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Review article

# Aggressive behavior in transgenic animal models: A systematic review

Amanda Jager[a,*,1], Dorien A. Maas[a,b,1], Kim Fricke[a], Rob B. de Vries[c], Geert Poelmans[b,d,1], Jeffrey C. Glennon[a,1]

[a] Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands
[b] Department of Molecular Animal Physiology, Donders Institute for Brain, Cognition and Behaviour, Radboud Institute for Molecular Life Sciences (RIMLS), Radboud University, Nijmegen, The Netherlands
[c] SYRCLE at Central Animal Laboratory, Radboud University Nijmegen Medical Center, The Netherlands
[d] Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

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ABSTRACT

Aggressive behavior is often core or comorbid to psychiatric and neurodegenerative disorders. Transgenic animal models are commonly used to study the neurobiological mechanisms underlying aggressive phenotypes and have led to new insights into aggression. This systematic review critically evaluates the available literature on transgenic animal models tested for aggression with the resident-intruder test. By combining the available literature on this topic, we sought to highlight effective methods for laboratory aggression testing and provide recommendations for study design as well as aggression induction and measurement in rodents that are translational to humans, taking into consideration possible confounding factors. In addition, we built a molecular landscape of interactions between the proteins encoded by the aggression-linked genes from our systematic search. Some molecular pathways within this landscape overlap with psychiatric and neurodegenerative disorders and the landscapes point towards a number of putative (drug) targets for aggression that need to be validated in future studies.

## 1. Introduction

### 1.1. Natural aspects of aggression

Aggression is a common inherent natural phenomenon that is observed across species with a high translational value. Human aggression is similar to aggression in other mammalian species such as mice and rats, such as the basis for aggressive acts like biting and scratching and many types of aggression. Often, aggression is referred to as a negative act but aggressive acts can contribute to the survival of individuals and groups. Within groups, aggression is a form of social interaction that is necessary for group survival and dynamics. For example, a hierarchy in which the strongest animal is the leader can only be established through competition between animals of the group (Shimoji et al., 2014; Wong and Balshine, 2011). In humans, this is reflected in the fact that physical size is positively related to the number of aggressive interactions (Archer, 2009; Pellegrini et al., 2007). Hence, distinct types of aggression can be defined in animals: play fighting, offensive aggression, defensive aggression, maternal aggression and predatory aggression (Blanchard et al., 2003). Whereas offensive, defensive and maternal aggression share many characteristics, play fighting and predatory aggression seem distinct. The frequency of play fighting is not predictive for the state of dominance in adult animals. Adult dominant animals display less play fighting compared to subordinate animals (Blanchard et al., 2003). Predatory aggression is distinct in that drug treatments that reduce the other forms of aggression often have no effect on predation (Blanchard et al., 2003). This systematic literature review focuses on aggression in rodents and in the next section, different forms of aggression will be discussed.

Aggression and aggressive acts differ between rodents. Therefore, in laboratory science it is important to take into account how aggression occurs in the natural environment of the species. Wild rats live in groups in burrows in the ground. Most often, a group consists of one dominant male, a couple of females and their offspring. Males are only allowed in the group if they have not reached adulthood and adult intruder rats are excluded (de Boer et al., 2017; Koolhaas et al., 1980). A colony is constituted of multiple of these territories and neutral areas where groups avoid each other and only minimal fighting occurs. Competitive fighting may occur between familiar animals within the colony and is often motivated by hunger, thirst or frustration of ongoing

* Corresponding author at: Kapittelweg 29, 6525 EN Nijmegen, The Netherlands.
*E-mail address:* Amanda.Jager@radboudumc.nl (A. Jager).
[1] Equal contribution.

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activity and appears more in female than in male rats (Adams 2006). Stability within a colony is established by suppressing aggressive conflicts among group members. However, animals have shown to become more tolerant toward each other when population densities increase (de Boer et al., 2017). Aggressive behavior observed in rats is highly adaptive and dependent on subtle factors such as time of the day, the presence of food or recognition of the individual. Lactating females may show aggressive behavior towards intruders but this decreases when no pups are present. In contrast, wild mice live in solitary. Male mice have a large territory compared to female mice, and sometimes the female territory is part of the male territory. Normally, a male mouse will behave in a sociable way to a female that comes into this territory, but male intruders will be attacked and driven away (Bronson, 1979).

As described by de Boer et al. (2017) rats and mice kept under laboratory conditions are highly adaptive in their social behavior and hence, differences exist between aggressive behavior of wild and laboratory animals. Rats in the wild as well as in the laboratory show a range of attack and defensive behaviors and both react defensively when attacked (de Boer et al., 2017). However, wild rats show defensive behavior prior to being attacked and are, as a consequence, less successfully assaulted by the attacker (Adams, 2006; Blanchard and Blanchard, 1977; Blanchard et al., 1986; 2003). Importantly, comparing laboratory Wistar rats to the wild Groninger rat demonstrated that highly aggressive phenotypes are not present in the Wistar population (de Boer et al., 2003). In Norway rats, it was demonstrated that domestication of wild rats leads to higher levels of serotonin (5-HT) in the brain with a decrease of defensive, but not of predatory aggression (Nikulina, 1991).

In contrast to their wild, solitary behavior, laboratory mice are often housed in groups of males which results in less respectively more aggression in certain mouse strains (Smith et al., 2005; Van Loo et al., 2001, 2003). The latter is e.g. the case for CD1 mice, as they will stay aggressive even when they are group housed (Miczek et al., 2001). Further, housing males with females is shown to increase aggression levels (Flannelly et al., 1982). When comparing wild mice with Swiss mice it has been shown that while patterns of defensive behavior are comparable, freezing and flight behavior are decreased in Swiss mice (Blanchard et al., 1998). When exposed to the anti-aggressive drug fluprazine, both wild and Swiss mice show decreases of aggressive behavior in the same domains (Ferrari et al., 1996).

Even though there are substantial differences in the aggressive behavior of wild and laboratory rats, and less so in mice, studying aggression in a laboratory setting is a valuable tool for deducing the neurobiology of aggression. It is very interesting to study why various strains of rats and mice have different aggression levels under defined conditions. This could provide insight into the neurobiological mechanisms and genetic background of aggression as a trait.

### 1.2. Inducement of aggression in laboratory animals: resident-intruder test

Most aggression research is performed in rats, hamsters and mice and utilizes the aggressive interaction between two animals. Examining more than two animals in an aggressive reaction is often avoided, as an increase in number can influence the aggressive behavior (Flannelly and Flannelly, 1987). Various paradigms are used to induce and measure aggression in a laboratory setting. Here, we shortly describe the most common aggression tests that involve two animals in an interaction.

Pain-elicited aggression is a method that induces aggression by exposing animals to painful stimuli (e.g. an electric shock). This method induces behavioral patterns observed in defensive rather than offensive aggression (Blanchard and Blanchard, 1977). Offensive aggression can be measured in food-motivated competition or tube fighting which was introduced in 1961 by Lindzey (Lindzey et al., 1961). Here, food is placed at the opposite ends of a single runway tube, the test animals meet in the middle and the 'dominant' animal pushes the 'subordinate' animal away. However, it was found that 'winning' a tube fight is poorly related to victories in other dominance or attack situations and cannot be used as a general measure of dominance or offensive behavior (Blanchard and Blanchard, 1977; Miczek and Barry, 1975). However, some more recent studies showed that animals that won in the social dominance test were also the most aggressive in the resident-intruder test (Shin et al., 2016; Yang et al., 2015). Isolation-induced aggression is another method that has shown to increase general aggressiveness in mice (Grant and Mackintosh, 1963; Valzelli and Bernasconi, 1979). Although in rats, there was a general tendency for greater aggression, it appears that aggression is milder and it does not lead to tissue damage (Grant, 1963). Studies into predatory aggression are, due to ethical considerations, not common practice anymore. Mouse killing by rats, or muricide, has often been studied in the past but the close phylogenetic link between mice and rats makes muricide behavior a mixture between predation and offensive aggression (Blanchard and Blanchard, 1977).

The last category of aggression is territorial aggression. This type of aggression is most often tested with the resident-intruder test, which was first proposed by Krsiak (1975), and has been extensively described in rats and mice (de Boer et al., 2003; Koolhaas et al., 2013; Koolhaas et al., 1980; Krsiak, 1975). In the resident-intruder test, the resident is provided with an arena in which to establish its own territory. To determine the level of aggressiveness of the resident, an intruder will be introduced into the territory of the resident and the animals are allowed to interact for a certain period of time (Koolhaas et al., 2013). The usual pattern of territorial aggression, or offensive aggression, is that the resident moves towards the intruder and secures a specific area for the intruder (Adams, 2006; Blanchard and Blanchard, 1977). The resident will aim for a homeostatic environment and successful re-establishment of the homeostasis will enhance aggressive behavior (Koolhaas et al., 1980). As a reaction, the intruder shows typical defensive aggressive behavior, with the exception of lactating females that show a combination of offensive and defensive behavior, depending on the gender of the opponent (Adams, 2006; Parmigiani et al., 1998). While offensive aggression involves an adaptive response to a change in the environment, defensive aggression can be assumed a protective mechanism of the bodily integrity. Typical defensive attacks are targeted towards the snout of the opponent, whereas offensive attacks are more targeted towards the back and flanks. The proportion of the bites targeted towards the back of the intruder increases when the interaction area is expanding due to increased chasing behavior (Blanchard et al., 2003). On other occasions, the resident will manipulate the position of the intruder so that the resident can attack the intruder, or the resident will target the closest part of the intruder. Studies with anaesthetized opponents indicate that specific target areas for offensive aggression exist independently of the reachability of the particular area (Blanchard et al., 2003). However, when the defender is unconscious or dead, the snout becomes an additional target of an offensive attack (Blanchard et al., 2003). Attacking the snout can also be considered as an attack driven by high levels of motivation and is observed in escalatory forms of aggression (de Almeida et al., 2005; Kruk, 1991; Miczek et al., 2001).

There are differences in the behavior of rats *versus* mice in the resident-intruder test. For example, it is more common that the resident keeps down the intruder. This is less often seen in mice since they tend to attack the ventrum, and keeping an opponent down enables attacking of the ventrum (Blanchard et al., 2003). Differences between mouse strains and amongst rat strains have also been found and, depending on variations in the resident intruder paradigm, even differences between mice and rats from the same strains have been identified. In this systematic review, we will describe these differences between and within strains of rats and mice extensively.

### 1.3. The translational value of the resident-intruder test

Aggression can be found in psychiatric disorders and clinical