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The effects of smoking on whisker movements: A quantitative measure of exploratory behaviour in rodents

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ABSTRACT

Nicotine, an important component of cigarette smoke, is a neurotransmitter that contributes to stress, depression and anxiety in smokers. In rodents, it increases anxiety and reduces exploratory behaviours. However, so far, the measurements of exploratory behaviour in rodents have only been semi-quantitative and lacking in sufficient detail to characterise the temporal effect of smoking cessation. As rodents, such as mice and rats, primarily use whiskers to explore their environment, we studied the effect of 3 months smoking with 1 and 2 weeks smoking cessation on whisker movements in mice, using high-speed video camera footage and image analysis. Both protraction and retraction whisker velocities were increased in smoking mice ($p < 0.001$) and returned to normal following just one week of smoking cessation. In addition, locomotion speeds were decreased in smoking mice, and returned to normal following smoking cessation. Lung function was also impacted by smoking and remained impaired even following smoking cessation. We suggest that the increased whisker velocities in the smoking mice reflect reduced exploration and impeded tactile performance. The increase in whisker velocity with smoking, and its reduction following smoking cessation, also lends support to acetylcholine being involved in awareness, attention and alertness pathways. It also shows that smoking-induced behavioural changes can be reversed with smoking cessation, which may have implications for human smokers.

1. Introduction

Tobacco smoking is a serious health problem and one of the major causes of death worldwide (Vella and Di Giovanni, 2013). While smoking can reduce anxiety and relieve stress (Picciotto et al., 2002), nicotine in cigarette smoke also has noxious effects, such as increasing anxiety and depression following chronic use and withdrawal (Casarrubea et al., 2015; Picciotto et al., 2002). Despite its potential noxious effects, nicotine intake is reinforced via the dopaminergic system (Corrigall et al., 1992; Di Chiara, 2000; Maskos et al., 2005; Tolu et al., 2013; Faure et al., 2014). It acts by binding with the nicotinic acetylcholine receptors (nAChRs), which mediate dopamine release and other neurotransmittors, such as serotonin and glutamate (Pierucci et al., 2004; Lester, 2014). Different patterns of neurotransmitter release occur depending on the course of nicotine administration (acute, chronic and withdrawal) and this partly accounts for the complex behavioural effects of nicotine on anxiety and depression. In addition, the distribution of nAChRs throughout the brain also means that nicotine administration can cause a variety of behavioural responses in both animals and humans (Mcdermott et al., 2013; Casarrubea et al., 2015).

In rodents, the administration of nicotine to regions of the brain that are associated with reward, such as the central amygdala (Zarrindast et al., 2008), lateral septal nucleus (Ouagazzal et al., 1999), dorsal raphe nucleus (Cheeta et al., 2001) and different areas of the mesolimbic dopaminergic system (Picciotto et al., 2002), has induced behaviours associated with anxiety, including a reduction in exploratory behaviours (Bättig et al., 1976; Casarrubea et al., 2015; Mesa-Gresa et al., 2013). Exploratory behaviours are usually approximated by measuring the duration and frequency of a range of movements, including rearing, head-dipping, grooming,

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climbing, sniffing and licking, during open field or hole-board tests (Casarrubea et al., 2015). In particular, head-dipping has been found to reduce significantly in rodents treated with nicotine in hole-board tests (Casarrubea et al., 2015; Piri et al., 2011; ve Yöntem and Albino, 2014), and is thought of as a reduction in exploration of the holes and floor. In healthy rodents, head-dipping (or "dabbing") has been associated with whisker exploration of the floor (Arkley et al., 2014; Grant et al., 2009, 2012b), as the whiskers are the primary tactile organ in nocturnal rodents (Roohbakhsh et al., 2016). Measuring duration and frequencies of exploratory behaviours, such as head-dipping is thought to not be sufficient to wholly characterise the complex effects of smoking and smoking cessation on behaviour (Casarrubea et al., 2015). Rather, an enhanced quantification of exploration is needed, and we propose that measuring precise changes in whisker movements in rodents might well offer this alternative.

Whiskers in rats and mice move backwards and forwards in a behaviour known as whisking, which occurs up to 25 times per second (Vincent, 1912). Studies have found that rodents use their whiskers to guide many tasks such as locomotion, navigation, foraging and hunting (Grant and Arkley 2016). With the development of high-speed video cameras and analysis programs, it has become apparent that rodents do not just make simple sweeping movements with their whiskers. Rather, they can precisely change the amplitude, velocity and position of their whiskers during locomotion and object exploration (Arkley et al., 2014; Carvell and Simons, 1995; Grant et al., 2009; Hartmann, 2001; Kleinfeld et al., 2006; Mitchinson et al., 2007; Szwed et al., 2003; Towal and Hartmann, 2008; Welker, 1964). For example, object exploration is generally associated with slower whisker movements at lower amplitudes (Carvell and Simons, 1995; Grant et al., 2009). Following an object contact, sensory information from the whisker shaft, such as force and direction, is transmitted in the follicle and passed through multiple neural pathways to the cortex (Grant and Arkley 2016). The organisation of cholinergic neurons throughout whisker-related sensorimotor areas in rodents (Beak et al., 2010), including brainstem, thalamus, (Timofeeva et al., 2005; Bosman et al., 2011), cortex (Bosman et al., 2011), cerebellum (Timofeeva et al., 2005), zona incerta and amygdala (Bosman et al., 2011) indicates that nicotine may well have an effect on whisker sensorimotor integration.

Finding a quantitative way to measure exploratory behaviours, by measuring whisker movements, would offer the ability to capture the complex effects of smoking and nicotine administration on rodents. As nicotine has been found to affect general exploratory behaviours in rodents (Bättig et al., 1976; Casarrubea et al., 2015; Mesa-Gresa et al., 2013), it is to be expected that whisker movements, being the primary mode of exploration, will also be affected by nicotine and smoking. This study will, for the first time, explore the effect of chronic smoking, the most important source of nicotine in humans, on whisker movements in mice. Previous studies have documented that nicotine results in a reduction in general exploratory behaviours in rodents (Bättig et al., 1976; Casarrubea et al., 2015), which we predict to be represented here by faster moving whiskers (Carvell and Simons, 1995; Grant et al., 2009; Mitchinson et al., 2007). A novel behavioural system that tracks and non-invasively measures whisker movements (Grant et al., 2014) will be used to obtain a quantitative measure of the impact of smoking and smoking cessation on exploratory whisker movements in mice.

2. Methods

All experimental procedures were approved by the Ethical Committee of Animal Experiments of the KU Leuven.

2.1. Animals

Forty male C57Bl6 mice were used in this study. Animals were housed on a 12-hour light-dark cycle and supplied with pelleted food and water *ad libitum*.

2.2. Smoking procedures

Animals were randomly assigned to the following groups: Control (C: n = 10), Smoking (S: n = 11), Smoking cessation for 1 week (S1W: n = 9) and Smoking cessation for 2 weeks (S2W: n = 10). Smoking was selected as the nicotine administration technique, as it is the most common way people are exposed to elevated levels of nicotine. Smoking animals were exposed to cigarette smoke (3R4F research cigarettes with filter purchased from Kentucky Tobacco Research and Development Center, University of Kentucky) using a nose-only exposure system (InExpose System, Scireq). Mice were placed in soft restraints and connected to an exposure tower. A cigarette puff was generated every minute, leading to 10 s of cigarette smoke exposure followed by 50 s of fresh air. Mice were acclimatized to the cigarette smoke exposure during the first week of the experiment. Afterwards, animals were exposed daily to four cigarettes, twice a day, 5 days per week, over 3 months (Rinaldi et al., 2012). Control animals were treated similarly, but were exposed to filtered air for the same duration. Animals in the smoking cessation groups stopped smoking for 1 or 2 weeks. As nicotine withdrawal behaviours are usually absent from 5 to 6 days (Damaj et al., 2003), the one-week time-point was selected as a minimum, and the two-week time-point was selected as an additional measure. Smoking and control mice were exposed to cigarette smoke or filtered air, respectively on the morning of their behavioural assessment and tested approximately 2 h after the smoking or filtered air treatment. Any stress caused by restraint in the experimental set-up was, therefore, equivalent between the smoking and control groups. The total particle density concentration of the cigarette smoke in the tower was measured weekly and was on average 149.5 mg total particulate matter per m^3. Mice were weighed weekly to ensure they maintained a healthy body mass for inclusion in the study. Two mice in the S1W group did not survive the smoking protocol.

2.3. Recording and measuring behaviour

Each mouse was placed in to a transparent, Perspex, rectangular arena (20 × 30 × 15 cm) (Fig. 1a), which was lit from below by a bright, normal-spectrum light box (PHLOX LEDW-BL-400/200-SLLUB-Q-1R–24 V). The mouse was filmed from above using a digital high-speed video camera (Phantom Miro ex2) recording at 500 frames per second with a shutter-speed of 1 ms and a resolution of 640 × 480 pixels. Multiple 1-s video clips were collected opportunistically (by manual trigger) when the animal moved in the field of view of the camera. Approximately 16 clips were collected from each animal. Four to six clips from each mouse were selected and trimmed based on to the following selection criteria developed in Grant et al. (2014): i) the mouse was clearly in frame; ii) both sides of the face were visible; iii) the head was level with the floor (no extreme pitch or yaw); iv) the whiskers were not in contact with a vertical wall; and v) the mouse was clearly moving forward. Six of the eleven smoking animals (S) could not be included in the study as their whiskers were barbered by a conspecific and thus could not be imaged. Barbering is not usually associated with stress, but rather caused by a particularly dominant animal in the home cage (Bresnahan et al., 1983). While barbering is relatively rare, to overcome this in future studies it is recommended to remove the dominant individual from the home cage, or to house mice singularly, a month before filming. This left a sample size of 32 animals