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Urine from stressed rats increases immobility in receptor rats forced to swim: Role of 2-heptanone

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Abstract

The present study was aimed to determine whether the urine from donor rats, which were physically stressed (UD-PS) by unavoidable electric footshocks, produces despair in receptor partner rats (RP) in the long-term. For each trial, an RP rat was placed during 10 min once per day for 21 days in a small non-movement-restricting cage impregnated with the urine collected from a UD-PS rat. Control rats, free of stimulation, maintained their locomotion and immobility scores at basal values throughout the 21-day test. After 21 days of stressing experience [$F(2,90)=15.22$, $P<0.0001$] locomotion significantly increased in RP rats ($r=0.938$, $P<0.01$), whereas in the UD-PS group locomotion decreased ($r=-0.606$, $P<0.05$). The RP and UD-PS groups displayed the longest time of immobility [$F(2,90)=8.83$, $P<0.001$] in the forced-swim test (RP, $r=0.886$, $P<0.05$; UD-PS, $r=0.962$, $P<0.001$) compared with the control group ($r=-0.307$, NS). We conclude that the RP became similarly despaired as the UD-PS group through the action of 2-heptanone, a ketonic compound identified in UD-PS urine by HS-GC/MS techniques. This ketone was found to be increased [$F(2,15)=3.50$, $P<0.05$] from the 1st day of unavoidable electric footshocks, and to induce despair, an effect reverted [$F(2,21)=16.5$, $P<0.0001$] by imipramine (5.0 mg/kg) in another group of rats.

Keywords: Alarm odors; Emotional stress; Forced-swim test; Physical stress; 2-Heptanone

1. Introduction

The odors emanating from physically stressed (PS) rats are recognized by non-stressed receptor conspecifics [1,2]. Once receptor partners (RP) perceive the chemical signal, their immunological system undergoes significant changes, since T-killer cells are suppressed and B-cells proliferate [3], rats develop hyperthermia [4], Fos protein expression is increased in the mitral cell layer in the accessory olfactory bulb [5], reproductive behavior and sexual maturation initiate [6] and social hierarchy may be modified [7].

These signals alert RP to the presence of a potential danger, promoting dispersion and the adoption of defensive actions in the group [8]; nevertheless, the chemical nature of these substances of alarm has not been identified. Usually, mice escape from places in which the odors from the urine of a PS mouse are present [9].

Urinary odors from rodents contain information about the species, sex, sexual state, dominance status, maternal state and individual identity of each animal [10]. The complexity of mouse urinary volatile profiles includes genetic background, sex, endocrine status and environmental conditions. All seem to influence the quantitative proportions of various urinary constituents [11,12].

Kiyokawa et al. [13] proposed the neural circuit of the alarm pheromone perception. When a rat is placed in a test box containing any alarm pheromone, the chemical sign is detected

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by the vomeronasal system, it is transmitted through the accessory olfactory bulb to the medial amygdaloid nucleus, and then to the bed nucleus of the stria terminalis. The main olfactory system may also transmit some volatile information. The information perceived by the main olfactory system is transmitted from the main olfactory bulb to the lateral and basolateral amygdaloid nuclei, and to the bed nucleus of the stria terminalis. Here, the information from both systems is integrated, and then the pheromone signal is transmitted to several nuclei in the hypothalamus and some brain stem nuclei such as the paraventricular nucleus, the dorsomedial hypothalamic nucleus, the periaqueductal gray and locus coeruleus that are involved in the regulation of anxiety as well as in the stress response [14].

Odor communication coming from a PS organism induces anxiety in an RP non-footshocked conspecific individual in mice [9] and rats [15,16]. It should be noted that anxiety and despair are interrelated entities [17,18]. In the pretest session of the forced-swim test [19], rats or mice swim vigorously at first, apparently looking for an exit. Some minutes later, they forsake their efforts to find an escape. The behavioral scheme is interpreted as despair, characterized by immobility, which is reduced by the administration of clinically effective antidepressants [20,21]. Imipramine among many other clinically effective antidepressants decreases immobility in the forced-swimming test [22] and also decreases head shaking, during swimming [23].

Given the key role of alarm substances in animal communication and behavior, it is of interest to identify the different classes of these molecules. Most of the stressor substances reputed as alarm cues in the honeybee, are volatile 6–8 carbon chains containing a ketonic group [24]. Among other ketones, 2-heptanone is normally present in the urine of mice *Mus musculus* females and strain C57BL/10 [12,25] as well as in human beings [26]. However, it is unknown if odors from PS rats produce despair in RP rats in the long-term; hence, we also explored the content of volatile components in the urine of stressed rats by static Head-Space and Gas Chromatography–Mass Spectrometry (HS-GC/MS) and determined the capacity of 2-heptanone to induce despair in rats forced to swim, as well as the capability of imipramine to reduce some of the thus provoked behavioral changes.

2. Materials and methods

2.1. Animals and housing

We included a total of 93 male Wistar rats, aged 3 months, weighing 300–350 g at the beginning of the experiments. The rats were maintained in translucent acrylic boxes (45.0×30.0×30.0 cm) in groups of 8 animals per box, with a light (below 100 lx)/darkness cycle of 12×12 h (lights ON at 7:00 AM), and with *ad lib* access to water and food. They were manipulated once per day, beginning 1 week before the experiment to reduce some of the possible stress produced by handling during the tests. In a first experiment, 48 rats were randomly assigned to any of three different experimental groups and underwent control, RP or UD-PS experiences in a two-compartment cage during 21 days. The urine was collected from another control, RP and UD-PS groups (N=21) and analyzed by static Head-Space and Gas Chromatography–Mass Spectrometry (HS-GC/MS). In a third experiment, 24 male Wistar rats were exposed to long-term inhalation with 2-heptanone during 14 days. All experimental procedures were carried out between 9 AM and noon. We strictly followed the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health.

2.2. Apparatus

A modified version of the two-compartment box was used as described by van den Berg et al. [27]. The glass box (base 30.0×25.0 cm, height, 30.0 cm) contained a stainless steel (0.5 cm diameter bars, spaced 1.3 cm) electrified grid floor and two compartments (15.0 cm×12.5 cm and 30.0 cm) divided by an opaque Plexiglas plate (0.2 cm in thickness). A plate of Plexiglas covered the floor of the safe compartment to prevent electric footshocks. The grid received the output of an electronic stimulator (Grass Instruments S44, Quincy, Mass, USA) coupled in series to a stimulus isolation unit (SIU5, Grass Instruments, Quincy, Mass, USA) and to a constant current unit (CCU1A, Grass Instruments, Quincy, Mass, USA).

2.3. Behavioral tests

2.3.1. Open-field test

Since changes in motor activity influence the immobility of animals forced to swim [28], the rats were subjected to the open-field test about 2 min before each swim session. Each rat was individually placed in an acrylic box (44.0×33.0×20.0 cm), with the floor divided into 12 plain squares (11.0×11.0 cm each). A 5-min habituating pretest session was carried out 24 h prior to the first 5-min video-taped test session. Later, on the 7th, 14th and 21st days they were only subjected to the 5-min test session. Two independent observers counted the number of squares crossed by the animals (crossing) in each 5-min video-taped test session. Crossing was assumed when an animal passed from one square to another with its rear legs. After each test, we carefully cleaned and deodorized the box with a cleaning solution (ammonia 0.5%, ethanol 15%, extran 10%, isopropyl alcohol 5%, pinol® 19% and water 50.5%). Immediately after the open-field test, the rats were forced to swim. All tests were performed during the light period. The room was illuminated with white light (40 lx) by a tungsten lamp placed 2 m above the open-field and forced-swim-test devices.

2.3.2. Forced-swim test

The general procedure used in the forced-swim test has been described in previous reports [29]. The test consisted of placing each rat individually in a rectangular pool (50.0×30.0 base area, ×60.0 cm height) with water (25±1 °C) 24 cm in depth. Following the model proposed by Porsolt et al. [19], 24 h after a first 15-min pretest session (discarded from the data analysis), each rat underwent the 5-min video-taped forced-swim-test