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Behavioral differences in three Wistar Han rat lines for emotional reactivity, cognitive processing and ethanol intake

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HIGHLIGHTS

- We compare three outbred Wistar rat lines in a range of behavioral paradigms.
- Differences are found in locomotion and anxiety-related behavior.
- Differences are found in cognitive processing and for intake of ethanol.
- The results show significant variations in the behavioral phenotypes of rat lines.

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ABSTRACT

Many laboratories obtain their experimental animals from commercial suppliers and are therefore dependent on their conditions and breeding schedules. A breeding stop or the substitution of a particular rat line by the supplier forces the customers to abandon their conventional test animals and to re-establish all behavioral paradigms with a new rat line. Therefore, it is vital to know whether behavioral differences emerge in various breeding lines of the same rat strain. In a recent case, the commercial supplier Harlan Laboratories GmbH is substituting the previous HsdHan:WIST line of Wistar rats with the RccHan:WIST line descending from a different breeding stock. We therefore tested animals of both lines (RccHan:WIST and HsdHan:WIST from Harlan Laboratories GmbH) as well as Wistar rats of the same line but obtained from a different supplier (Janvier) in a broad range of behavioral paradigms. We observed differences in locomotor activity, in classical anxiety-related paradigms (elevated plus maze and light/dark emergence test), as well as in object recognition memory and prepulse inhibition (PPI) of the acoustic startle reflex (ASR). We also found differences in ethanol intake and preference, but not regarding the intake of a palatable food reward and a bitter solution (quinine). These results demonstrate considerable variations in the behavioral phenotype between different breeding lines of the same Wistar rat strain and aim to increase the awareness of behavioral scientists for line and supplier differences affecting animal behavior.

1. Introduction

Most rat strains used in experimental research today are derived from the albino Wistar strain, one of the currently most popular rat strains for laboratory research. Initially developed at the Wistar Institute in 1906 by director Milton J. Greenman, neurologist Henry H. Donaldson and geneticist Helen Dean King, Wistar rats are now used worldwide in research for many different purposes (reviewed by [1]). Outbred Wistar rats originate from strains derived from *Rattus norvegicus* and the classic Wistar albino was also supplemented by Brown Norways and other strains. Since this time commercial suppliers continued the breeding and offer a whole variety of Wistar lines. According to Sabourdy [2], a breeding stock refers to the collection of animals of a given strain (e.g. Wistar Han®) kept for breeding by a specific supplier (e.g. by Harlan Laboratories or Janvier), which may be further divided into different lines such as RccHan:WIST (henceforth referred to as W[rcc]) and HsdHan:WIST (henceforth referred to as W[hsd]).

These different rat lines might not only differ in their genetic profile but also in their behavior. Besides the multitude of studies investigating behavioral, morphological and molecular differences in different rat strains (e.g. [3–6]) some studies also examined possible behavioral variations in the same line obtained from different suppliers [7–12].

Abbreviations: ASR, acoustic startle reflex; BW, body weight; EMT, light/dark emergence test; EPM, elevated plus maze; ITI, intertrial interval; PPI, prepulse inhibition; SCM, sweetened condensed milk; SPL, sound pressure level; W[hsd], HsdHan:WIST; W[Jan], RjHan:WI; W[rcc], RccHand:WIST.

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For example, Langer et al. [9] observed alterations in their model of temporal lobe epilepsy after they had to change their source of Sprague–Dawley and Wistar rats from Harlan-Winkelmann, Germany to Harlan Netherlands due to the closure of the German subsidiary in 2008. Possible explanations for such differences could be genetic variations, as well as different housing and handling conditions (e.g. staff, noise or architecture; [13,14]). Most laboratories receive all or parts of their animals from commercial breeders and are therefore at least partially dependent on their conditions and breeding schedules. Many experimenters tend to use the same line of animals and in this way gain a lot of expertise about the behavior of a particular line for the testing paradigms they are specialized in. If however the supplier decides to abandon the breeding of that particular line, the experimenter is forced to switch to another line and the expertise gained in many years of past research must be transferred to a new animal line.

For researchers it is important to be aware that there are differences between rat strains and lines and this needs to be considered when selecting the appropriate line for one's experimental hypothesis. Increasing the knowledge about behavioral differences between rat lines will help to make comparison between various studies easier. It is therefore important to share observations of occurring behavioral differences of different animal lines to add valuable information about line-specific behavioral profiles across multiple testing paradigms.

The Wistar rat line W[rcc] from Harlan Laboratories GmbH is derived from an original colony at Zentralinstitut für Versuchstierzucht, Hannover. In 2004 Harlan acquired a new breeding stock (RccHan:WIST) from RCC Ltd which was transferred to Harlan Laboratories in 2008. According to Harlan Laboratories, the W[rcc] animals display a smaller body weight gain and a higher life expectancy than other rat lines. This would improve efficiency of studies and reduce costs, and Harlan plans to replace all W[hsd] colonies with W[rcc] [15]. This prompted us to investigate whether these two lines differ in a variety of behavioral paradigms. Additionally, we wanted to test another Wistar line obtained from Janvier, RjHan:WI (henceforth referred to as W[Jan]), and compare the behavior of all three Wistar lines. The W[Jan] line also originates from the Zentralinstitut für Versuchstierzucht, Hannover.

The aim of the present study was to examine possible behavioral differences in Wistar lines (W[hsd] vs W[rcc] rats) derived from the same breeder (Harlan Laboratories) compared with a Wistar line derived from another breeder (Harlan Laboratories vs. Janvier). We therefore tested various behavioral paradigms to get a broad estimation of behavioral variety within these lines, including locomotor activity, emotional behavior, short-term mnemonic processing, sensorimotor gating and consummatory behavior of liquids with variable palatability and reinforcement value (sweetened condensed milk (SCM), ethanol and quinine).

2. Materials and methods

2.1. Subjects

A total of 36 male animals were used for the present study. 12 adult Wistar W[hsd] (HsdHan:WIST) and 12 adult Wistar W[rcc] (RccHan:WIST) rats were purchased from Harlan Laboratories GmbH (Horst, Netherlands). Additionally, 12 adult Wistar W[Jan] (RjHan:WI) rats were purchased from Janvier (Le Genest St Isle, France). The animals were housed in the same room in MakrolonTM cages (Eurostandard type IV) in groups of 6 on a 12 h light–dark schedule (lights on 7:00 am–7:00 pm). During the light phase, a radio provided background noise. Throughout all experiments the animals had free access to tap water and standard lab chow. Before undergoing behavioral testing, the animals were allowed to recover from the transport and to habituate to the new environment and experimenter for at least 7 days after arrival. All experiments were carried out in accordance with the guide for the care and use of laboratory animals as adopted and promulgated by the National Institutes of Health and were approved by the local animal care committee (Karlsruhe, Germany).

2.2. Behavioral testing

Behavioral testing was performed between 9:00 am and 5:00 pm during the light cycle and occurred in the sequence listed below. Animals were left undisturbed for at least 3–5 days between the different test sessions.

2.2.1. Open field

Locomotor activity was measured in an open field. The open field consisted of four equal compartments (50 cm × 50 cm × 45 cm) made of dark PVC. Two opposite walls of each box contained a sensor barrier about 15 cm above the ground to measure rearing behavior. Distance traveled [cm] and the rearing frequency were digitally recorded for 30 min at a light intensity of 50 lx by the observation program Viewer^2 (Biobserve GmbH, Bonn, Germany).

2.2.2. Light/dark emergence test (EMT)

The EMT took place in a light/dark box as described before [16]. The apparatus consisted of two different compartments, separated by a dividing wall with a 10 cm × 15 cm wide opening which enabled the test subjects to move freely between the compartments. The first compartment, with black walls (25 cm × 25 cm × 40 cm) could be closed by a lid and was used as a start box. The second compartment had gray walls (25 cm × 50 cm × 40 cm) and was brightly illuminated (80 lx). The rats were initially placed for 1 min in the dark, closed compartment and their behavior was recorded for 5 min after the start box was opened. Subsequent manual video analysis scored the latency of the animals to emerge from the dark compartment into the lit compartment [s] (an entry was defined when the animal entered the compartment with all four limbs), the emergence frequency, the duration of time spent in the light compartment [s], the amount of rearings and risk assessment behavior (only head or forepaws are placed in the open compartment without concomitant movement of the hind limbs, even if the rat subsequently entered the area). The apparatus was thoroughly cleaned with 70% ethanol between the sessions.

2.2.3. Elevated plus maze (EPM)

The EPM consisted of a plus-shaped apparatus made of dark gray PVC elevated 50 cm above the floor with two opposing open arms (12 cm × 50 cm × 50 cm) which were illuminated by 80 lx and two enclosed arms (12 cm × 50 cm × 50 cm) [16]. All arms extended from a central square (10 cm × 10 cm). At the beginning of each trial, the rats were placed in a closed arm of the EPM. Each rat was videotaped for 5 min and the following behaviors were analyzed: number of entries into open or closed arms (an entry was defined if all four paws were placed on that arm), time spent in open and closed arms [s], head dips (the whole head is lowered beneath the edge of an open arm), risk assessment (only head or forepaws are placed in an open arm without concomitant movement of the hind limbs, even if the rat subsequently entered the arm), self grooming frequency and self grooming time [s]. Percentage of open arm entries (open arm entries/(open + closed arm entries) × 100) and percentage of time spent in open arms (open arm time/(open + closed arm time) × 100) were calculated as well. The apparatus was thoroughly cleaned with 70% ethanol between the sessions.

2.2.4. Object recognition test

Short-term memory for objects was assessed with the object recognition test [17]. The recognition test was performed in the open field apparatus described above. The objects to be discriminated were made of metal, ceramic, or glass and existed in multiple copies. All objects and the test arena were cleaned with 70% ethanol and thoroughly dried before and during testing. Preliminary testing in our lab