ESTUDOS EM AGRESSIVIDADE: UM ESTUDO TEÓRICO EM PRIMATAS E HUMANOS E UM ESTUDO DE AGRESSIVIDADE EM RATOS APÓS PROVOCAÇÃO SOCIAL

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SUMÁRIO

RESUMO

Esta dissertação agrega estudos em agressividade em ratos, primatas e humanos. O primeiro artigo, uma revisão sistemática que aborda a agressividade em primatas e humanos, apresenta informações sobre diversas espécies de primata, com foco em símios, e humanos, enfatizando diferenças entre gêneros e apresentando bases moleculares e neuronais para este comportamento. O segundo artigo apresenta dados de um experimento utilizando o protocolo de Derrota Social, proposto por Miczek em 1979. O estudo também apresenta uma análise do comportamento de ratos Wistar, debatendo os padrões de comportamento observado e apresentando resultados de estatísticas descritivas e ANOVA de uma via dos dados encontrados. O artigo também discute a formulação de uma equação para auxiliar na escolha dos animais a serem utilizados como residentes, utilizando informações quantitativas relevantes para a análise do comportamento agressivo.

Palavras-chave: Agressividade, Primatas, Humanos, Ratos Wistar, Derrota Social.

ABSTRACT

This dissertation presents data regarding studies with humans, primates and rodents. The first article is a systematic review of aggressive behavior in primates and humans, presenting data about several species, focusing on apes and humans, and emphasizing gender differences, as well as molecular and neuronal bases for this behavior. The second article presents data of a study using the Social Defeat protocol, proposed by Miczek in 1979. The paper also contains analyses of aggression in Wistar rats, debating the behavioral patterns observed and presenting results from descriptive statistics and one-way ANOVA of the data found. The article also discusses the formulation of an equation, which uses quantitative information relevant to the analysis of aggression in order to improve the process of selecting resident males.

Keyword: Aggression, Humans, Primates, Wistar Rats, Social Defeat.

CAPÍTULO I

INTRODUÇÃO

Esta seção tem o intuito de apresentar o tópico central deste trabalho, bem como os temas centrais desta dissertação – o artigo teórico sobre agressividade em primatas e humanos e o estudo observacional com ratos.

Ambos os trabalhos envolvem a agressividade, que é um comportamento social complexo que surgiu evolutivamente com o intuito de se obter ou proteger recursos, tais como fontes de alimento, locais de esconderijo e parceiros para reprodução (Nelson & Trainor, 2007). É expressado por virtualmente todos os mamíferos e é de vital importância para a sobrevivência do indivíduo (Veenema, 2009) e desempenha uma função central na manutenção de estruturas hierárquicas em diversas espécies, tais como lobos, gorilas, leões e humanos. No entanto, a agressividade é extremamente dispendiosa em termos de energia e representa riscos para todos os indivíduos envolvidos.

Nas últimas décadas, a quantidade de estudos envolvendo o comportamento agressivo, seus mecanismos neurológicos e metabólicos, bem como as suas consequências para os indivíduos que o expressam, cresceu exponencialmente. A compreensão deste comportamento também é importante para que se possa encontrar possíveis tratamentos para condições psiquiátricas que o expressam de forma disfuncional. Além disso, a agressividade fora de contexto tem um custo severo para a sociedade: um relatório de 2002 da Organização Mundial da Saúde relata que, naquele ano, o número de vítimas de conflitos interpessoais se aproximou do dobro do número de vítimas de guerra.

Falhas nos mecanismos de regulação do comportamento agressivo são características de diversos transtornos de personalidade, conduta, depressão profunda, esquizofrenia e transtornos de ansiedade, entre outros (Veenema, 2009). Muitos destes transtornos apresentam desbalanceamentos em mecanismos neurológicos profundamente ligados à regulação do comportamento agressivo – depressão e transtornos de ansiedade apresentam, entre outros sintomas neurofisiológicos, uma redução no metabolismo de serotonina, neurotransmissor que está diretamente ligado à regulação da tomada de decisão (Rogers, 2010) e do comportamento agressivo através de, principalmente, dois de seus receptores (5-HT_{1A} e 5-HT_{1B}) (Olivier, 2004); a esquizofrenia apresenta como um de seus principais sintomas uma desregulação no metabolismo dopaminérgico (Maia & Frank, 2016), outro neurotransmissor envolvido na regulação da tomada de decisão (Rogers, 2010) e no comportamento agressivo (Chester et al., 2016). Os mecanismos serotonérgicos também estão profundamente associados à atividade de

GABA, o principal neurotransmissor inibitório do sistema nervoso de mamíferos e alvo de diversos antidepressivos (como os benzodiazepínicos).

A má regulação da agressividade pode levar à expressão de comportamento violento, que também pode ser definido como uma expressão de agressividade fora de contexto. Existem duas principais caracterizações de comportamento violento em animais - uma caracterização quantitativa, baseada em parâmetros como baixa latência de ataque e comportamentos frequentes e prolongados com o intuito de ferir o outro animal; e uma caracterização qualitativa, baseada em parâmetros como ataques em regiões vulneráveis do corpo do oponente e ataques independentes de contextos como o gênero do oponente ou as condições ambientais (Natarajan & Caramaschi, 2010).

Dentre os modelos animais utilizados para o estudo da agressividade, um dos mais comumente utilizados é o protocolo de derrota social. Este método consiste de uma exposição de um rato a um macho residente, de maior porte e que apresenta comportamento territorial. O protocolo foi estabelecido por Klaus Miczek, em 1979, e é amplamente utilizado devido à sua validade etológica. Os dados empíricos analisados no capítulo três deste trabalho foram obtidos a partir de cinco animais residentes, submetidos a este protocolo, após seu período de treinamento.

Este modelo contém muitas considerações éticas. Por exemplo, a exposição dos animais intrusos ao residente e os ataques sofridos por este animal podem, em muitos momentos, induzir quadros sintomáticos que se assemelham àqueles apresentados em condições como a depressão ou o transtorno de estresse pós-traumático. Até mesmo testemunhar a agressão pode ser o suficiente para induzir um quadro traumático (Patki, Solanki & Salim, 2014). No entanto, a análise do comportamento agressivo e de suas consequências, tanto no agressor quanto no animal atacado, faz deste modelo um dos mais utilizados na atualidade para estudos em agressividade.

O capítulo dois consiste de um artigo de revisão abordando primatas e humanos e apresenta dados sobre diversas espécies, com ênfase particular em símios (bonobo, chimpanzé, gorila e orangotango) e nas diferenças de comportamento entre os gêneros. Os estudos com estes animais são, em sua grande maioria, observacionais, com animais em seu ambiente natural ou em cativeiro. O estudo com humanos possui uma ênfase molecular e em mecanismos neurobiológicos da expressão do comportamento agressivo. O artigo também aborda diferenças da expressão do comportamento agressivo entre os gêneros.

O capítulo três consiste de um estudo observacional de derrota social e instigação do comportamento agressivo em ratos Wistar machos. Neste artigo, propusemos também uma equação para quantificar o comportamento e, com isso, observar seu aumento ou diminuição ao longo das diferentes sessões de treino, com o intuito de facilitar a aplicação deste protocolo. Também é proposta uma divisão entre comportamentos demonstrativos (*displays*) e comportamentos de ataque, para contribuir com a análise do comportamento agressivo dos animais.

O capítulo final discute as semelhanças entre o que pode ser observado nas diferentes espécies, além de apresentar perspectivas para estudos futuros na área.

CAPÍTULO II

ARTIGO 1 – BEHAVIOURAL, HORMONAL AND NEUROBIOLOGICAL MECHANISMS OF AGGRESSIVE BEHAVIOUR IN HUMAN AND NONHUMAN PRIMATES

Behavioural, hormonal and neurobiological mechanisms of aggressive behaviour in human and nonhuman primates

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CAPÍTULO III

ARTIGO DOIS - AGGRESSION IN MALE WISTAR RATS AFTER SOCIAL INSTIGATION

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Abstract

Aggression is a widely studied behavior, which can show itself both as a functional social behavior or a dysfunctional, escalated behavior that can be harmful to the individual and the hierarchic structure of its group. The Social Defeat protocol can be used as a model for both expressions of aggression and consists of exposures of an intruder rat to an aggressive resident male. Our study aimed to evaluate the aggressive behavior and patterns of Wistar rats. We discuss our findings on an experiment using the Social Defeat protocol, and propose a new way to assess and evaluate aggression. Our animals have shown statistically significant differences in clinches, keep down and upright postures, as well as their total aggressive score, calculated as described by Koolhaas, 2014. We also discuss the patterns of aggression that can be observed in low-aggressive Wistar rats, regarding to the preference of the animal towards attacking or showing aggressive displays.

Introduction

The aggressive behavior comprises behaviors that inflict harm or injury or even the threat of such stimuli (Berkovitz, 1983; de Almeida et. al., 2005). In a biological perspective, this behavior is crucial to individual's survival and reproductive successes and these features are interpreted as "individual fitness", and it is directly correlated to the proper demonstration of aggressive behaviors (de Boer et al. 2003).

In social hierarchies of the animal reign, males acquire higher positions when display aggressive behaviors; moreover, these aggressive show offs are useful to patrol and to defend territory REF. Maintaining a territory confers higher probability to find resources and sexual mates (Brain, 1981; Huntingford & Turner, 1988). Although this behavior represents a relevant effort on individual's survival, when expressed in exacerbated levels, it consists of threat to the own individual. Violence, as referred to displaced and inappropriate form of intense aggression, is considered a worldwide health major problem already attested on The World Report on Violence and Health (Krug, Mercy, Dahlberg, & Zwi, 2002). Exaggerated forms of aggressive behavior are of particular interest when endangers life of health professionals in clinic and psychiatric settings (de Almeida, Ferrari, Parmigiani, & Miczek, 2005). In spite of WHO's report health problem recognition, much of the knowledge about the topic on the neuroscience field still ignored. Successful studies on aggressive behavior, ranging from molecular, neurobiological and behavioral perspectives, presents important evidence for diagnosis, prevention, treatment and public and judicial policies on the theme (Miczek et al., 2007).

Different types of aggressive behaviors were characterized in laboratory context for experimental, clinic or veterinary purposes (Miczek & de Almeida, 2012). Studies in animal models began with the induction of aggressive behaviors in an exacerbated manner in individuals initially placid. The induction would happen through lesion or electric brain stimulation; applying painful stimulation to the animal; social isolation for long time periods; or exposing then to anticipated frustration (de Almeida et al., 2005). Ethologically inspired methods based on characteristic aggressive repertoire of each animal species took place to replace and rethink these methods (de Almeida et al. 2005). Nowadays, the study of aggressive behavior comprehends observation of acts, postures and communication signals that follow an intricate temporal sequential organization. These observations are sometimes more common to both males and females, and others are more sexually dimorphic (Miczek et al. 2007). Specific fundamental information about the species behavioral repertoire is crucial to predict the study success and its quality; moreover, it is crucial to determine the translational value of results to clinic and population settings.

Rats represent a social mammal species and they form hierarchies under territorial dominance in their populations (de Almeida et al., 2005). The distal function of the behavior in search of dominance represents an attempt to assure resources to a better genetic transmission; the proximal function in search of ritualization of the aggressive display contributes to lower injury and harm risks (de Almeida et al., 2005). In the case of mice, where the males tend to be solitary in adulthood, the laboratory research was developed based on territorial aggression as main feature for the protocol, generally a breeding mouse attacks a naïve intruder (Miczek & O'Donell, 1978; de Almeida et al., 2005).

A rodent laboratory social instigation protocol was developed aiming to increase the levels of aggressive behavior when the animal face an opponent previously presented in a short period protected from physical contact with the aggressors. Generally, hamsters, rats and mice initiate attack in short time with a high frequency if an intruder conspecific is placed in their territory, or even in a non-familiar territory, after being instigated by the presence of the opponent (Potegal, 1991; Fish, 1999; de Almeida et al., 2005, Da Veiga et al 2011). The repeated exposition to victories in such confrontations with intruders leads to a successive increase of aggressive responses. The neurobiology of this behavior was initially characterized in mice as a reflex of the rewarding nature of the aggressive behavior (de Almeida et al., 2005). In a particular manner, these evidence may be valid for rats, as studied by de Boer, van der Vegt &

Koolhaas (2003). This longitudinal study analyzed the aggressive behavior of feral strain lineages of *Rattus novergicus* and lineages of laboratory Wistar rats in a resident-intruder protocol. The results demonstrated a trimodal distribution of variations in the individual level of aggressive offensive behavior in the feral population; the individuals presented nonaggressive, low aggressive or high aggressive phenotypes. In the Wistar population, this distribution was bimodal, in which the high aggressive phenotype was absent (de Boer et al., 2003). Today, almost all rat species used in laboratory research have their behavioral aggressive traits (including underlying genetic components) compromised as result of artificial and inbreeding along the domestication time process (de Boer et al., 2003).

The objective of this study was to present evidence that, under controlled testing and housing conditions, the aggressive behavior of male Wistar rats may stablish or escalate. We hypothesized that the regular presentation of stimuli related to the offensive aggressive trait, once present on the individual, leads to establishment or reinforcement of the aggressive behavior. We also propose an equation to better assess aggressive behavior throughout all stages of training, allowing the research team to quantify aggression in order to improve results in further studies.

Method

Adult male Wistar rats (n = 24) and female Wistar rats (n = 6) from FEPPS (Fundação Estadual de Produção e Pesquisa em Saúde), Porto Alegre – RS, were housed in standard polycarbonate cages containing sawdust bedding and were provided with free access to food and water. The animals were divided into three different groups: i. Residents: 6 male rats, with a mean weight of 335.40 g and range of 44.20 g; ii. Instigators: 18 male rats, 287.60 g; iii. Females: 6 female rats, 256.30 g. The study began with the procedures of female sterilization.

Residents and females were housed together and were kept in separated room from instigators. The two cohorts were housed with controlled climatic conditions, with 20 to 22° C of temperature, 40 to 60 % of relative humidity and a controlled 12 h light-dark cycle (lights on at 7 am to 7 pm). This study was supervised and approved by the *Comitê de Ética para Uso de Animais do Hospital de Clínicas de Porto Alegre* (CEUA / HCPA) and it was executed following the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011) and Brazilian law 11794/2008.

After the operatory recovery period, the females were paired with resident males. The sterilization presents benefits related to preventing reproduction without disturbing hormonal cycles due to the preservation the ovaries. This culminates in females being sexually receptive without the need of experimenter controlling their hormonal system. Each couple was housed in custom-made acrylic boxes of 46x71x46 cm, the boxes were layered with sawdust and animals had free access to food and water. Pairing the residents provides the development of defensive territorial behaviors, this is achieved by raising the testosterone levels as result of copulatory behavior (Flannelly, Blanchard, Muraoka, & Flannelly, 1982). In addition, the cleaning routines needed to be planed; it had to obey a schedule that allowed the resident's scent and feces remain in the cage during the confrontation sessions. The odor is an important stimulus to the resident, and it is significant to the establishment of conditioned fear in intruder rats, for instance, in the social defeat protocol (Raab et al., 1986). After 21 days counted from the pairing day, equivalent to a complete gestation, it was possible to assure the success of female sterilization. Moreover, it was possible to assure that the male had several chances to copulate.

The residents' cages had partitions between them in order to keep the animals visually isolated from each other. The isolation is a stressful stimulus, in a short manner it leads to increased aggressiveness (Brain, Nowell, & Wouters, 1971) and contributes to the development of escalated aggressive states. The animals from the instigator group were housed in similar conditions to the residents and females, these animals were housed in pairs in a different room. Housing the animals in pairs facilitates the control of feeding from these individuals. They should weight 50 g less than resident rats, maintaining these animals in the minimum diet (15 g of chow / rat / day). Instead of an *ad libitum* diet, this method delays the weight gain preventing the instigators to achieve measures equal to the residents. The difference in weight and age between the animals is another factor to be considered within the resident-intruder paradigm, mainly when the goal is to exert control on the dominance of the larger and older animal over the intruder.

The training procedures were conducted as the following description. In the first hour of the light-cycle dark phase the females were removed from the resident cages, the instigators had their fur inked for posterior eye discrimination from the resident during interaction situations. The dark phase is the period of higher activity in rodents, the initial hours, paired with higher levels of corticosterone, are more suited for studying behaviors related to stress and aggression, usually the first four hours of the dark phase. The training sessions were conducted under red dim light determined to not disturb the dark phase of the light-cycle. A Latin square design was used to arrange one single encounter between resident and instigator in each session, this sampling method intends to avoid resident minimum contact with familiarized animals whenever still possible (fig. 1). These conditions are also necessary in the social defeat protocol, it is crucial to the establishment of unpredictability of the aggressive stimulus for the intruder animal. In the continuation of the study, it was possible to evaluate the resident ability to attack distinct intruders.

The residents were transferred to an appropriated behavioral testing room and the intruder living cage was introduced inside the resident home cage for 10 min, this is called pre-social instigation step. In this social instigation procedure the intruder remains protected from the resident, the cage allows visual, olfactory, and auditory stimuli to being exchange, without permitting physical contact between the animals. Once the stipulated time was over, the intruder was removed from its protective cage and directly exposed to the resident, this is called confrontation step. The resident should display aggressive behaviors until 10 min, and at least one bite attack was expected in order to consider the aggressive session as valid. Once the resident attacks, the session would end 5 min after the first bite, or the session would be finished earlier if the intruder was forced to assume a supine position for at least 5 seconds. Registered information consisted of first bite latency, bite frequency exhibited by the resident, and specific aggressive behaviors such as piloerection, upright posture, lateral threat, and chase. The training sessions lasted around 15 min, at the end of the session, the instigators and residents returned to their original housing rooms.

The scheduled training routine was initially with 48h of interval, three times a week. Training was rearranged for 78 and 96h time intervals after the reliable establishment of aggressive behaviors. Besides the presence of aggressive behavior, it was also tested three different conditions along the sessions. The first phase of training aimed to find which of the selected animals would present aggressive behaviors. Six residents and six instigators were tested following the before mentioned procedures. Six training sessions for each resident occurred in 14 days. According with the Latin Square sampling method the animals never met a familiarized mate during this phase. The second phase of testing aimed to answer which animals would show a stable pattern of aggression and consisted of 11 sessions throughout 21 days. A third phase was performed to assess which animals would show conditions of fighting more than once per night, and therefore the residents were exposed to two sessions each night, against two different provokers. The final stage intended to appraise if the animals would show aggression against unknown animals and more than once per night. Therefore, the animal would first be exposed to a known provoker and, in the following session, the resident would face an unknown animal as the provoker. To this end, 6 new provokers were added to the study. 12 trainings, consisting of 2 sessions per night, were repeated throughout a period of 14 days.

The behaviors and postures shown by both males during the interactions were visually recognized according to the descriptions and operational definitions presented by Koolhaas et al. (2013; 1980). Five behaviors were analyzed – chase, clinch, keep down, lateral threat and upright posture. Data analysis consisted of descriptive statistics and One-Way ANOVA tests to compare the prevalence of the aggressive behaviors. The latency of the first bite and the total number of bites were expressed through median and interquartile range of the obtained data, while the number of valid sessions (the ones in which the resident bit) was expressed in percentage.

When assessing the aggressive behavior of animals, we began by using just the score described by Koolhaas et al., 2004. However, the score did not consider session times, latency, nor the percentage of valid sessions, all criteria we considered to be important for our analyses. As our animals would be rated from “non-aggressive” to “low-aggressive” in Blanchard’s phenotype division, we still faced a duality in our data, as even our low-aggressive animals showed a consistently divergent pattern in their behaviors.

In order to better evaluate the amount of aggression in our animals, we first developed a simple equation taking into account the parameters that were labeled relevant to aggressive behavior. Those factors were: duration of the session, latency of the first bite, the total score of aggressive behaviors shown by the animals (upright posture, keep down, lateral threat, chase and clinch; as described by Koolhaas, 2004) and the percentage of valid sessions. This equation describes a standardized amount of aggression the animal shown in the time of sessions, and contains this information in the following order:

$$Standardized\ aggression = \frac{(Aggressive\ Score\ x\ percentage\ of\ valid\ sessions)\ x\ (600 - Mean\ Latency)}{Mean\ Session\ Duration}$$

Since we intended to express mathematically the amount of aggression our animals exhibited, we considered the values involved with aggression that were not time-related as our numerators; higher aggressive score implies more occurrences aggressive behavior and higher percentage implies more reliability on this behavior. Taking into account that higher session duration (considering the protocol has two conditions that would prematurely end the session – namely, 5 seconds of Keep Down or at 5 occurrences of clinch) and higher latency are both associated with lower levels of aggression, we used duration as denominator and subtracted the latency from the maximal duration of the session. Duration was particularly important as a denominator, since higher duration can result in higher aggressive score, but not in higher aggression, due to prolonged exposure, and therefore it was crucial to consider it. This equation showed us that our most aggressive resident was Resident 8, as will be discussed later.

Results

Behaviors

After the introduction of the provoker in the resident's territory, the animals have shown a repertoire of behaviors, actions and postures described as following. Initially, when the provoker is introduced, the resident will move towards it and then start behaviors of social and anogenital exploration, and attempts of sexual intercourse might occur. These behaviors are separated by short periods of self-grooming. On the other hand, the provoker will explore the resident's territory, show bipedal posture, smell the environment or assume a less exploratory state, in which it will stand still and analyze the surroundings. The aggressive behaviors of the resident will usually appear along with piloerection and lateral threats, to which the provoker will respond by fleeing or using its hind limbs to keep the resident away. Once the provoker takes a bipedal position in front of the resident, it will respond with the same posture, and both boxing and teeth tittering behaviors might occur. The aggression *per se* will appear as bites, usually aimed at the provoker's abdominal region or neck. The provoker will tend to flee, moving away from the resident, which might lead to a chasing behavior in the attacker. In other circumstances, the provoker may take a supine position, with its back against the substrate, expositing the ventral region. This position is usually imposed to the provoker by the resident.

As described by Boer et al. (2003), these behaviors are usually short-lasting and follow temporal sequences of investigation, threats and attacks, separated by long periods of inactivity or selfdirected behaviors. Throughout this interval, the resident can also walk, sit or lie down, for example.

Number of offensive behaviors

Latency times of the first attack of the resident and frequency of bites were registered. All sessions that contained at least one attack were considered valid. All sessions presented threatening and investigative postures associated with attacks, and even though some of the provokers have shown threatening behaviors and attempts of submitting the resident in our sessions, none of the residents were attacked by the stimuli animals. Data regarding the sessions are shown in Table 1.

During each training phase, it was possible to answer individual questions for the tested animals. Five of the six animal selected to the test developed reliably the aggressive behavior; the five most aggressive animals presented between 82% and 90% of their sessions considered valid. Only one animal did not show any attacks in all of his tested sessions. The first stage consisted of identifying which animals have shown aggressive traits in their behavioral repertoires in the environmental conditions planned and maintain along the study. On average, all five aggressive animals started to exhibit aggressive behavior after the third session and the percentage of valid sessions ranged from 33% to 83% throughout this stage. Phase two consisted of identifying which animals have shown stability in their attacks in two consecutive sessions; the percentage of valid sessions have shown a reduction in its range, varying from 73% to 100%. It was possible to observe an increase in the number of attacks per session for most animals, and this fact can be noticed by the increase in the range on the number of bites per session between phases one and two. One of the animals had its training interrupted due to not obtaining success in neither of the two phases. The following stages aimed to test the adequacy of the animals for their utilization in the social defeat protocol, as standardized and described by Miczek (1979; Miczek, Nikulina, Shimamoto, & Covington, 2011).

With that goal, the third phase was used to assess if the animals that showed stable aggressive behavior could attack two different provokers in the same night. One of the animals presented 67% of valid sessions in this phase, and the others ranged from 92% to 100%. The last phase were dedicated to the observation of whether the traits trained before depended on the subject the resident would face. The aim of this phase was to ensure that the residents would attack any opponent other than those he had already faced. In this phase, the percentage of valid sessions varied between 60% and 100%.

Data analysis with a one-way ANOVA test, using the behaviors as dependent variable, and not the animals, showed that there were statistically significant differences in clinch ($F(4,35)= 3{,}021$, $p=.031$), keep down ($F(4,35)=3{,}164$, $p=.025$) and upright ($F(4,35)=3{,}992$, $p=.009$) occurrences, as well as in the total aggressive score ($F(4,35)=2{,}686$, $p.=0{,}47$) and in the duration of the sessions ($F(4,35)=7{,}328$, $p < .001$). Chasing could not be analyzed in the oneway ANOVA due to one of the groups having a 0 variance (since one animal did not show any occurrences). Multiple comparisons, corrected by Bonferroni, showed that Resident 8 expressed more chases than any other animal (the behavior we considered to be the most aggressive of the repertoire), more clinches than Resident 5, more upright posture than Resident 3 (which was the only significant result of this behavior’s analysis) and shorter session durations than Residents 3 and 5. Lateral threat, keep down and overall aggressive score did not show any statistical differences in the multiple comparisons. No other differences between animals were observed.

Discussion and Conclusions

This study aimed to verify the hypothesis that, under a regular scheme of stimuli presentation, offensive aggressive trait-like features of a particular individual may turn stable or escalate. It was used a group of stimuli that would increase territoriality, aggressive behavior through natural hormonal features, and aggressive behavior through presentation of a controlled resident-intruder interaction setting.

The escalation of aggressive behavior after winning a fight is a well-known phenomenon, often called “the winner effect” (Kloke et al., 2011). In fact, this is one of the main processes involved in the Social Defeat protocol, and it is crucial that the aggressive behavior of the resident escalate from one stage of the training to another. Similarly, the intruder suffers from a contrary effect, but equally important to the protocol, called “the loser effect”, in which its aggressive behavior decreases after losing a session.

Aggressive behavior, generally, decreases with acute stress and increases in animals afflicted by chronic stress (Yohe, Suzuki & Lucas, 2012), but most studies do not describe an increase in the aggressive behavior of the intruders. This fact may, however, have more than one explanation – the absence of an escalation of the aggression from the intruder might not be the main interest of the study or, more likely, it does not happen due to the size difference of the animals. Still, it is known that animals in a higher hierarchic position are more likely to act aggressively towards their subordinates when afflicted by chronic stress and that even subordinate animals will show aggressive behavior towards their “superiors” if their opponent is either younger or smaller (Wommack & Delville, 2003). It is important to take into account that one of the reasons of the escalation in aggression that occurs might be related to the stress induced to the resident by the protocol itself - manipulation, confrontation, separation from the female and invasion of its territory are all known stressors. Additionally, the fixed schedule of confrontations (48 hours of interval) also points to an anticipation effect in both the resident and the intruder (Ferrari, van Erp, Tornatzky & Miczek, 2003).

Five of our six trained residents developed strong and reliable aggressive behavior. Although this is a study with pre-experimental design, with no control group for comparison, we accept the initial hypothesis, endorse the method as a valid procedure, and consider our findings as an example of our anticipated results.

The Groningen Group and Blanchard reported similar results of the establishment of reliable aggressive behavior with Wistar rats or albino laboratory rats. Longitudinal studies conducted in feral strains of *Rattus norvegicus* pointed out a trimodal distribution of individual's variability on aggressive offensive phenotypes during a 10 min. resident-intruder confrontation (de Boer et al., 2003). In the case of Wistar rats, these animals lacked the third highly aggressive phenotype, as previously anticipated by means of domestication. Our study demonstrated one animal in the range of non-aggressive phenotype and five animals that would be comparable with low-aggressive Groningen feral rats. The differences between the high and low phenotype reside on the basic evidence reported in old studies that feral strains presents killing and serious injuring while the Albino strain preserves the communication feature of aggressive behavior (Blanchard, Takahashi, & Blanchard, 1977). Ethologically, the animals from our study spent time investigating and exploring the intruder and consequently attacking the invader, while the high aggressive animals studied by de Boer et al. (2003) rapidly skip to the offensive attacks. This non-evaluative action could be also directed towards infants, females and anesthetized conspecifics, charactering some of these animals as presenting violent forms of aggression. In other words, this represents a maladaptive behavioral strategy in which preserving a colony or mate safety is absent.

The aggressive pattern of the animals varied, with some animals showing higher amounts of aggressive displays (such as lateral threat or upright posture) and others showing higher amounts of attack behavior (keep down, clinches and chasing). We believe that this difference highlights a binary pattern in low-aggressive animals – those who favor displays and those who favor attacks and submission. During our observations, the most aggressive animals displayed shorter session duration and a preference towards clinching and keeping down, while animals that presented more occurrences of upright posture and lateral threats have shown less occurrences the other two behaviors. Chasing was a very rare occurrence, but we treated it as an attack behavior, since it only occurred after clinches or submission attempts from the resident.

Albeit our sample size was not big enough to generate powerful statistical results, the observational data seemed reliable when we took these patterns into account. Our statistics showed differences between the expression of the behaviors in some animals, and while the small number of animals could undermine those results, most of them survived to ANOVA tests corrected by Bonferroni.

Our equation, as aforementioned, supported our statistical findings, which corroborates the equation as a useful tool to assess aggression. We analyzed solely the results of stage 4, but our findings support the hypothesis that the equation can work as well in the previous stages of our studies. The most relevant use of this formula would be to have a quantification of aggression to set as baseline for posterior studies from our and others groups that study aggressive behavior.

The Social Defeat protocol has been widely used to study both the resident and the intruder, and has provided valuable information on both the behavioral and molecular aspects of aggression. Studies using this model are recently being used not only to obtain data about aggression, both functional and dysfunctional, but also to induce Post-traumatic stress disorderlike symptoms (Patki, Solanki & Salim, 2014). However, it is crucial, for that end, that the aggressive behavior of the residents is stable and escalated. This study presents some insights on how to evaluate and distinguish different patterns of aggressive behavior to optimize results from the use of this model.

Tables

Table 1 – Latency, Frequency of clinches and number of valid sessions for each stage and throughout all training

Resident	Phase 1			Phase 2			Phase 3			Phase 4			General		
	Lat	Freq	Valid Sessions	Lat 229 (287)	Freq	Valid Sessions.	Lat 104,5 (319)	Freq	Valid Sessions	Lat 145 (335)	Freq 4 (5)	Valid Sessions	Lat	Freq	Valid Sessions
02R	- (0)	- (0)	0%		4 (5)	73%		3 (7)	67%			70%	195 (347)	4 (7)	59%
03R	229,5 (188)	1,5 (1)	67%	82,5 (426)	3 (6)	91%	67 (384)	3 (4)	92%	53,5 (60)	2,5 (3)	80%	72 (426)	2 (6)	85%
04R	111 (86)	2,5 (1)	67%	82 (126)	3 (6)	91%	120,5 (271)	2 (5)	100%	89 (173)	1,5 (3)	60%	93,5 (299)	2 (6)	82%
05R	155 (10)	2 (0)	33%	240 (333)	2 (9)	100%	81 (150)	2 (5)	92%	100 (127)	4 (7)	90%	131 (366)	2 (9)	85%
06R	- (0)	- (0)	0%	- (0)	- (0)	0%	- (0)	- (0)	0%	- (0)	- (0)	0%	- (0)	- (0)	0%
08R	130 (306)	2 (7)	83%	79 (375)	5 (7)	73%	106,5 (618)	4 (7)	100%	11 (365)	2,5 (6)	100%	88 (622)	4 (8)	90%

Note: Data regarding latency and frequency are shown as Median and Range Md (Rng)

Table 2 – Results from our equation of Standardized Aggression and data used in its application

Resident	% of Valid Sessions	Latency	Score	Duration	Total Aggression
2	0,7	145	24	189,5	40,3377
3	0,8	53	26	486	23,4107
4	0,6	89	21,5	415	15,8841
5	0,9	100	15,5	618	11,2864
8	1	11	14,5	108	79,0787

Figures

	1R	2R	3R	4R	5R
1ºS	6P	7P	8P	9P	10P
2ºS	10P	6P	7P	8P	9P
3ºS	9P	10P	6P	7P	8P
			…		
(n)S	6P	7P	8P	9P	10P

Figure 1: Latin square design for the sessions. R: Resident, S: Session, P: Provoker.

CAPÍTULO IV

DISCUSSÃO GERAL E CONCLUSÕES

Os artigos apresentados abordam alguns dos principais grupos cujo comportamento agressivo é amplamente conhecido: roedores, primatas e humanos. Roedores são o modelo mais clássico para estudos de comportamento, e muitas das bases neurológicas da agressividade foram descobertas graças a estudos com estes animais. O protocolo de derrota social simula de forma etologicamente válida os encontros agressivos entre ratos.

Estudos em laboratório com primatas são pouco comuns, devido às questões éticas e ao alto custo financeiro para manutenção deste tipo de pesquisa. Com estes animais, estudos etológicos observacionais, seja em cativeiro (como em zoológicos) ou na natureza, passam a ser a maior fonte de informações, desde análises indiretas da dieta a partir das fezes do animal até observações diretas das relações sociais em diferentes espécies. Dados sobre símios constituem uma parte considerável do corpo literário de agressividade neste grupo, ainda que os estudos com orangotangos sejam raros. Em muitos casos, dada a proximidade evolutiva entre símios e humanos, estes achados contribuem para a compreensão deste comportamento em humanos.

Estudos em agressividade com humanos normalmente usam análises comportamentais indiretas (como, por exemplo, análises de impulsividade) e medidas moleculares (níveis sanguíneos de testosterona, cortisol e serotonina, por exemplo). Um protocolo clássico para estudos de agressividade em humanos é o Jogo do Ultimato (Sanfey, Rilling, Aronson, Nystrom & Cohen, 2003), que funciona da seguinte forma: um dos participantes recebe um montante fictício de dinheiro e deve fazer uma oferta para o outro. Se o participante que recebeu a oferta aceitá-la, cada um dos jogadores recebe o montante combinado. Caso ele não aceite, então nenhum dos jogadores recebe nada. A premissa básica parte do princípio que, se no início do jogo nenhum dos participantes tinha nada, o participante que recebe o montante fará a menor proposta possível (com o intuito de manter o máximo de dinheiro para si), enquanto o outro jogador aceitará qualquer valor proposto, pois, inicialmente, não possuía nada – um raciocínio conhecido como “maximizador racional”. Uma adaptação deste método envolvendo chimpanzés - utilizando uvas-passa ao invés de dinheiro - mostrou que este é exatamente o resultado encontrado, até mesmo quando a oferta feita por um dos animais era de nenhuma uva-passa (Jensen, Call & Tomasello, 2007).

No entanto, os resultados deste protocolo com humanos não corroboram a hipótese inicial. Ao invés disso, os participantes mostravam a tendência de fazer ofertas mais altas do que somente o mínimo, com o intuito de ter a oferta aceita, e recusar ofertas baixas, para punir o outro participante (Crockett, Clark, Tabibnia, Lieberman & Robbins, 2008). Assim, suspeita-se que a intensa regulação do comportamento agressivo através dos mecanismos neurológicos envolvidos na interação social interfiram na análise contextual relacionada à expressão deste comportamento, levando o indivíduo a tomar uma decisão que não é favorável para nenhum dos indivíduos como forma de retaliação por ter recebido uma oferta baixa.

Uma das principais dificuldades para se realizar estudos experimentais diretamente com agressividade em humanos está relacionada às considerações éticas de se induzir uma situação de conflito com um ou mais indivíduos. A indução de emoções como a raiva prova-se particularmente complexa, pois o uso de o Termo de Consentimento Livre e Esclarecido deve apresentar de forma precisa às condições a que o indivíduo será submetido. Uma forma de se contornar essa dificuldade é através do uso de uma "*cover story*": uma história falsa que é contada para o indivíduo no início da pesquisa e esclarecida ao final dela. No entanto, esse método é considerado muito polêmico, por envolver ocultamento de informações no Termo de Consentimento.

Estudos com agressividade em diversas espécies desempenham uma função importantíssima, não somente para a compreensão das relações sociais entre estas espécies, como também para compreender os mecanismos que controlam e regulam estes comportamentos em humanos. Em mamíferos, diversos mecanismos mostram-se profundamente conservados (Veenema, 2009) e, a partir da compreensão de seu funcionamento, é possível elaborar intervenções farmacológicas para controla-los. Muitos transtornos psiquiátricos apresentam, dentre seus sintomas, uma má regulação do comportamento agressivo, causando sua expressão disfuncional e, como decorrência disso, danos consideráveis à sociedade. Assim, a elucidação dos mecanismos envolvidos com a agressividade passa a também ser de interesse da saúde pública. Estudos futuros podem se beneficiar dos artigos apresentados nesta dissertação como base para o desenvolvimento de intervenções mais eficazes para controlar este comportamento ou de fármacos mais eficientes para inibir a sua expressão disfuncional.

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