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1 **The effects of smoking on whisker movements: a quantitative measure of**  
2 **exploratory behaviour in rodents**

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18 **Keywords: smoking, mouse, vibrissae, velocity, active sensing, exploration**

## 19 **Abstract**

20 Nicotine, an important component of cigarette smoke, is a neurotransmitter that contributes to  
21 stress, depression and anxiety in smokers. In rodents, it increases anxiety and reduces  
22 exploratory behaviours. However, so far, the measurements of exploratory behaviour in  
23 rodents have only been semi-quantitative and lacking in sufficient detail to characterise the  
24 temporal effect of smoking cessation. As rodents, such as mice and rats, primarily use  
25 whiskers to explore their environment, we studied the effect of 3 months smoking with 1 and  
26 2 weeks smoking cessation on whisker movements in mice, using high-speed video camera  
27 footage and image analysis. Both protraction and retraction whisker velocities were increased  
28 in smoking mice ( $p < 0.001$ ) and returned to normal following just one week of smoking  
29 cessation. In addition, locomotion speeds were decreased in smoking mice, and returned to  
30 normal following smoking cessation. Lung function was also impacted by smoking and  
31 remained impaired even following smoking cessation. We suggest that the increased whisker  
32 velocities in the smoking mice reflect reduced exploration and impeded tactile performance.  
33 The increase in whisker velocity with smoking, and its reduction following smoking  
34 cessation, also lends support to acetylcholine being involved in awareness, attention and  
35 alertness pathways. It also shows that smoking-induced behavioural changes can be reversed  
36 with smoking cessation, which may have implications for human smokers.

## 37        **1. Introduction**

38 Tobacco smoking is a serious health problem and one of the major causes of death worldwide  
39 (Vella and Di Giovanni, 2013). While smoking can reduce anxiety and relieve stress (Piciotto  
40 et al. 2002), nicotine in cigarette smoke also has noxious effects, such as increasing anxiety  
41 and depression following chronic use and withdrawal (Casarrubea et al., 2015; Piciotto et al.  
42 2002). Despite its potential noxious effects, nicotine intake is reinforced via the dopaminergic  
43 system (Corrigall et al., 1992; Di Chiara, 2000; Maskos et al. 2005; Tolu et al. 2013; Faure et  
44 al. 2014). It acts by binding with the nicotinic acetylcholine receptors (nAChRs), which  
45 mediate dopamine release and other neurotransmitters, such as serotonin and glutamate  
46 (Pierucci et al., 2004; Lester, 2014). Different patterns of neurotransmitter release occur  
47 depending on the course of nicotine administration (acute, chronic and withdrawal) and this  
48 partly accounts for the complex behavioural effects of nicotine on anxiety and depression. In  
49 addition, the distribution of nAChRs throughout the brain also means that nicotine  
50 administration can cause a variety of behavioural responses in both animals and humans  
51 (McDermott et al., 2013; Casarrubea et al., 2015).

52 In rodents, the administration of nicotine to regions of the brain that are associated with  
53 reward, such as the central amygdala (Zarrindast et al., 2008), lateral septal nucleus  
54 (Ouagazzal et al., 1999), dorsal raphe nucleus (Cheeta et al., 2001) and different areas of the  
55 mesolimbic dopaminergic system (Piciotto et al., 2002), has induced behaviours associated  
56 with anxiety, including a reduction in exploratory behaviours (Battig et al. 1976; Casarrubea  
57 et al., 2015; Mesa-Gresa et al. 2013). Exploratory behaviours are usually approximated by  
58 measuring the duration and frequency of a range of movements, including rearing, head-  
59 dipping, grooming, climbing, sniffing and licking, during open field or hole-board tests  
60 (Casarrubea et al., 2015). In particular, head-dipping has been found to reduce significantly in

61 rodents treated with nicotine in hole-board tests (Casarrubea et al., 2015; Piri et al. 2011; ve  
62 Yontem et al. 2014), and is thought of as a reduction in exploration of the holes and floor. In  
63 healthy rodents, head-dipping (or “dabbing”) has been associated with whisker exploration of  
64 the floor (Arkley et al., 2014; Grant et al., 2009; 2012b), as the whiskers are the primary  
65 tactile organ in nocturnal rodents (Roohbakhsh et al. 2016). Measuring duration and  
66 frequencies of exploratory behaviours, such as head-dipping is thought to not be sufficient to  
67 wholly characterise the complex effects of smoking and smoking cessation on behaviour  
68 (Casarrubea et al., 2015). Rather, an enhanced quantification of exploration is needed, and we  
69 propose that measuring precise changes in whisker movements in rodents might well offer  
70 this alternative.

71 Whiskers in rats and mice move backwards and forwards in a behaviour known as whisking,  
72 which occurs up to 25 times per second (Vincent, 1912). Studies have found that rodents use  
73 their whiskers to guide many tasks such as locomotion, navigation, foraging and hunting  
74 (Grant & Arkley 2016). With the development of high-speed video cameras and analysis  
75 programs, it has become apparent that rodents do not just make simple sweeping movements  
76 with their whiskers. Rather, they can precisely change the amplitude, velocity and position of  
77 their whiskers during locomotion and object exploration (Arkley et al., 2014; Carvell &  
78 Simons, 1995; Grant et al., 2009; Hartmann, 2001; Kleinfeld et al., 2006; Mitchinson et al.,  
79 2007; Szwed et al., 2003; Towal & Hartmann, 2008; Welker, 1964). For example, object  
80 exploration is generally associated with slower whisker movements at lower amplitudes  
81 (Carvell & Simons, 1995; Grant et al. 2009). Following an object contact, sensory  
82 information from the whisker shaft, such as force and direction, is transmitted in the follicle  
83 and passed through multiple neural pathways to the cortex (Grant & Arkley 2016). The  
84 organisation of cholinergic neurons throughout whisker-related sensorimotor areas in rodents  
85 (Beak et al., 2010), including brainstem, thalamus, (Timofeeva et al., 2005; Bosman et al.,

86 2011), cortex (Bosman et al., 2011), cerebellum (Timofeeva et al., 2005), zona incerta and  
87 amygdala (Bosman et al., 2011) indicates that nicotine may well have an effect on whisker  
88 sensorimotor integration.

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

## 103 **2. Methods**

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

### 106 **2.1 Animals**

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

## 109 **2.2 Smoking Procedures**

110 Animals were randomly assigned to the following groups: Control (C: n=10), Smoking (S:  
111 n=11), Smoking cessation for 1 week (S1W: n=9) and Smoking cessation for 2 weeks (S2W:  
112 n=10). Smoking was selected as the nicotine administration technique, as it is the most  
113 common way people are exposed to elevated levels of nicotine. Smoking animals were  
114 exposed to cigarette smoke (3R4F research cigarettes with filter purchased from Kentucky  
115 Tobacco Research and Development Center, University of Kentucky) using a nose-only  
116 exposure system (InExpose System, Scireq). Mice were placed in soft restraints and  
117 connected to an exposure tower. A cigarette puff was generated every minute, leading to 10  
118 seconds of cigarette smoke exposure followed by 50 seconds of fresh air. Mice were  
119 acclimatized to the cigarette smoke exposure during the first week of the experiment.  
120 Afterwards, animals were exposed daily to four cigarettes, twice a day, 5 days per week, over  
121 3 months (Rinaldi et al., 2012). Control animals were treated similarly, but were exposed to  
122 filtered air for the same duration. Animals in the smoking cessation groups stopped smoking  
123 for 1 or 2 weeks. As nicotine withdrawal behaviours are usually absent from 5-6 days (Damaj  
124 et al. 2003), the one-week time-point was selected as a minimum, and the two-week time-  
125 point was selected as an additional measure. Smoking and control mice were exposed to  
126 cigarette smoke or filtered air, respectively on the morning of their behavioural assessment  
127 and tested approximately 2 hours after the smoking or filtered air treatment. Any stress  
128 caused by restraint in the experimental set-up was, therefore, equivalent between the smoking  
129 and control groups. The total particle density concentration of the cigarette smoke in the  
130 tower was measured weekly and was on average 149.5 mg total particulate matter per m<sup>3</sup>.  
131 Mice were weighed weekly to ensure they maintained a healthy body mass for inclusion in  
132 the study. Two mice in the S1W group did not survive the smoking protocol.

## 133 **2.2 Recording and Measuring Behaviour**

134 Each mouse was placed in to a transparent, Perspex, rectangular arena (20 x 30 x 15 cm) (Fig.  
135 1a), which was lit from below by a bright, normal-spectrum light box (PHLOX LEDW-BL-  
136 400/200-SLLUB-Q-1R-24V). The mouse was filmed from above using a digital high-speed  
137 video camera (Phantom Miro ex2) recording at 500 frames per second with a shutter-speed of  
138 1 ms and a resolution of 640x480 pixels. Multiple 1-s video clips were collected  
139 opportunistically (by manual trigger) when the animal moved in the field of view of the  
140 camera. Approximately 16 clips were collected from each animal. Four to six clips from  
141 each mouse were selected and trimmed based on to the following selection criteria developed  
142 in Grant et al. (2013): i) the mouse was clearly in frame; ii) both sides of the face were  
143 visible; iii) the head was level with the floor (no extreme pitch or yaw); iv) the whiskers were  
144 not in contact with a vertical wall; and v) the mouse was clearly moving forward. Six of the  
145 eleven smoking animals (S) could not be included in the study as their whiskers were  
146 barbered by a conspecific and thus could not be imaged. Barbering is not usually associated  
147 with stress, but rather caused by a particularly dominant animal in the home cage (Bresnahan  
148 et al. 1983). While barbering is relatively rare, to overcome this in future studies it is  
149 recommended to remove the dominant individual from the home cage, or to house mice  
150 singularly, a month before filming. This left a sample size of 32 animals (C: n=10, S: n=5,  
151 S1W: n=7, S2W: n=10), which is reflected in the individual averages in Figure 3.

152 In each selected clip, the mouse snout and whiskers were tracked using the BIOTACT  
153 Whisker Tracking Tool (Perkon et al., 2011). The tracker semi-automatically finds the  
154 orientation and position of the snout, and the angular position (relative to the midline of the  
155 head) of each identified whisker. Tracking was validated by manually inspecting the tracking  
156 annotations overlaid on to the video frames (Fig 1b) and a total of 166 clips, each of around  
157 0.5 seconds in length, were included in the analysis (C: n=51, S: n=33, S1W: n=35, S2W:  
158 n=47).



159 The movement of the entire whisker field was determined from the unsmoothed mean of all  
160 the tracked whisker angular positions for each side frame by frame (Grant et al. 2012; Figure  
161 1c, termed *naïve mean angle (nma)*). The following variables were calculated from the  
162 whisker angular position data. *Offset* is the mean angular position. To estimate the *amplitude*,  
163 the offset was removed from the whisking angle time series and the root mean square value  
164 was computed to give the root-mean-square (RMS) whisking amplitude. These time series  
165 were approximately sinusoidal, so the “peak-to-peak whisking amplitude” was estimated by  
166 multiplying the RMS whisking amplitude by  $2\sqrt{2}$  (Chatfield, 2003). This estimate of  
167 amplitude is reasonably robust to accommodate departures from a purely sinusoidal pattern.  
168 Whisk *frequency* was calculated using a discrete-fourier transform (FFT function in Matlab),  
169 with a peak frequency cut-off of 50 Hz, as anything above this would not be expected  
170 (Mitchinson et al. 2011). An auto-correlogram fitted each FFT curve to the original angular  
171 position signal and provided an indication of fit, or power; the FFT curve with the highest  
172 power was selected as the best frequency fit. Mean angular *retraction* and *protraction*  
173 *velocities* were calculated as the average velocity of all the backward (negative) and forward  
174 (positive) whisker movements, respectively. Offset, amplitude, retraction and protraction  
175 velocities were calculated individually for each whisker side, and then averaged between the  
176 left and right sides to give one value of each per clip.

177 As locomotion is a common behavioural measure, average *locomotion speed* was also  
178 calculated on a per-frame basis by tracking the nose tip and calculating the average number  
179 of metres moved per second. Each day the arena was calibrated, by taking an image of a  
180 ruler, to make the pixel to mm conversion.

### 181 **2.3 Pulmonary mechanics**

182 To verify that the dose and duration of smoking was such that it had physiological effects we  
183 also investigate pulmonary mechanics. The pulmonary system is directly exposed to cigarette  
184 smoke and effects should be seen there. Thereto, after filming, the mice were anesthetized  
185 with a intraperitoneal injection of a mixture of xylazine (8.5 mg/kg, Rompun®, Bayer,  
186 Belgium) and ketamine (13 mg/kg, Anesketin®, Eurovet, Belgium) and tracheotomized.  
187 Mice were then placed in a body plethysmograph and connected to a computer-controlled  
188 ventilator (Buxco-Force Pulmonary Maneuvers) to measure lung compliance (Cchord). Lung  
189 compliance, or more specifically chord compliance, measures the linear section of the lung  
190 Pressure-Volume Curve, and is strongly associated with lung volume. It has been suggested  
191 as a way of diagnosing a range of respiratory disorders (Harris 2005).

192 All the mice, including the barbered smoking mice were included in this section of the study.  
193 However, three control mice, one smoking mouse, one smoking cessation week 1, and one  
194 smoking cessation week 2 mouse were euthanized during procedures unrelated to this study  
195 prior to the extraction of these measurements, leaving a sample size of 32 (C: n=7, S: n=10,  
196 S1W: n=6, S2W: n=9), which is reflected in the individual averages in Figure 2.

#### 197 **2.4 Statistical considerations**

198 All data was distributed normally. Differences between groups for whisking measures and  
199 locomotion speed were analysed with linear mixed models. The treatment groups of mice  
200 (smoking, controls, smoking cessation week 1 and smoking cessation week 2) was a fixed  
201 between factor, and the individual mouse ID was a random between factor. Lung function  
202 data was analysed using a univariate ANOVA, with treatment group as a between factor.

203 As whisking variables can be altered by locomotion speed (Arkley et al. 2014; Grant et al.  
204 2012a), locomotion speed was also added as a covariate to the linear mixed models, but did  
205 not have a significant effect on the results and, therefore, was not included here.

206 A significance level of  $< 0.05$  was selected for all analyses. Tukey post-hoc tests were carried  
207 out on significant results and indicated with a \* on the subsequent graphs. Partial Eta Squared  
208 ( $\eta^2_p$ ) values are quoted for effect sizes throughout.

### 209 **3. Results**

210 Lung compliance was significantly increased in the smoking mice and remained impaired  
211 even after 2 weeks smoking cessation (ANOVA:  $F(3,164)=7.258$ ,  $p=0.001$ ,  $\eta^2_p = 0.500$ ,  
212 Tukey Post-hoc:  $C<S,S1W,S2W$ ). This can clearly be seen in Figure 2a, where the control  
213 mice have a significantly lower average Cchord compliance value than the smoking and  
214 smoking cessation groups. Indeed, the lowest Cchord compliance values can be seen in  
215 Figure 2b in the C2 and C5 control mice, and the highest values in the S9, S11 and S6  
216 smoking mice.

217 The smoking mice locomoted significantly slower than the control mice, however, after 1-  
218 week smoking cessation this difference had disappeared (mixed model:  $F(3,25.4) = 9.981$ ,  
219  $p<0.001$ ,  $\eta^2_p = 0.173$ , Tukey Post-hoc:  $S<C,S1W,S2W$ ). This can clearly be seen in figure  
220 3a, where the smokers have a significantly slower average locomotion speed than the control  
221 mice, and those in the smoking cessation conditions. Specifically, Figure 3b show that mouse  
222 S11 in the smoking condition had the lowest locomotion speed overall, with control mouse  
223 C10 having the fastest locomotion speed overall.

224 Example whisking traces from a smoking and control mouse can be seen in Figure 4. From  
225 Figure 5 it can be seen that smoking mice move their whiskers faster than all the other  
226 treatment groups in both the protraction and retraction stages of the whisk (protraction  
227 velocity mixed model:  $F(3,29.5) = 7.055$ ,  $p=0.001$ ,  $\eta^2_p = 0.092$ , Tukey Post-hoc:  
228  $S>C,S1W,S2W$ ; retraction velocity mixed model:  $F(3,31.6) = 6.486$ ,  $p=0.002$ ,  $\eta^2_p = 0.100$ ,  
229 Tukey Post-hoc:  $S>C,S1W,S2W$ ). Table 1 shows that the control mice held their whiskers

230 slightly further forward (with higher offset values) than those in the smoking cessation  
231 treatments, however this was not significant (mixed model:  $F(3,26.5) = 2.498$ ,  $p=0.081$ ,  $\eta^2p =$   
232  $0.055$ ). Likewise, smoking mice did tend to have larger amplitudes than control mice,  
233 however, this was also not significant (mixed model:  $F(3,26.8) = 2.417$ ,  $p=0.088$ ,  $\eta^2p =$   
234  $0.064$ ) (Table 1). Frequency was also not significantly altered between the smoking groups  
235 (mixed model:  $F(3,159) = 1.711$ ,  $p=0.167$ ,  $\eta^2p = 0.038$ ).

#### 236 **4. Discussion**

237 Results from this study show that there are measureable changes in exploratory behaviour in  
238 smoking mice, compared to control and smoking cessation conditions. In particular, whisking  
239 protraction and retraction velocities were both significantly increased (Fig. 5 and Table 1)  
240 and locomotion speed was significantly reduced (Fig. 3) in smoking mice (two hours post-  
241 smoking) and returns to normal following smoking cessation of just one week. Lung  
242 compliance was significantly increased in smoking mice, and did not recover following  
243 smoking cessation (Figure 2).

244 Smoking mice locomoted slower than non-smoking mice (Figure 3). Specific changes in  
245 locomotion have not yet been found in rodents treated with nicotine (Casarrubea et al., 2015);  
246 however, general activity has been found to decrease (Mesa-Gresa et al., 2013), which offers  
247 support for our observation. Other studies have reported increases (Battig et al., 1976;  
248 Calderone et al. 2008; Slawecki et al., 2003), or no changes (Casarrubea et al., 2015; Piri et  
249 al. 2011) in physical and locomotor activity levels in nicotine-treated mice, which differ from  
250 our own findings. Indeed, the association of nicotine and locomotion is complex in the  
251 literature and can be affected by gender (Calderone et al. 2008), and probably dosage as well.  
252 Whatever the cause of these discrepancies, the reduction in locomotion speed in our study  
253 was reversed after only a one-week period of smoking cessation (Figure 3). It is interesting to

254 note that smoking in humans is also often associated with reduced activity levels (Kaczynski  
255 et al. 2008; Larsson & Orlander 1984) and if our data in mice can be translated to humans,  
256 they suggest that a reduced drive for physical activity can be readily reversed by smoking  
257 cessation.

258 A decrease in exploratory behaviour following nicotine administration is a robust finding in  
259 rodents (Battig et al., 1976; Casarrubea et al., 2015; Slawecki et al., 2003). Specifically,  
260 nicotine-treated mice have been found to spend more time away from open areas and reduce  
261 the amount of time spent rearing and head-dipping (Casarrubea et al., 2015; Slawecki et al.,  
262 2003). Many studies have found a reduction in head-dipping during a hole-board task,  
263 following nicotine administration (Casarrubea et al., 2015; Piri et al. 2011; ve Yontem et al.  
264 2014). Head-dipping in exploring, healthy rodents has been found to be associated with  
265 whisker exploration of the floor (Arkley et al., 2014; Grant et al., 2009; 2012b), and we  
266 propose here that measuring whisker movements directly, rather than head movements, can  
267 offer a way to quantitatively measure exploratory behaviour in freely moving rodents.

268 Exploration in rodents, such as mice and rats, is primarily guided by their sense of whisker  
269 touch (Grant & Arkley 2016). Just like other sensory systems, tactile sensitivity is enhanced  
270 by moving the sensor in a certain way over an object (Carvell & Simons, 1995; Mitchinson et  
271 al., 2007; Grant et al., 2009; Towal & Hartmann, 2008). In particular, good performance on  
272 tactile tasks are often associated with slower whisker movements (Carvell & Simons, 1995),  
273 which allow the whiskers to contact surfaces for longer durations (Carvell & Simons, 1995;  
274 Grant et al., 2009). Rats and mice have the ability to change the velocity of their whiskers on  
275 a per-whisk basis, so they can respond quickly with changes in their whisking profiles (Towal  
276 & Hartmann, 2008). They are even able to speed up and slow down different phases of the  
277 whisk cycle, so that they can contact an object at an optimum speed (Moxon, 2008). The  
278 speed and amplitude of a whisker contact elicits different response profiles in thalamic and

279 cortical neurons (Pinto et al., 2000); for example, high velocity contacts elicit more spikes  
280 particularly in the thalamic and cortical neurons (Pinto et al. 2000; Shoykhet et al., 2000). As  
281 both velocity and amplitude information are used to code object position (Szwed et al., 2003;  
282 Ahissar & Arieli, 2001) the increased whisker speeds in the smoking mice may indicate that  
283 exploration abilities and tactile performance are somewhat impeded in these animals.

284 Whisker positions (offset) showed large inter-individual variability (Figure 5b) and did not  
285 differ significantly between smoking and non-smoking mice. Also amplitude and frequency  
286 were not significantly affected by smoking (Figure 5, Table 1), although amplitude did tend  
287 to show a general trend to be larger in the smoking mice than in controls and after smoking  
288 cessation. Whisking amplitude is usually decreased during close exploration of a surface  
289 (Carvell & Simons, 1995; Grant et al., 2009; Mitchinson et al., 2007); therefore, a reduction  
290 in exploration might well have caused the small increase in amplitude that we observed in  
291 smoking mice. In addition, perhaps the small sample numbers of smoking mice (n=5) have  
292 also contributed to the lack of significance in this result.

293 That whisking behaviour recovered in mice that have stopped smoking for only a week (Fig.  
294 5), without recovery of normal lung compliance (Fig. 2), suggests that behavioural effects are  
295 likely to improve well before lung recovery. In addition, the mechanism for the increase in  
296 whisking velocities is likely to be the interaction of nicotine with neuronal structures, rather  
297 than any change in lung function during smoking (Fig. 2). While smoking was selected as the  
298 nicotine administration technique in this study, as it is the most common way that people  
299 administer nicotine, and provides an efficient way of delivering it to the brain (Henningfield  
300 & Keenan 1993), future work could carry this study out using a direct nicotine delivery  
301 system, such as a patch. While the number of smoking mice included in the study was less  
302 than in the other conditions, we are confident that our statistical analyses represent our

303 findings, and we have manually examined the video footage and whisker traces to  
304 corroborate our findings for both locomotion and whisking data.

#### 305 **4.1 Links to brain and behaviour**

306 Due to the distribution of nAChRs throughout the brain and the complexity of behavioural  
307 pathways, it is hard to make strong inferences linking the effect of smoking and nicotine to  
308 any specific brain areas. Delivery of nicotine to specific brain areas, such as brainstem nuclei,  
309 cerebellum, primary motor cortex or primary somatosensory cortex, might help to improve  
310 understandings of the role of nicotine, and acetylcholine, on behaviour. A study by Shao and  
311 Feldman (2001) found that applying nicotine to the pre-Bötzinger complex (the brainstem  
312 pattern generator area for both breathing and whisking) caused neurons to fire at higher  
313 frequencies with lower amplitude spikes. Furthermore, Casarrubea et al. (2015) found that  
314 lesioning the lateral habenula, a structure associated with negative motivational signals,  
315 reversed nicotine-induced anxiety and reductions in exploratory behaviour. Cholinergic  
316 projections have been found to enhance whisker responses in primary motor cortex (M1)  
317 (Berg et al., 2005) and primary somatosensory cortex (Oldford & Castro-Alamancos, 2003;  
318 Eggermann et al. 2014), especially during alert states. Indeed, Eggermann et al. (2014)  
319 suggest that nicotinic signalling during whisking contributes to active states in the Primary  
320 Somatosensory Cortex. That exploration behaviours are reduced in smoking mice, may  
321 indicate a lack of attention to their surroundings, and gives support to the suggestion that  
322 acetylcholine is involved in awareness, attention and alertness pathways (Bosman et al.,  
323 2011).

#### 324 **5. Conclusions**

325 We quantified whisker movements in mice as a measure of exploratory behaviours following  
326 chronic smoking and cessation. We present here a quick, yet quantitative, method of

327 recording whisker movements, that does not require any animal training. We found that both  
328 protraction and retraction whisker velocities were significantly increased in smoking mice,  
329 and recovered following just one week of smoking cessation. As whisker velocities are linked  
330 with active sensing and object coding, we suggest that the smoking-induced increase in  
331 whisker velocity indicates a reduction in exploratory behaviour. The quick normalisation of  
332 smoking-induced changes in behaviour following smoking cessation may have implications  
333 for human health, as smoking-related anxiety behaviours may also recover in humans  
334 following cessation. As anxiety is strongly linked to the successfulness of smoking cessation  
335 (Pomerleau et al. 1978), an anxiety assessment conducted soon after smoking cessation may  
336 inform help to inform further smoking cessation plans.

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486

#### 487 FIGURE CAPTIONS

488 **Figure 1** Recording and tracking mouse behaviour. a) The experimental set-up. The high-speed video  
489 camera above the arena, which was illuminated from below by a light box. b) The tracked video  
490 footage showing head and whisker traces (left whiskers in red and right whisker in blue). c) Example  
491 of recording of whisker angles (nma: naïve mean angle) of the left (in red) and right (in blue) whisker  
492 fields.

493 **Figure 2:** Lung compliance in control, smoking and smoking cessation (one or two weeks) mice. a, is  
494 the mean plot for all animals with standard error bars, and b presents the data for individual mice. \*  
495 indicates difference of control from all other treatments, at  $p=0.001$ .

496 **Figure 3:** Locomotion speed in control, smoking and smoking cessation (1 or two weeks) mice. a, is  
497 the mean plot for all animals with standard error bars, and b presents the data for individual mice. \*  
498 indicates difference of smoking mice from all other treatments, at  $p<0.001$ .

499 **Figure 4:** Example of traces of whisker movement in smoking (a) and control (b) mice. The whiskers  
500 of the smoking mice move faster than those of the control mice. The blue trace shows the mean  
501 whisker movements on the right hand side, and the red trace corresponds to mean whisker movements  
502 of the left hand side.

503 **Figure 5:** Whisker velocities in control, smoking and smoking cessation (one or two weeks)  
 504 mice. a and c and e show the mean plot for all animals with standard error bars, and b and d presents  
 505 the data for individual mice. a,b: protraction velocity; c,d: retraction velocity. \* indicates a difference  
 506 in smokers from all other treatments, at  $p < 0.001$ ;

507 TABLE

508 **Table 1.** Measurements of whisker offset, amplitude and frequency in control, smoking and smoking  
 509 cessation (one or two weeks) mice. Table shows the Mean $\pm$ standard error data for the remaining  
 510 whisker measurements where no significant effect of smoking treatment was observed. Velocity  
 511 results can be seen plotted in Figure 5.

<b>Whisker Variables</b>	<b>C</b>	<b>S</b>	<b>S1W</b>	<b>S2W</b>
<b>Offset</b>	95.43 $\pm$ 0.98	93.16 $\pm$ 1.50	90.11 $\pm$ 1.06	89.36 $\pm$ 1.23
<b>Amplitude</b>	36.88 $\pm$ 1.12	43.61 $\pm$ 1.95	37.88 $\pm$ 1.06	40.27 $\pm$ 1.86
<b>Frequency</b>	13.37 $\pm$ 0.72	11.32 $\pm$ 0.74	11.38 $\pm$ 0.66	12.42 $\pm$ 0.78

512



Figure 1  
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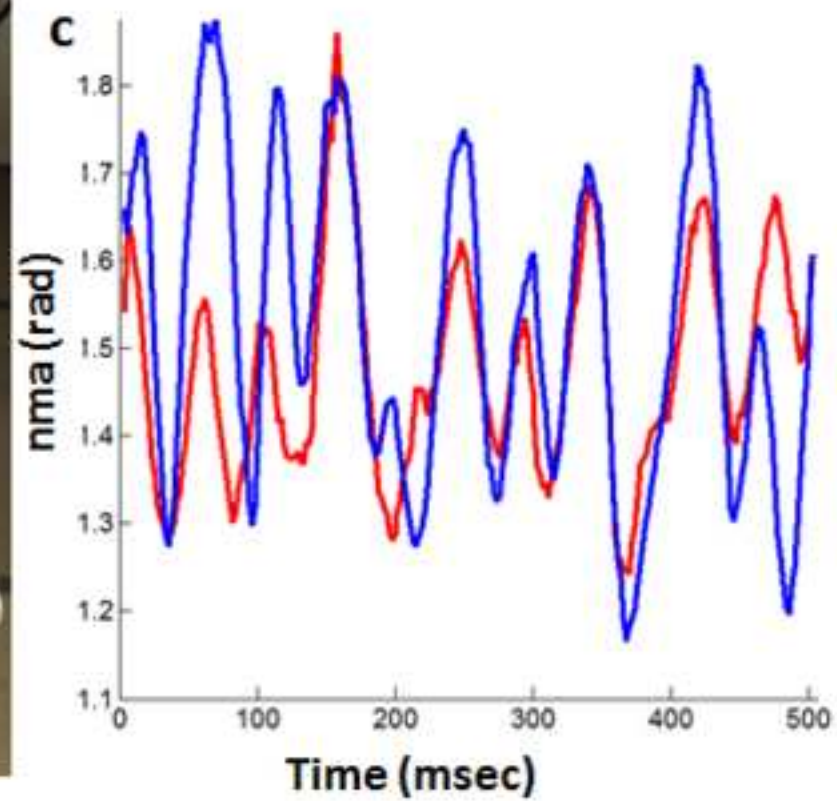
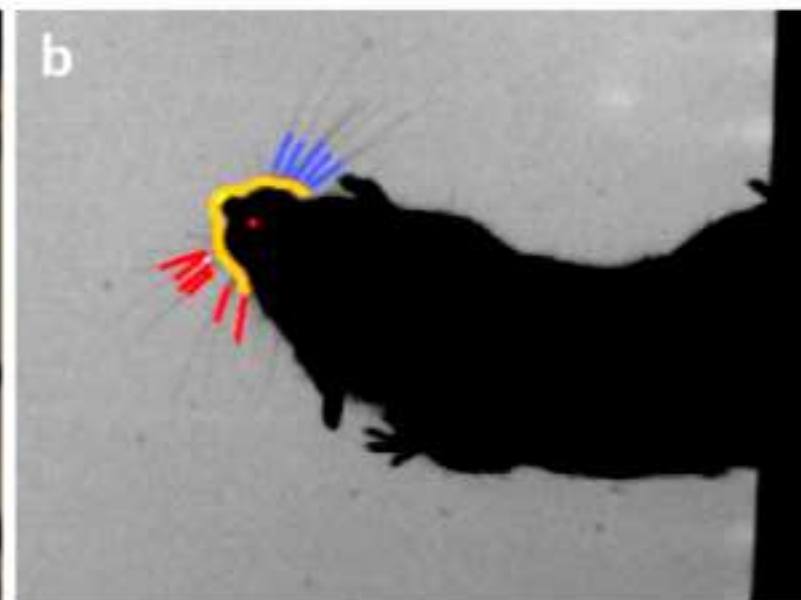


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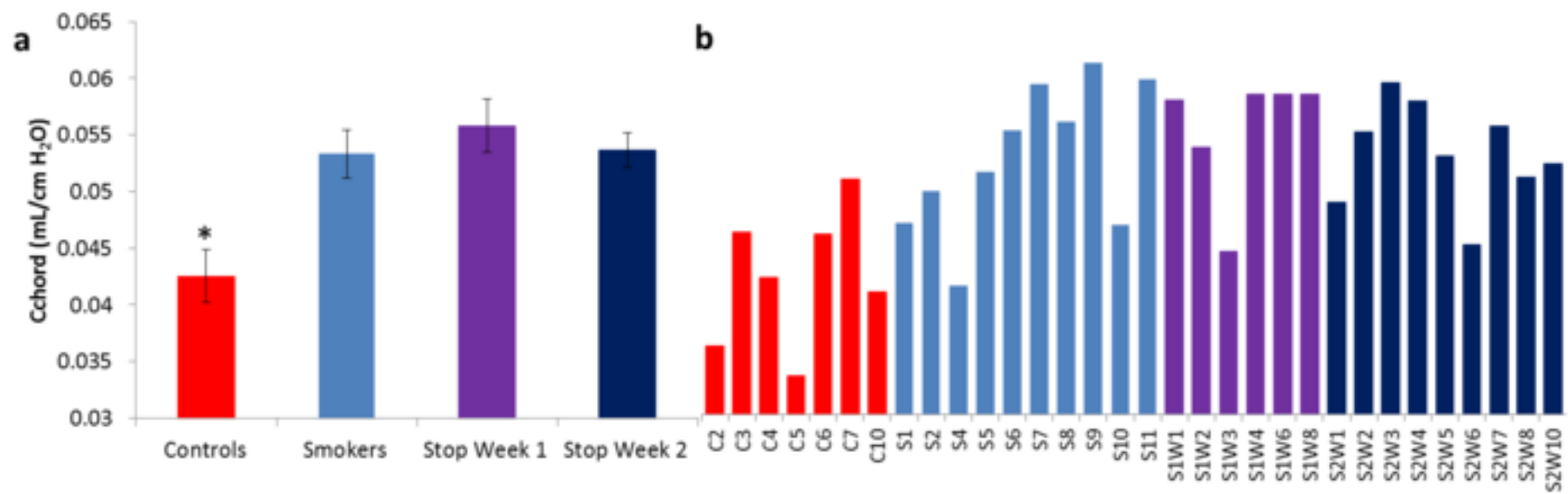


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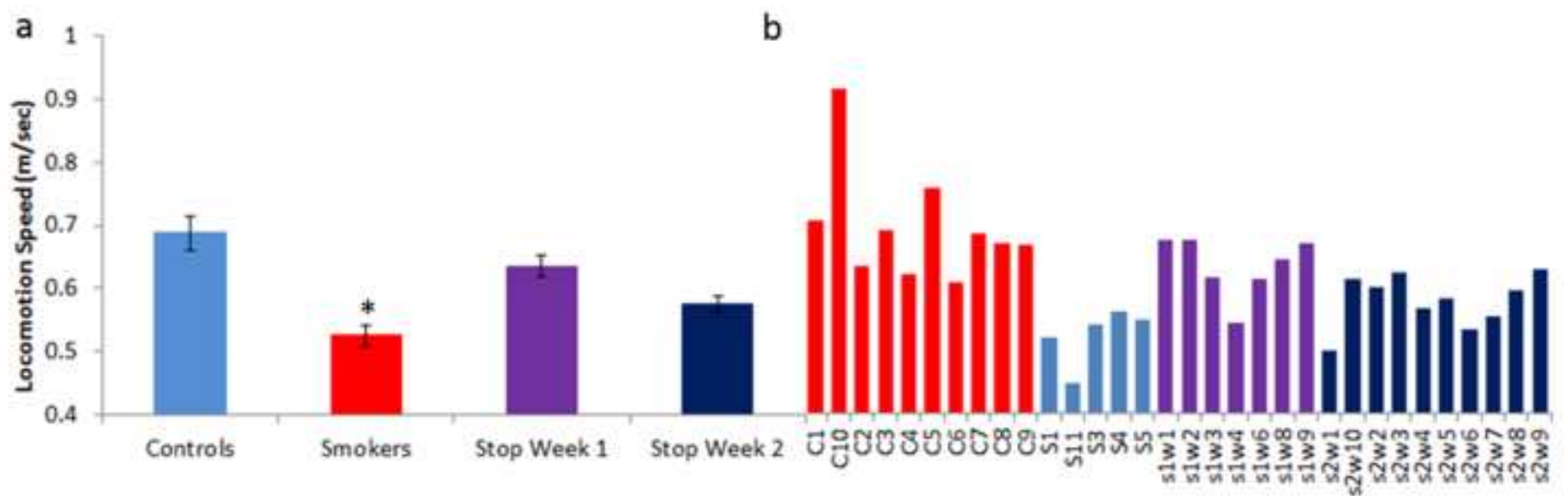
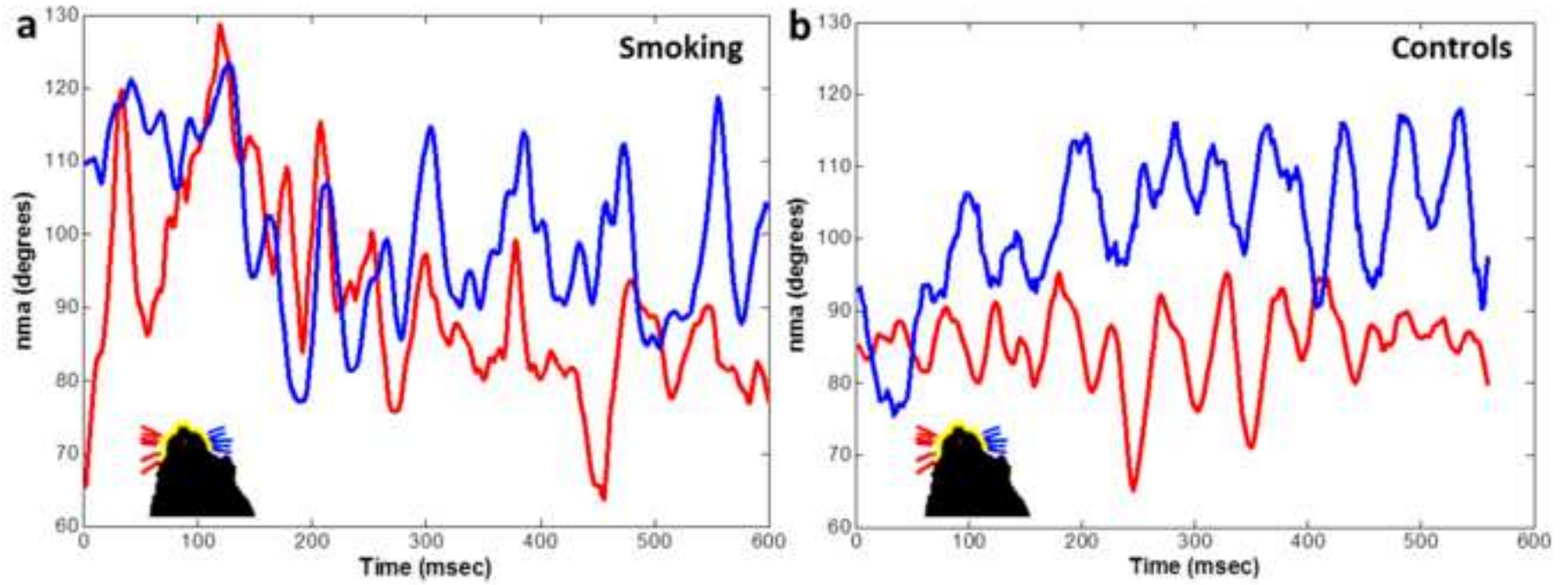


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**Figure 5**  
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