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Research report

Chronic variable stress exposure in male Wistar rats affects the first step of olfactory detection

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HIGHLIGHTS

- We investigated olfactory function in a rat model of chronic variable stress (CVS).
- CVS resulted in a depressive-like state and physical symptoms of chronic stress.
- Olfactory performance was reduced in depressed-like CVS rats.

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ABSTRACT

For most animal species, olfaction plays a paramount role in their perception of the environment. Odours are initially detected in neurons located in the olfactory mucosa. This tissue is regulated by several physiological signals and can be altered in pathology. A number of clinical studies suggest an association between depressive disorders and olfactory sensory loss. In rodents, depressive-like states can be observed in models of chronic stress. We tested the hypothesis that olfactory function might be altered in a rat model of depression, induced by chronic variable stress (CVS). While CVS rats exhibited several symptoms consistent with chronic stress exposure and depressive-like states (increased sucrose intake in sucrose preference test, increased immobility in forced swim test, hyperlocomotion), their odorant responses recorded at the olfactory mucosa level by electro-olfactogram were decreased. In addition we observed increased apoptosis markers in the olfactory mucosa using Western Blot. Our data are consistent with reduced olfactory capacities in a laboratory rat model of chronic stress and depression, in agreement with human clinical data; this warrants further mechanistic studies. Furthermore, this works raises the possibility that altered olfactory function might be a confounding factor in the behavioural testing of chronically stressed or depressed rats.

1. Introduction

Olfaction is widely conserved in evolution and allows animals to collect chemical information in their environment [1]. For most species, this sensory modality is essential for assessing social relationships or danger and finding a sexual partner or food. Odorant compounds in the environment are first detected at the peripheral level in a neuroepithelium, the olfactory mucosa (OM), through activation of olfactory sensory neurons [2]. The olfactory message is transmitted to the olfactory bulb and then to higher central nervous structures such as the piriform cortex. The OM is a highly dynamic tissue which undergoes constant remodelling and its function is under the control of diverse endocrine influences, in accordance with the physiological state of the organism [3,4].

In humans, alterations of olfactory perception at the peripheral level have been described in many psychiatric disorders, such as schizophrenia and depression [5]. Although there is no absolute consensus in the data [6,7], in almost all studies patients suffering from major depressive disorders tend to exhibit deficits in olfactory detection and signal processing. The heterogeneity of observations in the reported studies may be due to differences in patient sex, clinical diagnosis or drug treatment, such as antidepressant medication [6,7]. This association between depression and olfactory sensitivity is further strengthened by a correlation between a higher incidence of depressive symptoms and reduced olfactory sensitivity in healthy volunteers [8].

Abbreviations: CVS, chronic variable stress; EOG, electro-olfactogram; EPM, elevated plus maze; FST, forced swim test; OF, open field; OM, olfactory mucosa; OMP, olfactory marker protein; PCNA, proliferating cell nuclear antigen; SPT, sucrose preference test.

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Depression is a debilitating mood disorder where patients exhibit heterogeneous symptoms (as defined by the DSM-5) and is characterised, amongst other symptoms, by anhedonia and despair [9]. Some of the core symptoms of depression can be observed in several animal models, and these allow experimentation and investigation of aetiology and mechanisms [10]. Interestingly, one such model is the ablation of the olfactory bulb in rodents [11,12]. Upon bulbectomy, several physiological and behavioural parameters are affected; one symptom is increased locomotor activity when rats are placed in an open field [12]. In spite of some reported drawbacks, this model is highly predictive of antidepressant action [13] and can be used for drug screening.

Another, more physiological approach to induce depressive-like symptoms in rodents is the use of chronic unpredictable or variable stress [14,15]. For chronically stressed animals, physiological changes take place in order to adapt to regular challenges [16], through sustained stimulation of the adrenal glands that secrete catecholamines and glucocorticoids. The experimental protocols involve the use of various stressors, to avoid any habituation and increase the allostatic load [17]. In the case of chronic mild stress, animals are subjected to various mild stressors in an unpredictable manner over the course of several (5 to 9) weeks. This leads to anhedonia-like behaviour and the symptom can be reversed by antidepressant treatment [18,19]. Other researchers have used shorter protocols (3 to 4 weeks) and induced similarly depressed phenotypes in rodents [14,20,21].

These depression models however do not always lead to consistent effects among laboratories; indeed some published findings in the chronic mild stress model could not be replicated [15]. This may be due to genetic differences in chronic stress susceptibility, as its effects on anhedonia, as measured by sucrose preference, differed between various mouse strains [22]. This highlights the need to validate and characterise each chronic stress protocol.

To investigate the link between depression and olfactory function and the impact of increased allostatic load on olfactory epithelium biology, we hypothesised that chronic stress would reduce olfactory performance. We subjected male rats to a short chronic variable stress regimen (CVS), characterised their mood-related behaviour, and studied its impact on the first step of odorant detection by electrophysiology as well as on the cellular dynamics by protein markers. CVS treatment led to a reduction in the functional response to odorants and to an increase in apoptosis markers. Our findings reveal for the first time that exposing animals to chronic variable stress leads to functional and molecular alterations in the olfactory mucosa, the first step of olfactory detection.

2. Materials and methods

2.1. Rats and chronic stress protocol

All experiments were conducted in accordance with the European Union directive of 22nd September 2010 (2010/63/EU) and were approved by the local animal experimentation ethics committee (Comethea, no. 12/154). Male rats were all born from multiparous mothers and kept in pairs from weaning. They were bred and housed at our local animal care facility in a 12L:12D lighting schedule (lights on at 8:00) with free access to water and food (M25 Extralabo, Dietex France). Stressors were administered from 9:00 to 11:00 to avoid variability due to circadian rhythms, and all measurements were carried out during the light phase between 9:00 and 13:00. The chronic stress protocol and timing of the different behavioural procedures are described in Table 1; they were carried out from the age of 8 weeks. This age was chosen so that animals were sexually mature (as determined by visual inspection of the balano-preputial separation) yet young enough to allow dissection of the hemi head for electrophysiology measurements. This protocol was designed to maximise unpredictability of the applied stressors in order to prevent habituation. Body weight was monitored every 3 days. For organ dry weight measurements, adrenal glands and thymus were dissected, dried for 5 days at 60 °C and weighed to the nearest 0.01 mg. Organ weight data are expressed as 100 × (organ weight in mg)/(body weight in g). This experiment was repeated twice (described in Fig. 1; $n = 14$ control and 14 CVS, then $n = 10$ control and 10 CVS), results were similar for both cohorts and whenever possible data were pooled.

Table 1
Chronic variable stress schedule. Stressors were applied from 9:00 to 11:00 daily. Weighing of the matched control animals was done on the same days as for the stress treatment group. Unless otherwise specified, the animals were left in pairs during stressor exposure. Dates of sucrose preference test (SPT), elevated plus maze (EPM), electro-olfactogram (EOG) and forced swim test (FST) are also shown.

Day	Treatment
−5	SPT (pre-stress)
1	40 min obscurity (under a cardboard box), 40 min room light, 40 min obscurity
2	2 h in clean cage with no bedding
3	Alone, 2 h in clean cage with no bedding
4	20 min restraint
5	Overnight fast (16 h)
6	2 h in new cage with wet bedding
7	2 h on an oscillating plate (1 Hz)
8	2 h in new cage with dirty bedding and feces from unrelated Wistar males; SPT (mid-stress)
9	2 h in the dark in a cage tilted (30°), with no bedding
10	2 h alone in the dark, in clean cage with no bedding
11	EPM; Overnight fast (16 h)
12	2 h in clean cage with the bottom cooled to 4 °C
13	5 min in water at 22 °C
14	Alone, 2 h in clean cage with wet bedding and feces from unrelated Wistar males
15	2 h exposure to loud music ('hard techno mix' played in loop, 80 dB)
16	2 h alone on an oscillating plate (1 Hz), in a cage with dirty bedding and feces from unrelated Wistar males; SPT (late stress)
17	20 min restraint, alone
18	FST pre-test or OF or rest
19	FST or EOG

2.2. Behavioural analysis

2.2.1. Elevated plus maze (EPM)

This test was carried out on protocol day 11. The maze was a custom made Perspex apparatus in the shape of a plus, with one opposing pair of arms enclosed by high walls (closed arms) and the other opposing arms exposed (open arms). The EPM was raised 1 m above ground and the open arms illuminated with the direct light from neon lamps (100 ± 10 lx), whereas the closed arm were less

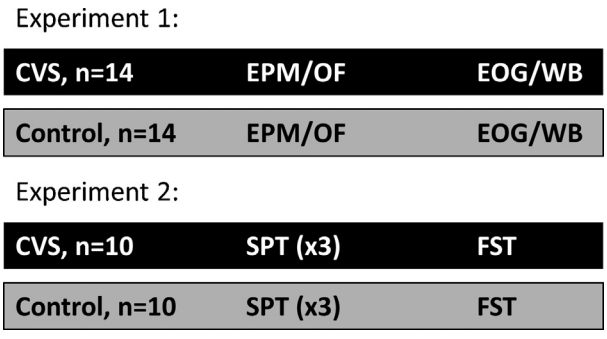

Fig. 1. Description of the two experimental cohorts used in this study. The experiment was carried out twice and is detailed in Table 1. Some data points do not appear in the final results, due to leaking bottles (SPT) or missing samples (adrenal and thymus weights; WB proteins).