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# Constitutive differences in glucocorticoid responsiveness to stress are related to variation in aggression and anxiety-related behaviors

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ABSTRACT

Glucocorticoids coordinate responses that enable an individual to cope with stressful challenges and, additionally, mediate adaptation following cessation of a stressor. There are important individual differences in the magnitude of glucocorticoid responsiveness to stressors. However, whether individual differences in glucocorticoid responsiveness to stress are linked to different behavioral strategies in coping with social and non-social challenges is not easily studied, owing to the lack of appropriate animal models. To address this, we generated three lines of Wistar rats selectively bred for the magnitude of their glucocorticoid responses following exposure to a variety of stressors over three consecutive days at juvenility. Here, we present findings following observations of a high level of variation in glucocorticoid responsiveness to stress in outbred Wistar rats, and the strong response to selection for this trait over a few generations. When challenged with different stressful challenges, rats from the three lines differed in their coping behaviors. Strikingly, the line with high glucocorticoid responsiveness to stress displayed enhanced aggression and anxiety-like behaviors. In addition, these rats also showed alterations in the expression of genes within both central and peripheral nodes of the hypothalamic-pituitary-adrenal (HPA) axis and enhanced reactivity to acute stress exposure. Together, these findings strongly link differences in glucocorticoid responsiveness to stress with marked differences in coping styles. The developed rat lines are thus a promising model with which to examine the relationship between variation in reactivity of the HPA axis and stress-related pathophysiology and could be employed to assess the therapeutic potential of treatments modulating stress habituation to ameliorate psychopathology.

## 1. Introduction

Glucocorticoids, the final products of the hypothalamus-pituitary-adrenal (HPA) axis, coordinate metabolic, behavioral and physiological responses that enable an individual to cope with stressful challenges. Glucocorticoids (primarily cortisol in humans; corticosterone in most rodents) are released from the adrenal glands into the circulation (Ulrich-Lai and Herman, 2009) and exert a multitude of effects, including the modulation of brain function and cognition, via their actions at mineralocorticoid (MR) and glucocorticoid (GR) receptors (de Kloet et al., 2008). Additionally, activation of GR in several brain areas acts to inhibit ongoing stress responses (Tasker and Herman, 2011), restore homeostasis and mediate adaptation, a process that has been termed 'allostasis' (McEwen, 1998). However, repeated, prolonged or inadequate stress responses may lead to physiological damage, termed 'allostatic load', thought to be the pathological basis of many stress-related disorders (McEwen, 2007).

There is substantial individual variability in the regulation of glucocorticoid responding to repeated exposure to stressful challenges (Federenko et al., 2004; Gerra et al., 2001; Pruessner et al., 1997; Kirschbaum et al., 1995; Wust et al., 2005; Foley and Kirschbaum, 2010) which has been associated with different vulnerability to develop psychopathologies (Kirschbaum et al., 1995; de Kloet et al., 2005; Kudielka et al., 2006; Pruessner et al., 1997). However, the possibility that individual variation in glucocorticoid responsiveness could be related to diverse behavioral strategies in coping with stressful challenges, regardless of lifetime exposure to stress, has been much less explored. If hormonal and behavioral responsiveness to stressors are intertwined, differences in coping styles might need to be integrated in the potential link between stress responsiveness and vulnerability to psychopathology.

Selective breeding has often proven a robust approach to obtain models useful for delineating the biological basis of stress-relevant behavioral traits (Bignami, 1965; Liebsch et al., 1998; Naumenko et al., 1989). Selection for differences in HPA axis activity has been successfully implemented across taxa (Pottinger and Carrick (1999) Satterlee

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and Marin, 2006; Touma et al., 2008) but to our knowledge, so far, not in rats. Here, we performed a selective breeding program with the aim to generate lines of rats enriched for either high or low corticosterone responsiveness to stressors, as well as those showing intermediate responses. Juvenility was selected as the period to focus our selection, as we aimed to investigate the impact of differential corticosterone responsiveness throughout adolescence on adult behavioral responses to social and non-social challenges. Specifically, the selection took into account individual differences in corticosterone responsiveness on a third day of exposure to diverse stressors taking place from post-natal days (p)28-30 (Tzanoulinou et al., 2014b). This stress procedure is the first part of a peripubertal stress protocol developed in our lab that leads to increased aggression and enhanced anxiety-related behaviors and passive coping responses at adulthood (Cordero et al., 2012; Marquez et al., 2013). The rationale for our selection criteria was based on the hypothesis that individual differences in behavioral responses at adulthood might be related to differences in corticosterone responsiveness to repeated exposure to stressors. This hypothesis was supported by evidence that enhancing corticosterone levels during the same peripubertal period leads to subsequent increases in aggressive behaviors (Veenit et al., 2013). Following the observation of high inter-individual variability in corticosterone response to stressors across the juvenility period in outbred Wistar rats, our data shows a strong response to selection for extremes in this trait. We additionally present a behavioral and endocrinological characterization of rats drawn from these selection lines.

## 2. Materials & methods

### 2.1. Animals

#### 2.1.1. Selective breeding procedure

Wistar Han rats were obtained from a commercial breeder (Charles River, France: 30 male and 30 female; parental generation; PG) and bred in our animal facility. The entire offspring of these pairings (F0) was subject to a 'stress adaptation test' (SAT). The SAT is a truncated version of the peripubertal stress protocol developed in our laboratory which, though evidently stressful, appears insufficient in begetting the range and magnitude of behavioral alterations associated with exposure to a longer protocol (Toledo-Rodriguez and Sandi, 2007; Tzanoulinou et al., 2014b). Specifically, the SAT involves consecutive exposures across p28-p30 to fear-induction procedures, in accordance with the protocol used by Tzanoulinou and colleagues [2014b]. On p28, rats were exposed to an open field (5 min) and then to an elevated platform (EP; 25 min). On p29, they were presented in a novel cage with the synthetic fox odor trimethylthiazoline (TMT; 25 min) followed by exposure to the EP (25 min). On p30, the presentation order of the p29 stressors was reversed. Tail blood samples were obtained following exposure to the stressors on p28 and p30.

Rats with extremely low (100 ng/ml being the upper limit) or extremely high (200 ng/ml being the lower limit) secretion of corticosterone on the last day of the SAT were selected for the 'low' and the 'high' breeding lines, respectively. A third breeding line, 'inter', was established consisting of animals with intermediate corticosterone values in the SAT ( $>$ 100 ng/ml and $<$ 200 ng/ml).

In each generation, 10 males and 10 females from each breeding line were selected as founder pairs to generate the following generation. Their offspring (e.g. F1 for F0) and animals from each subsequent generation were also exposed to the SAT and again selected according to their corticosterone response to the final stress session (p30). Selection was strictly within line, i.e. an animal from the low-line could only ever be selected to breed within the low-line. To minimize effects of genetic drift, the breeding scheme strictly excluded sibling mating. Moreover, to balance the potential contribution of each litter to the next generation, litter size was reduced to a maximum of 12 pups at p2. Care was taken to ensure as much variability in pairings as possible; for example, if two animals from the same litter went forward to breed the next generation then they were not paired with animals coming from the same alternate litter.

#### 2.1.2. Experimental subjects

Experimental subjects were male offspring taken from the first breeding of pairs from the lines in the specific generation under study, but not exposed to a stress procedure themselves. Female Wistar Han rats (used as cohabiting partners in the resident-intruder test) and male Wistar Han rats (used as intruders in the resident-intruder test) were purchased from a commercial breeder (Charles River, France). Experimental cohorts for this study were obtained as follows: from F4 for initial behavioral experiments (n = 12/line); from F6 for behavior replication (low- and high-line, n = 12/line; intermediate-line, n = 8); and from F8 for endocrine experiments (n = 12/line).

At weaning on p21, pairs of male rats from different litters were matched by weight and mixed among home cages. Rats were maintained on a 12 h light–dark cycle (lights on at 0700 h), in a temperature- and humidity-controlled environment (21 ± 1 °C; 55% humidity ± 5%), with *ad libitum* access to laboratory chow and water. They remained undisturbed, except for weekly cage changes, until experimental procedures began at adulthood (designated as p90). Experiments were performed between 0800 and 1200 h, the circadian trough in corticosterone production, except where otherwise stated. All procedures were conducted in accordance with the Swiss National Institutional Guidelines on Animal Experimentation and approved by a license from the Swiss Cantonal Veterinary Office Committee for Animal Experimentation.

### 2.2. Assessment of behavioral profile

#### 2.2.1. Elevated plus maze

Anxiety-like behavior was evaluated using a five-minute exposure to the elevated plus maze test, performed according to methods described by Tzanoulinou and colleagues [2014a]. The time spent in the open and closed arms, and distance moved, were automatically recorded (Ethovision, Noldus IT, Netherlands). Two animals (one intermediate- and one high-line rat) were removed from the analysis of the F6 experiment because they fell from the maze during testing.

#### 2.2.2. Resident-intruder test

Experimental rats cohabited with a female partner for 10 days to encourage territoriality. The female was removed 30 min before the onset of the test (between 1900 and 2200 h), and replaced afterwards. During the test, the male resident was exposed in its home cage to a slightly smaller (5–10% lighter), unfamiliar male intruder of the same strain for 30 min. Each intruder was used only once. Encounters were video-recorded and scored offline by an experimenter blind to the experimental group, assisted by Observer software (Noldus IT, Netherlands). The following parameters were quantified in frequency and duration: attack, offensive upright, lateral threat, keeping down, biting, social investigation, non-social investigation and auto-grooming. The cumulative duration of the first four behaviors were summed to provide a measure of total offensive behavior. Latency to first offensive act initiated by the resident was also recorded.

#### 2.2.3. Forced swim test

Coping responses to inescapable adversity were evaluated with a forced swim test. Animals were placed in a plastic beaker (25 cm diameter x 46 cm) containing 30 cm of water (25 °C) for 15 min. A second, 5 min session was performed 24 h later. Both sessions were recorded using a ceiling mounted video camera, and the time spent immobile, swimming or climbing was quantified manually with the aid of in-house software (Clicker; EPFL, Switzerland) by an experimenter blind to the animals' breeding line. One rat from the intermediate-line was removed from the analysis because of a technical problem with the video