novel object is placed (object segment)	D, L	With at least the forelegs in the segment in which the novel object is placed
Habitation in segment next to segment where the novel object is placed (object neighbouring segment)	L	With at least the forelegs in the segment next to the segment in which the novel object is placed

Definition and type of recording of the behaviours live-recorded during the novel-object test; D = duration (total time in s), F = frequency, L = latency (time in s until behaviour was first shown).

doi: 10.1371/journal.pone.0074579.t002

2.4: Statistical analysis

All statistical analyses were performed using SAS 9.3, SAS Institute Inc., USA. In a preliminary analysis, we checked for the influence of sex and weight on all behaviours and HRV measures using a one-way analysis of covariance model (ANCOVA, The GLM Procedure) with the fixed factor sex and the co-variable weight.

As main analysis, we performed a Principal Component Analysis (PCA), which described the relationship between new (latent) principal components (PC) and our 15 behaviours. A PCA is used to condense several correlated measures into a smaller number of principal components. The loadings of each measure on a principal component represent the correlation between the component and this measure. i.e., the loadings reflect the importance of each measure for the component. One of the main assumptions for using PCA or Factor Analysis for an analysis of data is a suitable correlation between all included measurements. A measure for this sampling adequacy (MSA) of the correlation matrix is the Kaiser-Meyer-Olkin criterion (KMO). As Budaev [33] mentioned "correlation matrices with KMO < 0.5 are entirely inappropriate whereas those with KMO below 0.6-0.7 must be treated with caution". We decided to use a PCA instead of a Factor Analysis, because many of our behaviour measurements were non-normally distributed [33], and because no a-priori theory or model exists [34]. The PCA was conducted with The FACTOR Procedure with the following parameter settings: method=PRIN, prior=ONE, rotation=VARIMAX. As input data set, we used a correlation matrix of all pairwise correlations of our 15 behavioural measures applying the non-parametric Spearman's rank correlation test (using The CORR Procedure), because some of the behaviours were not continuous and/or normally distributed (Table S1). One crucial point when using a PCA is the choice of the final number of extracted PCs [33]. Several methods are available for this decision. We performed four methods: Kaiser's number of eigenvalues > 1 [35], Cattell's scree-test [36], Horn's Parallel test [37] and Velicer's Minimum Average Partial (MAP) test [38]. For the Parallel test and MAP test, we applied the SAS syntaxes provided by O'Connor [39]. We decided for two PCs in the final PCA calculation, since three of these methods led to a two PC solution (except number of eigenvalues > 1). Corresponding PC scores for each calf were finally calculated with The SCORE Procedure. These scores were further used to determine score classes and to identify the calves with differing behaviour.

The influence of these score classes and sex on the HRV measures was tested by a two-way analysis of variance model (ANOVA, The MIXED Procedure) with the fixed factors score class, sex and their interaction. Post hoc tests were performed with a Tukey-Kramer correction for multiple testing.

The PC scores of the calves of the founder breeds (Holstein Friesian, Charolais) were calculated with The SCORE Procedure using the resulting loadings from the crossbreeds as it is no use to perform a PCA on such a low number of animals [40]. The influence of breed and sex on the score class was calculated with a two-way ANOVA (The MIXED Procedure) with the fixed factors breed, sex and their interaction. To analyse the stability of the scores over time, we applied Spearman's rank correlation test on scores of the two test ages (The CORR Procedure). For all analyses, we defined the significance level at 0.05 and treated p-values between 0.05 and 0.1 as tendency.

Results

3.1: Behaviour and heart rate variability

Descriptive statistics of the recorded behaviours of 361 crossbreed calves in the novel-object test are shown in Table S2. The HRV measures of 272 of these calves during base measurement and novel-object test are presented in Table S3. Weight had a significant influence on grooming duration with lighter calves grooming longer than heavier calves ($F = 4.25$, $p = 0.040$), but had no influence on any other behaviour. Sex had a significant influence on grooming duration and latency of activity ($F = 11.02$, $p < 0.001$; $F = 4.15$, $p = 0.042$) and tended to have an influence on change of segment ($F = 2.73$, $p = 0.099$), where male calves groomed longer, had a lower latency to show activity and changed segments less often than female calves. Weight had a significant influence on RMSSD/SDNN with lighter calves having higher measures than heavier calves ($F = 5.41$, $p = 0.021$), but none on any other HRV measure. Sex had an influence on HR, SDNN and RMSSD/SDNN ($F = 4.35$, $p = 0.038$; $F = 7.30$, $p = 0.007$; $F = 8.20$, $p = 0.005$), with male calves having a lower HR, lower SDNN and higher RMSSD/SDNN than female calves.

During the novel-object test, calves of the two breeds Charolais and Holstein Friesian did not differ in their behaviour except for the duration of running ($F = 4.25$, $p = 0.046$), with Charolais calves running longer than Holstein Friesian calves (least square mean 5.1 s vs. 1.4 s). Accordingly, Charolais calves had a higher HR and lower RMSSD than Holstein Friesian calves ($F = 12.6$, $p = 0.001$; $F = 5.1$, $p = 0.031$). Since SDNN did not differ between the breeds, the RMSSD/SDNN differed accordingly ($F = 6.4$, $p = 0.016$). None of the HRV measures differed between the breeds during base measurement.

3.2: Principal component analysis

The loadings of the behaviours in the two PCs gained from the PCA of the novel-object test are shown in Table 3. The loadings rated "excellent" (greater than 0.71 and lower than -0.71) and "very good" (greater than 0.63 and lower than -0.63) were accepted as explanatory variables [40]. PC 1 was most influenced by behaviours occurring in the novel-object context such as contact duration or the time spent close to the object, and PC 2 was most influenced by behaviours in context with the exploration of the open field (but not the novel object) and the inactivity of the animals. The measure of sampling adequacy (MSA) with 0.833 was "meritorious" [41] and our data therefore appropriate for PCA analysis [33]. The two PCs explained 46.8% and 11.2%, respectively, of the variation in the data.

For each animal, the scores in PC 1 and PC 2 were calculated from their standardised original data and the respective loadings as presented in Table 3. To distinguish animals from one another by their temperament, we divided the animals into score classes (SC) according to the level of their scores in the two PCs (as suggested by Mendl et al. [20]). We defined the intermediate level of the scores at ± 0.5 SD around the zero line to identify calves not showing distinct behaviour in one or both PCs. Using this procedure, we received nine SCs

Table 3. Principal component loadings of the behaviours.

Behaviour	PC 1	PC 2
Contact-D	**0.76457**	0.05006
Contact-F	**0.83250**	0.12250
Contact-L	**-0.89613**	-0.13959
Inactivity-D	-0.41347	**-0.85549**
Exploration-D	0.15037	**0.82661**
Exploration-L	-0.19767	*-0.63679*
Grooming-D	-0.08038	0.42876
Activity-D	0.56716	0.61371
Activity-L	-0.49598	-0.21244
Running-D	0.47287	0.34835
Vocalisation-F	0.38216	0.07442
Change of segment-F	*0.70109*	0.51393
Object segment -L	**-0.87210**	-0.15841
Object segment-D	**0.83888**	0.12061
Object neighbouring segment -L	**-0.73723**	-0.22699

Loadings of the behaviours in principal component (PC) 1 and PC 2 gained from the principal component analysis of the novel-object test; loadings above 0.71 in bold type, loadings above 0.63 in italics (cf. [40] loadings above 0.71 rated “excellent”, loadings above 0.63 rated “very good”); D = duration (total time in s), F = frequency, L = latency (time in s until behaviour was first shown).

doi: 10.1371/journal.pone.0074579.t003

(Figure 1). A plot with the scores of all 361 crossbreed calves is shown in Figure 1, where each dot represents one calf. The distribution of the male and female calves in the SCs is presented in Table 4.

Calves of SC IX had long contact to the novel object and hardly explored the open field, while those of SC I explored the open field a long time, made little or very late contact to the novel object and were highly active. However, using information from only behaviour made it difficult or impossible to describe some of the SCs and to understand the animals' temperament. We therefore analysed the heart rate variability measures for differences based on SC, sex and the interaction of SC and sex.

3.3: Development of temperament types (TT)

The interaction of SC and sex had no significant influence on the changes in any of the four HRV variables (HR:F = 0.52, p = 0.840; RMSSD: F = 0.34, p = 0.948; SDNN: F = 1.36, p = 0.212; RMSSD/SDNN: F = 0.48, p = 0.871). Sex had no significant influence (RMSSD: F = 0.04, p = 0.834; SDNN: F = 0.14, p = 0.707; RMSSD/SDNN: F = 0.66, p = 0.417) except for a tendency for HR change, where the female calves tended to have a higher increase in HR during the behaviour test compared to the male calves (t = 1.92; p = 0.056). SC had a significant influence on the changes in HR, SDNN and RMSSD/SDNN (HR:F = 5.19; SDNN: F = 6.64; RMSSD/SDNN: F = 5.04; all p < 0.001), but not on RMSSD (RMSSD: F = 0.73, p = 0.666). Most reliable information about the balance between sympathetic and parasympathetic nervous system is gained from the RMSSD/SDNN. The least square means, their standard error and the 95% confidence interval of the RMSSD/SDNN-ratio between test and base measurement are shown in Table 5. A value larger than 1.00 indicates a shift towards the parasympathetic nervous system during the test, while a value smaller than 1.00 indicates a shift towards the sympathetic nervous system. Confidence intervals not embracing 1.00 indicate a significant shift of the autonomous nervous system during the test compared to the base measurement. During the test, animals of SC III and VI showed a significant shift towards the sympathetic nervous system, whereas in those of SC I, II, IV, V, VIII, and IX the balance between the sympathetic and parasympathetic nervous system did not change (Table 4, Figure 2). Calves of SC VII had a 46% higher RMSSD/SDNN-ratio during the novel-object test compared to the base measurement (Table 4), i.e. they were on average strongly parasympathetically activated while calves of SC III and VI were sympathetically activated during the novel-object test. Therefore, we could describe the most distinct TT (SC I, III, VII, and IX) by characteristic terms for the displayed behaviour and the activated parts of the autonomous nervous system: “neophobic/fearful – alert” (SC I), “interested – stressed” (SC III), “subdued/uninterested – calm” (SC VII), and “neoophilic/outgoing – alert” (SC IX; Figure 2).

3.4: Stability over time

Each individual - intermediate or distinct – has its own specific temperament. Therefore, to evaluate stability of the scores, one needs to take all individuals into account.

3.4.1: Stability within the two-dimensional space. The distribution of the Charolais and Holstein Friesian calves was even within the scores plot (Figure S2) revealing no breed differences in the scores (PC 1: F = 0.13, p = 0.724; PC 2: F = 0.03, p = 0.867). During the repetition of the test procedure at 197 dpn (days *post natum*) 42.1% of the calves scored within 1 SD around their score at 90 dpn, 44.7% scored between 1–2 SD around their first score and 13.2% scored farther than 2 SD from their first score. Neither sex (F = 0.00, p = 0.973) nor breed (F = 0.22, p = 0.639) or interaction between sex and breed (F = 1.51, p = 0.228) influenced the difference in the score between the first and second test age. The animals scoring within 1 SD (n = 16) at both test ages showed various directions in the changes (Figure S2A), while 15 of 17 animals scoring between 1–2 SD from their first score showed a greater change in one PC (> 1 SD) and only a small change in the other (< 1 SD, Figure S2B). Of these 17 calves, 11 showed a lower score in PC 2 at the second test age, and 9 simultaneously showed a change of less than 1 SD in PC 1. Of the 17 calves, 5 showed a greater score (> 1 SD) in PC 1 at 197 dpn than at 90 dpn, 3 of which simultaneously showed a change of less than 1 SD in PC 2. Of the 5 calves scoring farther than 2 SD from the first score, one showed a small change (< 1 SD) in PC 1 and two showed a small change (< 1 SD) in PC 2 (Figure S2C).

3.4.2: Stability within each principal component. Figure 3 shows the stability over the two test ages separately for each PC with the solid black line indicating 100% stability. Scores of PC 1 significantly correlated between the test ages (r = 0.36, p = 0.028; Figure 3A) and scores of PC 2 tended to correlate between 90 and 197 dpn (r = 0.29, p = 0.079; Figure 3B). In the

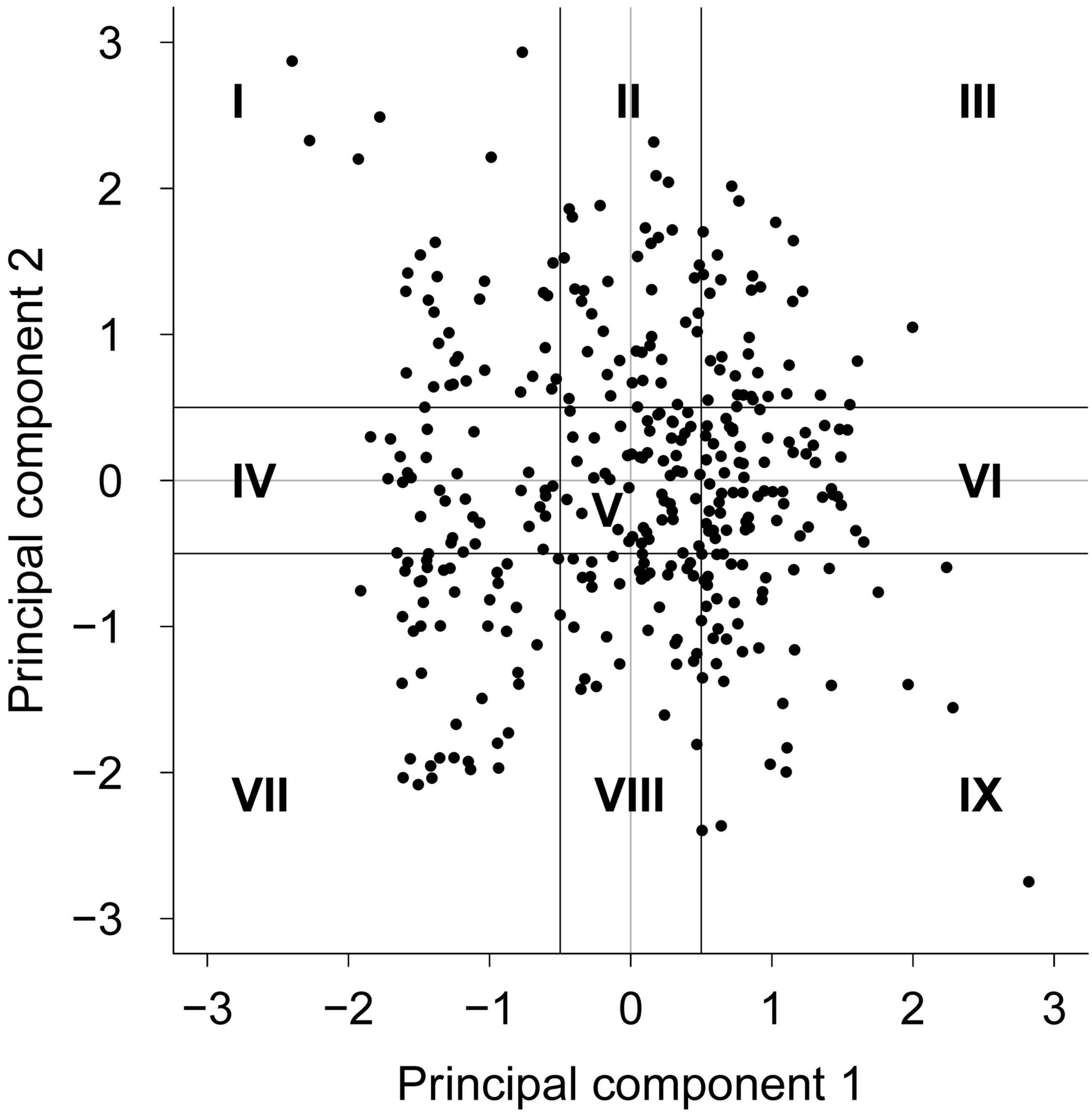


Figure 1. Scores plot of the crossbreed calves. Scores plot of 361 crossbreed calves gained from the standardised original data of the novel-object test and the respective loadings in the two PCs (Table 3), including the classification into nine score classes, numbered with Roman numerals; a range of ± 0.5 SD from the zero line was defined as threshold for the intermediate level.
doi: 10.1371/journal.pone.0074579.g001

same SC scored 21.1% of the calves, 39.5% stayed within the same score level in PC 1 (e.g., change from SC I to SC IV) and 10.5% in PC 2 (e.g., change from SC VII to SC IX). 28.9% of the calves changed SC on both score levels. However, no animal changed from SC I to SC IX or from SC III to SC VII and vice versa, meaning no animal changed from one extreme SC to the opposite SC.

Table 4. Distribution of the calves on the score classes.

SC	Male	Female
I	20	13
II	23	16
III	12	22
IV	16	17
V	22	28
VI	28	32
VII	20	22
VIII	15	18
IX	19	18

Distribution of crossbreed calves subdivided by sex on the score classes (SC) in the novel object test.

doi: 10.1371/journal.pone.0074579.t004

Table 5. Least square means of the nine score classes of the RMSSD/SDNN-ratio.

SC	LSM	SE	95% CI
I	0.93	0.10	0.73-1.12
II	0.92	0.08	0.75-1.08
III	0.82	0.09	0.64-1.00
IV	1.05	0.08	0.89-1.22
V	0.92	0.08	0.76-1.07
VI	0.83	0.06	0.70-0.96
VII	1.46	0.08	1.31-1.62
VIII	1.04	0.08	0.88-1.21
IX	0.97	0.09	0.80-1.15

Least square means (LSM), standard error (SE), and 95% confidence interval (CI) of the nine score classes (SC) of the RMSSD/SDNN-ratio; 1.00 indicates no change in the ratio during the novel-object test compared to the base measurement.

doi: 10.1371/journal.pone.0074579.t005

Discussion

Studies on animal temperament have often described temperament on a one-dimensional scale [11,16,17] while theoretical framework has suggested the necessity of two or more dimensions [6,18–20,42]. Only recently have studies started to present behavioural data condensed into two to four dimensions; however, except for Meager et al. [43] without characterising individual animals' temperament [44–46]. To our knowledge, the presented study is the first to support the claims of the above named theoretical framework with original data based on non-human mammals. Using a multivariate analysis we could condense numerous behaviours to fewer variables to display different dimensions of temperament, support the interpretation of the behaviours with physiological data and describe individual animals' temperament types. In context of cognitive enrichment, the approach of combining behavioural analyses with physiological data has successfully been used to test the animals' validation of the enrichment [47,48]. The high number of several hundred tested individuals in the presented study lets us provide foundational support for the theory of multidimensional temperament in non-human animals.

With an MSA of 0.833, the data was suited "meritoriously" [41] for the conducted PCA, which was therefore absolutely appropriate [33]. The fact that PC 2 of this analysis explains a much smaller percentage of variance in our data leads to the assumption that the high-loading behaviours in PC 2 explain less variance in the behaviour of the calves than the high-loading behaviours in PC 1. Therefore, we conclude that most variance in the data was caused by the animals' reactions to the novel object. It is unlikely that the calves would treat the open field as novel object, because they had time to acclimatise and to explore the open field prior to their exposure to the novel object. Réale et al. [6] define the category "exploration-avoidance" as independent of the category "boldness-shyness". Equally in our analysis, the exploration of the open field (shyness-boldness) loaded high in one PC, whereas seeking contact to and "exploring"' the novel object (exploration-avoidance) loaded high in the other PC. The exploration of the open field could, therefore, describe the activity of the calves. The data from the RMSSD/SDNN ratio clearly demonstrated that animals with similar scores in PC 1 did not necessarily respond similarly in their physiological reaction; this result is consistent for similar scores in PC 2. Hence, the perception or valence of the test situation was most likely different in different individuals [24], although they might have had similar scores in one of the PCs.

The multidimensional depiction of temperament or personality originates from human psychology [21,49] and was first implemented theoretically for animals by Koolhaas et al. and Mendl et al. [19,20]. If we compare the two-tier model suggested by Koolhaas et al. [19] with our plot, place it at a second level on top of our scores plot and turn it clockwise 45°, so the arousal dimension is aligned with our RMSSD/SDNN ratio results, we can see that our descriptions for the different TT are similar to those suggested by them. Also, if we compare our scores plot with the core affect model suggested by Mendl et al. [20] and turn it clockwise 45°, so that the arousal dimension in Mendl et al.'s [20] plot is aligned to our RMSSD/SDNN ratio results, and if we assume that the valence dimension in their plot remains orthogonal to its arousal dimension, we see that our descriptions for the different TT are similar to those suggested by them. We must emphasise, though, that neither of our PCs exactly represents any of the dimensions suggested by the two reviews [19,20]. Yet, one might argue that PC 1 might be consistent with the valence or coping dimension. One could assume that individuals approaching the novel object perceive the situation rather positively (hence have a positive valence of the situation) and are coping proactively; however, this cannot be scientifically supported with the by us conducted test alone. When attempting to fit our two dimensions "contact to novel object-related" and "exploration-activity-related" into the five categories of temperament traits defined by Réale et al. [6], we find PC 1 to be congruent to category 2: exploration-avoidance, reaction to among others novel objects. PC 2, though, cannot be easily fitted into this model. It could reflect the general

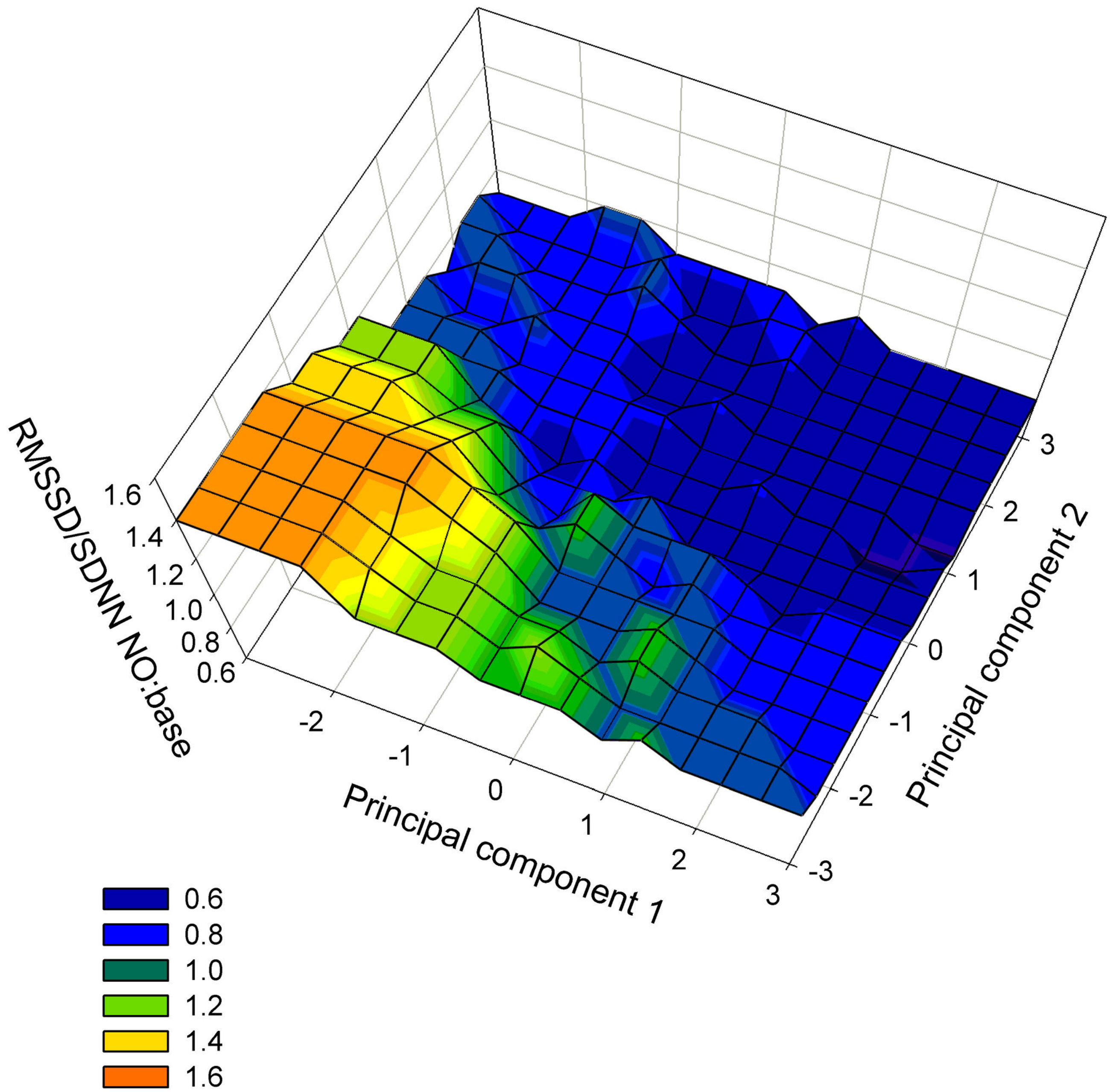


Figure 2. 3D scores plot including the RMSSD/SDNN-ratio. Smoothed 3D scores plot of 361 crossbreed calves during the novel-object test (NO) with the ratio of RMSSD/SDNN between NO and base measurement as the third dimension; colour spectrum from dark blue (strongly sympathetically activated) to red (strongly parasympathetically activated), smoother "running median", bandwidth method "nearest neighbours", and sampling proportion 0.100 (SigmaPlot 10.0, SysStat Software Inc., USA).
doi: 10.1371/journal.pone.0074579.g002

activity level of the test calf (category 3: activity), but the test situation could also be perceived as risky by the calves (category 1: shyness-boldness, this measurement can interfere with exploration-avoidance). The sociability of the animal (category 5: sociability, seeking presence of or avoiding conspecifics, by exploring the open field for a way back to the home pen) or a combination of the above mentioned categories are also possible explanations for PC 2.

When we used the loadings generated with data from 361 crossbreed animals on data of the animals from the two founder breeds at the same age and in a test repetition 4 months later, we received a similarly even distribution of those animals on the scores as of the scores of the crossbreeds. PC

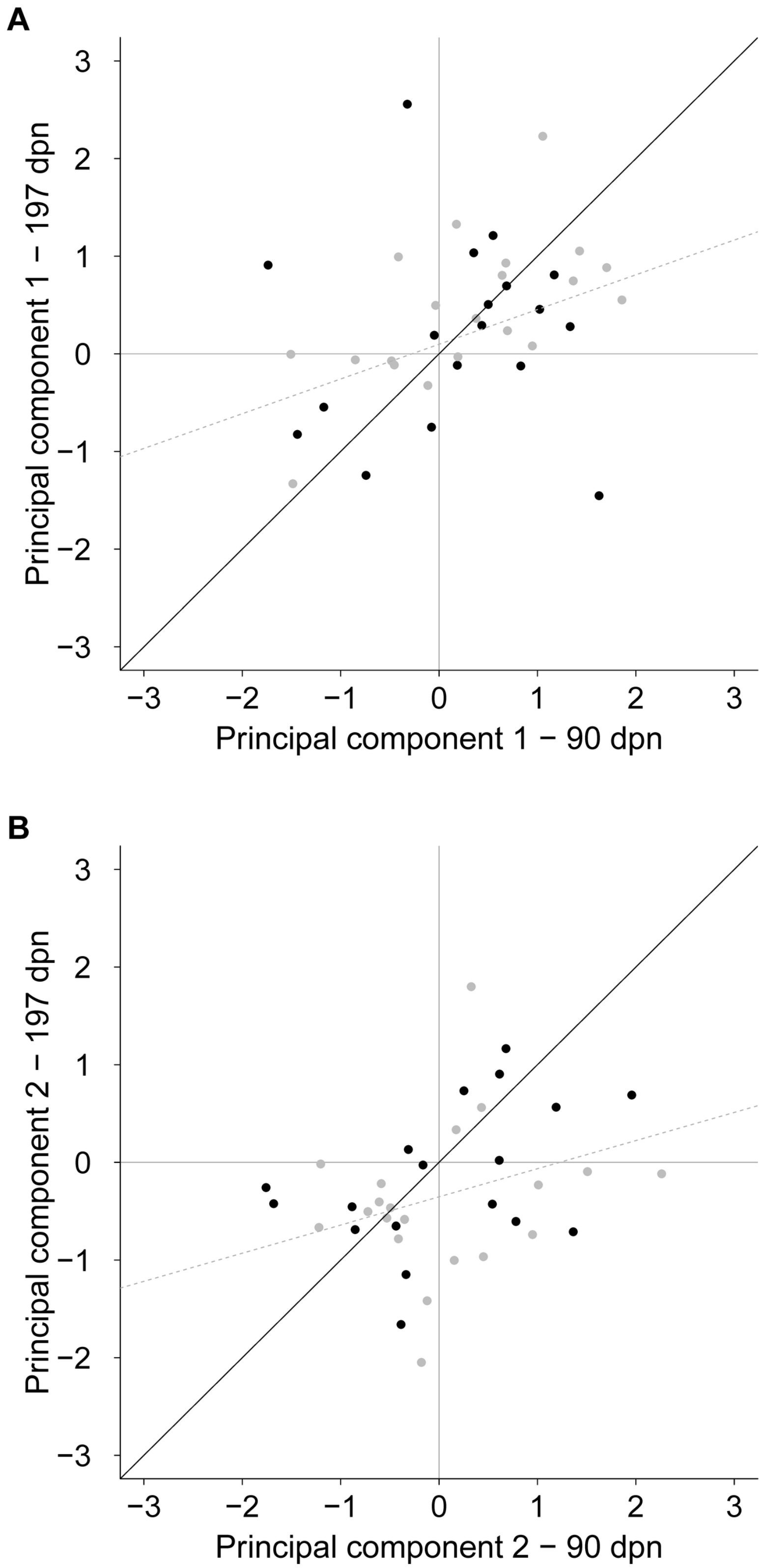


Figure 3. Score changes between the test ages separately for each principal component (PC). Changes in behavioural score between the test ages presented separately for (A) PC 1 and (B) PC 2 of 18 Charolais calves (black dots) and 20 Holstein Friesian calves (grey dots); solid black line marks 100% stability over time, dashed grey line marks the trend line.
doi: 10.1371/journal.pone.0074579.g003

1, which explained almost half of the variance in our data, correlated between the two test ages 90 and 197 dpn. Many animals were close to the 100% stability line in this PC. This result confirmed findings of other work, where individual differences were consistent over time in various species, some of which were tested at early ages [4,8,32,50–52]. PC 2 did not show similarly good results in terms of stability. As many animals with larger differences in that PC showed less exploration of the open field and more inactivity during the second test, one could argue for increased habituation to the open field or the test situation.

Interestingly, we could not find any differences in the scores between calves of the two breeds Charolais and Holstein Friesian. With the exception of Charolais calves running longer than Holstein Friesian calves, there were no differences between the breeds in the original data or the HRV base measurement. Breed differences in various behaviour tests have been reported; occasionally including relatively high heritability scores [53–56]. However, the conducted tests measured the reaction of cattle towards humans, which has been reported to be more influenced by management system than by breed [57]. We consider it likely, that the temperament traits measured in the presented study are evolutionary so profoundly important [6,20], that their expression does not differ between different breeds of the same species. Still, we cannot exclude the possibility that the two cattle breeds might develop differently in the measured temperament traits when they age past 7 months. Various temperament traits, though, have been reported to be already stable at the early age of 6-8 months in cattle and horses [32,50–52].

Conclusion

By using a principal component analysis to condense behaviours measured in calves in a novel-object test to two principal components (PC) and by correlating these PCs with heart rate variability measures, we could successfully describe four distinct temperament types that differed in behaviour and activity of the autonomous nervous system: “neophobic/fearful – alert”, “interested – stressed”, “subdued/uninterested – calm”, and “neoophilic/outgoing – alert”. During a repetition of the conducted novel-object test 4 months after the first test, more than 40% of the calves showed a similar behaviour pattern. In the remaining calves, the change was owed to a larger change in only one PC in nearly four-fifth of the animals. The novel object-related behaviours satisfactorily correlated between the two test ages. No differences in temperament scores could be found between sexes or breeds. Finally, we could describe distinct temperament types in calves based on behavioural and physiological measures emphasising the benefits of a multidimensional approach. The temperament-dependent assessment of a situation by the animals themselves should further be considered when trying to evaluate the housing and welfare of animals living under human care.

Supporting Information

Figure S1. Open field. Diagram of the open field (9.6 × 4.0 m) where the novel-object test was performed; circles indicate the alternative standing positions for the novel object, segment size 2.4 × 4.0 m.
(TIFF)

Figure S2. Score changes between the test ages. Changes in behavioural scores of 18 Charolais calves (black dots) and 20 Holstein Friesian calves (grey dots); arrow heads indicate the score of the same individual at the second test age of 197 dpn; for clarity (A) shows arrows for the 16 individuals scoring within 1 SD around their score at 90 dpn, (B) shows arrows for the 17 individuals scoring between 1–2 SD around their first score, and (C) shows arrows for the 5 individuals scoring farther than 2 SD from their first score.
(TIF)

Table S1. Correlation matrix with Spearman correlation coefficients of the 15 behaviours of the crossbreed calves during the novel-object test; D = duration (total time in s), F = frequency, L = latency (time in s until behaviour was first shown).
(DOCX)

Table S2. Mean ± SD, median, minimum, and maximum of behaviours of crossbreed calves during the novel-object test; duration and latency in s; D = duration (total time in s), F = frequency, L = latency (time in s until behaviour was first shown).
(DOCX)

Table S3. Mean ± SD, median, minimum and maximum of heart rate (HR in bpm), RMSSD (ms), SDNN (ms), and RMSSD/SDNN of crossbreed calves during base measurement in the home pen and during the novel-object test.
(DOCX)

Acknowledgements

We would like to thank Katrin Siebert and Ursula Engel for their precious help in executing the experiment with numerous animals, Heinz Deike for constructing and building the open field, Peter-Christian Schön for programming time-saving data processing tools and the animal keepers for helping to capture the calves.

Author Contributions

Conceived and designed the experiments: JL BP. Performed the experiments: KLG JL. Analyzed the data: KLG JL BP. Contributed reagents/materials/analysis tools: GN DR. Wrote the manuscript: KLG GN JL. Critical review and comments on data analyses and mansucript: GN DR BP.

References

1. Manteca X, Deag JM (1993) Individual differences in temperament of domestic animals: a review of methodology. Anim Welf 2: 247-268.
2. Locurto C (2007) Individual differences and animal personality. Comp Cogn Behav Rev 2: 67-78.
3. Wolf M, Weissing FJ (2010) An explanatory framework for adaptive personality differences. Philos Trans R Soc B 365: 3959-3968. doi: 10.1098/rstb.2010.0215.
4. Grandin T (1993) Behavioral Agitation During Handling of Cattle Is Persistent Over Time. Appl Anim Behav Sci 36: 1-9. doi: 10.1016/0168-1591(93)90094-6.
5. Grignard L, Boivin X, Boissy A, Le Neindre P (2001) Do beef cattle react consistently to different handling situations? Appl Anim Behav Sci 71: 263-276. doi:10.1016/S0168-1591(00)00187-8. PubMed: 11248377.
6. Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biol Rev 82: 291-318. doi:10.1111/j.1469-185X.2007.00010.x. PubMed: 17437562.
7. Forkman B, Boissy A, Meunier-Salaün MC, Canali E, Jones RB (2007) A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiol Behav 92: 340-374. doi:10.1016/j.physbeh.2007.03.016. PubMed: 18046784.
8. Range F, Bugnyar T, Schlögl C, Kotrschal K (2006) Individual and sex differences in learning abilities of ravens. Behav Processes 73: 100-106. doi:10.1016/j.beproc.2006.04.002. PubMed: 16675158.
9. Dingemanse NJ, Wolf M (2010) Recent models for adaptive personality differences: a review. Philos Trans R Soc B 365: 3947-3958. doi: 10.1098/rstb.2010.0221. PubMed: 21078647.
10. Bell AM, Sih A (2007) Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). Ecol Lett 10: 828-834. doi:10.1111/j.1461-0248.2007.01081.x. PubMed: 17663716.
11. Boersma GJ, Scheurink AJ, Wielinga PY, Steimer TJ, Benthem L (2009) The passive coping Roman Low Avoidance rat, a non-obese rat model for insulin resistance. Physiol Behav 97: 353-358. doi:10.1016/j.physbeh.2009.03.005. PubMed: 19296906.
12. Forkman B, Furuhaug IL, Jensen P (1995) Personality, Coping Patterns and Aggression in Piglets. Appl Anim Behav Sci 45: 31-42. doi: 10.1016/0168-1591(95)00601-N.
13. Trillmich F, Hudson R (2011) The Emergence of Personality in Animals: The Need for a Developmental Approach. Dev Psychobiol 53: 505-509. doi:10.1002/dev.20573. PubMed: 21866537.
14. Sulloway FJ (2011) Why siblings are like Darwin's finches: Birth order, parental investment, and adaptive divergence within the family. In: D BussPH Hawley. The evolution of personality and individual differences. Oxford: Oxford University Press.
15. Hudson R, Bautista A, Reyes-Meza V, Montor JM, Rödel HG (2011) The effect of siblings on early development: A potential contributor to personality differences in mammals. Dev Psychobiol 53: 564-574. doi: 10.1002/dev.20535. PubMed: 21866540.
16. Benus RF, Bohus B, Koolhaas JM, van Oortmerssen GA (1989) Behavioral Strategies of Aggressive and Nonaggressive Male-Mice in Active Shock Avoidance. Behav Processes 20: 1-12. doi: 10.1016/0376-6357(89)90008-9.
17. Koolhaas JM, Korte SM, de Boer SF, van der Vegt BJ, van Reenen CG et al. (1999) Coping styles in animals: current status in behavior and stress-physiology. Neurosci Biobehav Rev 23: 925-935. doi:10.1016/S0149-7634(99)00026-3. PubMed: 10580307.
18. Koolhaas JM, de Boer SF, Buwalda B, van Reenen K (2007) Individual variation in coping with stress: A multidimensional approach of ultimate and proximate mechanisms. Brain Behav Evol 70: 218-226. doi: 10.1159/000105485. PubMed: 17914253.
19. Koolhaas JM, de Boer SF, Coppens CM, Buwalda B (2010) Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. Front Neuroendocrinol 31: 307-321. doi: 10.1016/j.yfrne.2010.04.001. PubMed: 20382177.
20. Mendl M, Burman OH, Paul ES (2010) An integrative and functional framework for the study of animal emotion and mood. Proc R Soc Lond B 277: 2895-2904. doi:10.1098/rspb.2010.0303.
21. Posner J, Russell JA, Peterson BS (2005) The circumplex model of affect: an integrative approach to affective neuroscience, cognitive development, and psychopathology. Dev Psychopathol 17: 715-734. PubMed: 16262989.
22. Russell JA (2003) Core affect and the psychological construction of emotion. Psychol Rev 110: 145-172. doi:10.1037/0033-295X.110.1.145. PubMed: 12529060.
23. Veissier I, Aubert A, Boissy A (2012) Animal welfare: a result of animal background and perception of its environment. Animal Frontiers 2: 7-15. doi:10.2527/af.2012-0043.
24. Beauchaine T (2001) Vagal tone, development, and Gray's motivational theory: Toward an integrated model of autonomic nervous system functioning in psychopathology. Dev Psychopathol 30: 183-214. PubMed: 11393643.
25. van Reenen CG, O'Connell NE, van der Werf JTN, Korte SM, Hopster H et al. (2005) Responses of calves to acute stress: Individual consistency and relations between behavioral and physiological measures. Physiol Behav 85: 557-570. doi:10.1016/j.physbeh.2005.06.015. PubMed: 16081113.
26. Santucci AK, Silk JS, Shaw DS, Gentzler A, Fox NA et al. (2008) Vagal tone and temperament as predictors of emotion regulation strategies in young children. Dev Psychobiol 50: 205-216. doi:10.1002/dev.20283. PubMed: 18335488.
27. Calkins SD, Keane SP (2004) Cardiac Vagal Regulation across the Preschool Period: Stability, Continuity, and Implications for Childhood Adjustment. Dev Psychobiol 45: 101-112. doi:10.1002/dev.20020. PubMed: 15505799.
28. Boissy A, Manteuffel G, Jensen MB, Moe RO, Spruijt B et al. (2007) Assessment of positive emotions in animals to improve their welfare. Physiol Behav 92: 375-397. doi:10.1016/j.physbeh.2007.02.003. PubMed: 17428510.
29. von Borell E, Langbein J, Després G, Hansen S, Leterrier C et al. (2007) Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals - A review. Physiol Behav 92: 293-316. doi:10.1016/j.physbeh.2007.01.007. PubMed: 17320122.
30. Task Force of The European Society of Cardiology, The North American Society of Pacing and Electrophysiology (1996) Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. Eur Heart J 17: 354-381. doi:10.1093/oxfordjournals.eurheartj.a014868. PubMed: 8737210.
31. David M, Auclair Y, Cezilly F (2011) Personality predicts social dominance in female zebra finches, *Taeniopygia guttata*, in a feeding context. Anim Behav 81: 219-224. doi:10.1016/j.anbehav.2010.10.008.
32. Lansade L, Bouissou MF, Erhard HW (2008) Fearfulness in horses: A temperament trait stable across time and situations. Appl Anim Behav Sci 115: 182-200. doi:10.1016/j.applanim.2008.06.011.
33. Budaev SV (2010) Using Principal Components and Factor Analysis in Animal Behaviour Research: Caveats and Guidelines. Ethology 116: 472-480. doi:10.1111/j.1439-0310.2010.01758.x.
34. Gorsuch RL (1983) Factor Analysis. Hillsdale, NJ: Erlbaum.
35. Kaiser HF (1960) The Application of Electronic Computers to Factor Analysis. Educ Psychol Meas 20: 141-151. doi: 10.1177/001316446002000116.
36. Cattell RB (1966) The Scree Test For The Number Of Factors. Multivariate Behav Res 1: 245-276. doi:10.1207/s15327906mbr0102_10.
37. Horn JL (1965) A rationale and test for the number of factors in factor analysis. Psychometrika 30: 179-185. doi:10.1007/BF02289447. PubMed: 14306381.
38. Velicer WF (1976) Determining Number of Components from Matrix of Partial Correlations. Psychometrika 41: 321-327. doi:10.1007/BF02293557.
39. O'Connor BP (2000) SPSS and SAS programs for determining the number of components using parallel analysis and Velicer's MAP test. Behav Res Methods Instrum Comput 32: 396-402. doi:10.3758/BF03200807. PubMed: 11029811.
40. Comrey AL, Lee HB (1992) A First Course in Factor Analysis. New York, USA: Psychology Press.
41. Kaiser HF, Rice J (1974) Little Jiffy, Mark IV. Educ Psychol Meas 34: 111-117. doi:10.1177/001316447403400115.
42. Coppens CM, de Boer SF, Koolhaas JM (2010) Coping styles and behavioural flexibility: towards underlying mechanisms. Philos Trans R Soc B 365: 4021-4028. doi:10.1098/rstb.2010.0217. PubMed: 21078654.
43. Meager JJ, Fernö A, Skjæraasen JE, Järvi T, Rodewald P et al. (2012) Multidimensionality of behavioural phenotypes in Atlantic cod, *Gadus morhua*. Physiol Behav 106: 462-470. doi:10.1016/j.physbeh.2012.03.010. PubMed: 22465310.
44. Müller R, Schrader L (2005) Behavioural consistency during social separation and personality in dairy cows. Behaviour 142: 1289-1306. doi:10.1163/156853905774539346.

45. Tanaka S, Young JW, Halberstadt AL, Masten VL, Geyer MA (2012) Four factors underlying mouse behavior in an open field. Behav Brain Res 233: 55-61. doi:10.1016/j.bbr.2012.04.045. PubMed: 22569582.
46. Bergvall UA, Schapers A, Kjellander P, Weiss A (2011) Personality and foraging decisions in fallow deer, *Dama dama*. Anim Behav 81: 101-112. doi:10.1016/j.anbehav.2010.09.018.
47. Langbein J, Nürnberg G, Manteuffel G (2004) Visual discrimination learning in dwarf goats and associated changes in heart rate and heart rate variability. Physiol Behav 82: 601-609. doi:10.1016/j.physbeh.2004.05.007. PubMed: 15327907.
48. Zebunke M, Langbein J, Manteuffel G, Puppe B (2011) Autonomic reactions indicating positive affect during acoustic reward learning in domestic pigs. Anim Behav 81: 481-489. doi:10.1016/j.anbehav.2010.11.023.
49. Gerber AJ, Posner J, Gorman D, Colibazzi T, Yu S et al. (2008) An affective circumplex model of neural systems subserving valence, arousal, and cognitive overlay during the appraisal of emotional faces. Neuropsychologia 46: 2129-2139. doi:10.1016/j.neuropsychologia.2008.02.032. PubMed: 18440572.
50. Lansade L, Bouissou MF, Erhard HW (2008) Reactivity to isolation and association with conspecifics: A temperament trait stable across time and situations. Appl Anim Behav Sci 109: 355-373. doi:10.1016/j.applanim.2007.03.003.
51. Lansade L, Bouissou MF (2008) Reactivity to humans: A temperament trait of horses which is stable across time and situations. Appl Anim Behav Sci 114: 492-508. doi:10.1016/j.applanim.2008.04.012.
52. Kilgour RJ, Melville GJ, Greenwood PL (2006) Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity of humans, restraint and novelty. Appl Anim Behav Sci 99: 21-40. doi:10.1016/j.applanim.2005.09.012.
53. Gauly M, Mathiak H, Hoffmann K, Kraus M, Erhardt G (2001) Estimating genetic variability in temperamental traits in German Angus and Simmental cattle. Appl Anim Behav Sci 74: 109-119. doi:10.1016/S0168-1591(01)00151-4.
54. Hoppe S, Brandt HR, König S, Erhardt G, Gauly M (2010) Temperament traits of beef calves measured under field conditions and their relationships to performance. J Anim Sci 88: 1982-1989. doi:10.2527/jas.2008-1557. PubMed: 20154170.
55. Murphey RM, Duarte FAM, Torres Penedo MC (1980) Approachability of bovine cattle in pastures; breed comparisons and a breed x treatment analysis. Behav Genet 10: 171-181. doi:10.1007/BF01066267. PubMed: 7213302.
56. Murphey RM, Duarte FAM, Torres Penedo MC (1981) Responses of Cattle to Humans in Open Spaces - Breed Comparisons and Approach-Avoidance Relationships. Behav Genet 11: 37-48. doi:10.1007/BF01065826. PubMed: 7259721.
57. Boivin X, Le Neindre P, Chupin JM, Garel JP, Trillat G (1992) Influence of Breed and Early Management on Ease of Handling and Open-Field Behavior of Cattle. Appl Anim Behav Sci 32: 313-323. doi:10.1016/S0168-1591(05)80024-3.

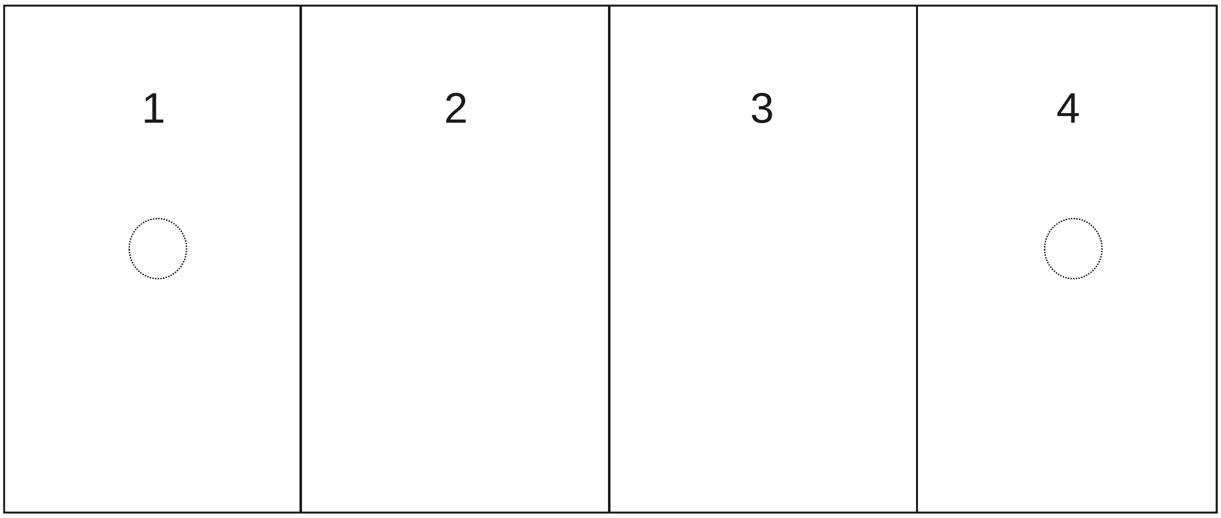


Figure S1. Open field. Diagram of the open field (9.6 × 4.0 m) where the novel-object test was performed; circles indicate the alternative standing positions for the novel object, segment size 2.4 × 4.0 m.

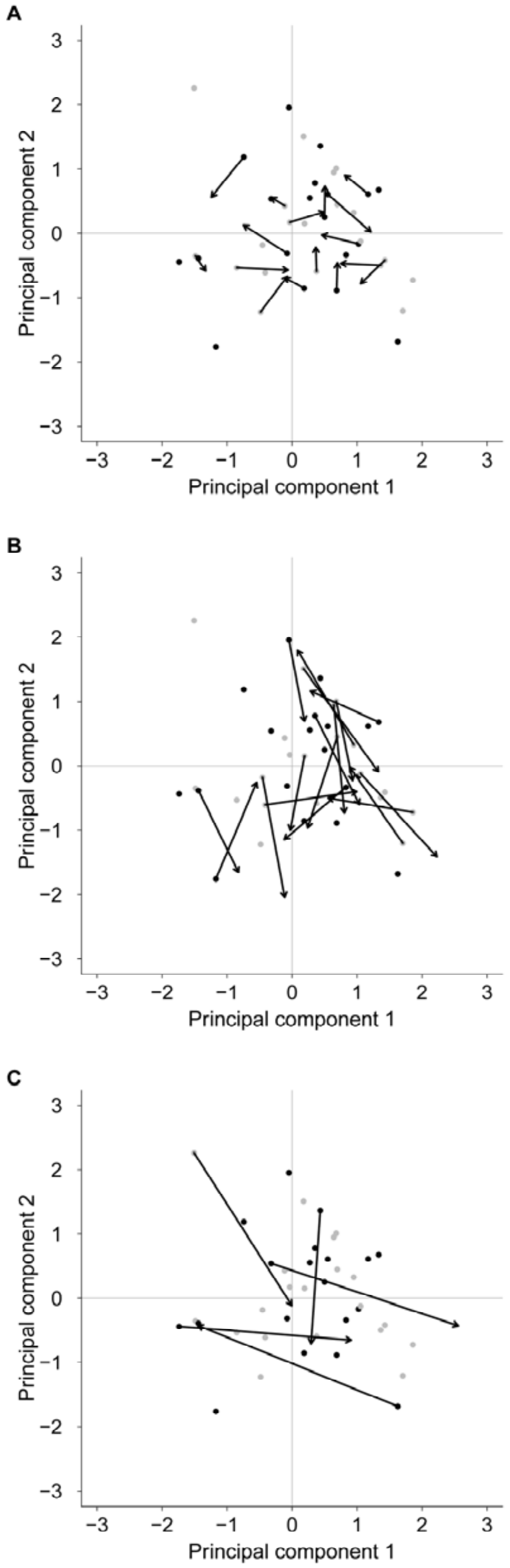


Figure S2. Score changes between the test ages. Changes in behavioural scores of 18 Charolais calves (black dots) and 20 Holstein Friesian calves (grey dots); arrow heads indicate the score of the same individual at the second test age of 197 dpn; for clarity (A) shows arrows for the 16 individuals scoring within 1 SD around their score at 90 dpn, (B) shows arrows for the 17 individuals scoring between 1-2 SD around their first score, and (C) shows arrows for the 5 individuals scoring farther than 2 SD from their first score.

Table S1. Correlation matrix of the 15 behaviours.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Contact-D														
2	Contact-F	0.825													
3	Contact-L	-0.618	-0.698												
4	Inactivity-D	-0.388	-0.461	0.481											
5	Exploration-D	0.257	0.320	-0.262	-0.820										
6	Exploration-L	-0.192	-0.278	0.304	0.494	-0.491									
7	Grooming-D	0.068	-0.012	-0.047	-0.252	0.185	-0.153								
8	Activity-D	0.424	0.511	-0.557	-0.802	0.447	-0.382	0.145							
9	Activity-L	-0.247	-0.309	0.436	0.329	-0.163	0.281	-0.092	-0.396						
10	Run-D	0.279	0.368	-0.416	-0.474	0.274	-0.186	0.022	0.427	-0.205					
11	Vocalisation-F	0.172	0.259	-0.306	-0.210	0.135	-0.043	0.009	0.253	-0.141	0.287				
12	Change of segment-F	0.469	0.609	-0.652	-0.724	0.432	-0.362	0.038	0.836	-0.399	0.667	0.349			
13	Object segment-L	-0.555	-0.623	0.926	0.478	-0.241	0.333	-0.059	-0.553	0.438	-0.430	-0.288	-0.646		
14	Object segment-D	-0.425	-0.483	0.698	0.455	-0.232	0.306	-0.140	-0.519	0.624	-0.359	-0.237	-0.597	0.747	
15	Object neighbouring segment-L	0.703	0.764	-0.719	-0.472	0.304	-0.268	0.014	0.501	-0.351	0.365	0.272	0.576	-0.700	-0.545

Table S1. Correlation matrix with Spearman correlation coefficients of the 15 behaviours of the crossbreed calves during the novel-object test; D = duration (total time in s), F = frequency, L = latency (time in s until behaviour was first shown).

Table S2. Behaviours during the novel-object test.

Behaviour	Mean	SD	Median	Min	Max
Contact-D	26.3	37.0	18.1	0.0	321.9
Contact-F	3.2	2.7	3.0	0	17
Contact-L	285.5	215.9	225.3	6.0	600.0
Inactivity-D	428.1	90.5	424.7	130.0	600.0
Exploration-D	69.1	51.1	59.5	0.0	250.6
Exploration-L	142.1	134.4	104.9	2.0	600.0
Grooming-D	5.8	12.7	0.0	0.0	114.8
Activity-D	80.5	47.9	74.9	0.0	317.5
Activity-L	60.8	95.7	24.5	0.0	600.0
Run-D	8.7	14.3	2.9	0.0	111.0
Vocalisation-F	15.3	14.9	11.0	0	76
Change of segment-F	17.5	12.7	15.0	0	65
Object segment-L	262.3	215.5	196.4	0.0	600.0
Object segment-D	174.6	189.1	123.7	0.0	591.4
Object neighbouring segment-L	131.9	110.4	103.9	0.0	600.0

Table S2. Mean ± SD, median, minimum, and maximum of behaviours of crossbreed calves during the novel-object test; D = duration (total time in s), F = frequency, L = latency (time in s until behaviour was first shown).

Table S3. Heart rate variability measures during base measurement and novel-object test.

HRV measures	Base measurement					Novel-object test				
	Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max
HR	113.9	16.4	113.4	70.2	186.4	125.0	17.9	125.0	68.0	190.5
RMSSD	7.86	7.00	5.64	2.14	68.06	8.71	6.01	6.39	2.93	37.25
SDNN	23.86	9.26	22.23	4.92	62.27	31.70	10.34	30.76	10.33	70.67
RMSSD/SDNN	0.35	0.19	0.28	0.12	1.15	0.31	0.18	0.25	0.13	1.16

Table S3. Mean ± SD, median, minimum and maximum of heart rate (HR, bpm), RMSSD (ms), SDNN (ms), and RMSSD/SDNN of crossbreed calves during base measurement in the home pen and during the novel-object test.

        - Usage Reports
    - Individual Membership
        - Individual Members List
    - Sponsorship
    - Advocacy
        - OA and PLOS Educational Materials
    - Shop The PLOS Store
    - Creative Reuse

Search for: Search

License

PLOS » About » Open Access » License

The Public Library of Science (PLOS) applies the Creative Commons Attribution License (CC-BY) to works we publish (read the human-readable summary or the full license legal code). Under this license, authors retain ownership of the copyright for their content, but allow anyone to download, reuse, reprint, modify, distribute, and/or copy the content as long as the original authors and source are cited. **No permission is required from the authors or the publishers.**

Appropriate attribution can be provided by simply citing the original article (e.g., Kaltenbach LS et al. (2007) Huntingtin Interacting Proteins Are Genetic Modifiers of Neurodegeneration. PLOS Genet 3(5): e82. doi:10.1371/journal.pgen.0030082).

For any reuse or redistribution of a work, users must also make clear the license terms under which the work was published.

This broad license was developed to facilitate free access to, and unrestricted reuse of, original works of all types. Applying this standard license to your own work will ensure that it is freely and openly available in perpetuity.

If you have a question about the Creative Commons License please use this contact form and choose “General Questions.”

Signup to get PLOS news

email address Sign up

Search PLOS articles for: Search

Tweets by @PLOS

- Home
- About
- Publications
- Publish
- Support Us
- Contact

Study III

Towards an easy applicable personality test

III

Connecting data of an automated restraint test and a standard behaviour test: An attempt in developing an easy applicable personality test for cattle

KATHARINA L GRAUNKE[1 3], JAN LANGBEIN[1], GERD NÜRNBERG[2], DIRK REPSILBER[2], PETER-CHRISTIAN SCHÖN[1] *

[1] Institute of Behavioural Physiology, Leibniz Institute for Farm Animal Biology (FBN), Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

[2] Institute of Genetics and Biometry, Leibniz Institute for Farm Animal Biology (FBN), Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

[3] University of Rostock, Faculty of Agricultural and Environmental Sciences (AUF), PHENOMICS office, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany

* Peter-Christian Schön (corresponding author)

Tel: +49-38208-68 805; Fax: +49-38208-68 802; E-mail: Schoen@fbn-dummerstorf.de

Abstract

As an animal's personality becomes more and more integrated into practice of breeding and selecting livestock, easy applicable behaviour tests to measure it are strongly demanded, yet very rare. We therefore aimed to develop an easy applicable procedure to measure personality in cattle (*Bos taurus*) with a maintained level of measure reliability. Data of 356 crossbreed calves tested at 90 and 91 dpn, respectively, in a newly developed automated restraint test (pulling test) was correlated with multidimensional personality types retrieved from a novel-object test (NO) including physiological measures of heart rate variability (HRV). With a principal component analysis (PCA) of the behaviours recorded in the NO and correlated with HRV, personality types (SC) were developed. These and the HRV-measure RMSSD/SDNN-ratio were correlated with a generalised linear mixed model (The MIXED Procedure, SAS 9.3, SAS Institute Inc., USA) to measures of the pulling test. Weight and sex had no influence on the pulling-test parameters tractive force, holding force, dwindling force, and total force, but on number of pulls (weight: $F = 4.27$, $p = 0.040$) and maximal tractive force (weight: $F = 48.6$, $p < 0.001$; sex: $F = 4.02$, $p = 0.046$). The behaviour parameters from the NO were combined to two principal components with a PCA. We divided the calves into nine score classes (SC), which significantly differed in the RMSSD/SDNN-ratio ($F = 5.04$; $p < 0.001$), hence in the activity of the autonomous nervous system. Tractive force was significantly influenced by the RMSSD/SDNN-ratio between NO and base measurement (F = 4.23, p = 0.041). SC tended to influence the parameters of the pulling test except for holding force and maximal tractive force (tractive force: F = 1.84, p = 0.070; dwindling force: F = 1.81, p = 0.075; total force: F = 1.84, p = 0.070; number of pulls: F = 1.74, p = 0.090). We found the candidate measure tractive force for further investigation in developing an easy applicable automated test for measuring cattle's personality on a large practice scale.

Keywords: behaviour; cattle; principal component analysis; heart rate variability; pulling test; temperament

1 Introduction

When trying to evaluate an animal's personality, one needs to rely on one or several behaviour tests. Probably the most common behaviour test is the open-field test, which has been used at least since the 1960s on mice to demonstrate behavioural differences between treatments or strains of mice (Thompson & Olian 1961). It has since been applied to various animal species, such as chickens, fish, pigs, cattle, etc. (Fraser 1974, Gallup & Suarez 1980, Yayou *et al.* 2010, Klefoth *et al.* 2012) and has been enhanced with novel stimuli to the classic novel-object test and equivalents with other novel stimuli like food or a human (Forkman *et al.* 1995, Kilgour *et al.* 2006, Sibbald *et al.* 2009). Several often used behaviour test have since been developed and used mostly in livestock. Some of these tests are the human-approach test, which is used in cattle or sheep (Murphey *et al.* 1981, Goddard *et al.* 2000), the back test (only pigs, Bolhuis *et al.* 2004), or the crush test, where observers rate the animals behaviour after pre-defined score categories (Behrends *et al.* 2009, Pajor *et al.* 2010). In cattle, the docility test has been developed to explicitly measure the individual's tameness towards humans (Boivin *et al.* 1992, Plusquellec *et al.* 2001). Together with the flight-time test, where the time leaving a scale or crush is taken (Müller & von Keyserlingk 2006, Gibbons *et al.* 2011), they are the two tests that have been included into breeding values of beef breeds (International Beef Recording Scheme 2012). These two included tests, however, only depict a very small part of an animal's personality. When one wants to select animals on other behaviour traits than docility or flightiness, there is no easy applicable behaviour test so far, that lets one select, e.g., curious animals for breeding.

Many of the aforementioned behaviour tests are always more or less differently executed and therefore rarely standardised. The open-field test, e.g., does not have a generally accepted duration, a standardised open-field size, or other generally applied set-ups such as the presence or absence of a start box and a protocol whether the test animal should be allowed to enter the open-field voluntarily or not (Boissy & Bouissou 1995, van Reenen *et al.* 2004). Recorded behaviours do often not resemble, let alone inter-observer dependent differences cannot fully be eliminated (Welfare Quality® 2009). In many tests, human interaction with the tested animals (Boissy & Bouissou 1988) is inevitable or part of the tests themselves, and observer-dependent rating are often fundamental parts of the tests (Grandin 1993). Some methods are therefore at least partly subjective and judgemental, especially when categories are used to classify the animals. Further, an easy applicable test used by anyone anywhere in the world, trained or untrained, educated or not, in any housing system (probably except for old-fashioned tied-barns) should yield objective comparable results. As some official breeders have added personality traits to their breeding goals (e.g., Zuchtbetrieb Zachert 2013, Traditional Herefords 2013), there is a strong request for an observer-independent standardised method of measuring personality traits with an easy applicable, practicable test with a high level of measure reliability.

We chose a commonly used behaviour test, the novel-object test, to identify different personality traits and to differentiate animals from one another. This test is known to provoke a reaction, which correlates with displayed behaviour during other tests (Lansade *et al.* 2008, David *et al.* 2011) or with social behaviour (David *et al.* 2011). The automated test was an easy applicable restraint test developed by us. Restraint test situations provoke either fear-related escape behaviour or the expression of the animal's reluctance of the situation, which describes an important part of personality (Kilgour *et al.* 2006, Benhajali *et al.* 2010), and are characterised by three main features: the number of escape attempts per time unit, the maximal force per attempt, and the force per time unit. The widely accepted crush test uses these features as well, but measures them indirectly by having observers judge them and rate the animals according to a predefined category scale (Grandin 1993, Kilgour *et al.* 2006, Benhajali *et al.* 2010, Gibbons *et al.* 2011).

The aim of this study was to take a first step in developing an easy applicable procedure to measure personality in cattle (*Bos taurus*) based on an automated restraint test that was evaluated with data of a multidimensional description of cattle temperament and simultaneously measured physiological data during a novel-object test. Taking both automatically measured data and ethological and physiological data, we meant to reveal a practicable way to test large numbers of individuals for their personality with maintained level of measure reliability.

2 Animals, materials, and methods

2.1 Animals and housing

We tested 361 calves (175 male, 186 female) of the F_2-generation of a running breeding project (Holstein Friesian × Charolais) with 90 and 91 dpn (± 3 dpn, days *post natum*) first in a novel-object test and then a pulling test. All calves were bred via embryo transfer into unrelated Holstein Friesian heifers as recipient mothers and were born and tested between 2004 and 2010. The calves were kept in various small groups of up to nine animals of similar age, apart from their recipient mothers from day one. Pens had a size of 6 m x 7 m and were covered with deep litter. Until 90 dpn, the calves were not subject to any other experimental procedures, and handling did not exceed routine handling by the animal keepers except in the case of animals requiring treatment for sickness.

After weaning, the animals were weighed at 111 dpn (± 3 dpn). The weight at the day of the restraint test was calculated based on the average daily weight gain from birth to weaning and the exact age at the experiment. On average, the calves weighed 119 kg (range: 74-160 kg, SD ± 14 kg) revealing no differences between male and female calves (male: 123 ± 14 kg, female: 116 ± 13 kg).

We further tested each 20 calves (10 male, 10 female) of the founder breeds Holstein Friesian and Charolais at 91 and 92 dpn (± 3 dpn) and a second time at 197 and 198 dpn (± 12 dpn). The calves were purchased from breeders and arrived at our facilities two days after birth at the latest. Male and female calves were housed together until the second test had been conducted. These calves were born and tested between 2008 and 2012. One male Charolais calf died, so there were 19 Charolais calves tested at the first test age. At the second test age, one male Charolais calf became extremely distressed during testing (novel-object test) and risked serious injury. The test was terminated; thus there is data of this test of 18 (8 male, 10 female) Charolais calves at 197 dpn.

All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Ministry of Agriculture, the Environment and Consumer Protection of the federal state Mecklenburg-Vorpommern, Germany.

2.2 Restraint test (pulling test)

To measure escape behaviour and reluctance in calves, we recorded the animals' expended power during a restraint situation (further called pulling test) applying the force transducer Megatron KT1400 (Megatron Elektronik AG & Co., Putzbrunn, Germany) when they were 91 dpn. The force transducer, which was connected to a PC, was built onto a metal bar of the home pen of the animals so that the cable to the calf elongated the force direction of the transducer. During the pulling test the tested calf was put on a halter, tied to the cable leading to the force transducer and stayed in one half of its home pen, while the pen mates were confined to the other half of the pen using metal bars to avoid falsifying the measurement. Visual, acoustic, and olfactory contact between the test calf and its pen mates therefore persisted in the hope of minimising effects and influences of isolation. The software recording the tractive force was then initiated and all human handlers left the barn. After a 30 min-recording period, a handler entered the barn, released the test calf, and reunited it with its pen mates.

A tractive force-time diagram was recorded and parameters from this diagram calculated using LabView 6.1 (National Instruments GmbH Germany, Munich, Germany).

The following parameters were calculated:

- Total force: an integral of the total tractive force diagram in kNs consisting of:
    - Tractive force: an integral of positive values of the derivation of the tractive force diagram (meaning only the upward tractive phases) in kNs (force multiplied by time that a calf pulls on the cable with elevating effort)
    - Holding force: an integral of zero values of the derivation of the tractive force diagram (meaning only the holding tractive phases) in kNs (force multiplied by time that a calf pulls on the cable with stable effort; i.e. “hanging” in the cable without moving)
    - Dwindling force: an integral of negative values of the derivation of the tractive force diagram (meaning only the downward tractive phases) in kNs (force multiplied by time that a calf pulls on the cable with declining effort; i.e. stop pulling on the cable)
- Number of pulls: all local maxima of the tractive force diagram above a threshold level of 60 N (empirical value)
- Maximal tractive force: the highest amplitude of the tractive force diagram (global maximum) in N

More detailed technical information on the appliance and recording can be found in Graunke *et al.* (2013a). Due to technical problems we lost data from one male and four female crossbreed calves.

2.3 Novel-object test

The behaviour test was performed at 90 dpn in an open field of 9.6 m × 4.0 m in size, which was unknown to the calves prior to testing. It was divided into four segments of 2.4 m × 4.0 m each. After allowing the test animal to acclimatise to the open field for 10 min, a novel-object test (NO) was conducted presenting an orange-white traffic pylon of 0.5 m height as novel object. It was let down into the outer segment, which was the farthest from where the calf stood. The NO lasted for 10 min. During the test, behaviour was live-recorded using The Observer 5.0 (Noldus, The Netherlands). Of in total 438 behaviour test sessions, 428 were conducted by three experienced observers whose observation highly correlated during a 90 min-test session (Pearson's Rho 0.973, $p < 0.001$). Recorded behaviours with their definitions and type of recording are listed in Table 1. For further analysis, the latency of the behaviours an individual did not show during the 10 min behaviour test was set to the maximum time of 600 s (10 min).

Table 1: Definition and type of recording of the behaviours live-recorded during the novel-object test; D = duration (total time in s during), F = frequency, L = latency (time in s until behaviour was first shown)

Behaviour	Type of recording	Definition
Contact with novel object (contact)	D, F, L	Physical contact with any part of the body with the novel object or sniffing the novel object while being closer than 0.1 m to it
Inactivity	D	At least three legs touch the ground, no forward movement
Exploration	D, L	Sniffing or licking the wall or floor of the open field
Grooming	D	Calf licking or scratching itself with one hind leg
Activity	D, L	Max. 3 legs touch the ground, forward movement
Running	D	Max. 2 legs touch the ground, fast forward movement
Vocalisation	F	Any kind of sound the calf makes
Change of segment	F	Leaving one segment and entering another with at least the forelegs
Habitation in segment where the novel object is placed (object segment)	D, L	With at least the forelegs in the segment in which the novel object is placed
Habitation in segment next to segment where the novel object is placed (object neighbouring segment)	L	With at least the forelegs in the segment next to the segment in which the novel object is placed

We applied a heart monitor system (Polar S810i, Polar Electro, Oy, Finland) to measure the heart beat activity during the test. The calves were fitted with flexible belts integrating two electrodes and a transmitter for wireless transmission of the R-R-interval data series to a separate storage device. One electrode was placed next to the sternum and the other behind the scapula, both on the left side of the most cranial part of the chest behind the forelegs. The coat under the electrodes was shaved and a conductive gel was used for better electrical conductivity. After the calves were fitted with the belts, they were then left alone with their pen mates in their home pen to gain base measurements. After 30 min, they were led into the open field for acclimatisation and testing. After testing, the R-R data series were corrected when necessary using Polar Precision Performance SW version 4.03 (Polar Electro, Oy, Finland) with the standard set-up. We accepted an error correction of up to 10 % per 5-min interval. In further processing of the data, neither differences between two R-R-intervals larger than 150 ms nor identical values of five or more consecutive R-R-intervals were accepted. With a program developed with LabView 2009 version 9.0 (National Instruments Germany GmbH, Munich, Germany) we detected complete 1-min intervals in the base measurements (starting 5 min after the experimenters left the barn) and the test, and calculated i. a. RMSSD/SDNN for each complete 1-min interval (see Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology (1996) for the exact calculation of the variable). The RMSSD/SDNN increases, when the parasympathetic nervous system's activity increases and/or when the sympathetic nervous system's activity decreases and therefore gives reliable information about the individual's perception of the situation. When there were at least seven complete 1-min intervals per base measurement and per test, the program further determined the mean of the first seven values. This was the case in 272 crossbreed calves (134 male, 138 female), 17 Holstein Friesian calves at 90 dpn (8 male, 9 female), all 20 Holstein Friesian calves at 197 dpn, all 19 Charolais calves at 90 dpn, and 17 Charolais calves at 197 dpn (8 male, 9 female). The ratio of RMSSD/SDNN of test and base measurement was used for further analyses.

2.4 Statistical analysis

All statistical analyses were performed using SAS 9.3, SAS Institute Inc., USA. In a preliminary analysis, we checked for the influence of sex and weight on the parameters of the pulling test using a one-way analysis of covariance model (ANCOVA, The GLM Procedure) with the fixed factor sex and the co-variable weight, and further the influence of breed, sex, and their interaction on the parameters of the pulling test. A Principal Component Analysis (PCA) was performed to condense the 15 behaviours recorded in the NO to new (latent) Principal Components (PC). It was conducted with The FACTOR Procedure with the parameter settings: method=PRIN, prior=ONE, rotation=VARIMAX. Since some of the behaviours were not continuous and/or normally distributed, we used a correlation matrix of all pairwise correlations of our 15 behavioural measures as input data set applying the nonparametric Spearman's rank correlation test (using The CORR procedure). We performed four methods to choose the appropriate final number of extracted PCs: Kaiser's number of eigenvalues > 1 (Kaiser 1960), Cattell's scree-test (Cattell 1966), Horn's Parallel test (Horn 1965), and Velicer's Minimum Average Partial (MAP) test (Velicer 1976). For the Parallel test and MAP test, we applied the SAS syntaxes provided by O'Connor (2000). We decided for two PCs in the final PCA calculation, since three of these methods led to a two PC solution (except for Kaiser's number of eigenvalues > 1). Corresponding PC scores for each calf were calculated with The SCORE Procedure. The results of the PCA were used to classify the calves into score classes (SC). The influence of score class and sex on the HRV measure was tested by a two-way analysis of variance model (ANOVA, The MIXED Procedure) with the fixed factors SC, sex, and their interaction. To correlate results from the NO and the pulling test, the influence of the RMSSD/SDNN-ratio, the PC scores, and the score class of the NO on parameters measured during the pulling test was tested by a two-way analysis of variance model (ANOVA, The MIXED Procedure) with the fixed factors RMSSD/SDNN-ratio, PC 1, PC 2 and SC. For the variables which were influenced by weight, sex, or both, the random effects of weight at the day of the experiment, sex, or both were included. Posthoc tests were performed with a Tukey-Kramer correction for multiple testing.

3 Results

3.1 Pulling test

Descriptive statistics of the recorded parameters of 356 crossbreed calves during the pulling test are shown in Table 2. Weight and sex had no influence on these parameters except for weight having a significant influence on number of pulls ($F = 4.27$, $p = 0.040$) and maximal tractive force ($F = 48.6$, $p < 0.001$) with heavier calves pulling more often and with more maximal tractive force, and sex having an influence on maximal tractive force with female calves pulling with greater force ($F = 4.02$, $p = 0.046$).

Table 2: Mean ± SD, median, minimum, and maximum of pulling parameters behaviours of 356 crossbreed calves during a 30 min restraint test; tractive, holding, dwindling, and total force in kNs and maximal tractive force in N

Pulling parameters	Mean	SD	Median	Min	Max
Tractive force	13.24	4.68	12.29	1.96	30.70
Holding force	17.59	7.09	16.32	1.98	44.62
Dwindling force	31.11	11.70	28.76	4.14	72.30
Total force	61.94	23.33	57.24	8.07	144.41
Number of pulls	102.9	70.1	84	0	424
Maximal tractive force	252.0	88.3	240.5	43.3	562.8

The two breeds Charolais and Holstein Friesian did not differ in their pulling behaviour except for the holding force ($F = 12.77$, $p < 0.001$), with Charolais calves holding stronger than Holstein Friesian calves (LSM 271.1 Ns vs. 154.3 Ns). Between the two test ages, the number of pulls and maximal tractive force tended to correlate ($r = 0.31$, $p = 0.056$; $r = 0.30$, $p = 0.067$).

3.2 Novel-object test

Descriptive statistics of the recorded behaviours of the crossbreed calves in the NO and their HRV measures during base measurement and NO can be found in Graunke *et al.* (2013b).

The factor analysis resulted in two factors whose loadings in the behaviours are shown in Table 3. PC 1 was most influenced by behaviours occurring in the novel-object context and PC 2 was most influenced by behaviours in context with the exploration of the open field (but not the novel object) and the inactivity of the animals (Table 3). The measure of sampling adequacy (MSA) with 0.833 was “meritorious” (Kaiser & Rice 1974) and the two PCs explained 46.8 % and 11.2 %, respectively, of the variation in the data. We divided the animals into score classes (SC) defining the intermediate level of the scores at ± 0.5 SD around the zero line and so received nine SC (Table 4). More detailed results of this analysis are presented in Graunke *et al.* (2013b).

The most reliable information about the balance between sympathetic and parasympathetic nervous system is gained from the RMSSD/SDNN. The calves of SC VII were strongly parasympathetically activated while calves of SC III and VI were sympathetically activated during the NO (Figure 1A). The balance between the sympathetic and parasympathetic nervous system either did not change or change very little in the other SC. Therefore, we could describe the distinct temperament types (TT; SC I, III, VII, and IX) by characteristic terms for the displayed behaviour and the activated parts of the autonomous nervous system: “neophobic/fearful – alert” (SC I), “interested – stressed” (SC III), “subdued/uninterested – calm” (SC VII), and “neophilic/outgoing – alert” (SC IX; Figure 1A).

Table 3: Loadings of the behaviours in principal component (PC) 1 and PC 2 gained from the principal component analysis of the novel-object test; loadings above 0.71 in bold type, loadings above 0.63 in italics (cp. Comrey & Lee (1992) loadings above 0.71 rated “excellent”, loadings above 0.63 rated “very good”), D = duration (total time in s), F = frequency, L = latency (time in s until behaviour was first shown)

Behaviour	PC 1	PC 2
Contact-D	**0.76457**	0.05006
Contact-F	**0.83250**	0.12250
Contact-L	**-0.89613**	-0.13959
Inactivity-D	-0.41347	**-0.85549**
Exploration-D	0.15037	**0.82661**
Exploration-L	-0.19767	*-0.63679*
Grooming-D	-0.08038	0.42876
Activity-D	0.56716	0.61371
Activity-L	-0.49598	-0.21244
Running-D	0.47287	0.34835
Vocalisation-F	0.38216	0.07442
Change of segment-F	*0.70109*	0.51393
Object segment-L	**-0.87210**	-0.15841
Object segment-D	**0.83888**	0.12061
Object neighbouring segment-L	**-0.73723**	-0.22699

Table 4: Level of scores in principal component (PC) 1 and PC 2 and the division into nine score classes numbered with Roman numerals

PC 2 \ PC 1	–	–/+	+
+	I	II	III
–/+	IV	V	VI
–	VII	VIII	IX

3.3 Connecting the pulling test and the novel-object test

The ratio of RMSSD/SDNN between NO and base measurement significantly influenced tractive force (F = 4.23, p = 0.041), but no other parameter of the pulling test, where calves with a higher RMSSD/SDNN-ratio pulling with more force than calves with a lower RMSSD/SDNN-ratio. Neither did PC 1 and PC 2 influence any measured parameter. SC tended to influence the parameters of the pulling test with the exception of holding force and maximal tractive force (tractive force: F = 1.84, p = 0.070; dwindling force: F = 1.81, p = 0.075; total force: F = 1.84, p = 0.070; number of pulls: F = 1.74, p = 0.090).

Figure 1 plastically illustrates how the different SCs gained from the NO reacted in the pulling test (Figure 1A) and how the tractive force of the pulling test correlated with the RMSSD/SDNN-ratio measured during the NO (Figure 1B).

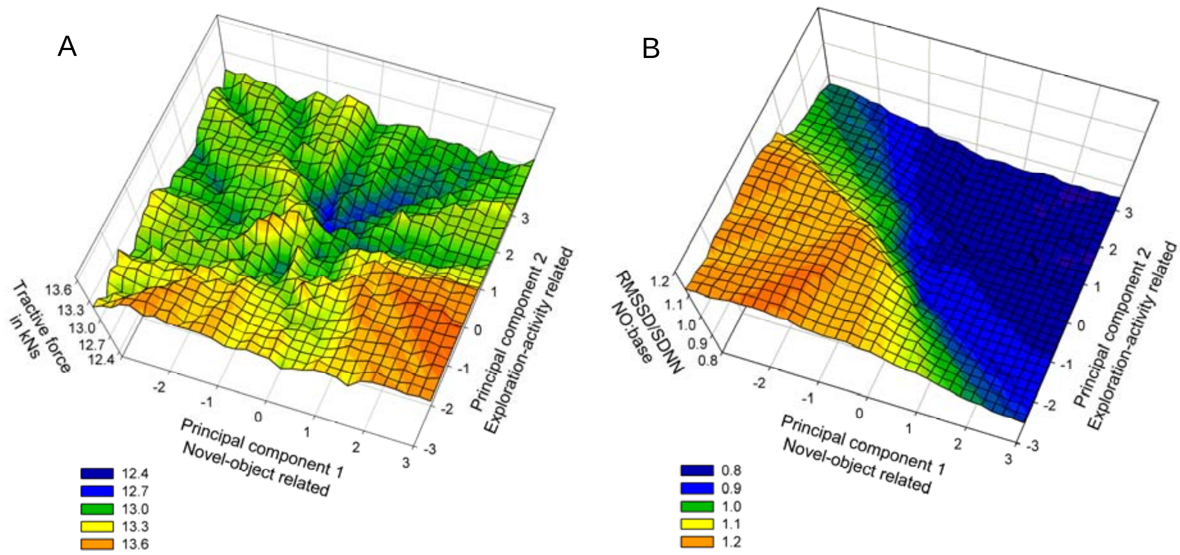


Figure 1: Smoothed 3D scores plot of crossbreed calves during the NO with (A) the tractive force in kNs of the pulling test as the third dimension (n = 356) and with (B) the ratio of RMSSD/SDNN between NO and base measurement as the third dimension (n = 272); colour spectrum from dark blue (low pulling effort/strongly sympathetically activated) to red (high pulling effort/strongly parasympathetically activated), smoother "running average", bandwidth method "nearest neighbours", and sampling proportion 0.500 (SigmaPlot 10.0, SysStat Software Inc., USA).

4 Discussion

The interest in personality or temperament in livestock has increased as its importance to animal welfare and breeding has gained acknowledgement from people working within these fields (Veissier *et al.* 2012, Zuchtbetrieb Zachert 2013). An easy applicable test that at best can be used by anyone anywhere in the world, trained or untrained, educated or not, in any housing system, and will yield objective comparable results, is strongly required by agricultural and breeding practice. In Graunke *et al.* (2013a) we have presented an appliance, which has the potential to become a part of such an easy applicable test. In this study, we have tried to evaluate this objective appliance with ethological and physiological data of a common behaviour test used to measure personality traits in animals, the novel-object test.

The stability of the calves' behaviour during the here presented pulling test could be shown with tendencies to correlation between the test ages in the two parameters number of pulls and maximal tractive force, both of which were influenced by weight in the crossbreeds. We assume it is likely that the heavier calves at 90 dpn continuously weighed more 3 months later at the second test, which is why weight should influence these parameters equally at both ages and should not interfere with the tendencies to correlation.

Correlations between parameters of the pulling test and the NO could be found, although not particularly distinct and appealing. The RMSSD/SDNN-ratio between the NO and base measurement correlated with the tractive force the animals were executing during the 30-min pulling test, and SC tended to correlate with tractive force, dwindling force, total force, and number of pulls. Calves of SC III pulled with least tractive force and were very active and stressed/sympathetically activated during the NO. Animals of SC IX pulled with greatest tractive force being alert/intermediate in their RMSSD/SDNN-ratio and very interested in the novel object during the NO, whereas animals of SC VII also pulled with high tractive force but were clearly parasympathetically activated/subdued in behaviour during the NO. This might be surprising, if one expected a sympathetically activated individual pulling with more effort than a parasympathetically activated individual. However, one should keep in mind that in the NO the test subjects could move freely and could choose freely to approach the novel object, explore the open field, or do anything in-between. The pulling test on the other hand forced them to stay at the spot, tied up, which they had not experienced earlier, and although in visual, acoustic, and olfactory contact with their pen mates not being able to approach them. This lack of controllability of the situation might have triggered a panic reaction in individuals parasympathetically activated during the NO resulting in higher tractive force. Animals sympathetically activated in the NO did obviously not react in the same way to the restraint in the pulling test; their reaction could be explained as much quicker surrender to the situation.

Tractive force is therefore a candidate parameter of the pulling test that could become a predictor of the calves' behaviour during a NO. So far, however, analyses and possibly even the experimental set-up of the pulling test need to be sharpened up in order to draw more conclusions from the presented results. A partial least square projection to latent structures could find a so far hidden correlation between the pulling test and the novel-object test, and if not the animals maybe could be tested solitary without any visual, acoustic, and olfactory contact to their pen mates.

In the literature, correlations between different behaviour tests used to measure personality traits have often been not very strong, inconsistent between different ages, contradictory between different studies, or lacking (van Erp-van der Kooij *et al.* 2002, van Reenen *et al.* 2004, Brown *et al.* 2009, Spake *et al.* 2012). Usually, correlations between different physiological and ethological parameters are found between behaviour tests confronting the animals with similar challenges, e.g., between a novel-environment test and a NO (van Reenen *et al.* 2005). Correlations have also been found between less obviously connected tests like a human proximity test or the flight distance and contact-to-novel-object-related behaviour during a novel-object-startle test, where the object is lifted up every time the animal gets close (Kilgour *et al.* 2006). Van Reenen *et al.* (2004) found a relatively good correlation (r = 0.48) between locomotion during a NO and a restraint test similar to the here presented pulling test in 6 months old calves, but not during earlier tests at younger ages.

Higher correlations between different behaviour tests have partly been found in the rare studies, which tested animals in groups (van Erp-van der Kooij *et al.* 2002, Brown *et al.* 2009). Van Erp-van der Kooij *et al.* (2002) found correlations of up to 0.44 and Zebunke *et al.* (unpublished manuscript) of up to 0.51 between NO, open-door test, and novel-human test in group-tested pigs. Possibly animals naturally living in groups should be tested in their established group in order to gain more reliable responses. Only recently, it has been shown that the presence, absence, and encouragement of an attachment figure significantly influenced the behaviour during a behaviour test in dogs in such way that the attachment figure served as a secure base from which a task can be fulfilled (Horn *et al.* 2013). Pen mates are the only attachment figures for most young livestock. The reaction to isolation from their pen mates and hence their secure base might overlap the animals' reactions towards, e.g., a novel object so much, that the fraction of novel-object-related behaviour one sees is not very reliable. There has also been reported a difference in behaviour between partial isolation and complete isolation, where completely isolated dwarf goats were generally less active than partially isolated individuals (Siebert *et al.* 2011). This may have influenced the NO, which did allow acoustic contact to pen mates, but could first and foremost apply to the pulling test, where the test animals could see, hear, and smell their pen mates. Possibly the attempt to minimise the stress of isolation by obtaining the maximal possible contact to the pen mates was counterproductive for this set-up and should be avoided in a future experimental set-up.

One also has to realize that animal personalities are most probably too complex, multidimensional constructs to be determined with one single test alone. Lacking or weak inter-test correlations do not necessarily diminish the involved tests, but may rather point out that they measure different aspects of personality. However, depending on the purpose one needs to select animals for, one or two tests might be enough to find out about the suitability of the tested animal for one’s purpose. Although not distinct, the correlations between the pulling test and the NO presented in this study should be worth looking for clearer connections between the two tests. This could be achieved with more complex statistical analyses like the partial least squares projections to latent structures or slight changes in the experimental set-up.

5 Conclusion

By correlating data on personality of young cattle with their behaviour during an automated restraint test, we meant to take a first step in developing an easy applicable procedure to measure personality in cattle. We found that animals who were differently activated of their autonomous nervous system during a novel-object test behaved differently during the automated restraint test. Additionally, the type of personality retrieved from the novel-object test tended to correlate with several measures of the restraint test, overall leaving us with at least one candidate measure, the tractive force, for advanced investigation. This study can therefore serve as a starting point for further development of an easy applicable automated test for measuring cattle’s personality on large practice scale.

6 Acknowledgements

We would like to thank Dieter Sehland and Kurt Wendland for their help in constructing the pulling appliance and Katrin Siebert, Ursula Engel, and Dieter Sehland for their precious help in executing the experiments with numerous animals. Furthermore, we thank Heinz Deike for constructing and building the open field and the animal keepers for helping to capture the calves. The first author Katharina L. Graunke was financed by the German agriculture network PHENOMICS, grant no. 0315536G, supported by the Federal Ministry of Education and Research, Germany.

7 References

Behrends S, Miller R, Rouquette F, Randel R, Warrington B, Forbes T, Welsh T, Lippke H, Behrends J, Carstens G, Holloway J, 2009. Relationship of temperament, growth, carcass characteristics and tenderness in beef steers. *Meat Sci* **81,** 433-438.

Benhajali H, Boivin X, Sapa J, Pellegrini P, Boulesteix P, Lajudie P, Phocas F, 2010. Assessment of different on-farm measures of beef cattle temperament for use in genetic evaluation. *J Anim Sci* **88,** 3529-3537.

Boissy A, Bouissou MF, 1988. Effects of Early Handling on Heifers Subsequent Reactivity to Humans and to Unfamiliar Situations. *Appl Anim Behav Sci* **20,** 259-273.

Boissy A, Bouissou MF, 1995. Assessment of individual differences in behavioural reactions of heifers exposed to various fear-eliciting situations. *Appl Anim Behav Sci* **46,** 17-31.

Boivin X, Le Neindre P, Chupin JM, Garel JP, Trillat G, 1992. Influence of Breed and Early Management on Ease of Handling and Open-Field Behavior of Cattle. *Appl Anim Behav Sci* **32,** 313-323.

Bolhuis JE, Schouten WGP, de Leeuw JA, Schrama JW, Wiegant VA, 2004. Individual coping characteristics, rearing conditions and behavioural flexibility in pigs. *Behav Brain Res* **152,** 351-360.

Brown JA, Dewey C, Delange CF, Mandell IB, Purslow PP, Robinson J, Squires EJ, Widowski TM, 2009. Reliability of temperament tests on finishing pigs in group-housing and comparison to social tests. *Appl Anim Behav Sci* **118,** 28-35.

Cattell RB, 1966. The Scree Test For The Number Of Factors. *Multivar Behav Res* **1,** 245-276.

Comrey AL, Lee HB, 1992. *A First Course in Factor Analysis*, Second edn. New York, USA: Psychology Press.

David M, Auclair Y, Cezilly F, 2011. Personality predicts social dominance in female zebra finches, *Taeniopygia guttata*, in a feeding context. *Anim Behav* **81,** 219-224.

Forkman B, Furuhaug IL, Jensen P, 1995. Personality, Coping Patterns and Aggression in Piglets. *Appl Anim Behav Sci* **45,** 31-42.

Fraser D, 1974. The vocalizations and other behaviour of growing pigs in an "open field" test. *Appl Anim Ethol* **1,** 3-16.

Gallup GG, Suarez SD, 1980. An Ethological Analysis of Open-Field Behaviour in Chickens. *Anim Behav* **28,** 368-378.

Gibbons JM, Lawrence AB, Haskell MJ, 2011. Consistency of flight speed and response to restraint in a crush in dairy cattle. *Appl Anim Behav Sci* **131,** 15-20.

Goddard PJ, Fawcett AR, Macdonald AJ, Reid HW, 2000. The behavioural, physiological and immunological responses of lambs from two rearing systems and two genotypes to exposure to humans. *Appl Anim Behav Sci* **66,** 305-321.

Grandin T, 1993. Behavioral Agitation During Handling of Cattle Is Persistent Over Time. *Appl Anim Behav Sci* **36,** 1-9.

Graunke KL, Langbein J, Repsilber D, Schoen PC, 2013a. Objectively measuring behaviour traits in an automated restraint-test for ungulates: towards making temperament measurable. *J Agric Sci* **151,** 141-149.

Graunke KL, Nürnberg G, Repsilber D, Puppe B, Langbein J, 2013b. Describing Temperament in an Ungulate: A Multidimensional Approach. *Plos One* **8,** e74579.

Horn JL, 1965. A rationale and test for the number of factors in factor analysis. *Psychometrika* **30,** 179-185.

Horn L, Huber L, Range F, 2013. The Importance of the Secure Base Effect for Domestic Dogs - Evidence from a Manipulative Problem-Solving Task. *Plos One* **8,** e65296.

International Beef Recording Scheme, 2012. *A Basic Guide to Breedplan EBVs*. Armidale, Australia: BREEDPLAN, University of New England.

Kaiser HF, Rice J, 1974. Little Jiffy, Mark IV. *Educ Psychol Meas* **34,** 111-117.

Kaiser HF, 1960. The Application of Electronic Computers to Factor Analysis. *Educ Psychol Meas* **20,** 141-151.

Kilgour RJ, Melville GJ, Greenwood PL, 2006. Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity of humans, restraint and novelty. *Appl Anim Behav Sci* **99,** 21-40.

Klefoth T, Skov C, Krause J, Arlinghaus R, 2012. The role of ecological context and predation risk-stimuli in revealing the true picture about the genetic basis of boldness evolution in fish. *Behav Ecol Sociobiol* **66,** 547-559.

Lansade L, Bouissou MF, Erhard HW, 2008. Fearfulness in horses: A temperament trait stable across time and situations. *Appl Anim Behav Sci* **115,** 182-200.

Müller R, von Keyserlingk MAG, 2006. Consistency of flight speed and its correlation to productivity and to personality in *Bos taurus* beef cattle. *Appl Anim Behav Sci* **99,** 193-204.

Murphey RM, Duarte FAM, Penedo MCT, 1981. Responses of Cattle to Humans in Open Spaces - Breed Comparisons and Approach-Avoidance Relationships. *Behav Genet* **11,** 37-48.

O'Connor BP, 2000. SPSS and SAS programs for determining the number of components using parallel analysis and Velicer's MAP test. *Behav Res Methods Instrum Comput* **32,** 396-402.

Pajor F, Muranyi A, Szentleleki A, Tozser J, Poti P, 2010. Effect of temperament of ewes on their maternal ability and their lambs' postweaning traits in Tsigai breed. *Arch Tierz - Arch Anim Breed* **53,** 465-474.

Plusquellec P, Bouissou MF, Le Pape G, 2001. Early predictors of dominance ability in heifers (*Bos taurus*, L.) of the Hérens breed. *Behaviour* **138,** 1009-1031.

Sibbald AM, Erhard HW, McLeod JE, Hooper RJ, 2009. Individual personality and the spatial distribution of groups of grazing animals: An example with sheep. *Behav Processes* **82,** 319-326.

Siebert K, Langbein J, Schoen PC, Tuchscherer A, Puppe B, 2011. Degree of social isolation affects behavioural and vocal response patterns in dwarf goats (*Capra hircus*). *Appl Anim Behav Sci* **131,** 53-62.

Spake JR, Gray KA, Cassady JP, 2012. Relationship between backtest and coping styles in pigs. *Appl Anim Behav Sci* **140,** 146-153.

Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996. Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *Eur Heart J* **17,** 354-381.

Thompson W, Olian S, 1961. Some effects on offspring behavior of maternal adrenalin injection during pregnancy in three inbred mouse strains. *Psychol Repts* **8,** 87-90.

Traditional Herefords, 2013. *Traditional Hereford Breeders Club - The original efficient beef breed.* http://www.traditionalherefords.org/#/the-breed/4565836802.

van Erp-van der Kooij E, Kuijpers AH, Schrama JW, van Eerdenburg FJCM, Schouten WGP, Tielen MJM, 2002. Can we predict behaviour in pigs? Searching for consistency in behaviour over time and across situations. *Appl Anim Behav Sci* **75,** 293-305.

van Reenen CG, Engel B, Ruis-Heutinck LFM, van der Werf JTN, Buist WG, Jones RB, Blokhuis HJ, 2004. Behavioural reactivity of heifer calves in potentially alarming test situations: a multivariate and correlational analysis. *Appl Anim Behav Sci* **85,** 11-30.

van Reenen CG, O'Connell NE, van der Werf JTN, Korte SM, Hopster H, Jones RB, Blokhuis HJ, 2005. Responses of calves to acute stress: Individual consistency and relations between behavioral and physiological measures. *Physiol Behav* **85,** 557-570.

Veissier I, Aubert A, Boissy A, 2012. Animal welfare: a result of animal background and perception of its environment. *Anim Front* **2,** 7-15.

Velicer WF, 1976. Determining Number of Components from Matrix of Partial Correlations. *Psychometrika* **41,** 321-327.

Welfare Quality®, 2009. *Training in the Welfare Quality® assessment protocols, Welfare Quality® Science and society improving animal welfare.* http://www.welfarequality.net/downloadattachment/43299/20259/2009-Nov_adapted_leaflet WQ Training Assessment Protocols.pdf.

Yayou K, Ito S, Yamamoto N, Kitagawa S, Okamura H, 2010. Relationships of stress responses with plasma oxytocin and prolactin in heifer calves. *Physiol Behav* **99,** 362-369.

Zuchtbetrieb Zachert, 2013. *Charolais + Blonde aus dem Havelland.* http://www.zachert-zucht.de/zuchtziel.php?id=3.