Video Article

The Resident-intruder Paradigm: A Standardized Test for Aggression, Violence and Social Stress

Jaap M. Koolhaas[1], Caroline M. Coppens[1], Sietse F. de Boer[1], Bauke Buwalda[1], Peter Meerlo[1], Paul J.A. Timmermans[2]

[1]Department of Behavioral Physiology, Center for Behavior and Neurosciences, University Groningen

[2]Radboud University Nijmegen

Correspondence to: Jaap M. Koolhaas at j.m.koolhaas@rug.nl

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Abstract

This video publication explains in detail the experimental protocol of the resident-intruder paradigm in rats. This test is a standardized method to measure offensive aggression and defensive behavior in a semi natural setting. The most important behavioral elements performed by the resident and the intruder are demonstrated in the video and illustrated using artistic drawings. The use of the resident intruder paradigm for acute and chronic social stress experiments is explained as well. Finally, some brief tests and criteria are presented to distinguish aggression from its more violent and pathological forms.

Video Link

The video component of this article can be found at https://www.jove.com/video/4367/

Introduction

Aggressive behavior belongs to the natural behavioral repertoire of virtually all animal species. From a biological point of view, aggressive behavior can be considered as a highly functional form of social communication aimed at active control of the social environment. It is characterized by a set of species-typical behaviors performed in close interaction with the opponent. Overt aggression and physical conflicts are potentially harmful not only for the victim but for the aggressor as well. Therefore, throughout the animal kingdom, mechanisms have been developed to minimize and control physical aggression in order to prevent its potentially adverse consequences. Such mechanisms include, for example, threatening behavior that often predicts and may thereby prevent physical attacks. Other mechanisms to keep aggression in control are taboos, ritualization, submission, reconciliation and appeasement. This holds in particular for offensive aggression, which is a form of aggressive behavior characterized by initiative of the offender and a range of introductory, often threatening, behavioral displays before attempting to reach the back and neck as non-vulnerable targets for the consummatory aggressive attack bites. Despite such highly adaptive control mechanism, examples exist of functional aggression turning into violence, which can thus be defined as an injurious form of offensive aggression that is out of control and out of context; it is a pathological form of offensive behavior that is no longer subject to inhibitory control mechanisms and that has no additive functional value to normal aggressive behavior in social communication [8]. Violence thus differs both quantitatively and qualitatively from normal adaptive offensiveness. This may include bites attacks targeted at vulnerable body parts such as the throat, belly and paws are normally off limits [5, 13, 20, 24].

Defensive aggression is the form of aggressive behavior performed in response to an attack by another individual. It is distinctly different from offense in terms of its behavioral expression and inhibitory controls [5]. Note that extreme forms of defensive behavior can have violent characteristics.

Much of the preclinical aggression research is conducted in territorial male resident rats or mice confronting an intruder conspecific. This so-called resident-intruder paradigm allows the spontaneous and natural expression of both offensive aggression and defensive behavior in laboratory rodents in a semi natural laboratory setting. By recording the frequencies, durations, latencies and temporal and sequential patterns of all the observed behavioral acts and postures in the combatants during these confrontations, a detailed quantitative picture (ethogram) of offensive (resident) and defensive (intruder) aggression is obtained. For extensive descriptions of the various behaviors see [3, 12, 18]. The paradigm is based on the fact that an adult male rat will establish a territory when given sufficient living space. Territoriality is strongly enhanced in the presence of females and/or sexual experience [1]. As a consequence of territoriality, the resident will attack unfamiliar males intruding in its home cage. Hence, offensive aggression can be studied by using the resident as the experimental animal. To determine the violent nature of aggression one can assess whether the offense is out of context and inhibitory control by using different types of intruders such as females or anesthetized males or a novel environment. A detailed quantitative analysis of the offensive behavioral repertoire is required to reveal to what extent the observed aggression is out of control.

The intruders in the resident-intruder paradigm will show defensive behavior in response to the offensive attacks by the resident. The paradigm therefore also allows one to study defensive behavior and social stress by using the intruder as the experimental animal. A form of chronic social stress can be created by repeatedly using the experimental animal as intruder, or by housing it in the cage (territory) of the resident, separated by a wire mesh screen [4].

Like any kind of stress paradigm, the resident-intruder paradigm is not free from ethical concerns. We therefore want to present a number of ethical considerations. Aggression, violence and social stress are serious problems in our human society. A report of the World Health Organization shows that interpersonal violence is not only a major source of death worldwide it is also a major source of serious health problems in the surviving victims of aggression [19]. Hence, there is a need to understand these behaviors in terms of their underlying causal mechanisms and modulating factors. Animal models are essential to obtain experimental support of the causal nature of physiological and environmental factors. From a biological point of view, aggression is a natural, biologically functional form of social behavior aimed at the establishment of a territory, social dominance and defense of resources. The resident-intruder paradigm brings this natural form of behavior into a laboratory setting that allows for controlled studies of both the aggressor and the victim. An issue of ethical concern is the question to what extent the animal's welfare is compromised when exposed to this paradigm. Several studies show that engaging in offensive aggressive behaviors and winning a fight are highly rewarding and/or reinforcing [11]. From that perspective, there is no suffering in the resident. However, an aggressive interaction requires an opponent as well. Defensive behavior and submission belongs to the natural repertoire to cope with dominance. Defeat and subjugation triggers an adaptive behavioral and physiological response aimed at adopting a subordinate position in a social group. From that point of view, the initial response during the defeat will lead to a well-adapted animal that does not necessarily suffer [15]. Only repeated exposure to a dominant and social isolation after the defeat may lead to a condition that goes beyond the adaptive capacity of the animal. This makes the paradigm suitable as an animal model for stress pathology with a high ecological validity [17]. Although the stress of social defeat is mainly of a psychosocial nature, physical harm and injury may occur. In a normal (non-violent) social interaction, this physical harm is limited. Biting occurs mainly at the back and flanks of the opponent; an area of the body with a thick and tough skin [5, 6]. Biting is in fact brief nipping of the skin, leaving behind small imprints of the incisors. This type of skin damage does not require any veterinary care. However, biologically functional aggression may change to a pathological, violent form of aggression which is out of control and out of context. In these situations, more serious wounds inflicted in particular at vulnerable body regions (belly, throat and paws) may occur [14]. To be clinically relevant, experimental model systems for violent aggressive behavior need to be valid, and this development poses a central ethical dilemma of this type of aggression research, namely harm and injury. Two countervailing principles govern this research: face validity is achieved when the behavior is potentially harmful and injurious, yet, at the same time, every ethical research guideline emphasizes the reduction and avoidance of the risk to be harmed or injured. Each research question and protocol needs to probe how much harm and injury is necessary or acceptable to generate scientifically valid information that can be translated into concerns of the public health system. When research on violence is the main aim of the experiment, it is self-evident that great care should be taken of the victim in terms of wound care or even euthanasia. The presence of serious wounding at vulnerable body regions should be the humanitarian endpoint of the intruder. When social stress is the main aim of the experiment, the interaction should be stopped when the resident shows signs of violence causing serious bite wounds at vulnerable body parts. After all, the psychosocial nature of the stress paradigm should not be mixed with the stress of severe physical injury. When residents show these signs of violence they should be excluded from the experiment.

Protocol

1. The Experimental Setup

1. Use for each resident a cage with a floor space of about half a square meter. Offensive aggression is a highly active form of behavior that requires sufficient space for its full expression. The cage should be made of sanitizable material.
2. House each resident with a female for at least one week before the start of the experiments, which will facilitate the development of territoriality. At the same time this will prevent social isolation, which is known to be stressful for social animals and may lead to reduced welfare and aberrant forms of social behavior.
3. Use companion females that are sterilized by ligation of the oviducts. In this way, the female stays hormonally intact and will be regularly receptive without becoming pregnant and developing maternal aggression.

2. Procedure

1. House the resident male and the companion female together in the resident cage for at least one week prior to testing.
2. Do not clean the bedding of the cage during that initial week or prior to later testing, since territoriality is strongly based on the presence of olfactory cues. These cues are both important for the resident in establishing its own territory and for the intruder to know that it is in the home cage of the resident. Please, notice that this deviation from the general animal care taking procedures may require special permission from the authorities.
3. Remove the companion female from the residential cage one hour before the test.
4. Introduce an unfamiliar male into the home cage of the resident at the start of the test. Preferably, the intruder should be slightly smaller than the resident and should not have been used in previous interactions with the same resident.
5. Record the behavior of the resident, preferably using a light sensitive video camera.
6. A test duration of 10 min is usually sufficient for the expression of the full offensive behavioral repertoire. For the purpose of standardization one may consider to continue recording for ten minutes after the first attack.
7. After completion of the test, remove the intruder male from the cage and reunite the resident male with its companion female.
8. Although aggression may occur at all times of day, it is best to test only during the dark phase; the rats' main activity phase.
9. Testing can be performed once or twice per day. The level of aggressive behavior often increases across the first couple of tests but generally stabilizes after three to four tests.

3. Offensive Aggressive Behavior

1. In principle, any strain of rats can be used as residents. However, strains may differ considerably for their absolute level of aggressive behavior. Moreover, there may be a considerable individual variation within strains.
2. Standardize intruders as much as possible in terms of strain, age and weight. Use rats of a non-aggressive strain that are slightly lower in body weight than the resident male.
3. Determine in the resident male the duration and frequency of the following behavioral parameters:
    i. Attack latency: the time between the introduction of the intruder and the first clinch attack
    ii. Move towards
    iii. Social exploration
    iv. Ano-genital sniffing
    v. Rearing
    vi. Lateral threat
    vii. Upright posture
    viii. Clinch attack
    ix. Keep down
    x. Chase
    xi. Non-social explore
    xii. Rest or inactivity

4. Analyses
    i. The behavioral parameters recorded should cover 100% of the observation time. This facilitates an unbiased interpretation of the results, *i.e.* when one behavior goes up, another has to go down.
    ii. Data reduction can be obtained by calculating scores of different behavioral categories; particularly:
        1. Total offense score: sum of lateral threat, upright, clinch, keep down and chase
        2. Social exploration score: sum of social explore, ano-genital sniffing and social groom

    iii. Data can be expressed as percentages of the total observation time

4. Violence

1. Follow the protocol explained under section 2
2. Behavioral tests and criteria:
    i. Determine in the resident male the attack latency. A very short latency is a first indicator of violence.
    ii. Calculate for the resident male the ratio between the frequencies of lateral threat and clinch. A ratio below 1 shows that animals attack without any introductory behavior and forewarning which is a reliable indicator of violence (out of control).
    iii. Bite target sites:
        1. Bites attacks targeted at vulnerable body parts such as the throat, belly and paws are a sign of violence.
        2. Bites targeted at the snout of the opponent indicate defensive behavior of the actor.

    iv. Out of context tests:
        1. Use an unfamiliar female as intruder. Attack of a female is a sign of violence.
        2. Use an anesthetized male as intruder. Attack of an anesthetized male is a sign of violence.
        3. Test the aggressive behavior of the resident in a novel environment. The absence of a change in aggression compared to the home cage is a sign of violence. The endpoint of this test is the first attack or ten minutes when the resident does not attack.

5. Defensive Behavior

1. Select a number of well trained, highly aggressive, non-violent resident males
2. Follow the protocol as explained under section 2
3. Determine in the intruder the duration and frequency of the following behavioral parameters (in addition to 3c):
    i. Submission latency
    ii. Submissive posture
    iii. Move away
    iv. Flight
    v. Defensive upright posture
    vi. Freeze
    vii. Non-social explore
    viii. Rearing

4. Analysis
    i. Data reduction can be obtained by calculating a defense score, which is the sum of the amount of time spent on flight, defensive upright posture, submission and freeze

6. Social Stress

1. Follow the protocol as explained under section 5
2. For intermittent social stress, repeat the protocol
3. For chronic social stress, keep the intruder in the resident's cage, but separated from the resident by a wire mesh screen
4. Continue step b and/or c for as long as the scientific questions and hypotheses require.

Representative Results

There is a considerable variation between strains and within strains in the level of offensive aggressive behavior. This is demonstrated in **Figure 1** which shows the frequency distribution of the offensive aggression score in a laboratory bred but originally feral strain of rats (Wild Type Groningen strain (WTG)) (**Figure 1a**) and a more common strain of laboratory rats (Wistar, **Figure 1b**). In the WTG strain, about one third of the animals is extremely aggressive whereas another third is not or very low aggressive. This is in contrast to the frequency distribution of a Wistar strain in which the highly aggressive phenotype is absent and about fifty percent of the animals can be considered as low or non-aggressive [16].

Figure 2 shows the distribution of different behavioral categories in the resident-intruder paradigm with the WTG strain as residents (**Figure 2a**) and the Wistar rats as intruders (**Figure 2b**). Shown is the average composition of offensive behavior in the WTG resident rat and the average composition of defensive behavior in the Wistar intruder in terms of the relative amount of time spent on the various behaviors.

Figure 3 shows an example of the use of the resident-intruder paradigm in behavioral pharmacology. The selective serotonin 1a receptor agonist Alnespirone induces a dose dependent reduction in offensive aggression, which is accompanied by a dose dependent increase in social exploration. The absence of any significant effects on non-social exploration and inactivity supports the view that the behavioral effects of this compound are specific for offensive aggression [9].

In some individuals offensive aggression may escalate into a violent form of aggression. The distinction between high levels of aggression and violence is illustrated in **Figure 4**. Despite the fact that there is no statistical difference in offense score, the violent form of aggression is characterized by a very short attack latency, attack of an anesthetized male or a female, serious wounding and a very low threat attack ratio [8].

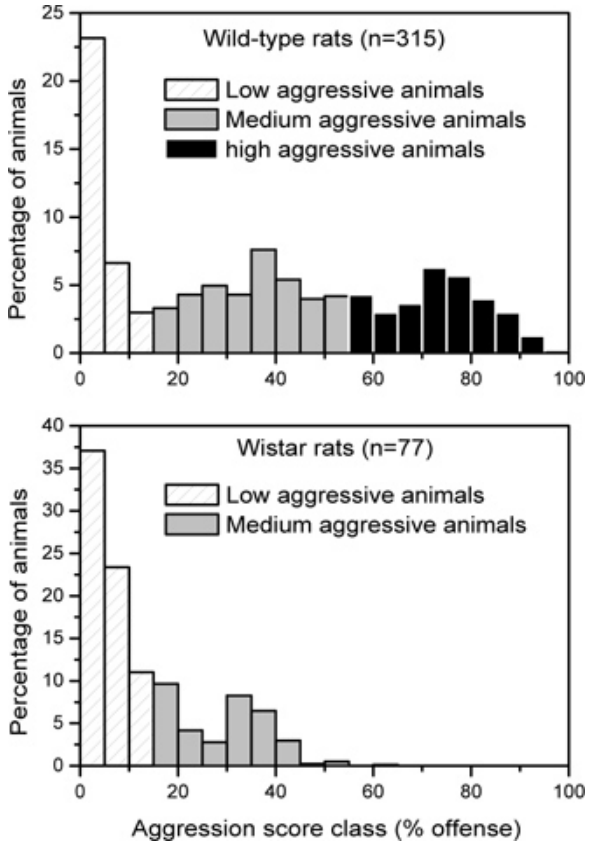

Figure 1. Frequency distribution of individual levels of offensive aggression in a laboratory bred, but originally feral rat strain (A - Top panel) and in a Wistar strain of male rats (B - Bottom panel) [16].

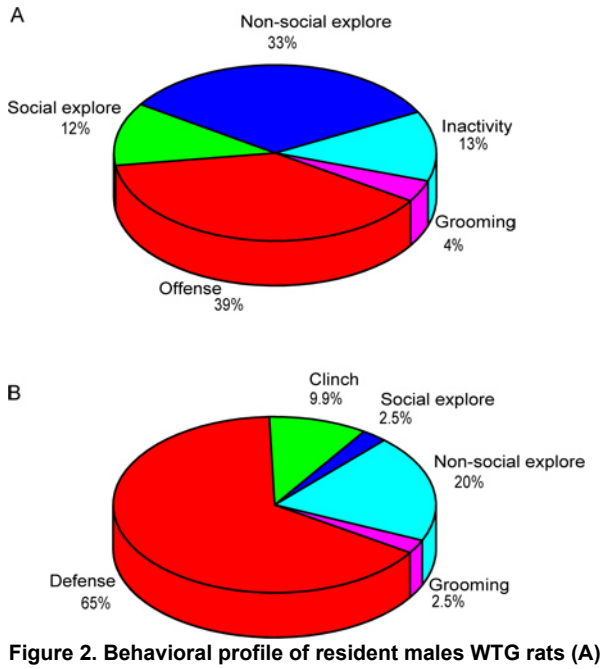

Figure 2. Behavioral profile of resident males WTG rats (A) and Wistar intruder rats (B) during a ten minutes resident intruder test.

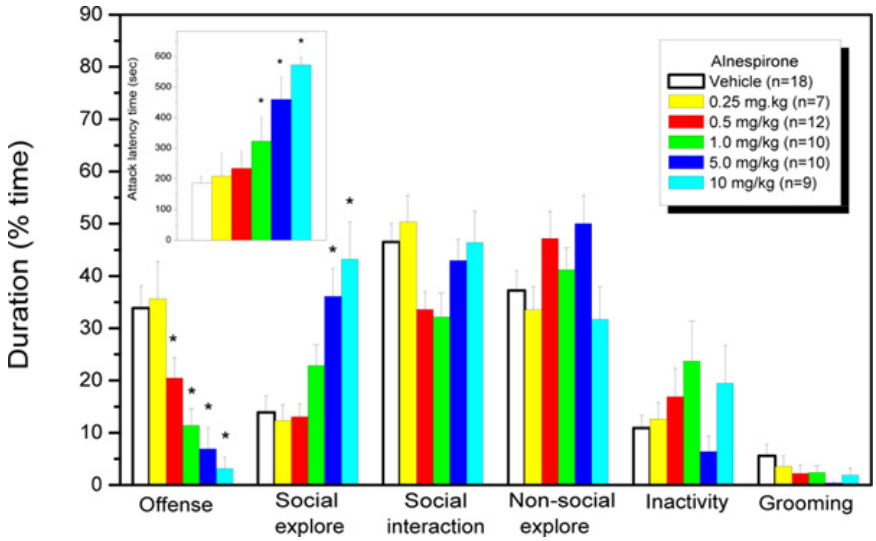

Figure 3. Dose dependent reduction of offensive aggression by the 5-HT1a agonist Alnespirone [9]. Click here to view larger figure.

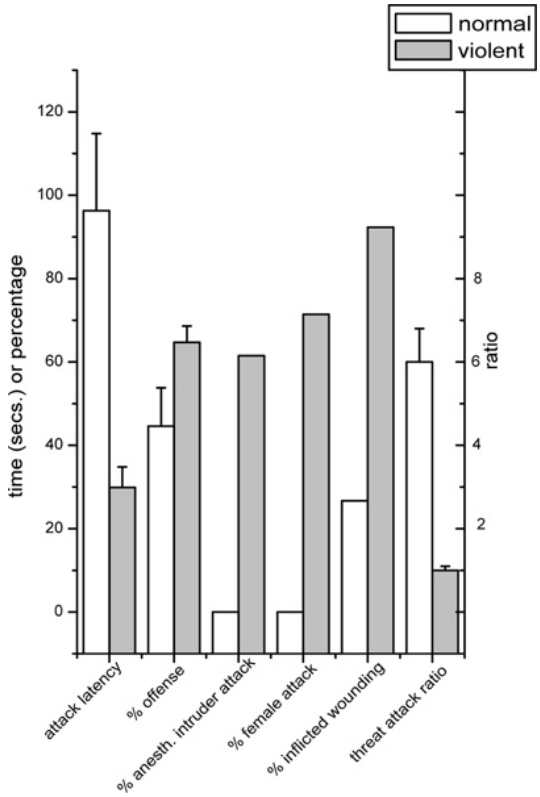

Figure 4. Comparison between highly offensive males and violent males [8].

Discussion

The resident-intruder paradigm can be used to study offensive aggression, defensive behavior, violence and social stress in rats and, with some small modifications for other rodent species as well. When studying aggression, principally all rat strains can be used. However, strains are not equally suitable. Depending on the exact purpose of the experiment, some specific characteristics of the animals should be considered. It is important to notice that there are large strain differences in the level and intensity of offensive aggression shown as a resident. **Figure 1** shows the frequency distribution of offensive aggression in an originally feral rat strain (**Figure 1a**) and in a standard laboratory Wistar strain (**Figure 1b**). The two strains differ considerably in the number of animals that will show aggressive behavior at all. Moreover, there is a large difference in the absolute scale of offense. The feral rat strain ranges from zero up to 80% offense in our standard 10 min test whereas the Wistar strain has a maximum of 25% offense; the highly aggressive phenotype is absent in this latter strain [16].

When the resident-intruder paradigm is used to study defensive behavior and social stress in the intruder, one needs these highly aggressive phenotypes as residents. After all, the resident has to reliably defeat any intruder entering his territory. Of course, the most aggressive individuals should be chosen and one should realize that even in a highly aggressive strain, not all individuals will be suitable for this purpose. True adult males of at least four months of age [10] should be used and one may consider using former breeder males. It is recommended to give the resident males some further experience with intruders during the days before the actual start of the social stress experiment. Any strain can be used as intruder. However, to guarantee clear winning by the resident and defeat of the intruder, we advise to use intruders with a slightly lower body weight than the resident male. Because olfactory cues are important in social communication and territoriality, cleaning the cage of the resident prior to testing will be a serious confounder.

It is recommended to videotape and record the full behavioral repertoire of the experimental animal during the test. This allows an unbiased analysis of the results, *i.e.* when one behavior goes up, another is likely to go down. For example, the results depicted in **Figure 3** shows that a reduction in offense following drug treatment is accompanied by an increase in social exploration and not by immobility. This provides evidence that the drug induced reduction in offense is not due to some kind of a sedative or motor inactivity effect. The total offense score is an index of the intensity of aggression and the latency of the first attack and number of attacking animals can be used as a measure of the readiness to attack [22].

For social stress experiments one should have a clear criterion for social defeat. When the intruder adopts a submissive posture (see above) and stays in this posture even when the resident moves away, this is a reliable criterion for social defeat. Notice that housing conditions of the intruders are extremely important in social stress research. First, the social stress should not be administered in the same room where the non-stressed controls are housed. Control animals witnessing (social) stress in other individuals may experience major stress themselves [7, 21]. Second, the consequences of social stress are sensitive to social buffering effects. Group housed, socially stressed animals do not show the same long-term consequences seen in socially stressed animals that are socially isolated afterwards [23].

Taken together, the resident intruder paradigm allows research on both the causes and the consequences of aggressive behavior. It is a model with a high face and construct validity that covers not only the adaptive biology of social behavior, but can be used to study maladaptive aspects as well in terms of violence and social stress pathology.

Disclosures

The authors declare that they have no competing financial interests.

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