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REAGRUPAMENTO EM PARES DE BOVINOS LEITEIROS: É IMPORTANTE SER REAGRUPADO COM UM PARCEIRO CONHECIDO?

Dissertação submetida ao Programa de Pós-Graduação em Agroecossistemas da Universidade Federal de Santa Catarina para a obtenção do Grau de Mestre em Agroecossistemas

Orientadora: Profa. Dra. Maria José Hötzel

Florianópolis, SC
2017

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Esta Dissertação/Tese foi julgada adequada para obtenção do Título de Mestre em Agroecossistemas e aprovada em sua forma final pelo Programa de Pós-Graduação em Agroecossistemas

Florianópolis, 30 de novembro de 2017.

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Este trabalho é dedicado a minha mãe, e a Michele.

“The cosmos is full beyond measure of elegant truths; of exquisite interrelationships; of the awesome machinery of nature. The surface of the Earth is the shore of the cosmic ocean. On this shore we've learned most of what we know. Recently we've waded a little way out, maybe ankle deep, and the water seems inviting. Some part of our being knows this is where we came from. We long to return. And we can. Because the cosmos is also within us. We're made of star-stuff. We are a way for the cosmos to know itself.”

Carl Sagan, *Cosmos* (1980)

AGRADECIMENTOS

Agradeço a minha Mãe, por todos os sacrifícios que ela fez para que eu possa estar onde estou hoje.

A Michele, pelo amor, pelo apoio incondicional durante todos estes anos de formação e por ser a minha guia neste mundo.

Ao Prof. Hector Ricardo Ferrari, que me inspirou para seguir este caminho.

À Prof.ª Maria José Hötzel, pela compreensão infinita e seus aportes essenciais para a materialização deste projeto.

Aos Profs. Marina von Keyserlingk e Daniel Weary, do Animal Welfare Program da UBC pela colaboração durante a minha estadia no Canadá.

Gostaria de agradecer também aos meus colegas da turma do nosso programa, que brindaram o maior apoio e “vigilância mutua” nos momentos onde as forças de vontade pareciam enfraquecer.

A Melissa Garcia Mendez, pela imensa ajuda no processamento dos vídeos. A José Bran Agudelo, pelos comentários referentes a estatística. Aos integrantes do LETA, pelas tardes de companhia e boas lembranças.

Às colegas da Cátedra de Bienestar Animal da Universidade de Buenos Aires, que me deram apoio moral e intelectual neste período.

Aos colegas e *farmers* da UBC Dairy Education and Research Centre, que me fizeram sentir como em casa durante a execução do projeto.

Aos membros da banca, Prof. Abdon Schmitt, Luciana Honorato, pelas cálidas palavras e os aportes para melhoras deste documento final.

A Global Affairs Canada, que a traves do seu Programa Emergent Leaders in the Americas, me concedeu a oportunidade de fazer o meu projeto de mestrado no Canadá.

À Coordenação de Aperfeicoamento de Pessoal de Nível Superior (CAPES) que me concedeu a bolsa durante o período de mestrado.

RESUMO

Os bovinos são animais gregários e formam vínculos seletivos com outros indivíduos do seu grupo, e estes vínculos reduzem os efeitos de situações estressantes. Devido aos reagrupamentos causados por diversos manejos na produção leiteira, esses vínculos são quebrados. A interrupção do tecido social dos animais pode ter impactos negativos no desenvolvimento e na produtividade futura. Além disso, reduz as oportunidades dos animais poderem realizar seus comportamentos específicos de espécies, o que leva a uma redução do seu bem-estar. Neste trabalho testamos as seguintes hipóteses: 1. uma novilha reagrupada com uma parceira desconhecida, em um grupo social desconhecido, mostraria maiores sinais comportamentais de angústia (por exemplo, menos tempo descansando e no cocho) em comparação com uma novilha reagrupada com uma parceira familiar; 2. as duas novilhas do par familiar passariam mais tempo juntas no período após a introdução no novo grupo; 3. as novilhas reagrupadas com uma parceira familiar seriam mais propensas a iniciar deslocamentos junto aos recursos primários nas baias - os cochos, bebedouros e camas. Assim, com o objetivo de demonstrar os efeitos do *buffering* social em novilhas leiteiras, 16 novilhas entre 12 e 16 meses foram submetidas a dois tratamentos experimentais. Em um tratamento (Familiar), uma novilha foi movida juntamente com uma parceira familiar (ou seja, da mesma baia original) para uma nova baia com um grupo de animais do mesmo tamanho da origem. Em outro tratamento (Desconhecida), a mesma novilha foi movida com uma parceira não familiar para uma nova baia com as mesmas características da anterior. As baias foram filmadas e, no primeiro período de 24 horas, alguns estados comportamentais (tempo gasto com o parceiro, no alimentador, o bebedor, as bancas) e eventos (deslocamentos, montagem) foram registrados. Um total de 768 horas de filmagens foram avaliadas por dois observadores, totalizando 6,946 eventos e 5,956 estados. A contagem dos eventos e a duração dos estados foi analisada utilizando o teste-t de Student para amostras pareadas. Encontramos para o tempo total gasto juntos e o tempo gasto juntos enquanto estavam deitadas diferiu entre os tratamentos ($p < 0,01$). Nessas ocasiões, os deslocamentos foram menos frequentes, sugerindo que os pares familiares de animais tendem a buscar-se mais do que pares desconhecidos em situações de estresse social. De todos os deslocamentos, os mais frequentes foram os que ocorreram nos corredores e na área de alimentação. Não encontramos nenhuma influência da ordem em que os tratamentos foram apresentados em nenhuma das variáveis estudadas. Estes dados preliminares apontam a que o reagrupamento das novilhas em pares familiares poderia melhorar a maneira em que os animais lidam com a mudança de ambiente e entorno social. Um melhor conhecimento das oportunidades para melhorar o manejo do gado leiteiro é uma solução de tecnologia imaterial que permite melhorar a qualidade de vida dos animais e aumentar sua produtividade.

Palavras-chave: Bem-estar animal. *Buffering* social. Gado leiteiro.

REGROUPING DAIRY HEIFERS IN PAIRS: DOES BEING REGROUPED WITH A FAMILIAR INDIVIDUAL MATTER?

ABSTRACT

Bovines are gregarious animals and form selective bonds with other individuals in their group, and these bonds reduce the effects of stressful situations. Due to the regrouping caused by various operations in dairy production, these bonds are broken. The interruption of the social fabric of animals can have negative impacts on development and future productivity. In addition, it reduces the opportunities in which animals can perform their species-specific behaviors, which in turn leads to a reduction in their well-being. In this work we test the following hypotheses: 1. a heifer regrouped with an unfamiliar partner, in an unknown social group, would show greater signs of distress behaviors (for example, less time resting and in the trough) in comparison with a heifer regrouped with a family partner. 2. the two heifers of the familiar pair would spend more time together in the period after the introduction to the new group; 3. the heifer grouped together with a familiar partner would be more likely to initiate displacements next to the primary resources in the pens – the feeders, water bins and stalls. Thus, with the objective of demonstrating the effects of social buffering in young heifers, 16 heifers between 12 and 16 months of age were subjected to two experimental treatments. In one treatment (Familiar), a heifer was moved together with a familiar partner (or, in other words, the same original pen) into a new pen with a group of animals of the same size from the original. In other treatment (Unfamiliar) the same heifer was moved with an unfamiliar partner to a new pen with the same characteristics of the previous one. The animals in both pens were filmed and, in the first 24-hour period, some behavioral states (time spent with partner, at the feeder, water bins and stalls) and events (displacements, mounting) were recorded. In total, 768 hours of footage were assessed by the two observers, totaling 6,946 events and 5,956 states logged. The event count and the states durations were analyzed with a paired samples Student's t-test. We found a significant difference ($p < 0.01$) between Familiar and Unknown treatments for total time spent together and time spent together while they were lying down. On those occasions, relocations were less frequent, suggesting that family members of animals tend to seek each other out more often than unknown ones in situations of social stress. We did not find any influence of the order in which the treatments were presented in any of the variables studied. These preliminary data suggest that the regrouping of heifers in familiar pairs could improve the way animals cope with the change of environment and social setting. A better knowledge of opportunities to improve the management of dairy cattle is a solution of immaterial technology that allows, at the same time, to improve the quality of life of animals and increase their productivity.

Keywords: Animal Welfare. Social Buffering. Dairy Cattle.

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1 INTRODUCTION

1.1 ANIMAL WELFARE

Different philosophical approaches form today the set of individual drivers for food consumption, mainly in the developed world. In some sectors of society, the values associated with consumption of food are bound to moral matters (DIETZ; ALLEN; MCCRIGHT, 2017). In addition to all this, there is the situation associated to the increasing demand for animal protein in countries with recent rise in surplus income (TILMAN; CLARK, 2014). Because of this there has been an expansion in world animal production, and along with that there has been a growing concern with the increase in greenhouse gas emissions (COMITTEE ON CONSIDERATIONS FOR THE FUTURE OF ANIMAL SCIENCE RESEARCH; FDA; NATIONAL RESEARCH COUNCIL, 2015; HÖTZEL, 2014). In some cases, GHG emissions can bring negative consequences to animal welfare (LLONCH *et al.*, 2017). The expansion of world animal production has raised the issue of how to define animal welfare, how to measure it, and what are the best and most efficient ways to improve it, and some have attempted this, with a variable degree of success. One of the first and more widespread definition of animal welfare is from BROOM (1986) and it states that a way to assess how good the welfare of an animal is, is to measure its attempts to face adverse situations, in the sense that it is a degree that varies according to “its state as regards its attempts to cope with its environment”.

The degree of welfare of an animal can be assessed through indicators that must reflect the quality of life, be scientifically sound and be practically feasible to acting professionals in this area of knowledge (WEMELSFELDER, F.; MULLAN, 2014). Two sources of information can be assessed to determine the degree of animal welfare: facilities and handling of the animals (provision), and animal-centered measurements that sum up the result of a long-term interaction between animal, facilities and stockpeople (WEBSTER, 2005). Animal-centered measures can be specific and objective, like presence/absence of lesions (e.g. TAUSON *et al.*, 2004), or more holistic indicators such as that obtained through Qualitative Behavior Analysis (QBA, WEMELSFELDER; LAWRENCE, 2001). In other cases, some tools help integrate the information from different methodologies that allow them to be synthesized in a score that reflects the global state of the human-facility-animal system (WELFARE QUALITY CONSORTIUM, 2009).

According to FRASER (2008), there are three theoretical frameworks in which animal welfare scientist base their studies: basic health and functioning, the ‘naturalness’ of the lives of animals, and their affective states. From the ‘basic health and functioning’ approach, good welfare means an animal that is productive and free of disease. Broom’s definition previously mentioned relates to this view. Another way to interpret this framework is based on physiological indicators of stress physiology, mainly the activation of the hypothalamus-pituitary-adrenal gland axis (HPA). This methodology has shown some limitations, since the activation of this axis may not reflect what is classically known as stress. Studies trying to correlate the increase in corticosteroids as a biomarker for stress have shown weak evidence that their concentration is always high during stressful events (OTOVIC; HUTCHINSON, 2015). Also, according to BOISSY et al. (2007), it is not the event by itself, but the *representation* that the animal makes of the event that determines the reaction. Finally within this framework, other authors focused on productivity and fertility of animals to judge their welfare (MCGLONE, J., 1993 cited by FRASER, D. *et al.*, 1997; MCGLONE *et al* 2004).

The second framework proposes that an animal has a good degree of welfare if it is allowed to live in tune with the natural environment in which that particular species has evolved (FRASER *et al.*, 1997). Some have criticized this point of view, arguing that it is not the naturalness of a behavior that defines the welfare of an animal, but the effects of that behavior has on the health of the animal and what the animal wants to do (DAWKINS; BONNEY, 2008).

The last framework is based on the emotions of the animals. Affective states are defined as the ‘behavioral and physiological responses (and in conscious beings, feelings) that can vary both in terms of valence (pleasantness/unpleasantness) and intensity (arousing or activating qualities) (MENDL; BURMAN; PAUL, 2010).

Traditionally, animal welfare has been associated with a reduction of stress in animals, i.e. the reduction of suffering, has been one of the main drivers of animal welfare research as a scientific and objective approach. Due to the recent impulse of positive psychology in humans, a positive welfare impulse has emerged in recent years trying to not only reduce suffering, but also bring positive well-being (BOISSY, Alain; LEE, 2014; GREEN; MELLOR, 2011; MELLOR, 2014; NAPOLITANO, Fabio *et al.*, 2009; YEATES; MAIN, 2008).

1.2 SOCIAL PREFERENCE IN ANIMALS

Pair bonding can be associated to kinship as it indirectly increases genetic fitness of the animal by helping a proportion of genes to pass on to the next generation. The collaboration between unrelated members in a group can be explained by the increase in fitness of the group as a whole, in the case that these individuals form stable social groups (HAMILTON, 1964a, 1964b).

Within these groups, the bonds formed between non-kin individuals are not random. Recent research into the distribution of social links suggests it might be associated with similar personality profiles (BRIARD; DORN; PETIT, 2015; CROFT *et al.*, 2009; KRAUSE; JAMES; CROFT, 2010). Personality traits such as boldness or shyness, might have an ecological role in the dispersal of individuals (COTE *et al.*, 2010). Trying to give an explanation of the probable cause of why this behavior occurs and how it evolved can be addressed by answering Tinbergen's "four questions" approach (1963). Some argue that group forming in animals is a pre-requisite for domestication (STRICKLIN, W. Ray, 2001), so the reason why this behavior has remained throughout generations of artificial selection by man implies that even after we have been caring for the survival of these animals, sociality still has some adaptive value. Following the same theoretical framework, there would be an adaptive value to have an intrinsic motivation to form bonds between non-kin conspecifics (WEST; GRIFFIN; GARDNER, 2007). Therefore, for a social animal such as cattle, the presence of a specific conspecific is a behavioral need that, when impaired, leads to an increase in behavioral and physiological indicators of suffering (MCLENNAN, 2013).

Animals distribute themselves in clusters for different reasons. In some cases, the clustering is the result of some resource which is of common interest of the group such as food (GRANT; ALBRIGHT, 2001), water (COIMBRA; MACHADO FILHO; HÖTZEL, 2012), mating partners, shadow of trees (BRAN AGUDELO, 2012), etc. Also, clustering may exist simply because the individual themselves prefer to maintain close proximity with each other (WHITEHEAD, 2008). In cattle, this non-random distribution manifests itself in terms of preferred partners in relatively small herds (ANDRIEU; BOUISSOU, 1978). This preference has been shown in feeding behavior, where pairs of cattle were observed together, independently of their position in the feed bunk (VAL-LAILLET *et al.*, 2009). This has also been observed in extensive conditions, where Japanese Black cattle spend more time with a familiar partner while grazing on a communal pasture (TAKEDA; SATO, Shusuke; SUGAWARA, 2000). Therefore, we chose to measure spatial proximity as an indicator of affinity. This technique is called the "gambit of the group" (WHITEHEAD; DUFAULT, 1999) as has been shown recently in horses (BRIARD; DORN; PETIT, 2015), kangaroos (BEST; BLOMBERG; GOLDIZEN, 2015) and in cattle (CHEN, Shi *et al.*, 2015). One of the factors that influence the strength of association between individuals is if they had contact from birth or early age (DUVE; JENSEN, Margit Bak, 2011). In cattle, the intensity of bonding between individuals might be related to the age where they started spending time together. According to ANDRIEU and BOUISSOU (1978), the strongest bond formation occurs when heifers were grouped from birth, and gets weaker as the age of association gets close to the first year of life, although there might be other critical periods later in their life that socialization might be facilitated (VEISSIER *et al.*, 1998).

1.3 SOCIAL BUFFERING

Human studies determined that social bonds reduce anxiety levels. An animal is then intrinsically motivated to form pairs in a group. In calves this behavioral need has been substantiated in studies of social isolation (for a review, see COSTA; KEYSERLINGK, VON; WEARY, 2016). This ability of a social partner to modulate the stress response on the recipient’s homeostasis has been defined by RAULT (2012), as social buffering. A neuroendocrinological basis of this phenomenon is related to the connection between the perception of the spatial proximity with a known partner and the reduction of the hypothalamic-pituitary-adrenal axis that mediates the classical stress response (HENNESSY; KAISER; SACHSER, 2009). Oxytocin is other hormone that might have a role on pair bonding by its effects on the hypothalamic-pituitary-adrenal axis and the reason why social contact is positively reinforced (DEVRIES, A. C.; GLASPER; DETILLION, 2003; GOBROGGE; WANG, 2015; RAULT, 2012).

There are several cues that, according to KIKUSUI; WINSLOW; MORI, (2006) trigger the buffering response of a partner. These include olfactory cues by pheromones and vocal cues. Visual cues can work even with the picture of the faces of their partners, as has been proven in goats (COSTA, A. P. DA *et al.*, 2004). Tactile cues are important in some species and oxytocin levels increase in such contexts. In cattle oxytocin is highly correlated with grooming behavior (CHEN, Siyu *et al.*, 2017), which in turn helps to create and maintain the social structure (SATO, S.; SAKO; MAEDA, 1991).

The familiarity of an animal with another animal can be a factor that modifies the behavior of this individual facing stressful situations in his life. During its lifecycle, dairy cattle individuals are regrouped for different purposes. The reasons these animals are regrouped includes: reproductive state (fertile, pregnant, dry, anestrous), different milk yields, and other reasons, such as disease, space allowance issues, etc. In some cases, adult cows are regrouped up to four times per lactations (SCHIRMANN *et al.*, 2011). This generates disturbances in the social structure of the groups that affect both the individual animal as well as the acceptor group.

Regrouping with unfamiliar conspecifics results in a triple challenge: bond breaking, a novel environment and a new social organization (for a literature review of regrouping experiments, refer to Table 1). The first challenge is due to the breakage of bonds form during the stay of the animal in the previous group. As previously shown in the separation of the calve from its dam (DARÓS *et al.*, 2014) separation of non-kin bonded animals has been proven to trigger an emotional response, manifested by increased heart rate, cortisol levels and number of vocalizations, an effect that was more intense when the contact prior to isolation was higher (BOISSY, Alain; NEINDRE, LE, 1997).

The second challenge is associated to the novelty of the environment. According to the appraisal theory of emotions, an individual affective state can be modified by the way it interprets the surrounding environment (ELLSWORTH; SCHERER, 2003). Novelty can be an important factor in the emotional response in animals and there is evidence that novel environment in ruminants triggers an increase in reactivity (COSTA, J. H. C.; KEYSERLINGK, VON; WEARY, 2016) and may even trigger an inflammatory response (RAZZUOLI *et al.*, 2016). The third and last challenge is the different social structure of the new location, and has been shown to result in acute behavioral changes in adult cows (KEYSERLINGK, VON; OLENICK; WEARY, 2008). In peripartum dairy cows, where the intensity of the regrouping is more intense due to weekly movements of individuals, regrouping had effects in their cortisol levels but not in their immunity (SILVA *et al.*, 2013). Some work has also reported that negative behavioral effects associated with comingling of heifers into new social groups can be mitigated if the heifer is moved with a familiar partner compared to being moved by alone (GYGAX; NEISEN; WECHSLER, 2009). In an experiment where lactating dairy cows were separated from their preferred partner, they showed higher heart rate and cortisol levels and more distress behaviors than the ones that were held with a known random individual (MCLENNAN, 2013). However, the degree to which a pair of heifers will cope with a regrouping event has been argued by some to be determined by the degree of familiarity between the conspecifics (RAULT, 2012). This experimental paradigm (regrouping alone vs. with a partner) has not taken into consideration that familiarity between partners might have a role in the stress-mitigating effects on the animal that is being regrouped.

The formation of dyadic bonds between individuals in a group has a role in the stabilization of social hierarchy, by means of the establishment of affiliative or agonistic relationships (KONDO; HURNIK, 1990).

We hypothesized that an individual originating from a pair regrouped with an unfamiliar partner would show greater symptoms of anxiety behaviors (for example, less time resting and in the trough) compared to individuals who were reunited with a familiar partner. We also hypothesized that the family pair would spend more time together in the period after the introduction of the new group. In addition, we hypothesized that heifers grouped together with a familiar partner were more likely to initiate displacements at the primary resources in the pens - feeders, water bins and stalls.

The aim of this study was to assess the behavioral responses of animals when they are introduced to a new pen (i.e. regrouping) with a familiar partner compared to when they are regrouped along with an unfamiliar partner.

Table 1. Literature review on the effects on social regrouping.

Authors	**Species (and type)**	**Type of regrouping**	**Measurement**	**Results**
BRAKEL; LEIS, 1976	Bovine (Dairy Cows)	4 groups moved into a group of 20	Milk yield, agonistic behavior	Reduction in 3% of milk yield, increased agonistic encounters (3x)
SOWERBY; POLAN, 1978	Bovine (Dairy Cows)	Shifting from 2 to 14% of an entire herd, of 7 different herds.	Milk yield	2.28% drop in the -3d to +2d period from regrouping event.
MENCH; SWANSON; STRICKLIN, W. R., 1982	Bovine (Beef Cows)	10 cows moved into each of 3 groups of 10	Behavior, plasma cortisol	Increased cortisol and agonistic interactions.
TENNESSEN; PRICE; BERG, 1985	Bovine (Dairy Cows)	2 bulls and steers in groups of 8 were regrouped so that each animal was penned with 6 strangers and 1 acquaintance	Agonistic behavior	High agonistic behavior returning to baseline after 5-10 days.
VEISSIER *et al.*, 1992	Bovine (Dairy Cows)	24 heifers exposed individually or along 3 familiar peers	Exploratory behavior, reactivity towards humans.	More exploratory behavior in the alone treatment. More approach to humans.
STOOKEY; GONYOU, H. W. W., 1994	Pig (Finishing pigs)	Mixing unfamiliar individuals in groups of 3. Mixing randomly in different sized pens.	Behavior, bodyweight gain	Increased agonistic interactions, lower weight gain vs control.
HASEGAWA *et al.*, 1997	Bovine (primiparous heifers)	Exchange of half of 2 group into each other, some according to social hierarchy.	Agonistic behavior, Milk production, social rank.	Increased agonistic behavior, decreased milk production and social rank.

Authors	Species (and type)	Type of regrouping	Measurement	Results
SEVI *et al.*, 2001	Sheep (lactating ewes)	Weekly regrouping of 5 animals into a new group.	Behavior, Immune response, Cortisol, Milk quality, Mastitis incidence.	Increased agonistic behavior, lower immune response, higher cortisol, reduction of milk quality, no difference of mastitis.
VEISSIER *et al.*, 2001	Bovine			
COUTELLIER *et al.*, 2007	Pig (Finishing pigs)	Mixing a member of a pair	Behavior, bodyweight gain, cortisol levels.	Increased agonistic interactions, cortisol levels and lower weight gain vs control.
FERNÁNDEZ; ALVAREZ; ZARCO, 2007	Goats (lactating)	Exchanging members of 2 groups, then merging them.	Milk production, aggression,	Increase in aggression, reduction of milk production on the first regrouping.
GUPTA *et al.*, 2008	Bovine (Holstein x Friesian Steers)	6 consecutive regrouping and relocation of a single steer.	Behavior, bodyweight gain	Increased time standing, no effect on bodyweight

2 MATERIALS AND METHODS

2.1 ANIMALS

The cows were managed according to the guidelines set by the Canadian Council on Animal Care (1993). Four groups of 10 heifers in four different pens (Figure 1 and Figure 2) were set up for this experiment: two pens termed "guest" (G1 and G2) and two pens termed "host" (H1 and H2). Of the guest groups, sixteen heifers were selected to be the subjects of the mixing (8 from each pen), and the host pens were the group that received these subjects. The heifers in the guest pens had been together for a period longer than a month, so we can assume that they were in a socially stable group (KONDO; HURNIK, 1990). The host pen consisted of a group of 10 heifers. The age difference within pairs was on average 0.8 (SD: 0.995) months, 0.1 (SD: 0.295) with the familiar regrouping and 1.7 (SD: 0.485) months in the unfamiliar regrouping. The youngest of any pair was on average 3.8 (SD: 0.836) months younger than the oldest of the guest pen. By type of regrouping, this difference is of -3.5 (SD: 0.996) months for the familiar and -4.2 (SD: 0.456) months for the unfamiliar regrouping. This age difference is representative of the age difference of a heifer that is being regrouped. The age structure of the heifers of each pen is summarized in Table 2.

Table 2. Age structure (in months) of the heifers in each pen

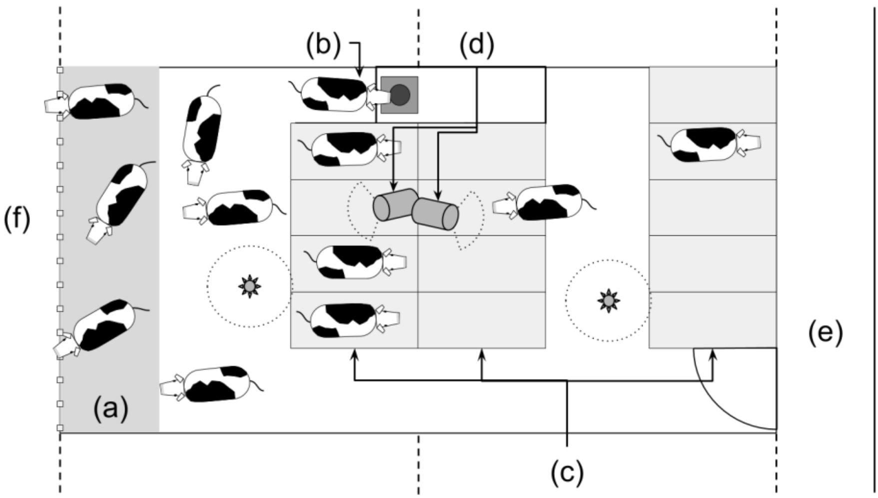
Group	Age average	Age SD	Age Min	Age Max
G1	6.85	0.192	6.70	7.17
G2	8.74	0.265	8.37	9.07
H1	8.74	0.366	8.37	9.77
H2	10.33	0.401	9.73	11.00
Grand Total	8.75	1.236	6.70	11.00

Source: Made by the author (2017).

2.1.1 Treatments

All selected individuals of the guest pens were subjected to the following treatments: one by one, each member was paired a) with a familiar partner (Familiar), and b) with an unfamiliar partner (Unfamiliar). Each animal was in

Figure 1. Schematics of the layout of the host pens: (a) Feeding area, (b) Water bin area, (c) Stalls area, (d) Camera positioning (overhead), (e) Outer corridor, (f) Inner (feeding) corridor. The dotted line circle shows the position of the red lights for night video recording.



Source: Made by the author (2017).

both treatments, either in Period 1 or in Period 2. In order to avoid carryover effects, the regroupings took place following a two-period two-treatment crossover design. To avoid order effect, the order of regrouping was pseudo-randomly determined.

Each member of the pair was naive to the host groups where they were moved into, in order to prevent effects of experience. In total, 16 mixings were done (i.e. 8 familiar pairs and 8 unfamiliar pairs). All regroupings occurred before the morning feeding, and the feed was provided immediately after the heifers are introduced into the pen.

2.1.1.1 Period 1

During the first week of period 1 a pair of each of the two guest pens, comprised of heifers belonging to the same pen (*familiar pair*), were moved to the host pens. They remained in the host pen for 3 days, and after that period they returned to their original guest pen. Until the next regrouping the following week, the host pens remained without guest heifers. During the following week, two new and different pairs were formed, consisting of members of different guest pens (*unfamiliar pair*). Each pair was moved to a different host pen. Both individuals of each pair entered the host pen simultaneously, and for that they had to be moved to a waiting area first. This same pattern was repeated each week until all the selected members of the guest group have experienced at least one regrouping. In total, there were 8 weeks of regroupings. See Appendix A for the complete regrouping schedule.

Figure 2. Camera image showing one of the host pens. In the digital version of this document, some parts are colored. *Yellow:* Feeding area. *Red:* Water bin area. *Cyan:* Stalls area.

Source: Made by the author (2017).

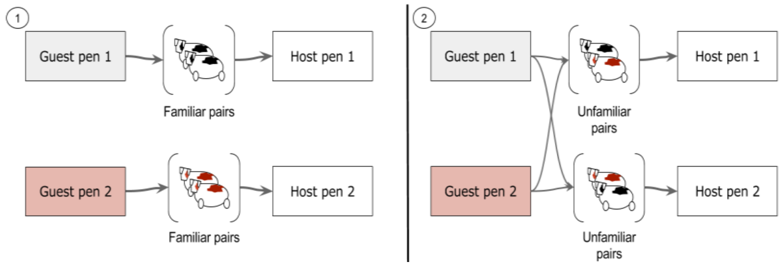
2.1.1.2 Period 2

During period 2, the individuals that were moved with a familiar member during period 1 were moved with an unfamiliar member, to a host group that none of the pair have experienced before. Likewise, the members that were moved with an unfamiliar partner during period 1 were moved with a familiar individual to a host pen, which they never visited before.

The average mixing interval (between the end of the regrouping of period 1 and the beginning of period 2) for each individual was 32.3 (SD 9.96) days (See Appendix 2 - Interval between regroupings for each individual). See Table 2 and

Figure **3** for details.

Figure 3. Regrouping example during period 1 of regrouping. 1. One week, two pairs of heifers belonging to the same guest pen were moved to different host pens. After a 3-day period stay, the moved heifers were returned to their respective guest pen. 2. The next week, a different set of heifers was selected, this time forming pairs of individuals coming from different groups. Although during period 1 the succession was 1-2, during period two, the regroupings of a certain week were adapted to meet the regrouping conditions, and might not follow these alternations.



Source: Made by the author (2017).

2.2 BEHAVIOR OBSERVATIONS

Two cameras were installed on top of each pen and were set to record continuously during the regrouping phases for the first 24 h. For specific behaviors scored please see the coding convention in Table 3.The videos were digitally stored for later behavior assessment using Remote Viewlog Software (GeoVision Inc., Taiwan).

After training and assessment of inter-observer reliability, two observers analyzed the video footage. The behaviors were assessed in a continuous sampling method, being one member of the pair the focal animal at the time. With the purpose of reducing subjectivity between observers, the behaviors were defined as much precise as possible, and practice sessions with example videos were put in place. The identification of individual focal cows was made through an image database consisting of pictures from above in order to recognize the fur pattern in the same angle that the surveillance cameras were recording the behaviors. The threshold for detection (logging of a certain behavior when in doubt if it really happened) was to favor specificity over sensitivity.

2.2.1 Behavior definitions

The social interactions were centered from the focal animal perspective. We classified the recorded behaviors according to its duration. States are behaviors that have variable duration and have a clear beginning and end, so their duration can be measured. Events have instantaneous duration and there is no clear beginning and end. Note that these definitions are built in order to be clearly identifiable with the resolution of the surveillance cameras on the night vision mode.

2.2.1.1 States

Pair proximity **(PP)**: The proximity (< 1 head length apart) of the head of the partner to any body part of the focal animal. In the case of being at the feeder, at a maximum of 1 head space in between, unless there is an animal in that middle head lock. Proximities that last less than 5 seconds were considered coincidental and disregarded, unless the interruption was caused by a displacement.

Pair proximity while lying **(PPLy)**: Idem (PP) The partner in the contiguous stall next to the focal animal.

Feeding bout **(F)**: Head across the headspace, down at the feed. If there was a brief (<30s) interruption that was not caused by a displacement in this behavior, it was considered as part of the bout instead of 2 separate bouts. It may be interrupted spontaneously or by displacement at the feeder **(DFi)**. In some special cases, they licked parts of the headlocks or the metal bars near the feeding space. These were not considered part of the feeding bout.

Lying **(Ly):** Sternal or lateral recumbence in the stalls or alleys.

At the water bin **(Dr)**: Head through the water bin bars. Due to camera placement, there was no way to confirm that the animal was necessarily drinking.

2.2.1.2 Events

Displacements

- Inbound **(i)**: by physical contact of other individual (a displacer), involving forward movement of the displacer, moving the focal animal of the headspace was occupying. The displacer may use the head or any other part of the body as means of exerting force to the focal animal. It is also considered a displacement when the event occurs in the feeding area.

- Outbound **(o)**: same as inbound, but the displacer being the focal animal.

Displacement modifiers

- At the feeder **(DF)**: When the feeding bout is interrupted
- At the alleys **(DA)**: Not to be confused with head butt playing where the sequence tends to begin with head scratching and there is no apparent displacement between the members of the pair, but alternation of displacements between them.
- At the drinker **(DDr)**
- At the stalls **(DS)**

Mount **(m)**

- Outbound **(o)**: the focal animal exerts the mounting.
  Inbound **(i)**: the focal animal receives the mounting.

2.2.2 Behavior coding reference

For ease of behavior logging in the spreadsheets, the behaviors were coded in a series of suffixes and prefixes, that combined together form the behavior. Table 3 summarizes the coding scheme.

2.3 DATA ANALYSIS

All tests and summaries were done with the R software (R CORE TEAM, 2017), and for reproducibility purposes (BARNES, 2010) the data and its scripts will be available online and in an appendix (Appendix B – Reproducibility). We consider each pair as our experimental unit.

With the displacements and mounting being a discrete (count) variable and the states being a continuous variable, the duration of each of the behaviors under study were treated separately.

Considering which treatment each individual was assigned to first, we proceeded to test if the order of treatments is in itself a factor that might cause differences in the treatment.

A normality test carried out with the Shapiro-Wilk Normality Test for both states and events.

We compared the amounts of displacements and mounts in each treatment using a Paired t-test for the variables that were complying with the theoretical requirements for a parametric test.

Table 3. Behavior coding

Code	Description
id	**Unique identifier for the heifer**
treatment	**Signals which treatment each heifer receives**
order	**A placeholder for the order effect testing**
d-	**Prefix for displacement**
-dr-	**Displacement at the water bin**
-f-	**Displacement at the feeder**
-a-	**Displacement at the alley**
-s-	**Displacement at the stalls**
m-	**Mounting**
-i	**Suffix indicating inbound action**
-o	**Suffix indicating outbound action**

Source: Made by the author (2017).

2.3.1 Normality test for events

The Shapiro-Wilk test showed mixed results for the different variables of the inbound and outbound displacements and mounting. The results are summarized in Table 4 for the familiar treatment and Table 5. for the unfamiliar treatment. The displacements at the feeder, alleys and the stalls did not follow a normal distribution, so non-parametric tests were done for these variables.

Table 4. Normality test for events in the familiar treatment.

	statistic	p value	Significance
Displacement water bin (inbound)	0.8307403	0.00718514	*
Displacement feeder (inbound)	0.9436061	0.3955538	
Displacement alleys (inbound)	0.9107636	0.1197167	
Displacement stalls (inbound)	0.9201399	0.1695593	
Mounting (inbound)	0.7469107	0.0005832578	*
Displacement water bin (outbound)	0.6531932	5.38286e-05	*
Displacement feeder (outbound)	0.6084203	1.945809e-05	*
Displacement alleys (outbound)	0.5277475	3.630517e-06	*
Displacement stalls (outbound	0.517499	2.969741e-06	*
Mounting (outbound)	0.2726549	4.553108e-08	*

Source: Source: Made by the author (2017).

Table 5. Normality test for events in the unfamiliar treatment.

	statistic	p value	Significance
Displacement water bin (inbound)	0.7855445	0.001761728	*
Displacement feeder (inbound)	0.8957842	0.06882319	
Displacement alleys (inbound)	0.9506958	0.5007893	
Displacement stalls (inbound)	0.8944199	0.06546477	
Mounting (inbound)	0.7648117	0.0009635513	*
Displacement feeder (outbound)	0.5462647	5.254141e-06	*
Displacement alleys (outbound)	0.562755	7.357529e-06	*
Displacement stalls (outbound	0.5946753	1.442587e-05	*
Mounting (outbound)	0.3787212	2.444198e-07	*

Source: Made by the author (2017).

2.3.2 Normality test for states

A Shapiro-Wilk normality test was carried out in the same manner as in the previous section (Table 4). Of the variables under study, only the time spent at the water bin, and the total time spent next to the regrouping partner along with the pair proximity with its partner while lying at the stall were significantly similar to a normal distribution.

Table 6. Shapiro-Wilk normality test for durations

	p value	Significance
Time spent at the water bin	> 0.05	*
Time spent at feeder	0.2390944	
Time spent lying	0.5821949	
Pair proximity not lying down	0.09085666	
Pair proximity while lying down	> 0.01	**
Total pair proximity	> 0.01	**

Source: Made by the author (2017).

Table 7. Shapiro-Wilk normality test for durations

	treatment	p.value	Significance
Time spent at the water bin	Familiar	0.47	
	Unfamiliar	> 0.05	*
Time spent at feeder	Familiar	0.96	
	Unfamiliar	0.23	
Time spent lying	Familiar	0.97	
	Unfamiliar	0.58	
Pair proximity not lying down	Familiar	0.95	
	Unfamiliar	0.56	
Pair proximity lying	Familiar	0.076	
	Unfamiliar	> 0.05	*

Source: Made by the author (2017).

2.3.3 Paired t-test for the difference between treatments

We proceeded to do a paired t-test for the difference in time spent at the water bin, while lying down next to a partner, and the total time spent in proximity with its partner.

3 RESULTS

In total, 768 hours of footage were assessed by the two observers, totaling 6,946 events and 5,956 states logged.

3.1 EVENTS

Overall, each animal that was regrouped received more displacement than they exerted. Most of the inbound displacements occurred in the alleys and at the feeder (Table 8). A greater variability in the outbound displacements was found (i.e., a higher frequency of outliers), probably because they were much less frequent than the inbound displacements Source: Made by the author (2017).
Figure 4 and Figure 5).

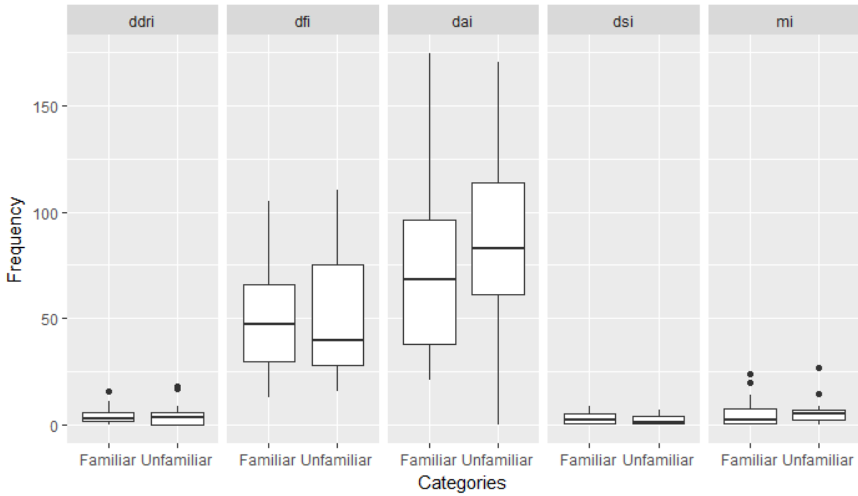
Table 8. Summary of inbound displacements and mounting, per animal, under different treatments.

Displacement	Treatment	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
Water bin	Familiar	0	1.75	3.0	4.375	6.25	16
	Unfamiliar	0	0.00	3.5	4.750	6.00	18
Feeder	Familiar	13	30.00	47.5	51.250	66.25	105
	Unfamiliar	16	28.25	40.0	49.690	75.25	110
Alleys	Familiar	21	38.25	68.5	76.060	96.25	174
	Unfamiliar	0	61.50	83.0	88.060	113.80	170
Stalls	Familiar	0	1.00	2.5	3.188	5.25	9
	Unfamiliar	0	0.75	1.5	2.312	4.00	7
Mounting	Familiar	0	0.75	2.5	5.625	7.50	24
	Unfamiliar	0	2.75	5.5	6.312	7.00	27

Source: Made by the author (2017).

Figure 4. Box and whiskers plot of inbound displacements and mounting. Reference: *ddri:* Displacements at the water bin, inbound. *dfi:* Displacement at the feeder, inbound.

dai: Displacements at the alleys, inbound. *dsi:* Displacements at the stalls, inbound. *mi:* mounting, inbound.



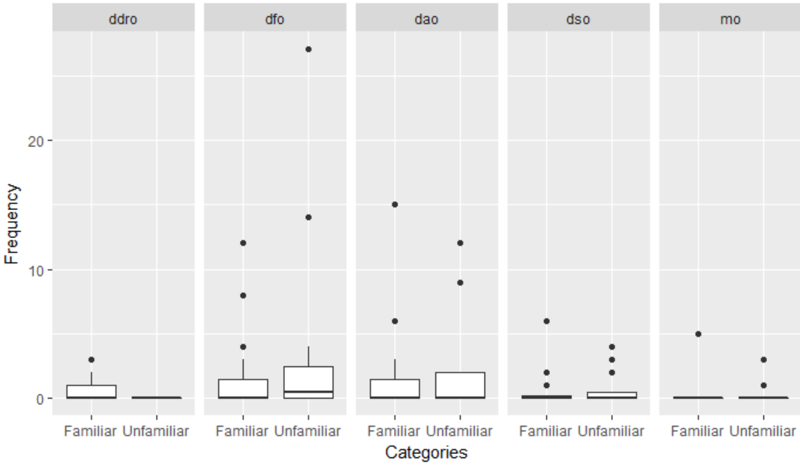
Source: Made by the author (2017).

Table 9. Summary of outbound displacements and mounting, per animal, under different treatments.

Displacement	Treatment	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
Water bin	Familiar	0	0	0.0	0.5625	1.00	3
	Unfamiliar	0	0	0.0	0.0000	0.00	0
Feeder	Familiar	0	0	0.0	1.8120	1.50	12
	Unfamiliar	0	0	0.5	3.4380	2.50	27
Alleys	Familiar	0	0	0.0	1.7500	1.50	15
	Unfamiliar	0	0	0.0	1.7500	2.00	12
Stalls	Familiar	0	0	0.0	0.9375	0.25	6
	Unfamiliar	0	0	0.0	0.7500	0.50	4
Mounting	Familiar	0	0	0.0	0.3125	0.00	5
	Unfamiliar	0	0	0.0	0.2500	0.00	3

Source: Made by the author (2017).

Figure 5. Box and whiskers plot of outbound displacements and mounting. Reference: *ddri:* Displacements at the water bin, inbound. *dfi:* Displacement at the feeder, inbound. *dai:* Displacements at the alleys, inbound. *dsi:* Displacements at the stals, inbound. *mi:* mounting, inbound.



Source: Made by the author (2017).

3.1.1 Order effect

Except for the displacements at the water bin outbound ($p<0,05$), there was no order effect in all the variables. For the significant variable, the order of presentation of the treatments significantly affected the number of displacements each heifer received.

3.1.2 Displacements

There was a significant difference of displacements at the water bin that the focal heifer exerted on other individuals ($p < 0.05$). However, given the low total amount of displacements, we can conclude that it is not biologically significant. There was no significant difference for the other events studied.

3.2 STATES

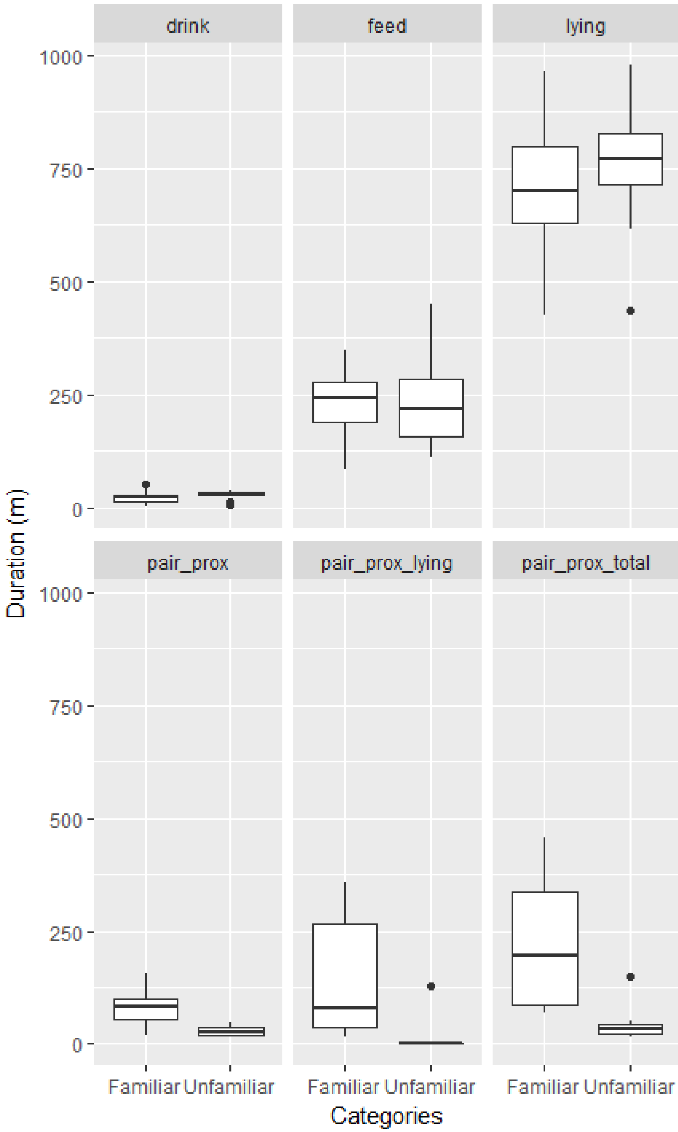
On average, the heifers spent most of the time lying down in both treatments (around 11 hours, see Table 10). For a complete descriptive summarization of the minutes that each heifer spent on each of the variables, see Table 10 and**Figure 2** Figure 6.

Table 10. Basic summarization for events. Values are expressed in hours spent.

Behavior	Treatment	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
At water bin	Familiar	0.05	0.2	0.4	0.36	0.45	0.84
	Unfamiliar	0.1	0.43	0.48	0.46	0.58	0.63
At feeder area	Familiar	1.38	3.15	4	3.87	4.61	5.82
	Unfamiliar	1.86	2.64	3.61	3.77	4.76	7.48
Lying down	Familiar	7.11	10.47	11.66	11.68	13.32	16.07
	Unfamiliar	7.27	11.89	12.85	12.6	13.78	16.3
Pair proximity w/ partner not lying	Familiar	0.31	0.9	1.38	1.35	1.7	2.64
	Unfamiliar	0.24	0.31	0.45	0.48	0.64	0.81
Pair proximity w/ partner lying	Familiar	0.24	0.62	1.29	2.37	4.41	5.94
	Unfamiliar	0	0	0.01	0.28	0.01	2.16
Pair proximity total	Familiar	1.12	1.44	3.25	3.72	5.58	7.62
	Unfamiliar	0.25	0.39	0.55	0.76	0.73	2.49

Source: Made by the author (2017).

Figure 6. Box-and-whiskers plot of durations of each of the behaviors in study, for the different treatments.



Source: Made by the author (2017).

3.2.1 Time spent lying down

There was a significant difference between treatments for the pair proximity while lying down ($p < 0.01$) and the total time spent in proximity ($p < 0.01$).

4 DISCUSSION

We tested whether the presence of a familiar conspecific would influence how the heifers interacted socially with a host group, in comparison to being regrouped in the company of an unfamiliar partner. We found that the heifers in the familiar treatments spent more total time in proximity and more time lying next to each other, when compared to the heifers that were unrelated. However, we did not see any significant difference in the time spent together in other contexts such as at the feeder or in the alleys, both of which were the places where there was a relative increase in displacements. This suggests that during a stressful event, heifers might seek a familiar partner as a result of the rewarding effects of social support (DEVRIES, A. C.; GLASPER; DETILLION, 2003; NELSON; PANKSEPP, 1998). The apparent lack of significance of the other variables under study can be explained by the fact that the effects on these heifers may not be strong enough to be detected with this experimental design.

Of all displacements and in all the treatments, we observed a higher count of them in the feeder and in the alleys. The difference of displacements in these locations can be explained as a resource protection mechanism of the food (GRANT; ALBRIGHT, 2001). The increase in displacements in the alleys can be explained by the greater exposure time to these locations in the pen. KEYSERLINGK, VON; OLENICK; WEARY, (2008) have shown that upon entering a new group, cows experience increased displacements in the feeding area and a reduction in the time they spent eating and lying, the number of lying bouts, and allogrooming events. This is coherent with the observed avoidance of conflict of the heifers in the present experiment, which might have found that the place in the pen where they could perform affiliative behaviors was where the competitive pressure was the least. A similar phenomenon was observed in dairy calves, which distribute themselves randomly when grain was offered, but spontaneously stayed close to a preferred partner when there was a lower-value resource offered, such as hay (CHEN, Shi *et al.*, 2014).

The order of the treatments did not affect the number of displacements or mounts. In virtually all the cases, there was no difference in the amount of displacements the heifers suffered when they were presented with a treatment first or second. The difference of displacements that the focal heifers made in the water bin was statistically significant, but not a practically significant one. We can conclude by these results that the crossover design was successful.

Previous work has shown that resting time decreases in stressful situations such as competition for food (HUZZEY, J.M. *et al.*, 2006; OLOFSSON; WIKTORSSON, 2001). However, contrary to what we hypothesized, there was no significant difference in overall resting time between treatments. Thus, regrouping with a familiar or unfamiliar partner may not have an impact on the risk of lameness (PROUDFOOT; WEARY; KEYSERLINGK, VON, 2010) and ketosis (ITLE *et al.*, 2015), a condition for which standing time is a risk factor.

Contrary to what we hypothesized, the heifers with the familiar treatment did not initiate more displacements than the ones with the unfamiliar treatment. Although a previous work confirmed that there is an overall increase in outbound displacements within subjects around the time of regrouping (KEYSERLINGK, VON; OLENICK; WEARY, 2008; SCHIRMANN *et al.*, 2011) to our knowledge no study assessed how being regrouped with a familiar companion influences agonistic interactions in cattle.

Although we were able to find differences in the time that the both heifers of the dyad spent together while lying down, due to the very design of this experiment we cannot establish a real preference of the animal for its familiar conspecific, but rather a correlation between the type of situation and the heifers' behavioral response. However, in a study done with calves, it was observed that, depending on the group size, individuals prefer a familiar partner to be close together in their resting places, in comparison to an unfamiliar one (FÆREVIK *et al.*, 2007). For this study we chose groups of individuals that were close in age; according to the management practices of the farm, it is inferred that they were raised in the same group from birth. Thus, for this study we assumed that the proximity of births of individuals to be regrouped implied social familiarity (DUVE; JENSEN, Margit Bak, 2011).

Direct visual observation has its advantages over automated techniques because there are no alternative technologies available to date to objectively detect complex behaviors in animals. However, thresholds for the detection of behaviors by humans are found to be subject to failure, particularly in observations involving continuous sampling of observations (LEHNER, 1998). There is also a trade-off between the need to continuously record observations and the intrusiveness of recording conditions by night vision cameras. As far as we know, cattle do not detect the red light that was used for the experiments (JACOBS; DEEGAN; NEITZ, 1998). Today's technology allows individual animals to be traced in a field, but that requires recording and lighting conditions that are not possible in real-life scenarios. There are currently artificial intelligence and artificial vision efforts that allow cataloguing position and state (seated, thrown, etc. FARAH; LANGLOIS; BILODEAU, 2011; KONIAR *et al.*, 2016) but until now these have not been validated. In addition, measuring other behaviors such as the number of vocalizations (BOISSY, Alain; NEINDRE, LE, 1997) or social licking (TRESOLDI *et al.*, 2015) might have made it possible to detect more subtle changes in behavior. Individual vocalizations were difficult to sort out in the heifer barn, and it might have required digital audio loggers and microphones mounted on the animals. The size and level of activity of these animals would have required strapping and restraining methods that might have altered the normal behavior of them. Additionally, sources of stress other than social stress, such as confinement or machinery noise, were closely similar between host and guest pens, so the differences in the measured behavioral indicators of stress (displacements, mounting and time spent in each area) may not have been strong enough to detect differences. In conclusion, the use of additional parameters, like the previously mentioned, measured in conjunction with events and behavioral states could have increased the sensitivity to the effects of social buffering in these animals.

For a long time, academia focused on issues of hierarchy and agonism rather than affiliation and its positive effects (YEATES; MAIN, 2008). Certainly, in the coming years and thanks to new technologies, we will have more information about the phenomenon of social buffering, which may improve fitness in intensive housing systems (FERGUSON, 2014). Undoubtedly, these discoveries will allow the development of new regrouping techniques that may have a low impact on the social fabric of dairy cattle. Recent advances in animal cognition have opened a window to their internal representations that animals have of the world (BOISSY, Alain; LEE, 2014; DÉSIRÉ *et al.*, 2004; PAUL; HARDING; MENDL, 2005). Domestic cattle and other production animals can form social and rewarding bonds, resulting in the formation of real animal societies, product of millions of years of evolution during their non-domestic life history (WHITEHEAD, 2008, chap. 1). Emulating in the best way the social dynamics of the species may allow us to improve the quality of life of their animals (FRASER *et al.*, 2013), satisfying the requirements of a population that day by day has greater concerns about the origin of their food (CARDOSO *et al.*, 2016).

In adult lactating cows, social disruption has significant impacts in milk production on the first day after regrouping (KEYSERLINGK, VON; OLENICK; WEARY, 2008). One of the factors that most seriously impacts the social skills in dairy cattle is the early social environment and previous experiences (BØE, Knut Egil; FÆREVIK, 2003). The impacts of a traumatic experience might only occur years after the stressful social event has occurred. The current state of knowledge is that social isolation on calves has consequences on the long term (and probably a lifetime), such as better coping abilities in isolation or a reaction to novelty (COSTA, J. H. C.; KEYSERLINGK, VON; WEARY, 2016; WAGNER *et al.*, 2012, 2015). The fact that we found signs of behavioral need for keeping a preferred partner close, considering practices that reduce to a minimum the separation of preferred partners might be a good preventative measure. These results should also should raise awareness that social dynamics might be a confounding factor in the experimental design of other types of trial not necessarily related to animal behavior, in which moving groups of individuals is part of the daily routine of the experimental facility.

5 CONCLUSIONS

Dairy heifers regrouped in pairs spent more time together, both in total time and while lying at the stalls when they are familiar to each other. This suggests that one of the mechanisms that they have during the stressful situation of a novel social environment, is to seek a member of the group which they have more affinity. The results of this study might help develop alternative regrouping methods taking into account the social affinities with individuals in the previous group.

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Appendix A – Regrouping schedule

Stage	Week	Start	End	Pair 1	Pen pair 1	Prev Dest	Pair 2	Pen pair 2	Prev Dest.	Type of pair	Destination
1	1	02-04	05-04	6091	G1	-	6094	G1	-	Familiar	H1
1	1	02-04	05-04	6070	G2	-	6071	G2	-	Familiar	H2
1	2	09-04	12-04	6095	G1	-	6072	G2	-	Unfamiliar	H1
1	2	09-04	12-04	6098	G1	-	6073	G2	-	Unfamiliar	H2
1	3	16-04	19-04	6099	G1	-	6100	G1	-	Familiar	H2
1	3	16-04	19-04	6079	G2	-	6075	G2	-	Familiar	H1
1	4	23-04	26-04	6101	G1	-	6076	G2	-	Unfamiliar	H1
1	4	23-04	26-04	6102	G1	-	6077	G2	-	Unfamiliar	H2
2	1	08-05	11-05	6091	G1	H1	6079	G2	H1	Unfamiliar	H2
2	1	08-05	11-05	6098	G1	H2	6102	G1	H2	Familiar	H1
2	2	14-05	17-05	6094	G1	H1	6075	G2	H1	Unfamiliar	H2
2	2	14-05	17-05	6099	G1	H2	6070	G2	H2	Unfamiliar	H1
2	3	21-05	24-05	6100	G1	H2	6071	G2	H2	Unfamiliar	H1
2	3	21-05	24-05	6095	G1	H1	6101	G1	H1	Familiar	H2
2	4	28-05	31-05	6072	G2	H1	6076	G2	H1	Familiar	H2
2	4	28-05	31-05	6073	G2	H2	6077	G2	H2	Familiar	H1

Appendix B – Reproducibility

1.1. Data manipulation and analysis in R – Source files.

The complete set of data files and R scripts can be found online in the following link: https://www.dropbox.com/sh/niu3f070vrulp22/AADnSkoj9tCbXN5qJWRE-z2Na?dl=0

1.2. Data manipulation and analysis in R – Source code.

1.2.1. Events analysis

```
table <-load("tabla.Rdata")
kable(tabla, caption = "\\label{tab:tab1}Age structure of each pen")
```

Age structure of each pen

Group	Age average	Age SD	Age Min	Age Max
G1	12.7	0.270	12.5	13.2
G2	14.3	0.380	13.7	14.8
H1	15.3	0.457	14.8	16.5
H2	16.5	0.475	15.9	17.4
Grand Total	14.8	1.423	12.5	17.4

```
events <- read_csv("events.csv")
```

The raw data is organized in the following way:

```
head(events)
## # A tibble: 6 x 13
##      id  treatment order  ddri   dfi   dai   dsi    mi  ddro   dfo   dao
##   <int>      <chr> <int> <int> <int> <int> <int> <int> <int> <int> <int>
## 1  6070   Familiar     1     2    49    53     6     3     1     4     3
## 2  6070 Unfamiliar     2     6    40    62     0     1     0     0     0
## 3  6071   Familiar     1     3    54    90     2     3     0     1     1
## 4  6071 Unfamiliar     2     6    30     0     0     0     0     0     0
## 5  6072   Familiar     2    16    67   115     5    20     0     0     0
## 6  6072 Unfamiliar     1     5    23    62     5     4     0     4    12
## # ... with 2 more variables: dso <int>, mo <int>
```

1.2.1.1. Cleaning and setting up the data

For further statistical treatment, some of the imported variables need to be converted in order to represent the correct variable type. This is achieved by the command as.factor in R.

```
events$id <- as.factor(events$id)
events$treatment <- as.factor(events$treatment)
events$order <- as.factor(events$order)
```

1.2.1.2. Basic summarization

In the following section we will summarize the data into the classic central tendency indicators (mean, standard deviation, inter-quantile range, and range).

```
kable(inbound.summary, caption = "\\label{tab:summary_inbound}Summary of inbound di
splacements and mounting")
```

Summary of inbound displacements and mounting

variable	treatment	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
ddri	Familiar	0	1.75	3.0	4.375	6.25	16
ddri	Unfamiliar	0	0.00	3.5	4.750	6.00	18
dfi	Familiar	13	30.00	47.5	51.250	66.25	105
dfi	Unfamiliar	16	28.25	40.0	49.690	75.25	110
dai	Familiar	21	38.25	68.5	76.060	96.25	174
dai	Unfamiliar	0	61.50	83.0	88.060	113.80	170
dsi	Familiar	0	1.00	2.5	3.188	5.25	9
dsi	Unfamiliar	0	0.75	1.5	2.312	4.00	7
mi	Familiar	0	0.75	2.5	5.625	7.50	24
mi	Unfamiliar	0	2.75	5.5	6.312	7.00	27

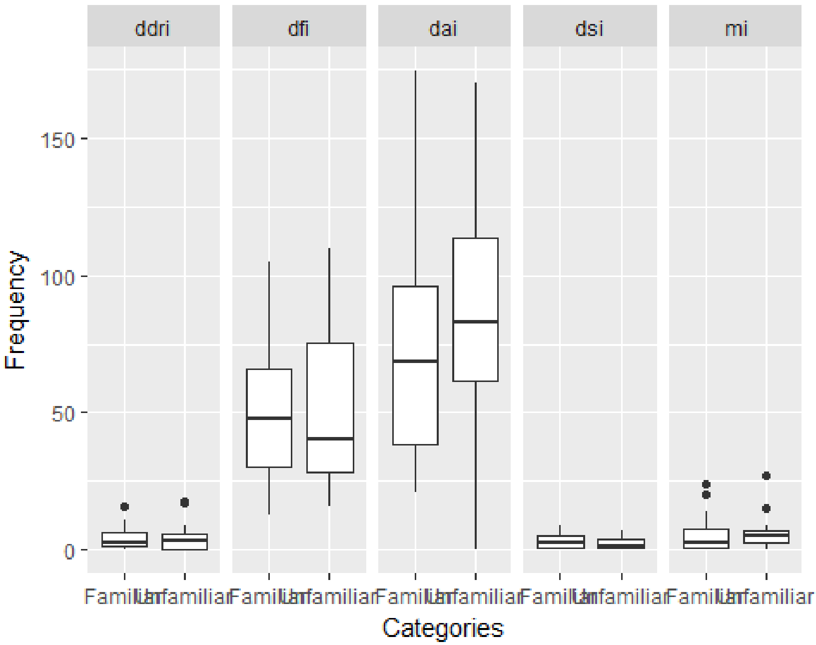
```
kable(outbound.summary, caption = "\\label{tab:summary_outbound}Summary of outboun
d displacements and mounting")
```

Summary of outbound displacements and mounting

variable	treatment	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
ddro	Familiar	0	0	0.0	0.5625	1.00	3
ddro	Unfamiliar	0	0	0.0	0.0000	0.00	0
dfo	Familiar	0	0	0.0	1.8120	1.50	12
dfo	Unfamiliar	0	0	0.5	3.4380	2.50	27
dao	Familiar	0	0	0.0	1.7500	1.50	15
dao	Unfamiliar	0	0	0.0	1.7500	2.00	12
dso	Familiar	0	0	0.0	0.9375	0.25	6
dso	Unfamiliar	0	0	0.0	0.7500	0.50	4
mo	Familiar	0	0	0.0	0.3125	0.00	5
mo	Unfamiliar	0	0	0.0	0.2500	0.00	3

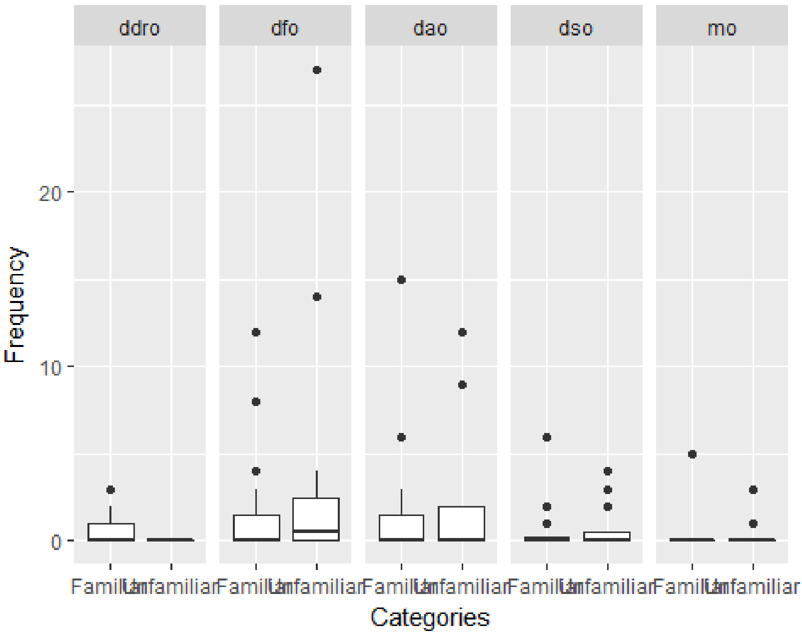
1.2.1.3. *Frequency distribution*

```
theming <- scale_fill_grey(start = 0.1,
    end = 0.4,
    na.value = "red")
labelss <- labs(x="Categories", y="Frequency")


df1 <- events %>%
  dplyr::select(treatment, ddri, dfi, dai, dsi, mi) %>%
  melt( id.var = "treatment")
df2 <- events %>%
  dplyr::select(treatment, ddro, dfo, dao, dso, mo) %>%
  melt( id.var = "treatment")
ggplot(data = df1, aes(x=treatment, y=value)) +
  geom_boxplot() + facet_wrap(~variable,ncol = 5) +
  theming +
  labelss
```



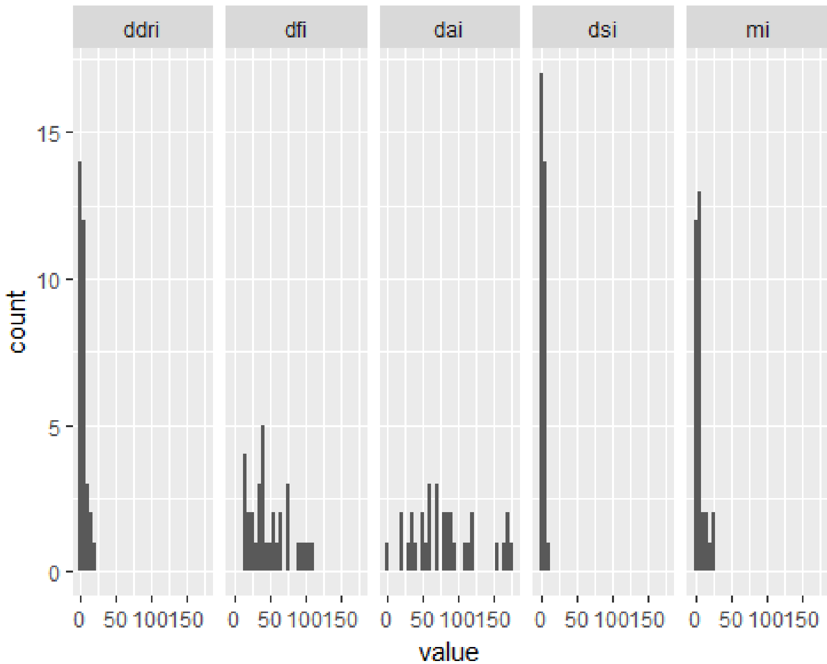
Box-and-whiskers plot of inbound displacements and mounting. **The majority of displacements occured at the feeder which can be explained as a resource protection and in the alleys, which is expected due to the time exposure to these locations in the pen.**

```
cap <-"\\label{fig:plot_inbound}**Box-and-whiskers plot of inbound displacements and mounting. ** The majority of displacements occured at the feeder which can be explained as a resource protection and in the alleys, which is expected due to the time exposure to these locations in the pen."
ggplot(data = df2, aes(x=treatment, y=value)) +
  geom_boxplot() + facet_wrap(~variable,ncol = 5) +
  labelss
```



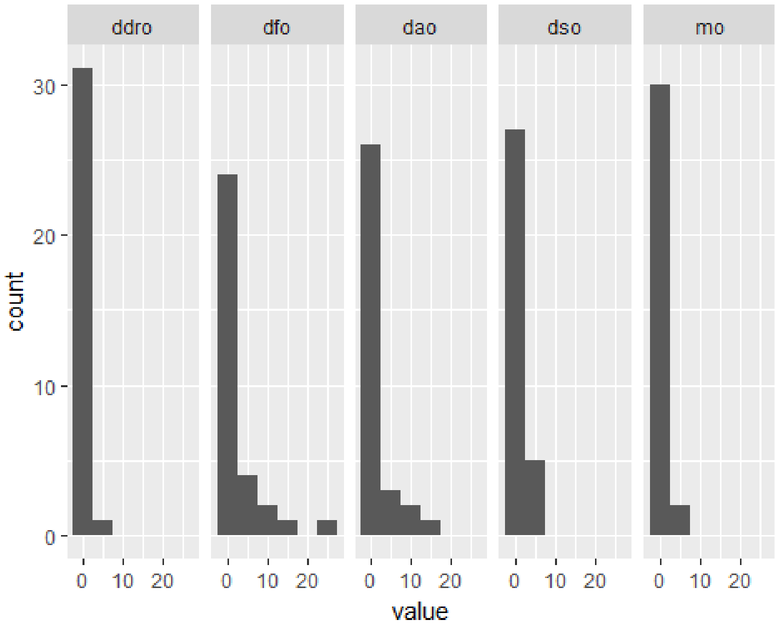
Box-and-whiskers plot of outbound displacements and mounting. **There is great variability in the outbound displacements, probably because they were much less frequent than the inbound displacements.**

```
cap <- "\\label{fig:plot_outbound}**Box-and-whiskers plot of outbound displacements and mounting.** There is great variability in the outbound displacements, probably because they were much less frequent than the inbound displacements."
ggplot(data = df1, aes(x=value)) +
  geom_histogram(stat = "bin", binwidth = 5) +
  facet_wrap(~variable, ncol = 5)
```



Frequency distribution for the inbound displacements and mounting

```
cap1 <- "\\label{fig:plot_histogram_inbound}Frequency distribution for the inbound displac
ements and mounting"


ggplot(data = df2, aes(x=value)) +
  geom_histogram(stat = "bin", binwidth = 5) +
  facet_wrap(~variable, ncol = 5)
```



Frequency distribution for the outbound displacements and mounting

```
cap2 <- "\\label{fig:plot_histogram_outbound}Frequency distribution for the outbound displ
acements and mounting"
```

1.2.1.4. Statistical tests

1.2.1.5. Normality test

```
events.fam <- events %>%
filter(treatment == "Familiar")

events.unfam <- events %>%
filter(treatment == "Unfamiliar")

lshap.familiar <- events %>%
  filter(treatment == "Familiar")  %>%
  dplyr::select(-id, -treatment, -order) %>%
  lapply(shapiro.test)

lres.familiar <- sapply(lshap.familiar, `[`, c("statistic","p.value"))
lres.familiar <- t(lres.familiar)
```

```
lres.familiar.sig <- lres.familiar %>%
  as.data.frame() %>%
  mutate(Significance = ifelse(p.value < 0.05, "*",""))

row.names(lres.familiar.sig) <- rownames(lres.familiar)


kable(lres.familiar.sig, digits = 2, caption = "Results") %>%
  kable_styling(full_width = F)
## Currently generic markdown table using pandoc is not supported.
```

Results

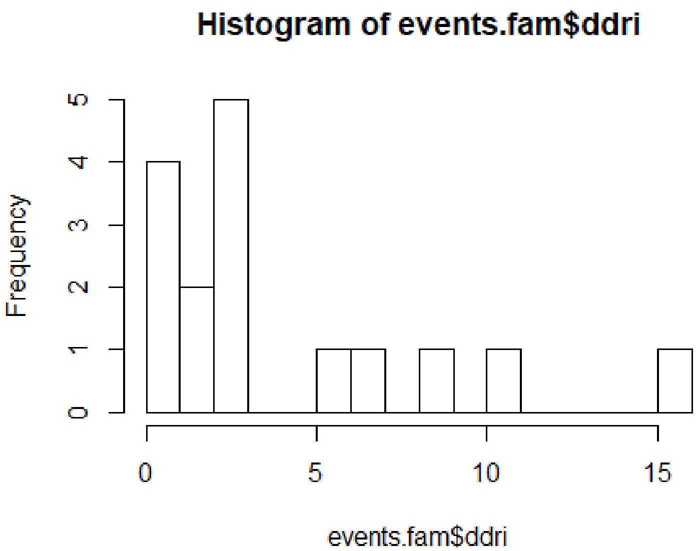
	statistic	P value	Significance
ddri	0.8307403	0.00718514	*
dfi	0.9436061	0.3955538	
dai	0.9107636	0.1197167	
dsi	0.9201399	0.1695593	
mi	0.7469107	0.0005832578	*
ddro	0.6531932	5.38286e-05	*
dfo	0.6084203	1.945809e-05	*
dao	0.5277475	3.630517e-06	*
dso	0.517499	2.969741e-06	*
mo	0.2726549	4.553108e-08	*

```
lshap.unfamiliar <- events %>%
  filter(treatment == "Unfamiliar")  %>%
  dplyr::select(-id, -treatment, -order, -ddro) %>% #ddro is ommited because all 0's
  lapply(shapiro.test)

lres.unfamiliar <- sapply(lshap.unfamiliar, `[`, c("statistic","p.value"))
lres.unfamiliar <- t(lres.unfamiliar)
lres.unfamiliar.sig <- lres.unfamiliar %>%
  as.data.frame() %>%
  mutate(Significance = ifelse(p.value < 0.05, "*",""))

row.names(lres.unfamiliar.sig) <- rownames(lres.unfamiliar)


kable(lres.unfamiliar.sig, digits = 2, caption = "Table with kable") %>%
  kable_styling(full_width = F)
## Currently generic markdown table using pandoc is not supported.
```

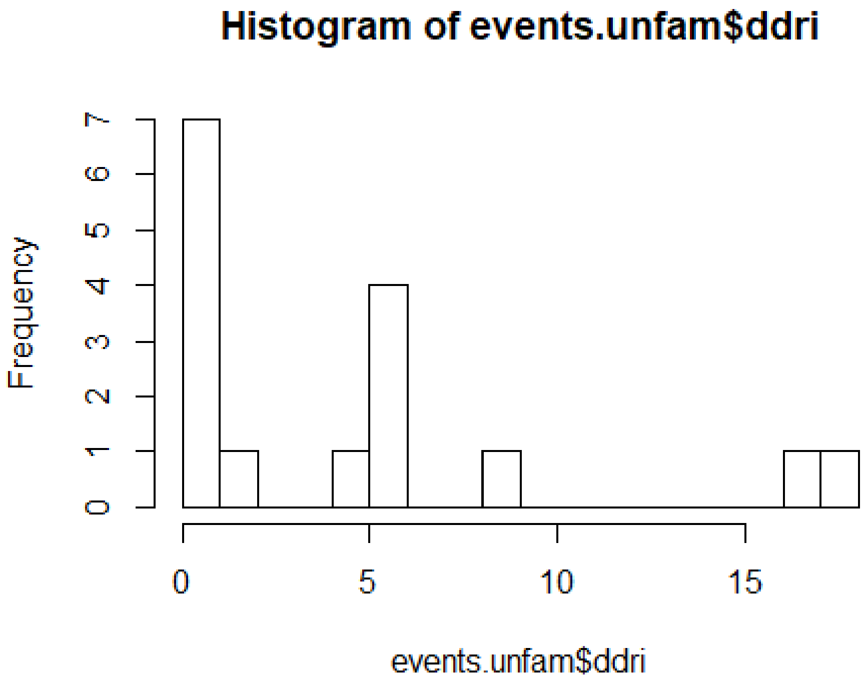
Table with kable

	statistic	P value	Significance
ddri	0.7855445	0.001761728	*
dfi	0.8957842	0.06882319	
dai	0.9506958	0.5007893	
dsi	0.8944199	0.06546477	
mi	0.7648117	0.0009635513	*
dfo	0.5462647	5.254141e-06	*
dao	0.562755	7.357529e-06	*
dso	0.5946753	1.442587e-05	*
mo	0.3787212	2.444198e-07	*

```
hist(events.fam$ddri, breaks = 16)
```




```
hist(events.unfam$ddri, breaks = 16)
```



1.2.1.6. Testing for order effect

```
# now test for an order effect -- did counterbalancing work?

# for a paired-samples t-test we must use a wide-format table; most
# R fns do not require a wide-format table, but the dcast function
# offers a quick way to translate long-format into wide-format when
# we need it.

dfi.order<-t.test(events.unfam$dfi[events.unfam$order == 1], events.fam$dfi[events.fam$or
der == 1], paired = FALSE, var.equal = TRUE)$p.value
ddri.order<-t.test(events.unfam$ddri[events.unfam$order == 1], events.fam$ddri[events.fa
m$order == 1], paired = FALSE, var.equal = TRUE)$p.value
dai.order<-t.test(events.unfam$dai[events.unfam$order == 1], events.fam$dai[events.fam$o
rder == 1], paired = FALSE, var.equal = TRUE)$p.value
dsi.order<-t.test(events.unfam$dsi[events.unfam$order == 1], events.fam$dsi[events.fam$o
```

```
rder == 1], paired = FALSE, var.equal = TRUE)$p.value
mi.order<-t.test(events.unfam$mi[events.unfam$order == 1], events.fam$mi[events.fam$or
der == 1], paired = FALSE, var.equal = TRUE)$p.value

dfo.order<-t.test(events.unfam$dfo[events.unfam$order == 1], events.fam$dfo[events.fam$
order == 1], paired = FALSE, var.equal = TRUE)$p.value
ddro.order<-t.test(events.unfam$ddro[events.unfam$order == 1], events.fam$ddro[events.fa
m$order == 1], paired = FALSE, var.equal = TRUE)$p.value
dao.order<-t.test(events.unfam$dao[events.unfam$order == 1], events.fam$dao[events.fam
$order == 1], paired = FALSE, var.equal = TRUE)$p.value
dso.order<-t.test(events.unfam$dso[events.unfam$order == 1], events.fam$dso[events.fam$
order == 1], paired = FALSE, var.equal = TRUE)$p.value
mo.order<-t.test(events.unfam$mo[events.unfam$order == 1], events.fam$mo[events.fam$
order == 1], paired = FALSE, var.equal = TRUE)$p.value

order.colnames <- c( "dfi", "ddri", "dai", "dsi",  "mi", "dfo" , "ddro",  "dao",  "dso" , "mo")
order.pvalues<- c(dfi.order, ddri.order, dai.order, dsi.order, mi.order, dfo.order, ddro.order,
dao.order, dso.order, mo.order)

ordereffect_table <- data.frame(Variables = order.colnames, "p-values" = order.pvalues)


ordereffect_table <-ordereffect_table %>%
  mutate(Significance = ifelse(p.values < 0.05, "*",""))



kable(ordereffect_table, digits = 2, caption = "Table with kable") %>%
  kable_styling(full_width = F)
## Currently generic markdown table using pandoc is not supported.
```

Table with kable

Variables	P values	Significance
dfi	0.29	
ddri	0.08	
dai	0.11	
dsi	0.14	
mi	0.34	
dfo	0.65	
ddro	0.03	*
dao	0.79	
dso	0.79	

mo	0.45

1.2.1.7. T-test

These variables satisfy the conditions for a t-test, so here are the results:

```
tddri <- t.test(events.fam$ddri, events.unfam$ddri, paired = TRUE, var.equal = TRUE)

tmi <- t.test(events.fam$mi, events.unfam$mi, paired = TRUE, var.equal = TRUE)

tddro <- t.test(events.fam$ddro, events.unfam$ddro, paired = TRUE, var.equal = TRUE)

tdfo <-t.test(events.fam$dfo, events.unfam$dfo, paired = TRUE, var.equal = TRUE)

tdao <-t.test(events.fam$dao, events.unfam$dao, paired = TRUE, var.equal = TRUE)

tdso <-t.test(events.fam$dso, events.unfam$dso, paired = TRUE, var.equal = TRUE)

tmo <- t.test(events.fam$mo, events.unfam$mo, paired = TRUE, var.equal = TRUE)

pvalues <- c(tddri$p.value,tmi$p.value,tddro$p.value,tdfo$p.value,tdao$p.value,tdso$p.valu
e,tmo$p.value)


pvalues_names <- c('ddri', 'mi','ddro', 'dfo', 'dao', 'dso', 'mo')

tablettest <- data_frame(pvalues_names, pvalues) %>%
  rename(Variables = pvalues_names, "p-values" = pvalues) %>%
  mutate(Significance = ifelse(pvalues < 0.05, "*",""))


kable(tablettest, digits = 2, caption = "Table with kable") %>%
  kable_styling(full_width = F)
## Currently generic markdown table using pandoc is not supported.
```

Table with kable

Variables	p-values	Significance
ddri	0.85	
mi	0.67	
ddro	0.03	*
dfo	0.46	
dao	1.00	

dso	0.64
mo	0.87

1.2.1.8. Repeated-measures ANOVA

```
m = ezANOVA(data = events, dv = dfi, within = treatment, wid = id)
m$ANOVA
##      Effect DFn DFd          F         p p<.05          ges
## 2 treatment   1  15 0.01709871 0.8977012       0.0007775189
```

1.2.1.9. Checking for alternative distributions

5.1.1.1.1.1.1.1.1 Poisson distribution

```
fit = fitdist(events.fam$dfi, "pois", discrete=TRUE)
gofstat(fit) # goodness-of-fit test
## Chi-squared statistic:  2598222
## Degree of freedom of the Chi-squared distribution:  3
## Chi-squared p-value:  0
##    the p-value may be wrong with some theoretical counts < 5
## Chi-squared table:
##         obscounts   theocounts
## <= 19 3.000000e+00 3.463969e-06
## <= 40 3.000000e+00 9.981241e-01
## <= 49 3.000000e+00 5.593952e+00
## <= 66 3.000000e+00 9.091309e+00
## > 66  4.000000e+00 3.166111e-01
##
## Goodness-of-fit criteria
##                                1-mle-pois
## Akaike's Information Criterion   334.4717
## Bayesian Information Criterion   335.2442
fit = fitdist(events.unfam$dfi, "pois", discrete=TRUE)
gofstat(fit) # goodness-of-fit test
## Chi-squared statistic:  53770.87
## Degree of freedom of the Chi-squared distribution:  3
## Chi-squared p-value:  0
##    the p-value may be wrong with some theoretical counts < 5
## Chi-squared table:
##         obscounts   theocounts
## <= 23 4.000000e+00 3.047604e-04
## <= 37 3.000000e+00 5.960687e-01
## <= 42 3.000000e+00 1.859651e+00
## <= 76 4.000000e+00 1.354081e+01
## > 76  2.000000e+00 3.160855e-03
##
## Goodness-of-fit criteria
```

```
##                          1-mle-pois
## Akaike's Information Criterion   341.1270
## Bayesian Information Criterion   341.8996
```

1.2.1.10. Non parametric testing

```
# Wilcoxon signed-rank test on dfi

wilcoxsign_test(dfi ~ treatment | id, data=events, distribution="exact")
##
##  Exact Wilcoxon-Pratt Signed-Rank Test
##
## data:  y by x (pos, neg)
##   stratified by block
## Z = 0.28449, p-value = 0.8013
## alternative hypothesis: true mu is not equal to 0
```

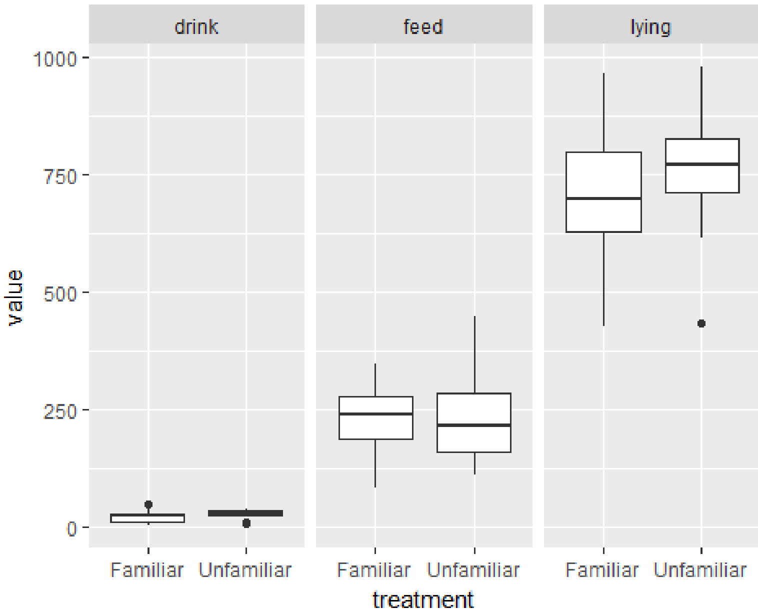
1.2.2. States analysis

1.2.2.1. Importing data

```
# DEBUG: set path
setwd("~/UFSC/Proyecto Social Network/R/Working Paper/")
# /DEBUG

load("states.RData")

states$treatment <- as.factor(states$treatment)
library(dplyr)
```

1.2.2.2. Summarizing data

```
##   variable  treatment   Min. 1st Qu. Median   Mean 3rd Qu.   Max.
## 1    drink   Familiar   3.00   12.18  23.86  21.90   26.93  50.45
## 2    drink Unfamiliar   5.87   26.06  28.67  27.81   34.74  38.02
## 3     feed   Familiar  83.00  188.80 240.10 232.20  276.90 349.00
## 4     feed Unfamiliar 111.70  158.60 216.80 226.40  285.70 448.80
## 5    lying   Familiar 426.60  628.20 699.40 700.70  799.40 964.40
## 6    lying Unfamiliar 436.00  713.30 771.30 756.10  826.50 978.10
# Transforming the data into a long table format


ggplot(data = states.long, aes(x=treatment, y=value)) +
  geom_boxplot() + facet_wrap(~variable,ncol = 3)
```



Box-and-whiskers plot of **Ads**

```
cap <-"\\label{fig:plot_states}**Box-and-whiskers plot of  ** Ads"
```

1.2.2.3. Statistical tests

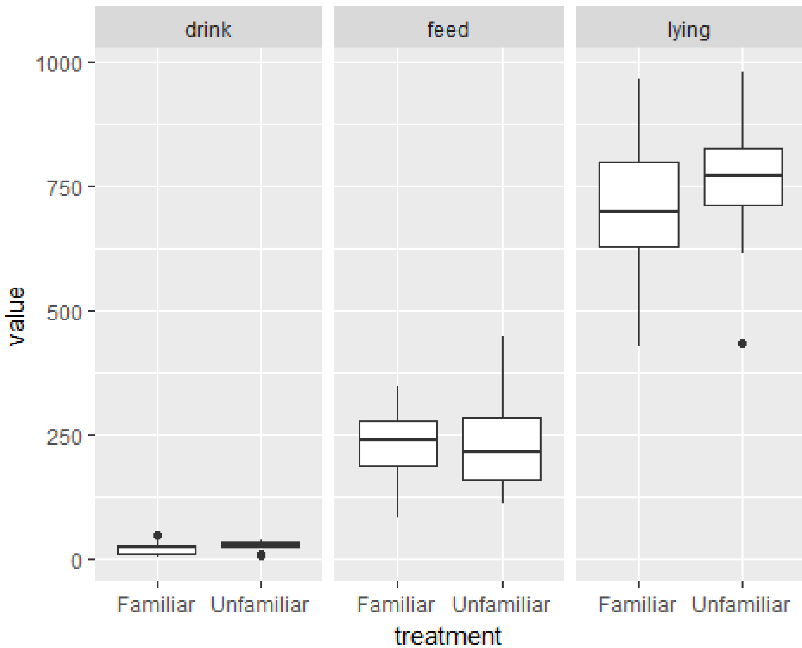
```
# DEBUG: set path
setwd("~/UFSC/Proyecto Social Network/R/Working Paper/")
# /DEBUG


load("states.Rdata")

# Transforming the data into a long table format

states.long <- states %>%
  select(treatment, drink, feed, lying) %>%
  melt( id.var = "treatment")

# Summarizing the values

states.summary <- ddply(states.long, ~variable * treatment, function(data) summary(data$v
```

```
alue))
kable(states.summary, digits = 2, caption = "Table with kable") %>%
  kable_styling(full_width = F)
## Currently generic markdown table using pandoc is not supported.
```

Table with kable

variable	treatment	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
drink	Familiar	3.00	12.18	23.86	21.90	26.93	50.45
drink	Unfamiliar	5.87	26.06	28.67	27.81	34.74	38.02
feed	Familiar	83.00	188.80	240.10	232.20	276.90	349.00
feed	Unfamiliar	111.70	158.60	216.80	226.40	285.70	448.80
lying	Familiar	426.60	628.20	699.40	700.70	799.40	964.40
lying	Unfamiliar	436.00	713.30	771.30	756.10	826.50	978.10

```
ggplot(data = states.long, aes(x=treatment, y=value)) +
  geom_boxplot() + facet_wrap(~variable,ncol = 5)
```



```
cap <-"\\label{fig:plot_states}**Box-and-whiskers plot of  ** Ads"


# Normality test

states.fam <- states %>%
  filter(treatment == "Familiar")

states.unfam <- states %>%
  filter(treatment == "Unfamiliar")

lsh.familiar <- states.fam %>%
  dplyr::select(-S, -W, -D, -Pen , -datapoints, -id, -treatment) %>%
  lapply(shapiro.test)

lresult.familiar <- sapply(lshap.familiar, `[`, c("statistic","p.value"))
lresult.familiar <- t(lresult.familiar)
lresult.familiar.sig <- lresult.familiar %>%
  as.data.frame() %>%
  mutate(Significance = ifelse(p.value < 0.05, "*",""))

row.names(lresult.familiar.sig) <- rownames(lresult.familiar)
View(lresult.familiar.sig)

kable(lres.familiar.sig, digits = 2, caption = "Results") %>%
  kable_styling(full_width = F)
## Currently generic markdown table using pandoc is not supported.
```

Results

	statistic	P value	Significance
ddri	0.8307403	0.00718514	*
dfi	0.9436061	0.3955538	
dai	0.9107636	0.1197167	
dsi	0.9201399	0.1695593	
mi	0.7469107	0.0005832578	*
ddro	0.6531932	5.38286e-05	*
dfo	0.6084203	1.945809e-05	*
dao	0.5277475	3.630517e-06	*
dso	0.517499	2.969741e-06	*
mo	0.2726549	4.553108e-08	*

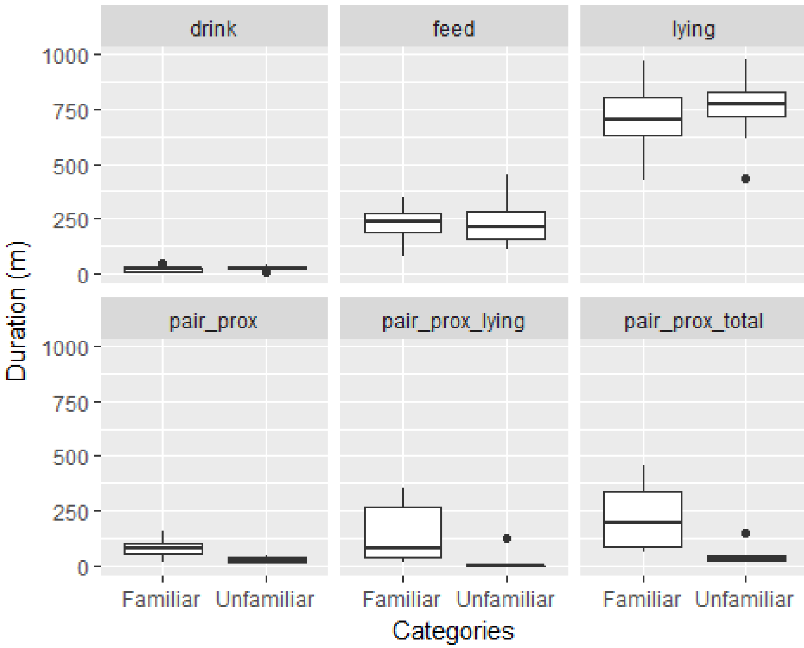
```
lsh.unfamiliar <- states %>%
  filter(treatment == "Unfamiliar")  %>%
  dplyr::select(-S, -W, -D, -Pen , -datapoints, -id, -treatment) %>%
  lapply(shapiro.test)

lresult.unfamiliar <- sapply(lsh.unfamiliar, `[`, c("statistic","p.value"))
lresult.unfamiliar <- t(lresult.unfamiliar)
lresult.unfamiliar.sig <- lresult.unfamiliar %>%
  as.data.frame() %>%
  mutate(Significance = ifelse(p.value < 0.05, "*",""))

row.names(lresult.unfamiliar.sig) <- rownames(lresult.unfamiliar)
View(lresult.unfamiliar.sig)

kable(lresult.unfamiliar.sig, digits = 2, caption = "Table with kable") %>%
  kable_styling(full_width = F)
## Currently generic markdown table using pandoc is not supported.
```

Table with kable

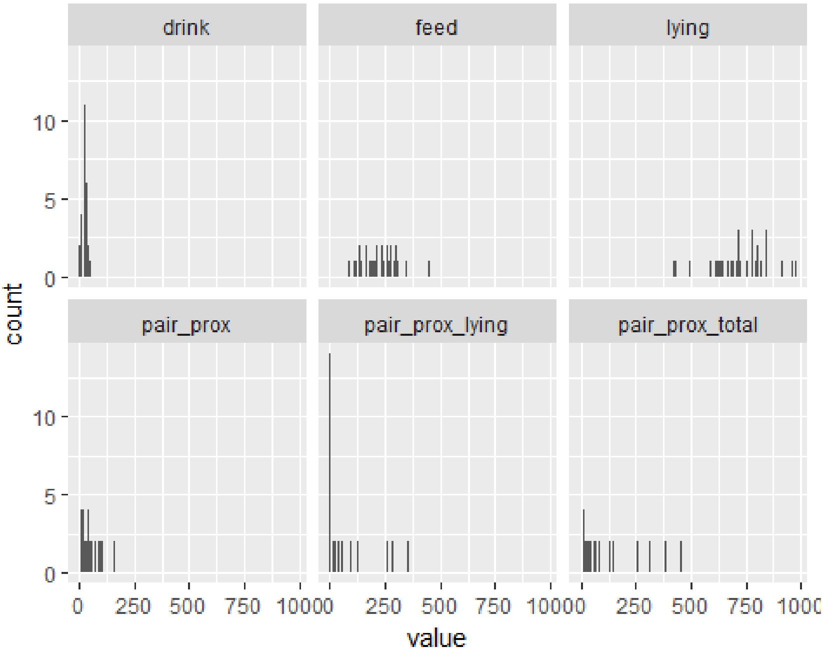
	statistic	P value	Significance
drink	0.8618011	0.02041831	*
feed	0.9294673	0.2390944	
lying	0.9555426	0.5821949	
pair_prox	0.9033217	0.09085666	
pair_prox_lying	0.4043742	3.770454e-07	*
pair_prox_total	0.6523726	5.280082e-05	*

```
### Distribution

theming <- scale_fill_grey(start = 0.1,
                           end = 0.4,
                           na.value = "red")
labelss <- labs(x="Categories", y="Duration (m)")


df1 <- states %>%
  dplyr::select(treatment, drink, feed, lying, pair_prox, pair_prox_lying, pair_prox_total) %>
%
  melt( id.var = "treatment")

ggplot(data = df1, aes(x=treatment, y=value)) +
  geom_boxplot() + facet_wrap(~variable,ncol = 3) +
```

```
  theming +
  labelss
```




```
ggplot(data = df1, aes(x=value)) +
  geom_histogram(stat = "bin", binwidth = 5) +
  facet_wrap(~variable, ncol = 3)
```




```
### T-testing
normal_variables <- c("drink", "pair_prox_lying", "pair_prox_total")

t_drink <- t.test(states.fam$drink, states.unfam$drink, paired = TRUE, var.equal = TRUE)
t_pair_prox_lying <- t.test(states.fam$pair_prox_lying, states.unfam$pair_prox_lying, paire
d = TRUE, var.equal = TRUE)
t_pair_prox_total <- t.test(states.fam$pair_prox_total, states.unfam$pair_prox_total, paired
= TRUE, var.equal = TRUE)

states_t_results <- c(t_drink$p.value, t_pair_prox_lying$p.value, t_pair_prox_total$p.value)

tablettest2 <- data_frame(normal_variables, states_t_results) %>%
  rename(Variables = normal_variables, "p-values" = states_t_results) %>%
  mutate(Significance = ifelse(states_t_results < 0.05, "*",""))

kable(tablettest2, digits = 2, caption = "Table with kable") %>%
  kable_styling(full_width = F)
## Currently generic markdown table using pandoc is not supported.
```

Table with kable

Variables	p-values	Significance
drink	0.16	
pair_prox_lying	0.00	*
pair_prox_total	0.00	*