

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

3 Robyn A Grant^{1,2*}, Nele Cielen³, Karen Maes³, Nele Heulens³, Gina LJ Galli⁴, Wim
4 Janssens³, Ghislaine Gayan-Ramirez³ & Hans Degens^{2,5}

5 1. * Conservation, Evolution and Behaviour Research Group, Manchester Metropolitan
6 University, Chester Street, Manchester UK

7 2. Neuromuscular and Skeletal Ageing Research Group, Manchester Metropolitan
8 University, Chester Street, Manchester UK

9 3. Laboratory of Respiratory Diseases, Katholieke Universiteit-Leuven, Herestraat 49,
10 Leuven, Belgium

11 4. Manchester University, Faculty of Medicine and Human Sciences, Core Technology
12 Facility, 46 Grafton St, Manchester, UK,

13 5. Sports Science and Innovation Institute, Lithuanian Sports University, Kaunas,
14 Lithuania

15 * Corresponding author: Robyn A Grant: robyn.grant@mmu.ac.uk

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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 m³.
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 (η^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.

Whisker Variables	C	S	S1W	S2W
Offset	95.43 \pm 0.98	93.16 \pm 1.50	90.11 \pm 1.06	89.36 \pm 1.23
Amplitude	36.88 \pm 1.12	43.61 \pm 1.95	37.88 \pm 1.06	40.27 \pm 1.86
Frequency	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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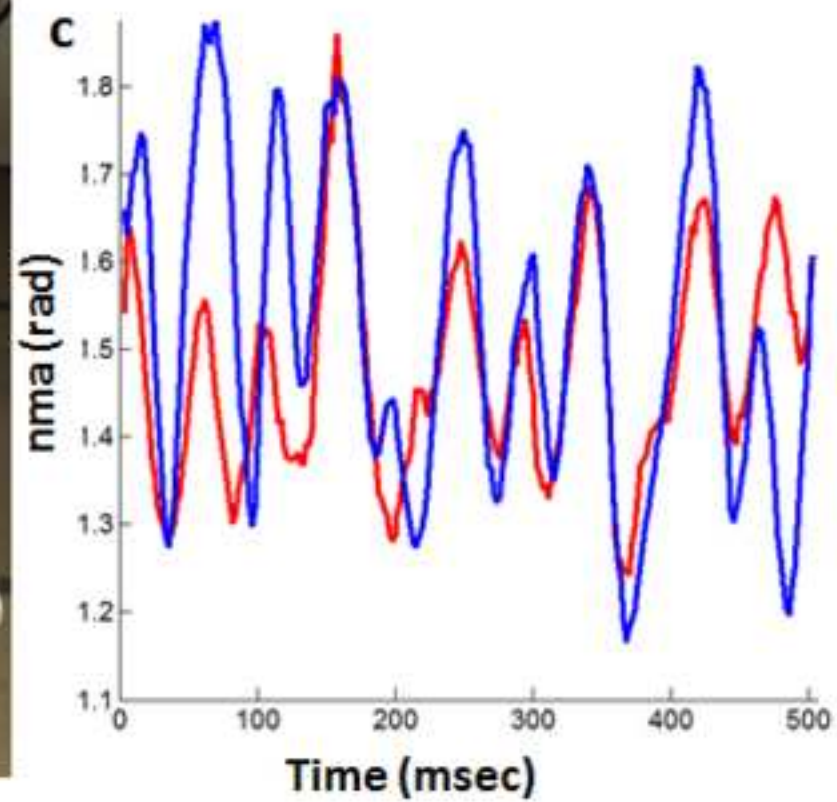
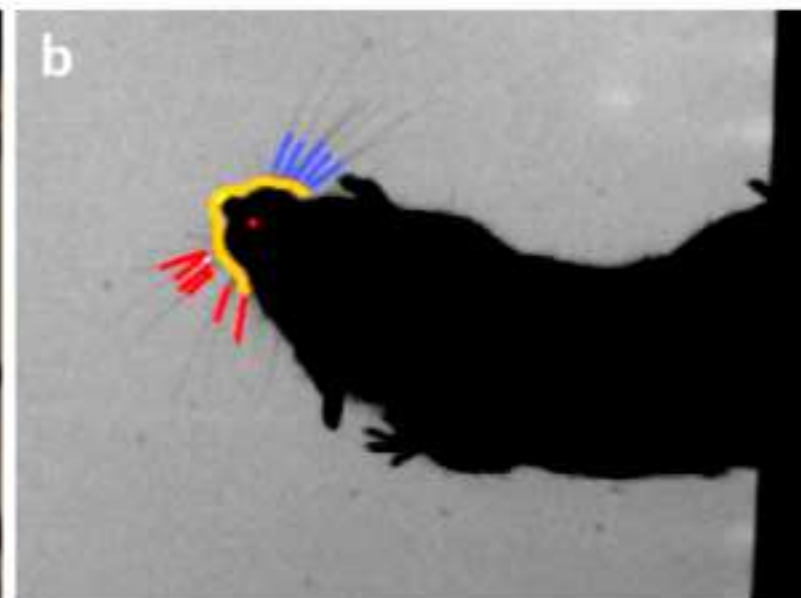


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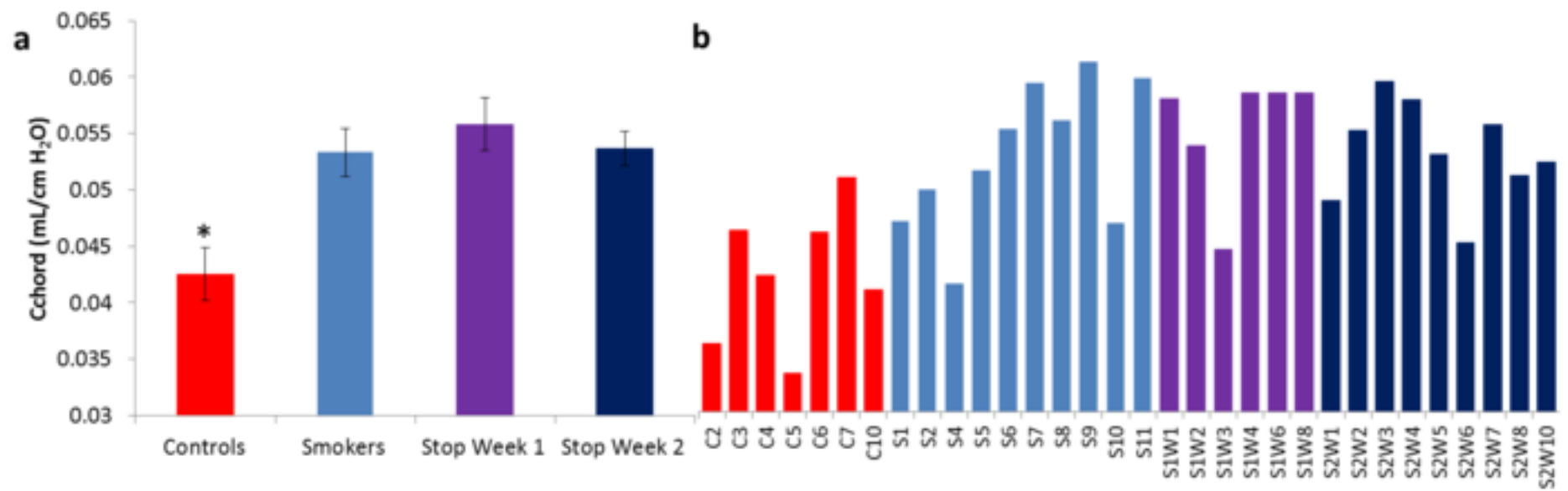


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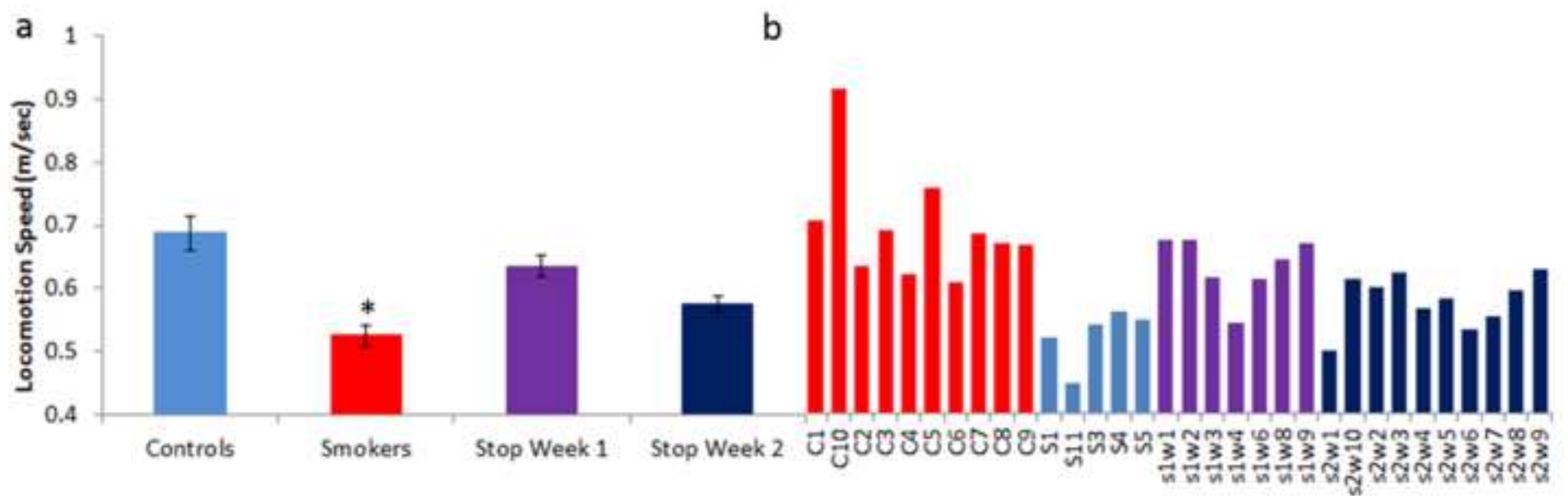


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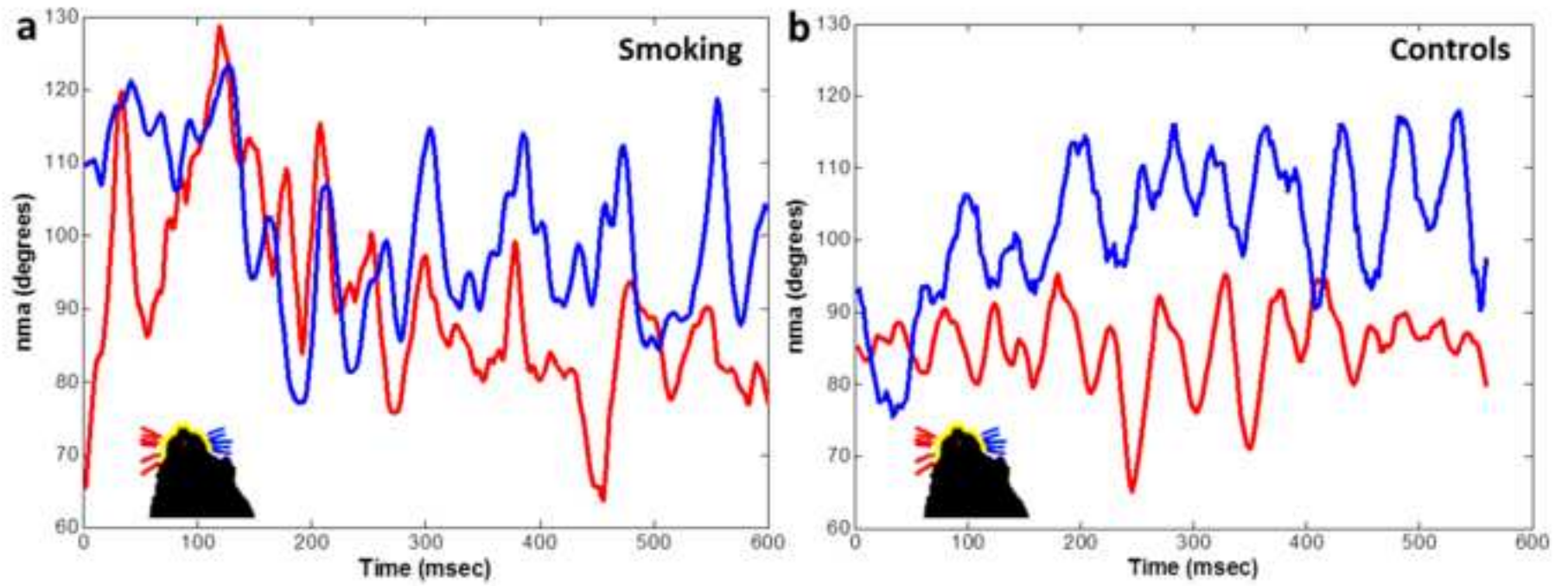


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