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Respiratory patterns reflect different levels of aggressiveness and emotionality in Wild-type Groningen rats☆

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ABSTRACT

Respiratory patterns represent a promising physiological index for assessing emotional states in preclinical studies. Since disturbed emotional regulation may lead to forms of excessive aggressiveness, in this study we investigated the hypothesis that rats that differ largely in their level of aggressive behavior display matching alterations in respiration. Respiration was recorded in male high-aggressive (HA, $n = 8$) and non-aggressive (NA, $n = 8$) Wild-type Groningen rats using whole-body plethysmography. Subsequently, anxiety-related behaviors were evaluated in the elevated plus maze and social avoidance–approach tests. During respiratory testing, HA rats showed elevated basal respiratory rate, reduced sniffing, exaggerated tachypnoeic response to an acoustic stimulus and a larger incidence of sighs. In addition, HA rats spent less time in the open arms of the plus maze and displayed higher levels of social avoidance behavior compared to NA rats. These findings indicate that HA rats are characterized by alterations in respiratory functioning and behavior that are overall indicative of an anxiety-like phenotype.

1. Introduction

The centrality of breathing at the interface between emotional/personality characteristics and the human physiology has been increasingly recognized in the last two decades in the field of psychophysiology (Boiten et al., 1994; Timmons and Ley, 1994; Wilhelm et al., 2001a). Mounting evidence suggests that interactions between emotional states, alterations in respiratory function and physiological changes at the level of chemical blood composition and autonomic nervous system regulation play an important role in the development of a variety of medical conditions, including hyperventilation syndrome (Folgering, 1999), panic disorder (Caldirola et al., 2004; Niccolai et al., 2009; Wilhelm et al., 2001c), chronic pain syndrome (Wilhelm et al., 2001a) and cardiovascular disorders (Anderson et al., 1996; Floras, 2014; Oldenburg and Horstkotte, 2010).

Respiratory research in animals can potentially contribute to a better understanding of the pathophysiology and neural pathways that relate breathing changes to emotional states. Using whole-body plethysmography, it has been recently demonstrated that rats with high levels of baseline anxiety show specific patterns of respiration (such as elevated basal respiratory rate, exaggerated respiratory responsiveness to repetitive stressful stimuli and frequent sighing) (Carnevali et al., 2013a) that resemble those that are commonly described in individuals affected by anxiety disorders (Abelson et al., 2001; Papp et al., 1997; Schwartz et al., 1996; Wilhelm et al., 2001c). Other studies have documented that respiratory parameters in rats (especially the respiratory rate) are strongly affected by conditioned and unconditioned aversive stimuli and by novelty (Frysztak and Neafsey, 1991; Hegoburu et al., 2011). Furthermore, a series of investigations in rats have demonstrated that neonatal maternal separation brings about long-term alterations in the respiratory system (such as altered responses to hypoxia (Genest et al., 2004) and hypercapnia (Genest et al., 2007b)) that are mediated by alterations in the chemoreflex circuitry in the lower brainstem (Kinkead et al., 2008) and descending influences from the hypothalamus (Genest et al., 2007a). Collectively, these findings indicate that respiratory parameters represent a promising physiological marker of different levels of emotionality in rats.

The present study was designed to characterize the respiratory patterns in two groups of male Wild-type Groningen rats (*Rattus*

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norvegicus) that differed largely in their level of aggressive behavior. In male rodents, aggressive behaviors are elicited in response to unavoidable contact with a proximal threatening stimulus (e.g., the presence of a male conspecific intruder in their own cage). In this regard, rats of the Wild-type Groningen strain show a wide and consistent individual variation in the levels of aggressive behavior, ranging from individuals that readily attack the intruder opponent (i.e., high-aggressive rats) to individuals that show no overt aggression at all (i.e., non-aggressive rats) (de Boer et al., 2003; Carnevali et al., 2013b). It has been hypothesized that high levels of aggressive behaviors in rodents may reflect a higher state of acute emotional response to sources of threat, and may develop as a consequence of abnormalities in the complex emotion regulation circuitry of the brain, which includes cortical, amygdaloid, hypothalamic and septo-hippocampal regions (Davidson et al., 2000; Nehrenberg et al., 2009; Neumann et al., 2010).

Therefore, by means of whole-body plethysmography, in this study we tested the hypothesis that high levels of aggressive behavior in rats are associated with alterations in respiration. In addition, based on previous investigations documenting a link between aggression and anxiety in rodents (Nehrenberg et al., 2009; Neumann et al., 2010), we examined whether high-aggressive rats displayed anxiety-related behaviors in the elevated plus maze and social avoidance–approach tests.

2. Methods

2.1. Ethics statement and animals

Experimental procedures and protocols were approved by the Veterinarian Animal Care and Use Committee of Parma University, with animals cared for in accordance with the European Community Council Directives of 22 September 2010 (2010/63/UE).

In this study we used 4-month-old male Wild-type Groningen rats (*R. norvegicus*) weighing approximately 350 g. This rat population, originally derived from the University of Groningen (The Netherlands), is currently bred in our laboratory under conventional conditions, at ambient temperature of $22 \pm 2\,^{\circ}C$ and on a reversed 12:12 light–dark cycle (light on at 19:00 h), with food and water available *ad libitum*.

2.2. Preliminary behavioral testing for aggressiveness

Seventy Wild-type rats were assessed for the display of aggressive behavior toward male unfamiliar conspecific intruders using a standard resident-intruder aggression test (Koolhaas et al., 2013). Ten days before the test, each rat was housed with a conspecific oviduct-ligated female partner to stimulate territorial behavior (Koolhaas et al., 1980; Lore and Flannelly, 1977). Fifteen min before the start of the test, the female partner was removed and an unfamiliar male Wistar rat was introduced into the home cage of the experimental rat. The intruder Wistar rats weighed on average 250 g (3 months old) and were socially housed. The test was repeated on three consecutive days, using a different intruder every time, in order to avoid familiarity between the opponents and obtain a reliable characterization of aggressive traits (de Boer et al., 2003). All tests lasted 10 min and the latency to the first attack toward the intruder (in s) was measured. The attack latency (average of 3 tests) was used as an index of individual aggressive behavior (Carnevali et al., 2013b). As commonly seen in this rat strain (de Boer et al., 2003; Carnevali et al., 2013b), individual male resident rats differed widely in their level of aggression toward unfamiliar intruder males. The eight most aggressive rats (average attack latency = 96 ± 7 s; average number of attacks = 7.2 ± 0.4) were selected and classified as high-aggressive (HA) rats. Eight rats did not attack the intruder during the 600-s confrontations and were selected and classified as non-aggressive (NA) rats. HA and NA rats were then used for the following experimental procedures.

2.3. Experimental protocol

All experiments were carried out under red light during the dark phase of the light/dark cycle to permit video recordings of animals' behavior.

2.3.1. Whole-body plethysmography

On day 1, HA and NA rats were placed into a custom-built whole-body plethysmograph, which consisted of a sealed Perspex cylinder (i.d. 9.5 cm, length 26 cm, volume 2.5 l) with medical air constantly flushed through it at a flow rate of 2.5 l/min (Kabir et al., 2010; Carnevali et al., 2013a). The output flow was divided into two lines using a T-connector. One line was attached to a differential pressure amplifier (model 24PCO1SMT, Honeywell Sensing and Control, Golden Valley, MN, USA), while the other line was open to the room air. Rats' behavior during the test was recorded using a video camera positioned close to the plethysmograph. For semi-quantitative assessment of animals' motor activity, a piezoelectric pulse transducer was placed under the plethysmograph. Rats were left undisturbed in the plethysmograph for 40 min. Subsequently, our purpose was to determine the respiratory arousal responses to two natural stressful stimuli. First, a predator (hawk) call was played back for 50 s (starting from min 40). Second, a piece of rat feces was placed in a syringe and the air with the rat odor was quickly injected into the input line (through which the plethysmographic chamber was constantly flushed with medical air) (min 50). These two sensory stimuli were presented when animals were in a quiet but awake state (i.e., no motor activity, eyes opened, slow regular breathing), and were chosen because they represent non-intrusive natural stressors (threat of predation and presence of an unknown conspecific). Finally, we aimed at determining the respiratory responses to a prolonged stressor (restraint). For this, animals were removed from the plethysmograph (min 60) and introduced into a restrainer (wire-mesh tube; inner diameter: 6 cm, length: 18 cm), which was immediately placed back into the plethysmograph for 15 min. Such stressor was selected because it has been shown to provoke prominent autonomic and neuroendocrine responses in the Wild-type Groningen rat strain used in the present study (Sgoifo et al., 1997; Carnevali et al., 2013b), and marked respiratory responses in other rat strains (Carnevali et al., 2013a; Bondarenko et al., 2014).

2.3.2. Elevated plus maze test

On day 4, HA and NA rats were tested on the elevated plus maze. The elevated plus-maze test, validated for measuring anxiety (Pellow et al., 1985), is based on creating a conflict between the rat's exploratory drive and its innate fear of open and exposed areas. The plus maze consisted of 4 elevated arms (100 cm above the floor, 50 cm long and 10 cm wide) arranged in a cross-like position, with two opposite arms being enclosed (by means of 40 cm high walls) and two being open, including at their intersection a central square platform (10 cm × 10 cm) which gave access to the four arms. Each rat was initially placed on the central platform facing one closed arm and behaved freely for 5 min. The behavior during the test was recorded using a video camera positioned above the maze.

2.3.3. Social approach–avoidance test

On day 6, HA and NA rats were submitted to a social approach–avoidance test, which is considered a reliable procedure to measure experimental anxiety in a social context (Haller and Bakos, 2002; Haller et al., 2003). The apparatus consisted