CELL INJURY, REPAIR, AGING, AND APOPTOSIS

Damage to Olfactory Progenitor Cells Is Involved in Cigarette Smoke—Induced Olfactory Dysfunction in Mice

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Exposure to cigarette smoke is a major cause of olfactory dysfunction. However, the underlying mechanisms by which cigarette smoke interferes with the highly regenerative olfactory nerve system remain unclear. To investigate whether cigarette smoke induces olfactory dysfunction by disrupting cell proliferation and cell survival in the olfactory epithelium (OE), we developed a mouse model of smoking that involved intranasal administration of a cigarette smoke solution (CSS). Immunohistological analyses and behavioral testing showed that CSS administration during a period of 24 days reduced the number of olfactory marker protein—positive mature olfactory receptor neurons (ORNs) in the OE and induced olfactory dysfunction. These changes coincided with a reduction in the number of SOX2⁺ ORN progenitors and Ki-67⁺ proliferating cells in the basal layer of the OE, an increase in the number of caspase-3⁺ apoptotic cells, and an increase in the expression of mRNA for the inflammatory cytokines IL-1 β and IL-6. Notably, the proliferating ORN progenitor population recovered after cessation of treatment with CSS, resulting in the subsequent restoration of mature ORN numbers and olfaction. These results suggest that SOX2⁺ ORN progenitors are targets of CSS-induced impairment of the OE, and that by damaging the ORN progenitor population and increasing ORN death, CSS exposure eventually overwhelms the regenerative capacity of the epithelium, resulting in reduced numbers of mature ORNs and olfactory dysfunction. (*Am J Pathol* 2016, 186: 579–586; <http://dx.doi.org/10.1016/j.ajpath.2015.11.009>)

Cigarette smoke represents a major source of exposure to toxic chemicals for humans and causes a diverse range of preventable illnesses.^{1,2} The numerous chemical irritants contained in cigarette smoke trigger the generation of reactive oxygen and nitrogen species and expression of inflammatory mediators, such as IL-1 β , IL-6, and tumor necrosis factor, in the respiratory tract.³ Because these mediators damage epithelial tissue and induce inflammatory responses in the respiratory tract, a long history of cigarette smoking significantly increases the risk of various pulmonary diseases, such as chronic obstructive pulmonary disease and lung cancer.^{3,4} In addition to lower respiratory diseases, cigarette smoke causes upper respiratory disorders, such as nasal polyps⁵ and chronic rhinosinusitis, and also impairs olfaction (hyposmia/anosmia).^{6–8} However, the

cellular and molecular mechanisms by which cigarette smoke disrupts olfaction remain largely unclear.

Olfaction is mediated by the olfactory system, which is composed of olfactory receptor neurons (ORNs) in the nasal cavity and the olfactory bulb in the forebrain.⁹ Olfactory dysfunction is associated with damage to ORNs and/or the olfactory bulb, which can occur because of a variety of causes, such as exposure to toxic chemicals, airway allergy, upper-airway viral infections, head trauma, and neurodegenerative diseases.^{10,11} Cigarette smoke is a major cause of

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hyposmia and anosmia.^{6–8} Cigarette smoke decreases the thickness of the olfactory epithelium (OE)¹² and increases apoptosis in the OE.¹¹ However, ORNs have regenerative potential through the olfactory epithelial stem cell system,¹³ and thus the extent to which apoptosis causes olfactory dysfunction remains unclear.

Two types of cells in the basal layer of the OE, globose basal cells and horizontal basal cells, act as ORN stem and progenitor cells and produce mature ORNs expressing olfactory marker protein (OMP), which is expressed exclusively in mature ORNs and modulates olfactory signal transduction.¹⁴ Recently, it was suggested that globose basal cells are actively proliferating multipotent progenitor cells that display increased proliferation after OE damage,¹⁵ whereas horizontal basal cells are quiescent stem cells that contribute to OE regeneration only after severe OE damage.^{16,17} SOX2 is a transcription factor that is widely expressed in stem cell populations, including in neural stem cells, and that plays a role in maintaining cells in an undifferentiated state.¹⁸ In the OE, SOX2 is expressed by progenitor cells and regulates homeostasis.^{10,13,19–21} Accumulating evidence suggests that SOX2 expression is suppressed by inflammatory cytokines, such as IL-6, resulting in a loss of stemness in multipotent cells.²² In addition, we recently demonstrated that SOX2⁺ ORN progenitors are damaged by viral infection, alone or in combination with airway allergy, leading to the eventual loss of ORNs.¹⁰ We, therefore, hypothesized that cigarette smoke–induced inflammation might also disrupt the olfactory progenitor cell system.

Herein, we explored the effects of cigarette smoke on ORNs and ORN progenitors using a newly established mouse model of smoking that involved administration of a cigarette smoke solution (CSS). We also investigated the extent to which CSS-induced damage to the ORNs recovers after cessation of exposure to CSS. Using histological analyses, olfactory habituation/dishabituation tests, and quantitative RT-PCR (RT-qPCR) analyses, we found that cigarette smoke impairs not only OMP⁺ mature ORNs, but also SOX2⁺ ORN progenitors. Decreased numbers of ORNs and their progenitors were associated with olfactory disorder that gradually recovered as inflammatory cytokine levels decreased after cessation of exposure to CSS.

Materials and Methods

Mice

Eight-week-old male C57BL/6 mice were purchased from Saitama Experimental Animals (Saitama, Japan). Mice were housed in a temperature-controlled environment under a 12-hour light-dark cycle with access to food and water *ad libitum*. All animal experiments were conducted in accordance with institutional guidelines and with the approval of the Animal Care and Use Committee of the University of Tokyo (approval number P14-086; Tokyo, Japan).

Mouse Model of Smoking

The mouse model of cigarette smoking was adapted from a guinea pig model that used CSS.²³ CSS, prepared by bubbling a stream of the smoke of 40 Hi-Lite cigarettes (Japan Tobacco Inc., Tokyo) through 40 mL of saline, was purchased from CMIC Bioresearch Center Co, Ltd (Tokyo, Japan). We defined the day of final CSS administration as day 0. CSS (20 μ L per animal per time) was administered intranasally once a day on days –23 to –19. This cycle of four consecutive CSS administrations, followed by 1 rest day, was then repeated, with cycles 2 to 5 conducted over days –18 to –14, –13 to –9, –8 to –4, and –3 to 0, respectively (Figure 1). Control mice received saline intranasally, according to the same schedule as CSS mice. CSS mice were sacrificed on days 1, 7, 14, and 28 after the final administration of CSS. Control mice were sacrificed on day 1 after the final administration of saline.

Tissue Preparation

Septal nasal mucosa was harvested on days 1, 7, 14, and 28 after the final intranasal administration of CSS for histological and RT-qPCR analyses. Immediately after sacrificing the mice, the nasal cavities were gently irrigated with 4% paraformaldehyde to minimize mechanical damage to the OE. The mandibles were discarded, and trimmed heads were skinned, fixed in 4% paraformaldehyde for a further 24 hours, and then decalcified for 7 days using Decalcifying Solution B (Wako Pure Chemical Industries, Ltd, Osaka, Japan). After decalcification, the tissues were dehydrated in a series of graded ethanol solutions, followed by embedding in paraffin.

Histological Analysis

All samples were cut at the level of the anterior end of the olfactory bulb, as described previously.¹⁰ Paraffin sections (4 μ m thick) were deparaffinized in xylene and rehydrated in ethanol before hematoxylin and eosin staining or immunostaining. Hematoxylin and eosin staining was used for evaluation of whole tissue structure. For immunostaining, deparaffinized sections were treated with 3% hydrogen

Figure 1 Experimental timeline. Mice were administered intranasally with cigarette smoke solution (CSS) between day –23 and 0 (five cycles, each including four daily doses of 20 μ L per mouse and 1 rest day). Subsequently, the olfactory epithelium was collected for immunohistochemistry (IHC) and quantitative RT-PCR (qPCR) on the days indicated.

peroxide to block endogenous peroxidase activity and incubated in Blocking One (Nacalai Tesque, Kyoto, Japan) to block non-specific immunoglobulin binding. Sections were then incubated with primary antibodies against mouse SOX2 (1:300 dilution; rabbit monoclonal; Abcam, Tokyo, Japan; clone EPR3131), Ki-67 (1:200 dilution; rabbit monoclonal; Novus Biologicals, Littleton, CO; number NB600-1252), OMP (1:8000 dilution; goat polyclonal; Wako, Tokyo, Japan; number 544-10001), cleaved caspase-3 (1:300 dilution; rabbit polyclonal; Cell Signaling, Tokyo, Japan; number 9661), appropriate peroxidase-conjugated secondary antibodies, and a diaminobenzidine substrate. Analysis of histological sections was restricted to the OE to minimize variation between specimens. Three different microscopic fields (dorsal, middle, and ventral) of each bilateral septal olfactory neuroepithelium were captured using a digital microscope camera (BZ-9000; Keyence, Osaka, Japan) with a 40 $\times$ objective lens. The number of OMP⁺ ORNs was quantified by averaging the number of cells in each of the three microscopic fields between sections. The number of SOX2⁺ ORN progenitors, Ki-67⁺ cells, and caspase-3⁺ apoptotic cells per mm of basal layer length were counted manually for each of the three different fields using Photoshop CS6 digital imaging software version 6.1 (Adobe Systems, San Jose, CA).

RT-qPCR

Total RNA was isolated from the septal nasal mucosa using TRIzol reagent (Life Technologies, Tokyo, Japan) on days 1, 7, 14, and 28 after the final intranasal administration of CSS, then converted to cDNA using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo, Tokyo, Japan), according to the manufacturer's instructions. qPCR analysis was performed using THUNDERBIRD Probe qPCR Mix or THUNDERBIRD SYBR qPCR Mix (Toyobo) and an ABI 7500 sequence detector system (Life Technologies). The gene-specific primers and probes used were as follows: *Rps3* as endogenous control (Life Technologies assay number Mm00656272_m1); *Il1b* (forward, 5'-AGGCAGGCAGTA-TCACTCATTGT-3'; reverse, 5'-CGTCACACACCAGC-AGGTTATC-3'); and *Il6* (forward, 5'-CTGCAAGAG-ACTTCCATCCAGTT-3'; reverse, 5'-AGGTCTGTTGG-GAGTGGTATCC-3'). The expression levels of each gene were normalized to the level of *Rps3* expression for each sample.

Behavioral Testing to Evaluate Olfactory Function

Olfactory sensitivity was evaluated by an olfactory habituation/dishabituation test²⁴ that was performed on days 1, 7, and 14 after the final intranasal administration of CSS. Mice were allowed to acclimatize in a clean plastic cage (46 $\times$ 23.5 $\times$ 20 cm) without wood chip bedding for 30 minutes, then presented with a piece of filter paper soaked in odorless mineral oil (in a 35-mm polystyrene dish) for 3

minutes. This procedure was repeated a total of four times at 1-minute intervals. However, for the fourth exposure, the filter paper was soaked in propyl propionate instead of mineral oil. Throughout each test, mouse behavior was recorded using a digital video camera. The nose of the mouse being within 1 mm of the filter paper was considered to represent investigative behavior. The duration of investigative behavior was compared between the third and fourth exposures. Mice with normal olfaction display gradually reduced durations of investigative behavior from the first to third exposures (habituation), but display reinstatement of investigative behavior when an odor is presented with the filter paper. A lack of reinstatement indicates reduced or absent olfactory sensitivity.

Statistical Analysis

Statistical comparisons between groups or time points were performed by paired *t*-tests, the *U* test, or one-way analysis of variance using GraphPad Prism software version 6.0 (GraphPad Software Inc., San Diego, CA). qPCR data were subjected to logarithmic transformation before analysis. *P* < 0.05 was considered to be statistically significant.

Results

CSS Exposure Impairs ORNs

We first examined the effect of continuous intranasal administration of CSS on ORNs (Figure 1). Immunohistochemical staining revealed that OMP⁺ mature ORNs formed thick layers in the OE of saline-treated mice (Figure 2A). In contrast, the number of OMP⁺ cells in the mice that received 20 doses (five cycles) of CSS administration was approximately 70% less than the numbers observed in saline-treated mice 1 day after the final treatment (Figure 2, A and B). In mice that received 10 doses (2.5 cycles) of CSS administration, no reduction in the number of OMP⁺ mature ORNs was observed. We next examined whether the OMP⁺ mature ORN population would recover after cessation of CSS administration. A time course analysis revealed that the reduction in OMP⁺ ORN numbers became more severe on day 7 after the final intranasal administration of CSS, but then gradually recovered during days 14 to 28 (Figure 2, C and D). This delayed recovery suggests that CSS-induced damage to the OE is not simply because of acute damage to mature ORNs, but rather that additional long-term factors are involved in impairment of ORNs by CSS.

CSS Exposure Induces Olfactory Dysfunction

We next examined whether the CSS-induced reduction in OMP⁺ mature ORN numbers is associated with impaired olfaction by using a habituation/dishabituation test to evaluate olfactory sensitivity. In this test, propyl propionate,

Figure 2 **A** and **B**: Immunohistochemical staining (brown) of olfactory marker protein (OMP)⁺ cells in the olfactory epithelium 1 day after the final dose of cigarette smoke solution (CSS). Mice received either 10 doses (2.5 cycles) or 20 doses (5 cycles) of CSS. **C**: Immunohistochemical staining of OMP⁺ cells in the olfactory epithelium in saline-treated mice and on various days after the final CSS dose. Representative images from two independent experiments. **D**: Number of OMP⁺ olfactory receptor neurons per field in saline- or CSS-treated mice. Data are pooled from two independent experiments. **E**: Olfactory habituation/dishabituation test. Mice were presented with a piece of filter paper soaked in odorless mineral oil three times for 3 minutes, at 1-minute intervals. For the fourth exposure, the filter paper was soaked in the odorant propyl propionate instead of mineral oil. Data are representative of two independent experiments. No significant differences were detected between exposures 3 and 4 for CSS-treated mice on day 1 ($P = 0.07$) or day 7 ($P = 0.53$), suggesting a decrease in olfactory sensitivity. Data represent means $\pm$ SEM (**A**, **B**, **D**, and **E**). $n = 6$ (**A–D**); $n = 4$ to 7 (**E**). ** $P < 0.01$ (*U* test); ††† $P < 0.001$, †††† $P < 0.0001$ versus saline-treated mice (one-way analysis of variance); †† $P < 0.01$, exposure 3 versus exposure 4 (paired *t*-test).

which is predominantly perceived by the OE,²⁴ was used as the odorant. Saline-treated mice displayed decreasing durations of investigative behavior when repeatedly exposed to a piece of filter paper soaked with odorless mineral oil, which is consistent with habituation (Figure 2E). On the fourth exposure, when the filter paper was soaked with propyl propionate, the duration of investigative behavior was significantly longer than that on the third trial, suggesting that the mice were capable of smelling the odorant (dishabituation). In contrast, although CSS-treated mice displayed a similar pattern of habituation as saline-treated mice, there was no significant difference in the duration of investigative behavior between the third and fourth trials on days 1 or 7 after the final CSS administration, suggesting a

decrease in olfactory sensitivity. The loss of olfactory sensitivity was most severe on day 7 after the final CSS administration, but recovered by day 14. These results demonstrate that olfactory function is impaired by CSS administration, but that olfactory function recovers by day 14 after the final CSS administration.

CSS Exposure Impairs ORN Progenitor Cells

The similarity in the timing of the recovery of OMP⁺ ORN numbers and olfaction after cessation of CSS administration suggested that damage to the OMP⁺ ORNs may underlie CSS-induced hyposmia and anosmia. Because the number of OMP⁺ mature ORNs is determined by the balance

between the cell death of ORNs and the *de novo* generation of ORNs from ORN progenitors, we next investigated the effects of cigarette smoke on the number of ORN progenitors and cell proliferation, and on cell death, by examining SOX2 expression, Ki-67, and cleaved caspase-3. Caspase-3 is a crucial mediator of programmed cell death (apoptosis), which is cleaved during activation into an active form by apoptotic signaling and catalyzes the specific cleavage of many key cellular proteins.²⁵ Increases in cleaved caspase-3 expression in the OE have been associated with the clinical loss of sense of smell.²⁶ The Ki-67 protein is a specific marker of cell proliferation,²⁷ and Ki-67⁺ cells are detected throughout the depth of the epithelium (mainly in the basal layer of the OE).²⁸ In saline-treated mice, a large number of SOX2⁺ ORN progenitors and Ki-67⁺ proliferating cells were detected in the basal layer (Figure 3A). Although SOX2 expression was also observed in sustentacular cells in the luminal layer without progenitor activity, these cells were excluded from our quantification of SOX2⁺ ORN progenitors. Cleaved caspase-3⁺ apoptotic cells were scarcely detected in the OE, but were somewhat more frequent on days 1, 7, and 14 after final CSS administration. The number of SOX2⁺ ORN

progenitors in the basal layer was approximately 20% lower than in saline-treated mice on day 1 after final CSS administration, was elevated on day 7, but had returned to saline-treated mouse levels by day 14 (Figure 3B). The number of Ki-67⁺ proliferating cells in the basal layer changed with a time course similar to that of SOX2⁺ ORN progenitors. Given that OMP⁺ ORNs are maintained by the continuous proliferation and subsequent maturation of SOX2⁺ ORN progenitors in the steady-state, these results are consistent with reduced proliferation of ORN progenitors in mice exposed to CSS and the CSS-induced death of ORNs having had a considerable impact on ORN numbers and olfaction.

CSS Administration Induces Increases in IL-1 β and IL-6 Expression in the OE

Finally, because inflammatory cytokines influence the proliferation and differentiation of neural stem and progenitor cells, we examined the possible involvement of inflammatory cytokines in the CSS-induced disruption of the ORN progenitor cell population. Time course analyses revealed that Il1b and Il6 mRNA expression was significantly

Figure 3 **A:** Immunohistological staining (brown) of SOX2⁺ olfactory receptor neuron (ORN) progenitor, Ki-67⁺ proliferating, and cleaved caspase-3⁺ (Cas3⁺) apoptotic cells. Tissue sections were counterstained with the nuclear dye hematoxylin (blue). **Arrowheads** indicate Cas3⁺ apoptotic cells in the olfactory epithelium. Representative images from two independent experiments. **B:** Numbers of SOX2⁺ ORN progenitors and Ki-67⁺ actively proliferating cells per mm of the basal layer in saline (open circles) or cigarette smoke solution (CSS)-treated (closed circles) mice. Data are pooled from two independent experiments. Data represent means $\pm$ SEM (**B**). $n = 6$ (**A** and **B**). * $P < 0.05$, ** $P < 0.01$ (paired *t*-tests).

Figure 4 IL1b (A) and IL6 (B) mRNA expression in the nasal mucosa was quantified by quantitative RT-PCR in saline- or cigarette smoke solution (CSS)-treated mice (open and closed circles, respectively), and calculated relative to expression of the endogenous control gene *Rps3*. Data are representative of two independent experiments. Data represent means $\pm$ SEM. $n = 4$ to 6. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (U test).

elevated in the nasal mucosa on days 1 and 7 after final CSS administration, relative to expression in saline group mice (Figure 4, A and B). However, IL1b and IL6 expression returned to the saline-group levels by day 14 after final CSS administration.

Discussion

Herein, we demonstrate, for the first time, that long-term CSS administration damages not only OMP⁺ mature ORNs but also SOX2⁺ ORN progenitors in the OE. The reduction in mature ORN numbers was associated with olfactory dysfunction, but cessation of CSS administration resulted in restoration of ORN numbers and olfaction. Time course analyses revealed that the numbers of SOX2⁺ ORN progenitors and Ki-67⁺ proliferating cells increased transiently approximately 7 days after final CSS administration, preceding recovery of the OMP⁺ mature ORN population. Increased apoptosis is unlikely to be solely responsible for the reduction in ORN numbers, because the number of cleaved caspase-3⁺ apoptotic cells remained elevated 14 days after final CSS administration, even after the recovery of the OMP⁺ mature ORN population. Elevated expression of the inflammatory cytokines IL-1 β and IL-6 also persisted until day 7 after CSS administration, suggesting that persistent CSS-induced inflammation may influence ORN recovery.

Numerous studies have linked cigarette smoke exposure with olfactory dysfunction in humans,^{1,2,6–8,29} but there are also some studies that report only minimal effects of smoking on olfaction. For example, Yee et al³⁰ observed no adverse impact of smoking history on olfactory function in human patients with chronic rhinosinusitis. Similarly, Rawson et al³¹ reported a similar proportion of functional olfactory neurons in biopsy specimens from smokers and non-smokers. Differences in the duration and dose of smoking may account for some of these discrepancies.

It has proved challenging to establish an animal model of cigarette smoking that enables investigation of the mechanisms underlying cigarette smoke-induced olfactory dysfunction. In the field of pulmonary research, various experimental models of lung disorders have been described in which guinea pigs, rats, or mice are exposed to cigarette smoke for 3 months or longer.^{23,32–34} However, recently, it was reported that intratracheal CSS administration causes pulmonary impairment in a much shorter time period than exposure to cigarette smoke does.²³ Herein, we established a practical and reproducible model of cigarette smoking in which olfactory impairment develops after 20 doses of intranasal CSS administration over 24 days. The chemical irritant content of a single dose of CSS administration is approximately equivalent to the exposure a human receives from smoking approximately 40 cigarettes,²³ and thus our model corresponds to the exposure received by a heavy smoker and is likely to provide insights into the olfactory dysfunction experienced by these smokers. Nevertheless, daily CSS administration does not recapitulate all aspects of cigarette smoking, such as the prolonged period of exposure within each day (time spent smoking) or exposure to heat and ash. Our finding that mice exposed to only 10 doses of CSS did not experience any change in OMP⁺ mature ORN numbers suggests that a certain minimum period of exposure to cigarette smoke is required for the development of olfactory dysfunction. On the other hand, it is possible that longer periods of exposure or higher doses of CSS administration than those used in the present study could cause irreversible OE impairment and permanent hyposmia/anosmia.

The OE contains mature ORNs and their progenitors, which are part of the regenerative peripheral nervous system.¹³ The sense of smell is mediated by ORNs that are exposed to the nasal airway, which renders these cells vulnerable to environmental injury and death. As a consequence, a basal level of apoptotic ORN death occurs even in the absence of disease.³⁵ Dead ORNs are replaced by the mitosis and maturation of progenitors to maintain sufficient numbers of neurons throughout adult life. Disruption of this balance is proposed to be a common cause for clinical loss of sense of smell.⁸ In our CSS exposure model, we found that a decrease in the numbers of proliferating cells and ORN progenitors and an increase in ORN apoptosis were associated with a reduction in the number of mature ORNs in the OE of CSS-treated mice. The exact mechanism by which cigarette smoke disrupts the balance between proliferation and apoptosis in the OE remains unclear, and there are conflicting reports in the literature on this question. It has been reported that cigarette smoke decreases the thickness of the OE in humans¹² and increases apoptosis of the OE in rats.¹¹ On the other hand, in a study of patients with chronic rhinosinusitis and a history of smoking, metaplastic hyperplasia was observed, but ORNs and progenitors appeared to persist beneath the epithelium.³⁰ Thus, further studies are required to clarify the mechanisms of cigarette

smoke—induced pathological changes in the OE in rodents and humans. However, it seems likely that cigarette smoke could damage ORNs and their progenitors by inducing inflammation and immune responses in the OE, similar to the way in which cigarette smoke toxicity in the lungs is mediated largely by inflammation and immunotoxicity.^{3,4} Our observation that CSS induces expression of the inflammatory cytokines IL-1 β and IL-6 is consistent with this theory. In particular, IL-6 suppresses the expression of SOX2,²² suppresses OE regeneration after injury, and plays an important role in the development of hyposmia.³⁶ Considering that elevated levels of IL-1 β and IL-6 expression persisted for at least 7 days after final CSS administration and coincided with the reduced numbers of mature ORNs in the OE, these inflammatory cytokines might be involved in reducing SOX2⁺ ORN progenitor numbers, suppressing the maturation of ORNs from their progenitors, or inducing apoptosis. A better understanding of how inflammation affects the ORN progenitor cell system could guide the development of therapeutic strategies for cigarette smoke— or chemical irritant—induced hyposmia and anosmia.

Herein, we focused on the effects of CSS exposure on the OE. However, olfactory dysfunction can also be induced by damage to the olfactory bulb in the forebrain. The ORNs are anatomically linked to olfactory bulbs via the cribriform plate, which enables cigarette smoke and cigarette smoke—induced inflammation to directly impair the central nervous system and affect olfaction.³⁷ Accordingly, damage to the olfactory bulb could also be responsible for some olfactory dysfunction. Future neurophysiological studies that quantify signal transduction from the ORNs to the olfactory bulb are necessary to fully understand the pathogenesis of cigarette smoke—induced olfactory dysfunction.

In conclusion, we have demonstrated that by damaging the SOX2⁺ ORN progenitor population and increasing ORN death, CSS eventually overwhelms the regenerative capacity of the epithelium, resulting in reduced numbers of mature ORNs and olfactory dysfunction. However, the ORN progenitor population and olfaction recovered after cessation of exposure to CSS. Our findings provide a basis for future studies investigating the mechanisms underlying cigarette smoke—induced damage to ORN progenitor cells, and will help guide the development of preventive and therapeutic approaches for cigarette smoke—induced olfactory dysfunction.

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