

1 **Perception and emotions: on the relationships between stress and olfaction**

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4

5 **Abstract**

6

7 Adaptations to the ever changing social and physical environment are necessary, sometimes
8 involving the stress response. Olfaction is a chemical sense allowing the detection of
9 molecules in the air and many species rely on it for social recognition and fundamental
10 functions as feeding or reproduction. This non exhaustive literature review addresses three
11 topics at the intersection of sensory biology and stress biology, in order to raise awareness
12 and enlighten our understanding of animal behaviour. First we show that stress and
13 associated emotions, such as fear can alter odour perception, from the sensor (peripheral
14 nervous system) to the interpretation of the sensory input (brain). This can be of relevance
15 for understanding the behavioural consequences of negative experiences and depression-like
16 states. Second, odours involved in animal chemical communication can signal danger in the
17 form of predators or stressed conspecifics, and in turn induce stress in recipient animals, as a
18 form of chemical empathy. Finally, we review some of the evidence for the use of odours as
19 stress-relief agents. There is no clear evidence of treatment efficacy and it might be that
20 under the labels of inhalation aromatherapy, aromachology or pheromone therapy, a lack of
21 perspective has led to the conflation of various biological phenomena: an alleged
22 spontaneous olfactory effect on stress relief, environmental enrichment or the recall of
23 positive emotional states. Careful experimental design and critical analysis are paramount to
24 clearly evaluate this promising intervention. The world around us shapes our emotions,
25 which in turn affect our perception. There might be a huge animal welfare potential to
26 harvest from improved knowledge in the studies of stress and olfaction interactions, this will
27 eventually allow us to show more empathy to animals in our care.

28

29

30 **Keywords:** stress; welfare; olfaction; odours

31

32 1. Introduction

33 We live in a world populated with more competitors and predators than friends, some of those
34 interactions change over time and adaptation is often necessary. Sometimes, this requires action of
35 the stress axis. Sensory information can guide and help animals adapt to their environment, in order
36 to make sense of the ever changing world they live in. Food, sexual partners, friends or foes and the
37 way home can be identified using various sensory modalities. Here, I will discuss examples of the
38 reciprocal relationships between the olfactory perception of the environment and some of the
39 emotions experienced by animals (in the wild or those managed by humans). This literature review
40 addresses three topics at the intersection of sensory biology and stress biology, where an improved
41 awareness can enlighten our understanding of animal behaviour and empower our efforts to
42 improve animal welfare. There is a wealth of information on the topics addressed in this paper and
43 for further general reading on olfaction biology please refer to specialised reviews (Apfelbach et al.,
44 2005; Ihara et al., 2013; Li and Liberles, 2015; Nielsen et al., 2015a; Meunier and Rampin, 2017). The
45 purpose here is not to exhaustively synthesise all the existing literature, but rather to give an
46 overview of areas relevant to applied ethologists as a first step for people who might wish to work in
47 this field, to widen perspectives and stimulate critical conversations and debates.

48

49 Humans can smell too!

50 First of all, in order to improve the reader's empathy towards the subject at hand, and emphasise
51 how accessible the world of odours can be, let us discuss the capacity of detecting odours in *Homo*
52 *sapiens*. Humans are allegedly microsmatic (low olfactory capabilities), whereas a large number of
53 animal species are traditionally considered to be macrosmatic, they rely on odours and have higher
54 sensory abilities. This notion may be based on culture rather than scientific observation, possibly
55 based on hypotheses from the 19th century neuroanatomist Paul Broca (McGann, 2017) and
56 stemming from a time when animals and humans were seen as fundamentally different. However,
57 human microsmia has now fallen out of favour, with recent work highlighting sometimes comparable
58 sensitivity between humans and other animals (McGann, 2017). The implication is that, although
59 there is a degree of individual variability in our ability to perceive odours, experimental scientists and
60 anyone involved in the keeping of animals should use their nose.

61

62 Perception and olfaction

63 Perception occurs when stimuli are detected and interpreted into a meaningful pattern. Odours are
64 volatile molecules diluted in the air (or water), that are perceived by animals as olfactory sensation
65 whose first step takes place through activation of sensory neurons. These are located in specialised
66 tissues called neuroepithelia, at the interface between the environment and dedicated sensory
67 structures in the brain. In most species this task is carried out by the main olfactory epithelium (OE,
68 also known as olfactory mucosa), with contributions from the accessory olfactory system, composed
69 of the vomeronasal organ (Mucignat-Caretta, 2010) or Grueneberg ganglia (Brechbuhl et al., 2008).
70 The OE is generally considered to be involved in general odour recognition. The detection of odorant
71 molecules consists in the translation of chemical signals into electric nervous message, this
72 peripheral signal is then transmitted through the olfactory bulb to various brain structures that
73 process emotion (e.g. amygdala) and learning (e.g. hippocampus). In comparison to other sensory
74 modalities, the route of information is direct (Soudry et al., 2011). In contrast, audition and vision are
75 connected to those emotional and cognitive structures via relays in specific thalamic nuclei and

76 subject to further processing and regulation (reviewed in (Wijesinghe et al., 2015). This closer
77 neuroanatomical link between olfactory and limbic regions is deemed of great importance, when
78 considering the impact odours can have on animal behaviour. Olfactory detection is a regulated
79 process, it is likely to be altered by various endogenous signals, such as certain feeding-related
80 hormones (Palouzier-Paulignan et al., 2012) and phases of the circadian cycle (Amir et al., 1999). The
81 mechanisms through which the olfactory system and odour perception exhibit plasticity are diverse
82 and some have been reviewed elsewhere (Wilson et al., 2004). Depending on the time scale and the
83 nature of the event considered, they may involve rapid molecular events in response to leptin or
84 insulin affecting olfactory neurone response in the OE (Palouzier-Paulignan et al., 2012), effects of
85 signalling peptides on OE olfactory neuron cell survival or proliferation (Laziz et al., 2011), neural
86 network rearrangement in cortical regions (reviewed in McGann 2015), and in the longer term,
87 epigenetic alterations possibly affecting olfactory gene expression patterns have been identified in
88 response to learning paradigms (Jones et al., 2008; Dias and Ressler, 2014). From the rapid effect of
89 hormones within minutes, to the several days necessary for tissue level alterations, those factors
90 contribute to modulate detection and response to all or specific odorant molecules. Individual
91 variability in olfactory performance will therefore be the product of genetic factors (e.g. olfactory
92 receptor polymorphisms), physiological factors (e.g. hormone levels and tissue sensitivity) and life
93 history (previous exposure to odorants).

94 Odours can elicit various neuroendocrine processes that affect animal behaviour in an innate and/or
95 learnt fashion, when an association between odours and other stimuli is created. Olfactory cues are
96 important for the initiation and maintenance of feeding and reproductive behaviours, intra- or
97 interspecific recognition of social cues and predators. The molecules that make up odours can be
98 from environmental sources (e.g. food or plants) but can also originate from other animals and some
99 of these have been identified as semiochemicals, i.e. chemicals of animal origin that affect the
100 behaviour in a recipient. There are many types of semiochemicals, for instance pheromones are
101 substances that affect behaviour in the same species and allelochemicals affect behaviour in other
102 species. Further aspects of terminology and relevance to applied ethology have been discussed
103 elsewhere (e.g. see [reviews by Wyatt \(Wyatt, 2014; 2017\) or Nielsen \(Nielsen et al., 2015b\)](#)). Like any
104 sensory cues, odours can have different valence, they can be attractive or repulsive. This can vary
105 due the ecology of the species concerned (predator odour generally elicits fear and avoidance in
106 preys) and there are also individual preferences. Indeed, this can be altered by experience and
107 learning (reviewed in (Nielsen et al., 2015b) and the same odorant can be attractive at low
108 concentrations and aversive at high concentrations. For an accessible and more thorough primer on
109 the mechanisms of olfaction, readers can refer to another review (e.g. (Meunier and Rampin, 2017).

110

111 **Challenges of working with odours**

112 For scientific investigators, olfaction is a field of sensory biology that can be technically challenging.
113 The greatest difficulty lies with ensuring consistent stimulus delivery. Unlike other experimental
114 treatments, such as dietary manipulation or drug injection, it is difficult to ensure the animal has
115 smelled the test odour. The type of experimental paradigm used can affect the results, odour
116 delivery can be affected by the size of the experimental arena in which the odours are presented, the
117 distance to the odour source, and even the direction of the wind. Stressful experimental conditions
118 (e.g. neophobia, strong lights or unusual sounds) can lead to the animal not investigating or inhaling
119 the odorant source. Some researchers choose to ensure odorant exposure by diluting it in food or
120 drinking water, at the risk of inadvertently studying the pharmacological or even toxicological effects
121 of the chemical administered. Finally, depending on the type of animal testing facility used, physical

122 factors such as temperature, compound vapour pressure and air movement will affect odour
123 volatility and spatial distribution. This can affect stimulus delivery or potentially lead to
124 contaminations, such as when supposedly naïve animals might be exposed to test odour prior to the
125 experiment. In addition, contaminating stimuli might originate from outside (e.g. grass cutting) or
126 inside the facility (e.g. cleaning products adsorbed onto surfaces). Great care is therefore required
127 when testing the relationship between animal behaviour and odours.

128

129 **The stress response: a few reminders**

130 Whether challenges result from environmental factors or other beings, the body constantly requires
131 physiological adaptations to achieve homeostasis. Stressors are factors that constitute a real or
132 perceived threat to the homeostatic drive of the individual and its survival. The stress response is the
133 sum of psychic arousal and changes in affect associated with physiological reaction where resources
134 are mobilised, to sustain metabolic and behavioural adaptations, and allow the classical 'fight or
135 flight' reaction. It is induced by a vast range of psychological to physico-chemical factors. There is no
136 Cartesian duality of mind and body here, mental and physical resources work together to survive a
137 predator encounter or a chronic shortage of food.

138 This section is a brief reminder of stress biology notions and merely introduces some key players in
139 the three stories to come (for a more extensive review, see (Goldstein, 2010)). Briefly, environmental
140 stimuli that could threaten the animal modify the activity of various brain regions (e.g. hippocampus
141 or amygdala) which in turn activate neurones in the posterior region of the hypothalamus, causing
142 stimulation of the adrenal gland medulla via the splanchnic nerve, resulting in the rapid release of
143 catecholamines, adrenaline and noradrenaline. Those fast acting mediators are responsible for the
144 first signs of the stress response (increased cardiopulmonary function, sweating). In parallel, another
145 part of the hypothalamus is also activated, the paraventricular nucleus (PVN). This leads to secretion
146 of **corticotropin-releasing** hormone (CRH), that acts on the pituitary gland, which in turn sends
147 messages through release of adrenocorticotrophic hormone (ACTH), which travels via the bloodstream
148 and in turn stimulates the cortex of the adrenal glands to release glucocorticoid hormones
149 (predominantly cortisol in large mammals and fish; corticosterone in rodents and birds).
150 Glucocorticoids affect metabolism, as well as a vast range of processes pertaining to immunity or
151 brain function. Collectively those hormones are responsible for the adaptive effect of the stress
152 response, this is called acute stress. If the animal is subjected to repeated and inappropriate
153 stimulation of this stress response, pathologies can appear, this is called chronic stress (McEwen,
154 2007). The neuroendocrine response described above is accompanied by alterations in arousal and
155 emotions. Mechanistically, the neuroendocrine and physiological changes concomitant with the
156 stress response are causative of further behavioural effects, responsible for the fight of flight
157 response (de Kloet et al., 2008).

158 The question of the effects of stress is still a matter of investigation, but several consequences have
159 already been identified (some are reviewed in (Greenberg et al., 2002)). A large number of
160 behavioural effects have been identified and they are related to aggression and social interactions,
161 sex, food intake, cognition and exploration (Greenberg et al., 2002). Behaviour changes in stressed
162 animals are accompanied by increase in fear or anxiety, this is thought to prevent risk taking (Nettle
163 and Bateson, 2012) and alterations in memory (de Kloet et al., 2008).

164 Over time, chronic stress can lead to the development of depressive-like symptoms (McEwen, 2007)
165 with increased learned helplessness and loss of pleasure seeking (anhedonia, (Katz, 1982); inhibition
166 of social behaviour (Beery and Kaufer, 2015); alterations in body grooming (Kalueff et al., 2016) and

167 increased aggressiveness (Neumann et al., 2010). Repeated exposure to stressors may have an
168 impact on fearfulness, as shown in sheep exposed to six weeks of unpredictable stressors (Destrez et
169 al., 2013). Overall, stress impacts affective states as well as emotions.

170 Emotions have previously been linked to perception, for pain, vision and gustation. In several animal
171 models and human patients, associations have been reported between stress levels or mood
172 disorders and sensory perception. In the case of pain perception, acute stress may have analgesic
173 effects (Sorge et al., 2014), whereas chronic stress can cause hyperalgesia (Blackburn-Munro and
174 Blackburn-Munro, 2001). Using electrical measurements of retina activity in response to visual
175 stimuli, it was recently reported that depressed patients had lower retinal function, suggesting that
176 vision can be affected by depressive disorders (Bubl et al., 2010). In human, subjects displaying traits
177 of mild subclinical depression were asked to assess taste and fat content in test food, their ability to
178 discriminate subtle variations was blunted compared to healthy controls (Platte et al., 2013). Overall,
179 depressive-like mood and/or chronic stress appear to alter several aspects of sensory perception in
180 animals.

181

182

183 **2. The stress response impacts sensory perception**

184

185 **Olfaction is affected by acute stress**

186 Here we argue that olfactory tissues are stress hormone responsive and function differently in
187 people and animals with varying levels of stress, **or** when a negative association with an odour has
188 been created (conditioning), and when suffering from stress-related mood alterations.

189 Hormones can be seen as the regulators of homeostatic coordination of physiology and behaviour,
190 influencing a plethora of biological processes. In keeping with (and as an integral part of) this
191 integrated control, olfaction has been shown to be affected by several hormonal factors. Depending
192 on the state of satiety, food smells will not impact animal behaviour the same way, in part because
193 the odorant signals are interpreted differently in the brain but also because they are detected
194 differently; indeed, feeding-related hormones (e.g. insulin, NPY, leptin) affect olfactory function
195 (Palouzier-Paulignan et al., 2012). Although less established, there is evidence for an olfactory
196 regulation effect of stress-related glucocorticoid hormones (such as cortisol or corticosterone) whose
197 secretion has profound impact on physiology and behaviour. Hormones act on receptors, and the
198 presence of glucocorticoid receptors (GR) in a cell type or a tissue suggests the potential for
199 responsiveness to this hormone. Peripheral nervous system and brain regions associated with
200 olfactory response such as the olfactory epithelium, olfactory bulb and the lateral olfactory tract,
201 express such receptors (Morimoto et al., 1996; Robinson et al., 1998). Receptors for other mediators
202 of the stress response have also been identified in olfactory related regions: CRH (Garcia et al., 2016),
203 ACTH (Mountjoy et al., 1994) and adrenaline (Kawai et al., 1999).

204 Given that several stress hormones originate from adrenal glands, interesting functional information
205 can be gathered from situations when their function is altered. In patients suffering from adrenal
206 insufficiency, with reduced cortisol production, olfactory perception threshold was reduced: these
207 patients showed an increased olfactory sensitivity and this was reversed by steroid supplementation
208 treatment (Henkin and Bartter, 1966). Some of the earlier studies in rat models provide conflicting
209 evidence of adrenal hormone influence, following adrenalectomy olfactory performance was either

210 reduced (Sakellaris, 1972) or unaltered (Doty et al., 1991). Given that patients or animals with
211 reduced adrenal gland function suffer from far more debilitating issues than altered olfaction,
212 confounding factors are probably at play and clouding the issue.

213 More recently, research using subtler models has brought some elements of response to the
214 question of how stress might impact olfaction. **Some insights gained from human studies might**
215 **inform us on homologous animal emotional states.** In studies with healthy patients a link between
216 cortisol level and olfactory function was investigated. In women, when olfactory detection threshold
217 was measured across the menstrual cycle, increased cortisol was associated with improved odour
218 detection abilities (Pause et al., 1996). In a postpartum study, higher cortisol levels were associated
219 in new mothers with a greater attraction and a better ability to recognize their infants' odours
220 (Fleming et al., 1997). Cortisol levels can be experimentally manipulated in human test subjects, for
221 instance by inducing fear, such as in the Trier Social Stress Test where volunteers are tested following
222 public speaking (TSST, (Kirschbaum et al., 1993)). In men subjected to a modified TSST, plasma
223 cortisol levels were raised and the volunteers were found to experience more anger (Hoenen et al.,
224 2017). Olfactory sensitivity was enhanced in those subjects. Interestingly, in physiologically stressed
225 controls subjected to physical exercise and displaying similar signs of physical arousal, without the
226 experience of fear, changes in olfactory function were not detected. This suggests that, in this
227 paradigm, not all physiologically or metabolically stressful experiences lead to alterations in olfaction,
228 it might have been the experiencing of emotions concomitant with the stress response that affected
229 sensory perception. Although no attempts were made to accurately assess their emotional state,
230 subjects submitted to a stressful mental arithmetic task in public, leading to stress-related increases
231 in cardiopulmonary function and cortisol levels, performed better at detecting the rotten egg smell
232 of 2-mercaptoethanol (Pacharra et al., 2016). A study in healthy patients showed that the emotional
233 status affected olfactory function, high anxiety was significantly associated with reduced olfactory
234 functioning for both odour detection and identification (Takahashi et al., 2015). In some cases, odour
235 perception was not found to be affected, but the brain processes in response to odours presentation
236 differed. A **human** brain imaging study (Krusemark et al., 2013) showed that the induction of a
237 transient anxiety state in healthy patients altered activation response to odours in several brain
238 regions and the functional interactions between those regions were also affected. In one of the few
239 mechanistic studies of acute stress and olfaction in laboratory animal, adrenaline released during a
240 mild stressor exposure was shown to act on the olfactory bulb to repress the formation of olfactory
241 memories (Manella et al., 2013). **Thus,** a mildly stressed animal may not remember odours
242 associated with the **negative** event in an odour recognition task. **Altogether this** supports the idea
243 that behavioural responses to odours can be affected by stress and stress-related emotional states.
244 Acute stress and the hormones associated herewith have been shown to affect several aspects of
245 olfactory detection; overall it might be argued **from available data** that acute stress could enhance
246 olfactory detection (Pause et al., 1996; Fleming et al., 1997; Pacharra et al., 2016; Hoenen et al.,
247 2017). For practical reasons olfactory testing is primarily carried out with a limited number of
248 odorant molecules. It is important to bear in mind that odours differ in their pleasantness or valence,
249 and it is likely that stress affects the perception of different odours in different ways. This has not
250 been studied, and an obvious experiment would be to test the effect of stress on the perception of
251 aversive danger-related odours (predators), against more attractive odours with a **positive** valence
252 (social partners, food).

253

254 **Olfaction is affected by aversive learning**

255 Exposure to aversive unconditioned stimuli may generate an initial stress response and associated
256 emotions, such as fear, dependent on the degree of stimulus aversiveness. Associative learning may
257 take place if exposure is repeated. When odorant conditioned stimuli are associated with aversive
258 conditions, some studies show plasticity is induced in olfactory cells (Wilson et al., 2004; Jones et al.,
259 2008; Tong et al., 2014), and the relatively longer lasting encoding of the negative association in
260 tissue morphology (e.g. through DNA methylation marks, associated with change in numbers of
261 specific olfactory sensitive neurones in the OE) leads to altered responses to odours. The time
262 window for the creation of such association ranges from hours in the case of associative learning, to
263 days or weeks, when cell and tissue morphological alterations are necessary. In fact, fear-based
264 learning paradigms have been used to study the function and plasticity of the olfactory system. In a
265 mouse model, electric foot shocks associated with acetophenone, an odorant molecule for which the
266 olfactory receptor is known (M71), led to increased freezing in presence of acetophenone alone after
267 3 weeks of training, which is characteristic of Pavlovian conditioning (Jones et al., 2008). This learning
268 was accompanied by increased number of neurons expressing the cognate receptor to this odorant,
269 in the olfactory epithelium and olfactory bulb glomeruli. This morphological change is an example of
270 experience-dependent plasticity in the olfactory system, induced by learning, which requires further
271 validation in other models. Surprisingly, in this study, where the perception of a specific odorant was
272 increased in negatively conditioned mice (after 3 weeks of associative learning with foot shocks),
273 there was no indication that the mice were chronically stressed. Work from the same laboratory
274 went on to show this phenomenon was reversible, as the memory extinction was accompanied by a
275 loss of M71 overexpression (Morrison et al., 2015) and might possibly be epigenetically transmitted
276 through the paternal germline (Dias and Ressler, 2014): in other words, the naive offspring of
277 conditioned males displayed increased fear to acetophenone, through sperm-based transmission of
278 information. Some of those spectacular claims will have to be verified through reproduction in other
279 laboratories, as doubts about their validity have been cast due to an improbably high number of
280 reported significant p-values (Francis, 2014). Other investigators have also described examples of
281 plasticity in the mouse brain in response to fear-based learning. Following 3 days of the odour-foot
282 shock paradigm, olfactory bulb nerve outputs in response to the threat-predictive stimulus (i.e. the
283 odour) were altered, thereby facilitating learning of the aversive association (Kass et al., 2013). Using
284 a similar experimental set up in human volunteers (finger electric shocks paired with odours), the
285 hypothesis of aversive conditioning-mediated olfactory neuroplasticity was tested, and olfactory
286 detection threshold was lowered following repeated conditioning events (Parma et al., 2015). Finally,
287 using electro-olfactogram in human to study olfactory mucosa function, unpleasant electric shocks
288 on the wrist administered in the presence of odours led to peripheral alterations in odour sensitivity
289 (Cavazzana et al., 2018), thereby supporting some of the earlier findings in mice described above
290 (Jones et al., 2008). The mechanisms at play are not known but interestingly, to further describe this
291 phenomenology, it should be noted that appetitive (i.e. positive) conditioning might also affect
292 olfactory neuron numbers (Jones et al., 2008). Mice for whom acetophenone was paired with
293 cocaine exposure also had more M71 receptor expression, suggesting this plasticity might be at least
294 partly relevant to stimulus salience and the arousal associated.

295 The fast and efficient detection of odours learnt to be associated with threats could provide a
296 number of adaptive benefits (McGann, 2015). Associative learning is generally considered to affect
297 cortical brain regions involved in memory and emotion processing (e.g. hippocampus). Recent work
298 suggests that sensory systems might be subject to similar anatomical and functional plasticity, during
299 the learning process. The behavioural consequences of those adaptations reflect the changes in
300 olfactory sensitivity and signal processing. It would be interesting to test whether these mechanisms
301 occur in species other than laboratory rodents and humans.

302

303 **Olfaction is affected by chronic stress and associated emotions**

304 Repeated exposure to stressful situations has been shown to affect emotions and perception of
305 odours. This is especially in the case of unpredictable and heterospecific stressors, where little or no
306 learning and habituation can occur, and when there is a perceived lack of control (Weiss, 1972).
307 Chronic stress has global effects on metabolism and the immune system, which can shape and affect
308 the substratum for animal behaviour (the brain) and any epithelium of the body (including the
309 olfactory epithelium). Classically, chronic stress is known to affect brain structures involved in
310 emotion processing and memory formation: the prefrontal cortex, the hippocampus and amygdala
311 (fear associated region). Chronic stress induced alterations of those structures can lead to mood
312 changes and promote the onset of anxio-depressive symptoms in human (Anacker et al., 2011); this
313 can be used to induce anxious- and depressive-like symptoms in experimental animals.

314 Although not classical depression symptoms, a number of olfactory-related anomalies have been
315 identified in depressive patients, from loss of sensitivity (Buron and Bulbena, 2013) to alterations in
316 the pleasant sensation brought by odours (olfactory anhedonia, (Naudin et al., 2012)). A recent
317 meta-analysis finds most published studies agree on this olfactory loss in depressive patients, but the
318 heterogeneity in methods used to assess olfactory function hinders the identification of mechanisms
319 (Taalman et al., 2017). In patients suffering from panic disorders, although odour perception was not
320 reported to be affected, brain imaging studies showed that brain regions were differently activated
321 in response to odours, suggesting alterations in the way the information is processed (Wintermann
322 et al., 2013). Recent work has contributed to the understanding of those phenomena, using models
323 where mood was experimentally altered through chronic stress exposure in laboratory rats. In our
324 laboratory, we recently demonstrated that the first step of olfactory detection was functionally
325 reduced, in the olfactory epithelium following a regimen of chronic variable stress (CVS, with
326 unpredictable stressors), where rats developed depressive-like symptoms (Raynaud et al., 2015). This
327 loss of function was concomitant with a decrease in olfactory neuron cells lost by apoptosis. This
328 work was greatly extended by another team, using a similar model, where it was shown in several
329 tests that CVS led to a reduction of olfactory driven behaviours and changes to a brain region
330 involved in the transmission of olfactory information, the nucleus of the lateral olfactory tract (nLOT,
331 (Vaz et al., 2018)). CVS rats suffered from a loss in olfactory sensitivity towards social or food odours,
332 and exhibited deficiencies in behaviours induced by odours: for instance, there was a loss of
333 attraction and loss of aversion to odours that are respectively innately attractive and repulsive to
334 control rats. Interestingly, following a four-week recovery period, some of those deleterious effects
335 persisted, suggesting chronic stress might have lasting effects on olfactory function. It should be
336 noted that it is difficult to ascertain treatment effects on olfactory changes in depressed animals,
337 since loss of response to odours could indicate a loss of olfactory perception, but also a loss of
338 motivation and emotional reactivity, symptoms also encountered in those models. In addition to
339 those CVS models, the links between olfaction and anxiety/depression have been explored in
340 another model, where mice chronically treated with corticosterone in their drinking water developed
341 anxious and depressive-like disorders (Siopi et al., 2016). Those alterations were accompanied by
342 changes in cell survival in the olfactory bulb and a loss of olfactory discrimination, acuity and memory
343 at the behavioural level. The induction of olfactory loss has also been reported to induce depressive
344 symptoms. Olfactory bulbectomy, the surgical removal of rodent olfactory bulb, has been extensively
345 used as a depression model (Kelly et al., 1997; Song and Leonard, 2005). Although its mechanisms of
346 action are not fully understood, bulbectomy probably involves disruption of the cortico-
347 hippocampal-amygdalar circuit (Song and Leonard, 2005). Also, in rodents (Glinka et al., 2012; Chen

348 et al., 2014) and fish (Abreu et al., 2016) animal models where olfactory sensitivity is reduced
349 through genetic modification of the olfactory detection machinery, anxiety- and depressive-like
350 effects can be detected. One can hypothesise that the sensory deprivation these animals experience
351 might negatively impact their welfare and their mood.

352 Collectively these studies show that fear and negative experiences can alter odour perception, from
353 the sensor (peripheral nervous system) to the interpretation of the sensory input (brain). Bearing in
354 mind that the keeping and production of animals can lead to stress, the next obvious question is
355 whether these laboratory observations are of relevance to other species and whether captive animal
356 welfare could be improved by taking those findings into account.

357

358

359 **3. Odours can induce a stress response**

360 As described above, olfactory signals are potent conditioned stimuli in associative fear learning. In
361 certain species, olfactory signals can also induce fear and stress reactions in an innate fashion. Those
362 semiochemicals serve a wider purpose of communicating information about various aspects of
363 physiology, such as reproductive status, food preference, or the presence of danger. Animals can
364 infer much information from chemical cues emitted by others, and those contribute to many
365 adaptive behavioural responses, depending on the ecological niche and living style of the animal. For
366 a comparative review of those signals in species as different as mouse, insects and the worm
367 *Caenorhabditis elegans*, see a review by Ihara (Ihara et al., 2013).

368

369 **Odours as potential predation signal**

370 Unusual or abnormal environmental signals can cause alarm and induce a stress response. A strong,
371 unpleasant odour or the smell of something known to be dangerous (e.g. fire) will signal a potential
372 danger and can be physiologically translated into a stress response. But the reason why the annual
373 biology department BBQ is not usually a source of intense fear and acute stress is that other
374 contextual cues allow for a more thorough and accurate assessment of the situation (odour of
375 burgers and the sight of friendly colleagues).

376 Strong unpleasant smells can make us feel uncomfortable. For instance, they may signal rotten food,
377 and we seek to avoid them. Butyric acid is the product of fatty acid degradation by bacteria and is
378 well known for smelling like rancid butter or sweaty armpits. TMT (2,5-dihydro-2,4,5-
379 trimethylthiazoline) is also known for its unpleasant scent, and has long been considered to be the
380 main fear-inducing component of fox faeces odour, though this has recently been challenged
381 (Rampin et al., 2018). It is nevertheless recognised for its fear-inducing properties in rodent preys.
382 When rats were allowed to explore a test arena containing butyric acid or TMT in one corner, they
383 avoided spending time near the odorant source (Endres and Fendt, 2009). However, unlike butyric
384 acid, TMT also caused an increase in the amount of time the rats spent freezing. Freezing is a
385 transient immobilisation whilst the rat appears alert, thought to help reducing harm in threatening
386 situations and classically recognised as a fear-related behaviour. This finding therefore shows that it
387 is not only unpleasantness that drives seemingly innate fear behaviours but that certain compounds
388 specifically induce a fear reaction.

389 In addition to using fox faeces, scientist have induced fear in rodents using a variety of odours
390 sourced from diverse predator species (felidae, canidae, bear): cloth rubbed on fur, used bedding,
391 anal gland secretions, faeces and urine (Apfelbach et al., 2005). Preys sensitive to predator odour
392 include wild and domesticated animals from a wide range of species: rodents (mouse, rat, vole),
393 rabbit, hare, goat, deer, beaver, cattle and sheep.

394 Some fear inducing compounds have been purified in predator secretion, in addition to TMT, mostly
395 various pyridine analogues (Brechbuhl et al., 2015) including 2-phenylethylamine (PEA;(Ferrero et al.,
396 2011)). There is evidence that the fear-inducing properties of predator smell are dependent on their
397 meat-based diet, leading to sulphur and nitrogen-rich faeces (Nolte et al., 1994; Berton et al., 1998;
398 Apfelbach et al., 2015; Osada et al., 2015). Urine from coyotes fed melon did not cause the same
399 avoidance reaction as urine from predators fed meat in four prey species: the mountain beaver
400 (*Aplodontia rufa*), the house mouse (*Mus musculus*), the deer mouse (*Peromyscus maniculatus*), and
401 the guinea pig (*Cavia porcellus*) (Nolte et al., 1994). Faeces collected from cats fed either a vegetarian
402 diet or freshly killed mice: exposure to the faeces was tested in an exploratory task using mice, and
403 the meat faeces caused a reduction in exploration and induced more anxious-like behaviours (Berton
404 et al., 1998). The impact of predator scent on prey is also stronger when presented as a complex
405 bouquet of several odours, rather than as single isolated molecular odorants (Apfelbach et al., 2015).

406 Animals sensitive to predator odours display a hormonal stress response, as evidenced by increases
407 in blood pressure and plasma cortisol or corticosterone levels (e.g.(Takahashi, 2014). This stress
408 response will in turn affect various biological function, including brain functions. Behavioural
409 responses of prey to predator odours have been extensively reviewed elsewhere (Apfelbach et al.,
410 2005); these responses include freezing, avoidance behaviour, defensive burying, reduced or delayed
411 eating, and changes in the acoustic startle response (reaction to a sudden loud noise).

412 In rodents, and possibly other species, several structures are dedicated to odour recognition and are
413 involved in fear-related molecule detection (Takahashi, 2014): the main olfactory epithelium, the
414 vomeronasal organ or the Grueneberg ganglia (Brechbuhl et al., 2013; Brechbuhl et al., 2015).
415 Different brain regions will also be activated by different fear-inducing odorants and this may
416 translate into slightly different physiological and behavioural responses (Takahashi, 2014). The neural
417 pathway of TMT-induced fear and stress responses has recently been described in detail (Kondoh et
418 al., 2016). Upon odour detection, the olfactory bulb is directly connected to a brain nucleus related
419 to the amygdala, the amygdalo-piriform transition area, which in turn activates the paraventricular
420 nucleus of the hypothalamus and induces the endocrine stress response (corticosterone production).
421 This neuroendocrine activation pathway is an interesting illustration of the intimate neuroanatomical
422 links between olfactory and stress response pathways.

423 Responses to frightening predator odours vary between individuals. It is thought that antipredator
424 behaviour is primed by genetic factors, and triggered by life experiences. These variations are the
425 product of genetic variability (e.g. in emotional or endocrine responsiveness, or in different species)
426 and life experience (e.g. previous stress exposure). In rat, strain differences have been shown in fear
427 responses to TMT, for instance between Sprague-Dawley, Long-Evans and Wistar rats (all albino
428 laboratory rats), the latter being less responsive in terms of freezing (Rosen et al., 2015). In response
429 to fox faeces, Wistar rats and the pigmented Brown Norway strain differed in the duration and
430 occurrence of freezing behaviour (Rampin et al., 2018). There are several reports that TMT or cat
431 urine do not always induce freezing or avoidance in laboratory and field conditions (Apfelbach et al.,
432 2005). A rarely discussed but plausible explanation could be that over the decades of
433 experimentation with laboratory rats, which are bred captive in small plastic boxes and in the
434 absence of foxes or cats, the innate fear of TMT trait disappeared because of the lack of selective

435 pressure to maintain it. A recent study supports this hypothesis, where after 13 generation of being
436 geographically segregated on an island from their dingo predators (*Canis familiaris dingo*), Northern
437 quolls (*Dasyurus hallucatus*) marsupials were tested for their response to predator odour (Jolly et al.,
438 2018). In comparison to quolls born and raised in the vicinity of dingoes, island quolls exhibited no
439 signs of fear or recognition in response to predator fur, even though they had cohabited for millennia
440 prior to island segregation. Several studies point to the role of past experience. For instance in cows,
441 the response to wolf urine odour (together with auditory stimulation of recorded wolf howling) was
442 increased in animals bred in areas inhabited by wolves, in comparison to naïve animals who had not
443 experienced wolves (Cooke et al., 2013). In other ungulates, the heart rate of elk (*Cervus elaphus*
444 *canadensis*) exposed to various predator odours was highly variable between individuals (Chabot et
445 al., 1996). The effect of life experience also applies to attempts to repel rodents using odour sources:
446 they can become less effective, as there is evidence of habituation following repeated exposure to
447 commercial prey repellent (Apfelbach et al., 2005). Olfactory driven behaviours can be altered in sick
448 individuals. It has been reported that infection with the parasite *Toxoplasma gondii* can lead to
449 alterations in olfactory perception of fear related odours. Infected rats might become less fearful of
450 cat odours (Vyas et al., 2007), thereby leading to higher chances of an unfortunate final meeting with
451 the predator and increased transmission of the parasite. This phenomenon is still a matter of debate
452 (Worth et al., 2013).

453

454 **Odours that signal stress to conspecifics**

455 Many animal species express prosocial behaviours homologous to what is known as empathy in
456 humans. For instance, the prairie vole (*Microtus ochrogaster*) provides support, in the form of
457 grooming, to conspecifics that have suffered from adversity (Burkett et al., 2016). Farmed pigs have
458 been reported to show signs of emotional contagion, both for positive and negative emotions
459 (Reimert et al., 2013). Odorants, either as a single compound or as a mixture, can be used to transmit
460 information about emotional states; these are sometimes referred to as 'alarm pheromones'. This
461 olfactory-based empathy, a form of chemical communication through social signalling of arousal in
462 animal groups, provides an evolutionary advantage, as it warns individuals to possible threats
463 experienced by others. Although admittedly less efficient and slower than other visual or auditory
464 signals, those odorant molecules are the volatile homologues of sonic alarm calls and one could
465 hypothesise they are stealthier (if they do not spread too far), preventing the advertisement of one's
466 presence to predators.

467 Some of those olfactory signals can be detected in faeces and urine. Naïve rats can distinguish
468 between faeces from chronically or acutely stressed and non-stressed conspecifics (Valenta and
469 Rigby, 1968; Mackay-Sim and Laing, 1980; Bombail et al., 2018a). In addition, rats can also distinguish
470 faeces from stressed chickens (Bombail 2018), suggesting the potential for heterospecific recognition
471 of distress. Urine also contains distress signals, transmitting negative experiences to naïve
472 conspecifics, as has been demonstrated in species of agricultural interest. In pigs, when a food
473 dispenser was sprayed with urine from a restrained animal, the latency to feed from it was longer
474 than when sprayed with control urine, a sign of distress (Vieuille-Thomas and Signoret, 1992). In an
475 experiment with cows, naïve individuals also exhibited a longer latency to feed in the presence of
476 urine from a heifer subjected to electric shocks (Boissy et al., 1998). The smell of stressed heifer urine
477 also induced a rise in cortisol levels in the exposed animals. Alarm pheromones are not limited to
478 animal dejections: various stressors have been shown to cause the production of an airborne signal
479 inducing a number of physiological and behavioural stress responses in naïve rats (Kiyokawa et al.,

480 2013). In anaesthetised rats, electric stimulation of the perianal gland lead to the production of this
481 alarm pheromone (Kiyokawa et al., 2013; Inagaki et al., 2014).

482 As seen above, some of those odorant signals may originate from glands. Analytical chemistry has
483 allowed the identification of some alarm substances. In the case of the rat alarm pheromone, a
484 mixture of two compounds (4-methylpentanal and hexanal) is reported to be responsible for the
485 stress information transfer (Inagaki et al., 2014). Stressed mice secrete a sulphur-containing volatile
486 compound that shares chemical features with compounds from the urine of meat-eating predators
487 (2-sec-butyl-4,5-dihydrothiazoles). Mice use it to signal non-specific dangers to others, as this alarm
488 signal is detected by the Grueneberg ganglia (Brechbuhl et al., 2013). It has been hypothesised that
489 some of these odorants have been evolutionarily borrowed from predator species, hence their
490 categorisation as not only pheromones but also kairomones, a substance benefiting a receiver from
491 another species (Brechbuhl et al., 2013). Additionally, adrenaline might induce sweating, affecting
492 body odour, thereby leaving chemical cues about emotional states. Stress is known to increase
493 micturition (Smith et al., 2011a) and defecation (Sanger et al., 2000), perhaps promoting the
494 spreading of such olfactory messages. Chronic stress alters immunity at the level of epithelial tissue
495 (e.g. digestive tract or skin), thereby altering the qualitative and quantitative properties of the
496 microbial communities living there and therefore the odorant metabolites they release. Stress
497 odours may thus arise from alterations in the balance of metabolite levels, rather than being the
498 appearance of novel compounds (Sakuma et al., 2013; Bombail et al., 2018a).

499 A meta-analysis of 26 studies suggests that humans may also be capable of communicating
500 emotional states and detecting stress-related emotions (fear and anxiety) via body odours (de Groot
501 and Smeets, 2017). However, the question of human pheromones is a matter of debate (Wysocki and
502 Preti, 2004) since, although some responses have been identified (some are referenced in de Groot
503 and Smeets, 2017), **the vomeronasal organ putatively involved in pheromone detection might only
504 be vestigial in humans** (Trotier et al., 2000; Liman and Innan, 2003; Zhang and Webb, 2003). If there
505 is a significant pheromone effect, it is still controversial and human fear might be better
506 communicated via auditory and visual signals.

507 Attempts have been made to harness some of this knowledge for a few practical applications.
508 Research has been carried out to attempt to regulate the impact of rodent pests to protect
509 agricultural crops and plantations. For instance exposing faecal predator odours (coyote) on sheep
510 and cattle might prevent grazing in certain areas (Pfister et al., 1990). But other aspects of the
511 predator-prey relationship can be explored. Studies of African wild dog (*Lycaon pictus*) in a zoo show
512 that an endocrine stress response can be induced by exposure to prey odour such as gazelle. Not all
513 manifestations of stress are detrimental, it is thought a **rise** in cortisol levels may also simply reflect
514 arousal caused by a novel situation, and that this could be used for enrichment purposes (Rafacz and
515 Santymire, 2014). There are clearly potential applications and exciting knowledge to be gained from
516 the world of semiochemicals.

517

518 **4. Can odours be calming and reduce the effect of the stress response?**

519 Although stress is an adaptive response that prolongs life in the wild, it can have negative
520 physiological and behavioural consequences. Several types of substances are available in the
521 pharmacopeia to modulate targets in the endocrine and nervous systems in order to treat
522 pathological manifestations and consequences of chronic, inappropriate or exacerbated stress
523 responses. Pharmacological treatments can sometimes provide relief and reduce anxiety or
524 depression. Beta blockers reduce the effects of adrenal catecholamines and can dampen

525 physiological and psychological effects of the acute stress response (Middlemiss et al., 1981).
526 Selective serotonin reuptake inhibitors (SSRI, aka Prozac) are reported to reduce in some cases the
527 depressive symptoms, often precipitated by chronic stress (Arroll et al., 2009). We have seen how
528 odours can influence animal mood and behaviour in the case of innate and learnt negative
529 associations. Can odours reduce the stress response and its manifestations? Historically, other
530 cultures with an alternative view of the medical process have used inhalation aromatherapy for the
531 treatment of diverse ailments. Many plant-derived products have remarkable pharmacological
532 effects when used topically or internally, but here I shall solely discuss inhalation of odorant
533 compounds and the treatment of exacerbated stress response by the odorant sensation. The practice
534 is sometimes referred to as aromachology, defined as 'olfactory effects that have been scientifically
535 demonstrated to affect mood, physiology and behavior' (Herz, 2009). Several studies point towards a
536 role for odours in the amelioration of stress effects using various experimental models, and in the
537 following, evidence for and against these claims will be reviewed. It should be added, that the
538 phenomenon is distinct from social buffering where the presence of socially related animals appears
539 to provide anxiolytic effects in experimental animals, a process that (sometimes) involves olfactory
540 cues of animal origin (Kiyokawa and Hennessy, 2018).

541

542 **Evidence that odours act to attenuate the impact of stress and improve animal mood**

543 Oils extracted from citrus fruits have been a popular source of odorants in stress-relief research.
544 Essential oils from bergamot, *Citrus bergamia*, are reported to have beneficial effects on anxiety and
545 depression symptoms (Navarra et al., 2015). A study in rats describes that bergamot oil inhalation
546 reduced expression of anxiety in behavioural tests and reduced corticosterone level (Saiyudthong
547 and Marsden, 2011). Rats that were exposed to lemon oil vapours showed reduced immobility time
548 in the Porsolt forced swim test (Porsolt et al., 1977), a marker of antidepressant activity (Komori et
549 al., 1995). This reduction in immobility time was confirmed independently in mice (Komiya et al.,
550 2006). Lemon oil also had anxiolytic effects in an elevated plus maze task (Komiya et al., 2006). In
551 this latter study, lavender and rose oils did not produce any anti-depressant or anxiolytic effects.
552 However, rose oil had anxiolytic effects in rats in another report (de Almeida et al., 2004) and was
553 also shown to reduce the corticosterone induction response (Fukada et al., 2012). Besides essential
554 oils, another odour has been tested in several stress models: a mixture of trans-2-hexenal and cis-3-
555 hexenol called Green Odour (GO), which evokes in humans the smell of fresh cut grass. In models of
556 prenatal stress, application of stressors to gravid dams sometimes leads to depressive-like
557 behaviours in offspring (Fujita et al., 2010). Following 10 consecutive days of restraint stress during
558 the second half of gestation, dams gave birth to offspring that developed abnormal stress plasma
559 markers responses and depressive-like symptoms. A parallel group of stressed dams treated with GO
560 during the stress exposure regimen gave birth to pups that did not develop such stress-related traits
561 (Fujita et al., 2010), suggesting GO countered the effect of restraint stress. **In rats, chronic stress for
562 10 days in a 3-minute forced swim test increased immobility times, a marker of depressive-like
563 states.** GO exposure re-established locomotor behaviour, suggesting a loss in learned helplessness
564 and anti-depressant effects (Watanabe et al., 2011). Rats pre-exposed to GO before exposure to
565 stressors (TMT odours or electric foot shock treatment) showed a lower stress response than non-GO
566 exposed conspecifics (Nikaido et al., 2011). Finally, rats subjected to a fear conditioning paradigm
567 aimed at modelling Post Traumatic Stress Disorder (PTSD) display ameliorated symptoms following
568 GO exposure (Nikaido et al., 2016). There are also reports of odour-mediated stress relief in humans.
569 Men and women waiting for their dentist appointment were asked to fill questionnaires about their
570 inner state and emotions while sat in the dentist waiting room, in the unannounced presence or

571 absence of orange oil, *Citrus sinensis* (Lehrner et al., 2000). Men were not affected by the presence of
572 odours, but female patients reported less anxiety, a more positive mood, and a higher level of
573 calmness. There is anecdotal evidence that odour exposure during therapy sessions can help patients
574 suffering from PTSD (Daniels and Vermetten, 2016).

575

576 **Critical analysis of the literature**

577 With so many studies in peer-reviewed scientific journals, it seems surprising that the claim of an
578 odorant-based therapeutic strategy for acute and chronic stress relief has not been taken up by a
579 larger fraction of the scientific and medical community. The mechanisms of action are still unclear,
580 yet the claim that odours might have antidepressant and anxiolytic action in experimental animals
581 requires some mechanistic foundation. Components of the GO affected dopamine release in brain
582 slices and PC12 cell culture (Kako et al., 2008; Kobayashi et al., 2010). A great issue here is that these
583 are not olfactory effects, as the cells and tissues were isolated in a dish; these were pharmacology
584 experiments. In a follow up study, rats with brain cannulae that allowed *in vivo* dopamine sampling,
585 were exposed to GO and this induced neurotransmitter release (Kako et al., 2011). Studies in animal
586 models by use of pharmacological tools, showed **that** exposure to odours modulated dopamine and
587 serotonin release and their receptors *in vivo* (Komiya et al., 2006). However, in all these studies,
588 there is a lack of negative controls (a comparison to other odours that might not have any effect), so
589 it could just **be that any odour arousing** the animal leads to striatal dopamine release. Modulation of
590 neurotransmitter action would constitute a possible mechanism for odour stress relief, but this has
591 not yet been clearly demonstrated. What would be the mechanical basis for some commonalities in
592 reported **effects** for such different odours as GO (Fujita et al., 2010), rose (de Almeida et al., 2004)
593 and lemon (Komiya et al., 2006): would the same **olfactory detection and stress-regulating brain**
594 **pathways be activated**? These are not the only questions raised by this literature. There are
595 inconsistencies as to what odours have what effect (e.g. rose oil has anxiolytic effects in some
596 reports but not others), and differences in odorous compound volatility are not acknowledged as
597 possible confounding factor (Komiya et al., 2006). Why would the same odorant have differing
598 properties? There are inconsistencies in terms of protocols used: diversity of control odours, e.g.
599 triethyl citrate (Fujita et al., 2010) and butyric acid (Watanabe et al., 2011) or distilled water (Nikaido
600 et al., 2011), diversity of behavioural effects and endocrine endpoints. All **these factors** make
601 comparisons difficult. Also, it is challenging to preclude the spreading of odour treatments, therefore
602 experiments cannot be fully **blind**, a requisite for stringent scientific testing. Odorant concentrations
603 are difficult to ascertain from the methodology descriptions and can be difficult to standardise.
604 Sometimes the effect is attained with very high amounts of an odorant, at 10 and 100% odorant
605 saturation in the air (Tashiro et al., 2016). It might be that high doses of odorant in the air can enter
606 the bloodstream and therefore have pharmacological rather **than** olfactory effects; in this case, a
607 comparison where the compounds are administered by ingestion or injection would be necessary.

608 In human experiments, such effects of odours could be attributed to the pleasant element of odour
609 exposure, perhaps helping patients dealing with adversity and the awaited loss of control of, for
610 example, sitting in a dentist chair. In PTSD sufferers during a therapy session, pleasant odours might
611 have grounding and calming effects, a distraction from the negative experience of **recalling a**
612 **traumatic event. Experimental odours might simply cover the unpleasant environmental smells (the**
613 **eugenol from the dentist surgery or dirty cage litter for animals).** It could be that in humans, sensory
614 pleasure or aesthetics allow us to face adversity and to be more resilient to stress. **The hypothesis**
615 **that pleasantness acts in stress relief should also be tested in animals, taking into account individual**
616 **odour preference variability.** Could it also be that laboratory animals whose ancestors have not

617 experienced the outside world for generations feel soothed by smelling the odour of pleasant
618 essential oils or fresh cut grass? A plausible mechanism for the stress relief observed in experimental
619 animals may be that the presence of an odour act as an environmental enrichment during a stressful
620 situation, in an otherwise relatively barren experimental cage (Wells and Hepper, 2017). These
621 alternative explanations ought to be rigorously tested, in order to discriminate between **direct (the**
622 **odorant specifically acting on stress centres)** and indirect effects (e.g. masking effect or enrichment,
623 **memory recall).**

624 A meta-analysis of six aromatherapy studies failed to detect any treatment effect (Cooke and Ernst,
625 2000). Massages in conjunction with aromatherapy may have had a positive therapeutic effect, but
626 massage alone also did, and there was only evidence of a very modest transient anxiolytic effect.
627 Again, treatment blinding is impossible and there may have been flaws in study design (Cooke and
628 Ernst, 2000). The Cochrane collaboration concentrates on reviewing medical data through
629 comprehensive large-scale meta-analysis studies, in order to critically assess the efficacy of
630 treatments and promote evidence-based medical care. A recent search through the Cochrane
631 database (www.cochrane.org) failed to find any significant effect of inhalation aromatherapy. The
632 evidence was at best 'equivocal' (if not ineffective) for alleviating suffering in dementia and in labour
633 pain management (Smith et al., 2011b; Forrester et al., 2014). These meta-analyses are not
634 supporting measurable effects of inhalation aromatherapy, but absence of proof is not proof of
635 absence and further research, with adequate and stronger study design, is needed. Human studies
636 are complex, and because these are challenging and costly to conduct, it often leads to **low-power**
637 studies, which can further cloud the question. There is a strong tendency towards publication of
638 positive results (e.g. practices such as p-hacking, (Head et al., 2015), and several biases can have
639 detrimental consequences in terms of assessing the validity of scientific claims (Ioannidis, 2005), but
640 a number of recommendations have been made (Ioannidis, 2014; Head et al., 2015).

641

642 **Appeasing pheromones**

643 In addition to the use plant-derived odorant compounds described above, there have been attempts
644 at attenuating the consequences of the stress response using animal-derived volatile molecules with
645 alleged 'pheromone' properties. A few published studies support the efficacy of those pheromones,
646 which are commercially available in numerous formulations, and advertised for use in several farm
647 and pet species, for a large number of indications. In general, those studies consist in using
648 'appeasing pheromones' (usually synthetic analogue preparations of compounds found on the skin of
649 conspecifics) in a stressful situation using collars or sprays, and identify an effect on stress- or
650 performance-related indicators. A dog appeasing pheromone mimicking molecules found on the
651 mammary area of lactating bitches **(composed of myristic acid, lauric acid, pentadecanoic acid, and**
652 **stearic acid, (Pageat and Gaultier, 2003))** was used on Beagle dogs subjected to fear-inducing
653 thunderstorm recordings. This led to a reduction in behavioural fear and anxiety indicators, in
654 comparison to a control group (Landsberg et al., 2015). Appeasing pheromone also appeared to
655 provide relief in newly adopted dog pups, animals fitted with a pheromone collar exhibited fewer
656 vocal signs of distress (Gaultier et al., 2008). However, in another study, dog appeasing pheromone
657 did not have any effect on the behavioural effect of stress, in military dogs experiencing the
658 transition from foster care to military training (Broach and Dunham, 2016). A bovine appeasing
659 pheromone has also been described. Dairy cows who experienced the seasonal transition from an
660 indoor environment to outdoor pastures exhibited a mild decrease in milk production, possibly
661 through the mild stress induced by novelty. Application of bovine appeasing pheromone improved
662 milk production parameters (Osella et al., 2018). At the stressful time of weaning in pigs, exposure to

663 a compound mixture analogue to sow skin secretion, improved feeding and growth performance
664 within 48h (McGlone and Anderson, 2002). This commercial preparation is made of various fatty
665 acids: hexadecanoic acid, cis-9-octa decenic acid, 9,12-octyladecanoic acid, dodecanoic acid,
666 tetradecanoic acid, and decanoic acid (Pageat, 2001). Interestingly, pigs might also be affected by
667 rabbit-derived odours. A compound produced by the lactating doe (2-methyl-2-butenal, Schaal et al.,
668 2003) had similar effects on pigs as above, food intake and weight gain were improved by compound
669 exposure (McGlone et al., 2017). It should be noted that a pheromone is a signal involved in
670 intraspecific communication, if it has effects within one species, it does not necessarily mean that
671 this molecule has a biological effect on others. If those compounds are indeed a type of
672 semiochemicals (not pheromones), one should question the evolutionary history of chemical
673 communication between rabbits and pigs, and the significance such rabbit-derived compound might
674 have for the recipient. A possibility is that the olfactory properties of the compounds (fatty acid
675 mixtures) are similar, that they activate similar olfactory receptors, and might be appeasing across
676 species because they might be reminiscent of maternal odours, but this remains to be tested. In a
677 meta-analysis of various treatments used against cat urine spraying, a stress-related behaviour,
678 pheromone therapy was found to be effective using secondary outcome measures, albeit to a lower
679 degree of effectiveness than psychopharmacological treatment (Mills et al., 2011). The only
680 systematic study on the effect of pheromone treatment in dogs and cats identified several potential
681 design and data analysis flaws, indicating that out of 14 studies, only one provided adequate proof
682 for treatment efficacy (Frank et al., 2010). Further concerns have been voiced by others about the
683 validity of some of the claims for pheromone efficacy (Hewson, 2014). Commercial success differs
684 from clinical effectiveness and given the large economic interests at stake, the large scale and multi
685 centre, independent assessment of treatment efficacy should be carried out. There is no information
686 available on how those putative pheromone substances might act, improved mechanistic
687 understanding should shed light on this application of olfactory biology.

688 Overall there is no clear evidence so far to show odour exposure works spontaneously and effectively
689 for stress relief at the population level; However, are there specific phenotypes of individuals for
690 whom this treatment might work? Might this be associated to other variables e.g. stress resilience or
691 olfactory performance? As we have seen above, there is no such thing as a typical stress response, as
692 animals will differ in terms of physiology and life experience. It is perhaps illusory to use 'one size fits
693 all' solutions for such a polymorphic issue as stress relief. Perhaps it would also be interesting to
694 assess whether the alleged spontaneous effects of those substances differ in animals whose olfactory
695 perception might be altered, such as in the stress or mood –related disorders.

696

697 **Another way to use odours in stress relief?**

698 The lack of solid reproducible evidence warrants caution in generalising this alleged effect of
699 spontaneous odour exposure on stress. Besides the enrichment hypothesis (above), associative
700 learning could provide interesting tools for modulating animal mood. Negative association with an
701 aversive stimulus (e.g. foot shock and odour) that induces a stress response is a well-established tool
702 to study memory and stress biology. Can animals learn to associate a positive emotional state with
703 an odour and can this be used for stress relief? This positive conditioning has been shown in certain
704 cases for pigs (Oostindjer et al., 2011). Young animals whose mothers had been fed flavoured food
705 during late gestation and lactation showed lower behavioural and hormonal signs of stress response
706 upon weaning when the same odour was presented. Likewise, since maternal odour is critical in early
707 life attachment (Beery and Kaufer, 2015), it could be hypothesised that this reminiscence of the
708 maternal environment and early life experience through olfaction might be a mechanism through

709 which compounds of lactating female origin with putative 'appeasing pheromones' properties might
710 operate to reduce stress, rather than through intra-specific communication with semiochemicals (see
711 above). Critically, one should wonder about the evolutionary pressures having led to the selection for
712 this alleged phenomenon, consisting in chemical signals from the mother to the weaned or adult
713 offspring. One can question whether the conditioning of positive emotions is as strong as the
714 conditioning of negative emotions, given the strong evolutionary drive that favours the latter, for
715 species survival. It would be interesting to study whether other challenging situations can be made
716 less stressful through this seemingly comforting role of odour application. Attempts to associate a
717 positive affective state with an odour are currently underway (Bombail et al., 2018b).

718 Under the same label of inhalation aromatherapy, aromachology or pheromone therapy, it could be
719 that a lack of critical thinking has led to the conflation of various biological phenomena: an alleged
720 spontaneous olfactory effect on stress relief, environmental enrichment or the recall of positive
721 emotional states. It would be easy to believe that stressed animals can be soothed by odours as
722 easily as it is to light a patchouli candle, and statements such as '(aromatherapy) is a natural way of
723 healing a person's mind, body and soul' (Ali et al., 2015) can be misleading. To quote a great soul
724 music artist 'When you believe in things that you don't understand/Then you suffer/ Superstition
725 ain't the way' (Superstition by Stevie Wonder, 1972). There might be a case for the use of odours in
726 management of adverse and pathological effects of the stress response (e.g. (Salesse, 2017)), but a
727 better understanding of the phenomena and mechanisms at play is essential, and this requires
728 further systematic methodological appraisal.

729

730 5. Conclusion.

731 Animal species managed by humans use olfaction and exhibit stress responses. The inner state of
732 animals and their emotions alters their perception, while perceived odours can sometimes affect
733 their mood and well-being. Several fascinating questions and opportunities lie at the intersection
734 between the study of perception and emotions. Some of this knowledge should be incorporated into
735 research and animal care practices, to better improve animal welfare by understanding their
736 olfactory world. Examples have already been described elsewhere (Nielsen et al., 2015a) and some
737 will be reiterated, in the light of what has been covered here:

- 738 - If stressed or depressed animals perceive odour differently, then several aspects of olfaction-
739 driven behaviours will be impacted : their appetite or sex drive might be reduced, their social
740 recognition of conspecifics might be altered and this may lead to increased aggression,
741 odour-based parent-offspring recognition might also be affected. Could the use of olfactory
742 cues (e.g. to enrich the environment or train olfactory function) improve those behaviours?
- 743 - Stress-related odours could be detected in animal breeding facilities, as a non-invasive
744 indicator. Odours can also provide information on disease and reproductive status,
- 745 - Exposure to stress-related or predator odours should be avoided to prevent emotional
746 contagion,
- 747 - Olfactory enrichment and possibly appetitive olfactory conditioning could be used as ways to
748 diversify the sensory world of captive species and contribute towards their well being

749 I am confident this list will be expanded through our creativity and fearlessness to engage in cross
750 disciplinary thinking.

751

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757

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