Dexamethasone affects mouse olfactory mucosa gene expression and attenuates genes related to neurite outgrowth

Jun Tian, MD[1], Jayant M. Pinto, MD[2], Yi Xin, MS[1], Henghui Zhang, MD[3], Li Li, MD[4], Zhifu Sun, MS[1] and Yongxiang Wei, MD,PhD[1]

Background: **Olfaction is one of the important senses for humans. Systemic glucocorticoids are the most commonly used medications for olfactory loss because of their strong anti-inflammatory effects. However, their effect on olfactory function is still controversial and the precise mechanism is not clear. To gain a global view of the effect of systematic glucocorticoid treatment on gene expression in the olfactory mucosa (OM), we profiled these changes in a murine model of olfaction in order to identify underlying molecular mechanisms.**

Methods: **C57BL/6 mice were injected daily for 2 weeks (WK2) with dexamethasone (DEX, intraperitoneally, 1 mg/kg body weight) vs 1 day of DEX (D1) vs controls, which received saline (Ctrl) (n = 9/group). Total RNA from the OM was used to analyze global gene expression. Genes showing changes in expression were compared using the Database for Annotation, Visualization and Integrated Discovery (DAVID, v6.7) and the General Olfactory Sensitivity Database (GOSdb; http://genome.weizmann.ac.il/GOSdb).**

Results: **Between the WK2 and Ctrl groups, 3351 genes were differentially expressed, of which 236 genes were related to olfactory function. Genes involved in axon guidance, cell projection, and inflammation were enriched and overlapped significantly with those in the GOSdb.**

Conclusion: **Systemic glucocorticoids exert effects on transcription of a notable number of genes in the OM and appear to orchestrate changes related to axon guidance, cell projection, and inflammation. Further examination may allow targeted therapies that lack the side effects of this category of medication.** © 2015 ARS-AAOA, LLC.

Key Words:
corticosteroid use; olfactory disorders; olfaction; microarray; epithelial cell

***How to Cite this Article*:**
Tian J, Pinto JM, Xin Y, et al. Dexamethasone affects mouse olfactory mucosa gene expression and attenuates genes related to neurite outgrowth. *Int Forum Allergy Rhinol.* 2015;XX:1–12.

Olfaction is one of the most important senses for humans, affecting such basic processes as the appreciation of food and drink, the detection of hazards in the environment, the generation of feelings of pleasure, sexuality, and behavior, and the maintenance of mood.[1] Despite its critical importance, the significance of olfaction and its decline is frequently underappreciated by patients and physicians alike.

Olfactory dysfunction is common, with 4 major causes: aging, chronic sinonasal inflammation, head trauma, and upper airway viral infection. Sadly, what Donald Leopold said 14 years ago remains true: "Truly, it is difficult to sit in front of the patient who has just had testing to define the amount of loss and tell him or her that there is no therapy

[1]*Department of Otolaryngology–Head and Neck Surgery, Beijing Anzhen Hospital, Capital Medical University, Beijing, China;* [2]*Section of Otolaryngology–Head and Neck Surgery, Department of Surgery, The University of Chicago, Chicago, IL;* [3]*Peking University Hepatology Institute, Peking University People's Hospital, Beijing, China;* [4]*Department of Otolaryngology–Head and Neck Surgery, Beijing Chaoyang Hospital, Capital Medical University, Beijing, China*

Correspondence to: Yongxiang Wei, MD, PhD, Department of Otolaryngology Head and Neck Surgery, Beijing Anzhen Hospital, Capital Medical University, Beijing, China; e-mail: weiyongxianglc@126.com

Additional Supporting Information may be found in the online version of this article.

Funding sources for the study: National Natural Science Foundation, Government of China (No. 81271062); Beijing Natural Science Foundation, Government of China (No. KZ201310025029); and Beijing Municipal Science and Technology Commission (No. 2012218048).
Potential conflict of interest: None provided.
Correction added on 16-September, after first online publication: The Appendix has been moved to supporting information

Received: 22 April 2015; Revised: 27 May 2015; Accepted: 29 May 2015
DOI: 10.1002/alr.21586
View this article online at wileyonlinelibrary.com.

and no return to normalcy is expected."[2] Other than olfactory retraining, which has not been extensively studied,[3–5] the only therapy we have for olfactory loss is targeted at the 1 pathologic process that is reversible and affects olfaction: inflammation. Thus, systemic glucocorticoids are the most commonly used medications for olfactory loss because of their strong anti-inflammatory effects, but the precise mechanism of effect is not clear in this setting. For example, recent studies demonstrated that the systemic administration of corticosteroids rather than intranasal topical administration (standard administration or targeted via special maneuvers) appeared to have a stronger effect on olfactory dysfunction.[6,7] Most studies, however, lack an untreated control group, and there is a wide range (32-66%) of patients with spontaneous recovery of the sense of smell following upper respiratory tract infections,[8–10] so one must interpret these data cautiously.

The identification of glucocorticoid receptors in the olfactory mucosa (OM)[11] suggests that corticosteroids may have direct effects on the cells of the OM. Other data[12,13] suggest that olfactory sodium-potassium adenosine triphosphatase (Na^+K^+ ATPase) is regulated by aldosterone, a steroid analogue. Our studies showed that glucocorticoids enhance both the messenger RNA (mRNA) expression of cyclic nucleotide-gated (CNG) channels and the production of cyclic adenosine monophosphate (cAMP) by a dose-dependent effect.[14] These data provide some evidence support the speculation that steroids might have some direct effect on the olfactory transduction in addition to their well-known anti-inflammatory activity. This concept is supported by the clinical observation that systemic steroids sometimes are helpful in patients without obvious inflammatory disease. Direct evidence that steroids work in this manner on olfactory physiology is needed.

Glucocorticoids are involved in regulation of diverse cell processes and coordinate a variety of physiological responses.[15] Thus, long-term use even of low-dose steroids is of considerable concern because of numerous side effects, and high levels of systemic steroids can actually potentiate damage to the olfactory apparatus.[16] Therefore, understanding the precise mechanisms underlying their effects would allow for the design of targeted therapies that might minimize or eliminate these deleterious side effects.

To gain a global view of the effect of systematic glucocorticoid treatment on gene expression in the OM, we profiled these changes in a murine model of olfaction in order to identify underlying molecular mechanisms.

Materials and methods

Animals and treatment groups

Twenty-seven wild-type, 6- to 8-week-old, male, pathogen-free C57BL/6 mice (n = 27, 17 to 22 g; Vital River Laboratory Animal Technology Co. Ltd., Beijing, China) were handled in accordance with U.S. National Institutes of Health guidelines and maintained in the Animal Experimental Center of Bejing Anzhen Hospital. The studies

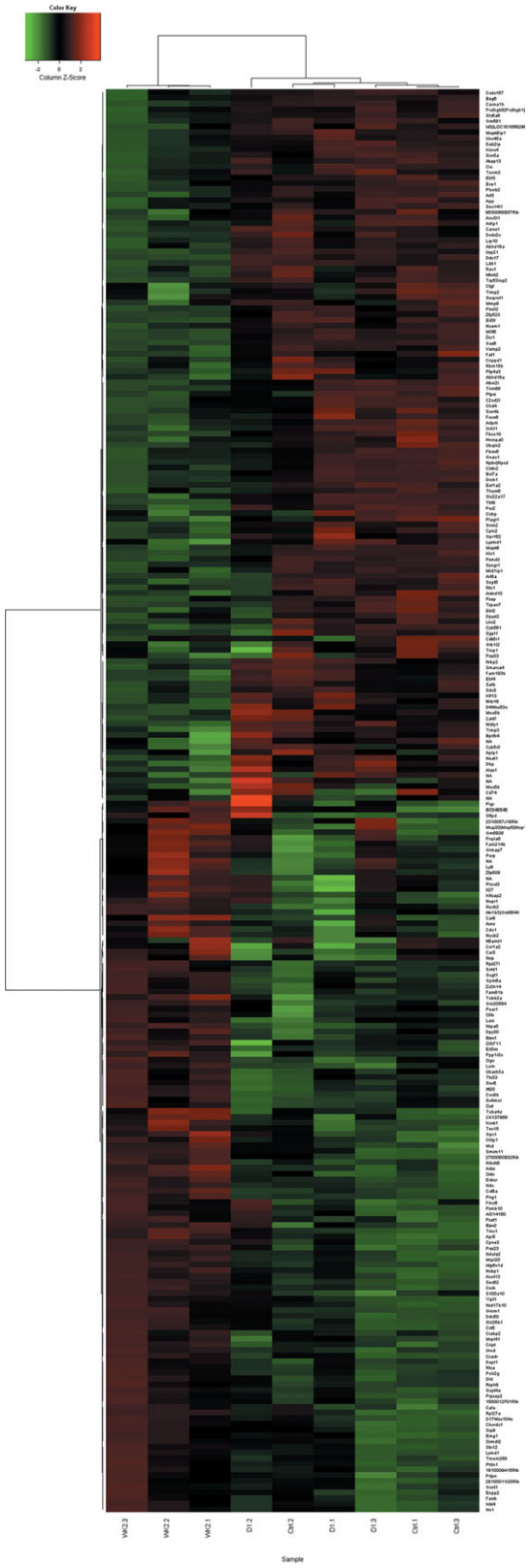

FIGURE 1. Unsupervised hierarchical clustering analysis of overall gene expression between each experimental sample. The top 250 differently expressed genes were included for analysis and demonstrated by rows. The color bar stands for the $\log_2$ values of the probe change folds, with red for upregulation and green for downregulation. Three arrays in the WK2, D1, and Ctrl groups were numbered separately and labeled by columns. Ctrl = control; D1 = 1-day group; WK2 = 2-week group.

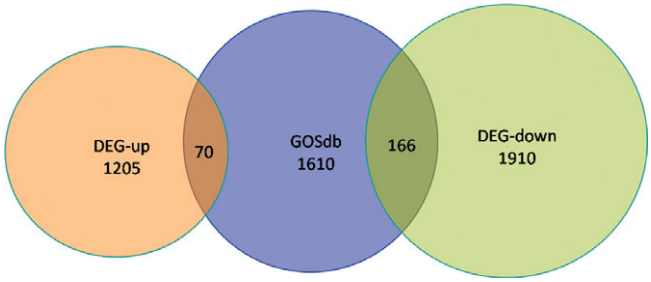

FIGURE 2. Genes overlap in the GOSdb and all the DEGs, with upregulated genes marked by red color (70/1275, 5.5%) and downregulated genes marked by green color (166/2076, 8.0%). DEG = differently expressed genes; DEG-down = downregulated DEGs; DEG-up = upregulated DEGs; GOSdb = General Olfactory Sensitivity Database.

followed the institutional and national guidelines for the care and use of animals and were approved by the Animal Care and Use Committee of the Capital Medical University, Beijing.

The animals were randomly divided into 3 groups with 9 mice in each group. In the day 1 group (D1), the mice were injected once with dexamethasone (DEX) intraperitoneally (1 mg/kg body weight); after 24 hours, the animals were euthanized for gene examination. In the 2-week group (WK2), the mice were injected with DEX (1 mg/kg body weight) once a day for 2 weeks. In control group (Ctrl), the mice were injected with an equivalent amount of normal saline and dealt with in the same way as the mice in 2-week group.

RNA preparation

Samples of olfactory epithelium were prepared according to the method described in Genter et al.[17] Briefly, mice were

TABLE 1. Top 10 enrichment pathways after 2 weeks treatment with dexamethasone

No.	Gene set name	Genes in gene set (K)	Genes in overlap (k)	k/K	*p*	Benjamini q
1	Pathways in cancer	323	63	0.195	0.000621	0.109
2[a,b]	Chemokine signaling pathway	182	39	0.214	0.00149	0.13
3[a,b]	ECM-receptor interaction	83	22	0.265	0.00151	0.0897
4[b]	Cytokine-cytokine receptor interaction	244	47	0.193	0.00439	0.185
5	Focal adhesion	198	39	0.197	0.00682	0.225
6[c]	Axon guidance	131	28	0.214	0.0082	0.225
7	TGF-beta signaling pathway	87	20	0.23	0.0134	0.302
8	Circadian rhythm	13	6	0.462	0.0186	0.354
9	Small cell lung cancer	85	19	0.224	0.0215	0.361
10[a]	Dilated cardiomyopathy	92	20	0.217	0.0237	0.36

[a]The pathways that were also identified in 1 of the other 2 enrichment analyses.
[b]These pathways indicate a role in inflammation, with wide effects such as inflammatory host defenses, cell growth, differentiation, cell death, angiogenesis, and development and repair processes aimed at the restoration of homeostasis.
[c]The pathway that was also identified in the other of 2 the enrichment analyses.
ECM = extracellular matrix; k = genes in overlap; K = genes in gene set; TGF = transforming growth factor.

TABLE 2. Top 10 enrichment pathways of differently expressed auxiliary olfactory genes after 2 weeks treatment with dexamethasone

No.	Term	Count	%	*p*	Genes
1[a]	Axon guidance	10	4.3	6.16E−05	UNC5B, PLXNA4, RAC1, PLXNB2,GNAI2, RGS3, FYN, EFNA2, CXCL12, NFATC1
2[b]	Dilated cardiomyopathy	6	2.5	0.007989605	ADCY4, ATP2A2, CACNG7, ITGB4, ITGB5, GNAS
3[b]	Long-term depression	5	2.1	0.01618072	GNAO1, GNAI2, ARAF, GNAS, PLA2G4E
4	ARVC	5	2.1	0.018543865	ATP2A2, CACNG7, ITGB4, ITGB5, ACTN1
5	HCM	5	2.1	0.026876882	ACE, ATP2A2, CACNG7, ITGB4, ITGB5
6[b]	Gap junction	5	2.1	0.028988964	GJD2, ADCY4, GNAI2, TUBA4A, GNAS
7	Prion diseases	3	1.2	0.081389021	NCAM1, FYN, HSPA5
8	Adherens junction	4	1.7	0.083986801	PTPRJ, FYN, RAC1, ACTN1

[a]The pathway that was also identified in the other of the 2 enrichment analyses.
[b]The pathways that were also identified in 1 of the other 2 enrichment analyses.
ARVC = arrhythmogenic right ventricular cardiomyopathy; HCM = hypertrophic cardiomyopathy.

TABLE 3. Enrichment pathway of top 450 auxiliary olfactory genes

No.	Term	Count	%	*p*	Benjamini
1[a]	Calcium signaling pathway[a]	30	7.246376812	2.15E−14	2.61E−12
2	GnRH signaling pathway[a]	20	4.830917874	9.56E−12	5.83E−10
3	Melanogenesis	20	4.830917874	1.69E−11	6.86E−10
4	Vascular smooth muscle contraction	20	4.830917874	4.63E−10	1.41E−08
5[a]	Long-term potentiation[a]	13	3.140096618	3.59E−07	8.75E−06
6[b]	Gap junction	14	3.381642512	5.25E−07	1.07E−05
7[a]	Taste transduction	12	2.898550725	5.55E−07	9.68E−06
8	Insulin signaling pathway	17	4.106280193	1.02E−06	1.56E−05
9[b]	Chemokine signaling pathway	19	4.589371981	2.22E−06	3.01E−05
10[b]	Dilated cardiomyopathy	13	3.140096618	7.21E−06	8.80E−05
11	Oocyte meiosis	14	3.381642512	1.47E−05	1.63E−04
12	Glioma	10	2.415458937	5.73E−05	5.83E−04
13	Progesterone-mediated oocyte maturation	11	2.657004831	1.05E−04	9.87E−04
14[a]	Apoptosis[a]	11	2.657004831	1.28E−04	0.001117311
15	Phosphatidylinositol signaling system	9	2.173913043	0.001016166	0.008234927
16	Type II diabetes mellitus	7	1.690821256	0.002149379	0.0162728
17[b]	Prion diseases	6	1.449275362	0.002541679	0.018097737
18[a]	ErbB signaling pathway	9	2.173913043	0.002659179	0.017885453
19[b]	Wnt signaling pathway[a]	12	2.898550725	0.002670885	0.017026222
20	Purine metabolism	12	2.898550725	0.004004524	0.024179515
21	Tight junction	11	2.657004831	0.00404596	0.02327758
22	Fc epsilon RI signaling pathway	8	1.93236715	0.007324944	0.039949745
23[a]	Neurotrophin signaling pathway[a]	10	2.415458937	0.009684818	0.050312241
24[a]	MAPK signaling pathway	15	3.623188406	0.014693699	0.07248579
25	HCM	7	1.690821256	0.028369097	0.131026687
26	Maturity onset diabetes of the young	4	0.966183575	0.034037723	0.149981312
27	Adipocytokine signaling pathway	6	1.449275362	0.037370321	0.158100401
28	Retinol metabolism	6	1.449275362	0.039468003	0.160923469
29[a,b]	Long-term depression[a]	6	1.449275362	0.048567487	0.188967171
30	Glutathione metabolism	5	1.207729469	0.055021087	0.205580411
31[a]	Neuroactive ligand-receptor interaction	13	3.140096618	0.056108174	0.203277978
32	Non-small-cell lung cancer	5	1.207729469	0.061642643	0.215389702
33[a,c]	Axon guidance[a]	8	1.93236715	0.070677032	0.237371297
34[b]	ECM-receptor interaction	6	1.449275362	0.079484719	0.257092447

[a]An obviously potential role in olfactory function.
[b]The pathways that were also identified in 1 of the other 2 enrichment analyses.
[c]The pathway that was also identified in the other 2 enrichment analyses.
ECM = extracellular matrix; GnRH = gonadotropin-releasing hormone; HCM = hypertrophic cardiomyopathy; RI = receptor.

TABLE 4. Gene Ontology analysis based on cellular component after 2 weeks treatment with dexamethasone

No.	Gene set name	Genes in gene set (K)	Genes in overlap (k)	k/K	*p*	Benjamini q
1[a]	Cell projection	575	112	0.195	4.35e−05	0.024
2[b]	Golgi apparatus	682	128	0.188	6.92e−05	0.0191
3	Cell junction	470	93	0.198	0.000116	0.0213
4	Plasma membrane	2906	451	0.155	0.000238	0.0326
5	Proteinaceous extracellular matrix	297	62	0.209	0.000444	0.0484
6	Plasma membrane part	1633	265	0.162	0.000515	0.0468
7	Anchoring junction	123	31	0.252	0.000768	0.0594
8	Heterochromatin	51	17	0.333	0.000769	0.0523
9	Extracellular matrix	309	62	0.201	0.00126	0.0753
10	Nuclear heterochromatin	35	13	0.371	0.00146	0.0782

[a]The pathway that was also identified in the other 2 enrichment analyses.
[b]The Gene Ontology that was also identified in 1 of the other 2 enrichment analyses.
k = genes in overlap; K = genes in gene set.

euthanized by CO_2 asphyxiation, and after decapitation, the skin and soft tissue were removed from the skull and the lower jaws were removed. An incision was made on the skull along the midline and caudal one-third of septal epithelium and the ethmoid turbinates were dissected free under stereomicroscopy. The bones were removed simultaneously. All samples were quickly frozen in liquid nitrogen and stored at −80°C until extraction of RNA. Once all samples were collected, total RNA was extracted from each mouse using the Trizol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The yield, purity, and quality of the total RNA for each sample were determined with a Nanodrop 2000 (Thermo Scientific, Waltham, MA) and RNA electrophoresis.

Gene expression

Real-time polymerase chain reaction (PCR) data for expression of olfactory marker protein (OMP) (a known marker of olfactory epithelium) mRNA levels and quality of RNA product were used to select 3 individual mouse samples from each treatment group with optimal expression of OMP. Then, these 9 total RNA samples (3 per group) were processed for gene expression analysis (performed by Phalanx Biotech Group, Hsinchu, Taiwan). The RNA concentration and purity were checked by standard methods (optical density (OD) A260/A280 [>1.8] and A260/A230 [>1.6]), and the yield and quality were accessed using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) (RNA integrity (RIN) > 6). Fluorescent

TABLE 5. Gene Ontology analysis based on molecular function after 2 weeks treatment with dexamethasone

No.	Gene set name	Genes in gene set (K)	Genes in overlap (k)	k/K	*p*	Benjamini q
1	GTPase regulator activity	361	86	0.238	6.98e−08	8.64e−05
2	Nucleoside-triphosphatase regulator activity	367	86	0.234	1.51e−07	9.37e−05
3	Small GTPase regulator activity	223	56	0.251	3.23e−06	0.00133
4	DNA binding	1781	301	0.169	3.56e−06	0.0011
5	Protein kinase activity	583	117	0.201	4.26e−06	0.00106
6[a]	Cation binding	3885	596	0.153	1.25e−05	0.00257
7[a]	Ion binding	3934	601	0.153	1.9e−05	0.00335
8	GTPase activator activity	192	48	0.25	2.02e−05	0.00312
9[a]	Metal ion binding	3850	588	0.153	2.71e−05	0.00373
10	Enzyme activator activity	249	55	0.221	0.000175	0.0215

[a]The Gene Ontology that was also identified in 1 of the other 2 enrichment analyses.
k = genes in overlap; K = genes in gene set.

TABLE 6. Gene Ontology analysis based on biological process after 2 weeks treatment with dexamethasone

No.	Gene set name	Genes in gene set (K)	Genes in overlap (k)	k/K	*p*	Benjamini q
1	Intracellular signaling cascade	915	174	0.19	1.5e−06	0.00567
2	Protein amino acid phosphorylation	640	128	0.2	3.5e−06	0.0066
3	Regulation of transcription	2227	365	0.164	1.77e−05	0.0221
4	Regulation of small GTPase mediated signal transduction	228	55	0.241	2.08e−05	0.0195
5	Regulation of Ras protein signal transduction	181	46	0.254	2.81e−05	0.021
6	Transcription	1772	296	0.167	3.54e−05	0.0221
7	Phosphorus metabolic process	866	157	0.181	7.26e−05	0.0385
8	Phosphate metabolic process	866	157	0.181	7.26e−05	0.0385
9	Protein kinase cascade	236	54	0.229	0.000112	0.0515
10	Enzyme-linked receptor protein signaling pathway	273	60	0.22	0.000146	0.0595

k = genes in overlap; K = genes in gene set.

amplified RNA (aRNA) targets were prepared from 1 μg total RNA samples using Amino Allyl MessageAmp™ II aRNA Amplification Kit (AM1753; Life Technologies, Carlsbad, CA) and Cy5 dyes (Amersham Pharmacia, Piscataway, NJ). Fluorescent targets were hybridized to the mouse Whole Genome OneArray® in triplicate with Phalanx hybridization buffer using the Phalanx Hybridization System (all from Phalanx Biotech Group).[18] This gene chip contains 27,295 DNA oligonucleotide probes, and each probe is a 60-mer designed in the sense direction. Among the probes, 26,423 probes correspond to the annotated genes in RefSeq v42 and Ensembl r59 database. Additionally 872 control probes are also included. The detailed descriptions of the gene array list are available from GEO platform GPL15523x (http://www.ncbi.nlm.nih.gov/geo/). After 16 hours of hybridization at 50°C, nonspecific binding targets were washed away by 3 different washing steps (Wash I: 42°C for 5 minutes; Wash II: 42°C for 5 minutes then 25°C for 5 minutes; Wash III: rinse 20 times), and the slides were dried by centrifugation and scanned by Agilent's High-Resolution C Scanner (Agilent Technologies)/Axon 4000B Scanner (Molecular Devices, Sunnyvale, CA). The Cy5 fluorescent intensities of each spot were analyzed by GenePix 4.1 software (Molecular Devices).

The signal intensity of each spot was loaded into Rosetta Resolver System® (Rosetta Biosoftware, Seattle, WA) for data analysis. We used the error model of the Rosetta Resolver System to remove both systematic and random errors from the data. Spots that passed the criteria were normalized by the 50% media scaling normalization method. Normalized spot intensities were transformed to gene expression $\log_2$ ratios between the control and treatment groups. The spots corresponding to loci with $\log_2$ ratio ≥ 0.585 or $\log_2$ ratio ≤ -0.585 (1.5-fold difference) and p value < 0.05 in gene expression were defined as differentially expressed genes and selected for further analysis.

Gene expression analysis

The differentially expressed genes were compared and annotated using the Database for Annotation, Visualization and Integrated Discovery (DAVID, v6.7), a free Web-based application, with the whole *Mus musculus* genome as the background, and gene ontology (GO) categorization was performed using a modified Fisher exact test.[19,20]

To determine whether these differentially expression genes have effect on olfaction, we compared these genes to those loci from an available database named the General Olfactory Sensitivity Database (GOSdb; http://genome.weizmann.ac.il/GOSdb) by Keydar et.al.,[21] which has identified a list of 1680 candidate auxiliary olfactory genes by a systematic exploration via literature survey as well as data mining, using DAVID (looking for homologous murine genes). To focus on the most reliable signals, only the top 450 candidate auxiliary olfactory genes were compared. Enrichment analysis of GO category (including biological process, molecular function, and cellular component) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway under the Functional Annotation Clustering Tool was calculated for all annotated genes in DAVID. High classification stringency was chosen and other DAVID default options settings were used. Genes that were both differentially expressed genes and listed in GOSdb were then chosen for enrichment analysis to provide further information on mechanism.

Validation studies

Expression changes in the genes of interest were confirmed using reverse-transcription polymerase chain reaction (RT-PCR). Briefly, mRNA amounts were measured in duplicate from samples from 9 mice in each of the groups using the SYBR® Premix Ex Taq™ II (TaKaRa, Shiga, Japan). To make complementary DNA (cDNA), reverse

TABLE 7. Gene Ontology analysis of differently expressed auxiliary olfactory genes after 2 weeks of treatment with dexamethasone

No.	Term	Count	%	*p*	Genes
	GO Analysis_CC				
1[a]	Cell projection	20	8.6	1.38E−04	ADCY4, FABP7, MAPK8IP1, TTLL3, CPLX2, RAC1, PROM1, NCAM1, CAPZB, MYO1C, PKHD1, HTT, TTLL10, CROCC, GNAS, PKD1, BBS1, RPGRIP1, FBXO10, SLC1A2
2	Plasma membrane part	37	15.9	6.57E−04	CYB5R3, KCNC4, RAB5B, PANX2, GRIP1, PKHD1, ITGB4, ITGB5, PANX3, KCNA5, CXADR, CAPZB, KISS1R, SLC1A2, WNK4, SYN2, RAC1, SV2A, SCN5A, KCNE3, GJD2, PHACTR1, GNAO1, MYO1C, FLOT2, ACTN1, HOMER2, LDLRAP1, NOTCH3, NCAM1, PROM1, IGSF5, RGS3, GNAS, CP, SYTL1, PLA2G4E
3	Plasma membrane	56	24.1	8.58E−04	CYB5R3, ADCY4, JPH3, KCNC4, PLXNA4, CLSTN2, RAB5B, GRIP1, EFNA2, CXADR, SHH, LPHN3, SLC1A2, KISS1R, WNK4, TAAR1, SV2A, LPHN1, GJD2, FLOT2, ACTN1, LDLRAP1, TRPM2, NCAM1, IGSF5, GNAS, TSHR, GNAI2, PANX2, PKHD1, ITGB4, ITGB5, NKAIN1, PANX3, KCNA5, CAPZB, APLP1, LINGO1, ACE, P2RY2, RAC1, SYN2, PKD1, SCN5A, KCNE3, PHACTR1, GNAO1, MYO1C, HOMER2, PROM1, NOTCH3, RGS3, FYN, CP, SYTL1, PLA2G4E
4	Cell fraction	17	7.3	0.004044793	CYB5R3, JPH3, GNAO1, GNAI2, HTT, FLOT2, ANPEP, SHH, APLP1, RDH10, ATP2A2, RAC1, ARAF, CELF2, GNAS, SYTL1, ATP8B3
5	Cell-cell junction	8	3.4	0.006830636	GJD2, IGSF5, PANX2, WNK4, PANX3, CXADR, CAPZB, SCN5A
6	Cytoplasmic membrane-bounded vesicle	13	5.6	0.006935327	PACS1, RAB5B, HTT, GRIP1, ACTN1, LDLRAP1, SYN2, RAC1, SEC22B, HSPA5, SV2A, SYTL1, ATP8B3
7	Cell junction	14	6.0	0.007344874	GJD2, PHACTR1, GRIP1, PANX2, ACTN1, PANX3, CXADR, CAPZB, HOMER2, IGSF5, WNK4, SYN2, SV2A, SCN5A
8	Membrane-bounded vesicle	13	5.6	0.007738331	PACS1, RAB5B, HTT, GRIP1, ACTN1, LDLRAP1, SYN2, RAC1, SEC22B, HSPA5, SV2A, SYTL1, ATP8B3
9	~Endoplasmic reticulum	19	8.1	0.021133129	CYB5R3, SGPL1, JPH3, CLSTN2, GRIP1, NAPA, RIC3, RTN1, RDH5, RDH10, SCFD1, ATP2A2, RYR3, NUCB2, MBOAT1, SEC22B, POFUT2, HSPA5, ALG13
10	Cell projection part	7	3.0	0.022051598	SLC1A2, TTLL10, MYO1C, PKHD1, CROCC, RPGRIP1, TTLL3
	GO Analysis_MF				
1[b]	Metal ion transmembrane transporter activity	13	5.6	1.60E−04	KCNC4, CACNG7, KCNA5, TRPM2, KCNJ4, ATP2C2, ATP2A2, RYR3, PKD1, SCN4B, KCNH3, SCN5A, KCNE3
2[b]	Cation channel activity	11	4.7	6.92E−04	KCNJ4, KCNC4, CACNG7, RYR3, SCN4B, PKD1, KCNA5, SCN5A, KCNH3, KCNE3, TRPM2
3[a]	Alkali metal ion binding	10	4.3	7.27E−04	KCNJ4, FXYD2, KCNC4, SLC4A8, SCN4B, KCNA5, SCN5A, KCNH3, KCNE3, TRPM2 (无特异性)
4[b]	Ion channel activity	13	5.6	8.47E−04	FXYD2, KCNC4, CACNG7, KCNA5, TRPM2, KCNJ4, CLIC3, RYR3, PKD1, SCN4B, KCNH3, SCN5A, KCNE3
5	Substrate specific channel activity	13	5.6	0.001107541	FXYD2, KCNC4, CACNG7, KCNA5, TRPM2, KCNJ4, CLIC3, RYR3, PKD1, SCN4B, KCNH3, SCN5A, KCNE3
6[b]	Voltage-gated ion channel activity	9	3.8	0.001237805	KCNJ4, KCNC4, CLIC3, CACNG7, SCN4B, KCNA5, SCN5A, KCNH3, KCNE3
7[b]	Voltage-gated channel activity	9	3.8	0.001237805	KCNJ4, KCNC4, CLIC3, CACNG7, SCN4B, KCNA5, SCN5A, KCNH3, KCNE3
8	Passive transmembrane transporter activity	13	5.6	0.00124619	FXYD2, KCNC4, CACNG7, KCNA5, TRPM2, KCNJ4, CLIC3, RYR3, PKD1, SCN4B, KCNH3, SCN5A, KCNE3

(*Continued*)

TABLE 7. Continued

No.	Term	Count	%	*p*	Genes
9[b]	Channel activity	13	5.6	0.00124619	FXYD2, KCNC4, CACNG7, KCNA5, TRPM2, KCNJ4, CLIC3, RYR3, PKD1, SCN4B, KCNH3, SCN5A, KCNE3
10	Ribonucleotide binding	36	15.5	0.001515222	ADCY4, GNAI2, RAB5B, PFKFB2, ADRBK2, TRIB3, CHD7, WNK4, DGKG, SYN2, RAC1, DCLK3, HSPA5, AGAP1, ABCA13, CDK14, ATP8B3, NWD1, GNAO1, MYO1C, EPRS, CARNS1, MAST4, ATP2C2, TTLL10, MAP3K15, ATP2A2, RAB36, FYN, DYRK1B, ARAF, TUBA4A, GNAS, SMARCA4, XYLB, CAMK1D
	GO Analysis_BP	14	6.03	1.39E−07	NOG, HTT, EMX2, SHH, APLP1, BBS1, HES1, NOTCH3, SLC1A2, FYN, LHX2, RAC1, FABP7, SMARCA4
1	Forebrain development*	17	7.32	1.11E−04	FXYD2, KCNC4, SLC8A2, CACNG7, KCNA5, TRPM2, KCNJ4, ATP2C2, ATP2A2, SLC4A8, SCN4B, PKD1, CP, KCNH3, SCN5A, KCNE3, NFATC1
2	Metal ion transport*	16	6.89	1.21E−04	LMX1B, HTT, EMX2, NEUROG1, RPGRIP1, NAPA, CXCL12, SHH, NOTCH3, BBS1, HES1, LINGO1, LHX2, RAC1, OLIG2, DSCAM
3[b]	Neuron differentiation	22	9.48	1.72E−04	FXYD2, KCNC4, SLC8A2, HTT, CACNG7, KCNA5, TRPM2, KCNJ4, SLC1A2, ATP2C2, CLIC3, ATP2A2, RYR3, WNK4, SLC4A8, SCN4B, PKD1, CP, KCNH3, SCN5A, KCNE3, NFATC1
4	Ion transport	7	3.01	2.16E−04	BBS1, PKHD1, BAIAP2L1, RAC1, ITGB4, CAPZB, TTLL3
5[a]	Cell projection assembly	7	3.01	2.52E−04	BBS1, SLC1A2, HTT, LHX2, EMX2, RAC1, SHH
6	Telencephalon development	6	2.58	5.59E−04	ADCY4, UCN3, GNAI2, TAAR1, GNAS, TSHR
7[b]	G-protein signaling, coupled to cAMP nucleotide second messenger	13	5.60	5.81E−04	BAIAP2L1, CROCC, PKHD1, ITGB4, CAPZB, CXCL12, SHH, TTLL3, BBS1, LINGO1, LHX2, RAC1, DSCAM
8[b]	Cell projection organization	17	7.32	6.11E−04	FXYD2, KCNC4, SLC8A2, CACNG7, KCNA5, TRPM2, KCNJ4, ATP2C2, ATP2A2, SLC4A8, SCN4B, PKD1, CP, KCNH3, SCN5A, KCNE3, NFATC1
9	Cation transport	6	2.58	8.36E−04	ADCY4, UCN3, GNAI2, TAAR1, GNAS, TSHR
10[b]	G-protein signaling, coupled to cyclic nucleotide second messenger	14	6.034482	1.39E−07	NOG, HTT, EMX2, SHH, APLP1, BBS1, HES1, NOTCH3, SLC1A2, FYN, LHX2, RAC1, FABP7, SMARCA4

[a]The pathway that was also identified in the other 2 enrichment analysis.
[b]The Gene Ontology that was also identified in 1 of other 2 enrichment analysis.

transcription was performed on 0.5 g of total RNA per the manufacturer's instructions using HiFi-MMLVcDNA kit (CWbio. Co. Ltd, Beijing, China). Primers were designed using Primer Premier 5.0 (PREMIER Biosoft, Palo Alto, CA). The amplification process was performed with ABI7500 (Applied Biosystems, Carlsbad, CA) and thermal cycler conditions were performed: 95°C for 30 seconds, 45 cycles of 95°C for 5 seconds, and 60°C for 45 seconds. Melting curves were performed on all samples. The quantitative RT-PCR results for all mRNAs tested were normalized to the amount of β-Actin RNA present in each sample.

The expression data obtained for all groups has been submitted to GEO (http://www.ncbi.nlm.nih.gov/geo/) as required by NIH guidelines and is available as record GSE68151.

Results

Analysis of differentially expressed genes in mouse olfactory epithelium

In order to systematically evaluate the effects of glucocorticoids on mouse olfactory epithelium, we compared the gene expression profiles in DEX-treated vs normal saline-treated olfactory epithelium at 2 time points. Comparative analysis revealed that 3351 genes were differentially expressed between the WK2 and Ctrl groups, of which 2076 genes were downregulated and 1275 genes were upregulated; 118 genes were differentially expressed between the D1 and Ctrl groups, of which 70 genes were downregulated and 48 genes were upregulated. Unsupervised hierarchical clustering analysis showed overall gene expression similarity within each experimental group (Fig. 1). Differentially

TABLE 8. Functional annotation clusters by GO TERM of top 450 auxiliary olfactory genes

	GO CC		GO MF		GO BP	
No.	Functional annotation clustering	Enrichment score	Functional annotation clustering	Enrichment score	Functional annotation clustering	Enrichment score
1	Cilium[a]	4.52	Ion channel activity[a,b]	8.70	Cell projection morphogenesis[a,c]	11.76
2	Membrane and cell fraction	2.34	Purine nucleotide binding	5.02	Inner ear development	10.28
3	Cation channel complex[a]	1.31	Voltage-gated channel activity[b]	3.87	Detection of abiotic stimulus	8.48
4	Synapse part[a]	0.52	Calcium:sodium antiporter activity	1.74	Regulation of neurogenesis[a]	8.33
5	Contractile fiber	0.44	Ion binding[a,c]	1.32	Neuron projection morphogenesis[a]	7.86
6	Cell-cell junction	0.20	Peptide receptor activity, G-protein coupled[a]	1.19	Regulation of cAMP metabolic process[a]	6.39
7	Golgi apparatus part[b]	0.11	Guanyl nucleotide binding	0.99	Cilium assembly[a]	4.80
8	Nucleoplasm part	0.08	Purinergic nucleotide receptor activity	0.90	Detection of mechanical stimulus	4.73
9	Lysosome	0.03	Ligase activity	0.27	Chemical homeostasis	4.57
10			ATPase activity, coupled	0.15	G-protein signaling\cAMP-/cyclic-nucleotide-mediated signaling[a,b]	4.07

[a]An obviously potential role in olfactory function.
[b]The Gene Ontology that was also identified in 1 of the other 2 enrichment analyses.
[c]The pathway that was also identified in the other 2 enrichment analyses.
cAMP = cyclic adenosine monophosphate.

expressed genes that were also in GOSdb (as well as the entire list) were also selected for the further analysis (Fig. 2)

To address the functional significance of these differentially regulated genes between the treatment group and the control group, the gene lists were input into DAVID as described above to identify any enriched pathways and GO functional annotations. Short-term treatment with DEX induced little change in gene expression (see Supporting Information A, B, and C). We therefore focused our analysis on comparison of differentially expressed genes between the WK2 and Ctrl group, which represent the "long-term" effect of glucocorticoids.

Of the top 10 enrichment pathways, most were inflammation-related (Table 1). The axon guidance pathway was also identified, which represents a key stage in the formation of neuronal network and may be an important in olfactory dysfunction and repair. In this pathway, 10 of 28 downregulated genes have been previously identified as auxiliary olfactory genes in GOSdb. Axons are guided by a variety of guidance factors, such as netrins, ephrins, slits, and semaphorins. These guidance cues are read by growth cone receptors, and signal transduction pathways downstream of these receptors converge onto the Rho GTPases to elicit changes in cytoskeletal organization that determine which way the growth cone will turn. The pathway was also identified by the enrichment analysis of DEGs that were listed in the GOSdb (Table 2). The enrichment analysis of the top 450 genes in the GOSdb shows that the following pathways are potentially important in olfaction (Table 3): calcium signaling pathway, GnRH signaling pathway, and Wnt signaling pathway; the axon guidance pathway was also enriched, albeit not as strongly.

To address functionality, GO analysis was done. Cell projection was identified in the Cellular Component part, indicating a prolongation or process extending from a cell; eg, a flagellum or axon (Table 4). As for molecular function, ion binding–related function was significant (Table 5). Biological processes significantly related to olfactory function were not demonstrated by the analysis (Table 6).

To understand how the differently expressed auxiliary olfactory genes work, which are overlap genes both in the GOSdb and all the differently expressed genes, GO analysis was done for these genes (Table 7). Cell projection, channel activity, ion binding, G-protein signaling, coupled to cAMP nucleotide second messengers are potentially olfactory-related. Cell projection was identified again, which suggested that the cell projection may play an important role in the effect of long treatment with DEX on olfactory function.

Although the GOSdb has identified candidate auxiliary olfactory genes, it is still not clear how these genes operate to affect olfactory function. Thus, the top 450 genes of the database were input for GO Analysis (Table 8) to help confirm our results. This analysis result demonstrated that these 450 genes take part in cell projection morphogenesis, cilium component, ion channel activity, ion binding, and others, consistent with our prior analysis.

Last, we analyzed olfactory receptor (OR) genes because they are a key component of olfactory sensory transmission. After 2-week treatment with DEX, a total 570

TABLE 9. Primers and probes used for qRT-PCR

Gene symbol	Forward/ reverse	Primer and probe sequence (5′–3′)	Amplicon size (bp)
ADCY4	Forward	CCCTATTTCTGCATTCCCACG	217
	Reverse	CGCTCCTGCCTCAGTTTCTTT	
MYO1C	Forward	AGCCTGAGTGATAGCCTATTTGTG	158
	Reverse	ATGCTGCCCTGGTTGATGTT	
GNAS	Forward	AGAAAGTCCTCGCTGGCAAAT	200
	Reverse	GCAGGTAAAGTGAGGGTAGCAGTA	
PKD1	Forward	CCTCCTGCGTTGGCGGTA	139
	Reverse	GCGGAACTCCTTGACCTTGCT	
BBS1	Forward	TACGGGCGAGAAGACAACACC	86
	Reverse	ACAAACACTGCTGTGCGCTTC	
HTT	Forward	GTTGCCACAGACTTCTACAGACACC	82
	Reverse	AGCCACCACCTCAAACACAGACT	
RPGRIP1	Forward	AGACGGTTCCTAAAGCCGATTC	173
	Reverse	TGGTTTCCTCAGGGACATCG	
FYN	Forward	GAGGAGCCCATTTACATCGTCA	236
	Reverse	CGAGCCAATCCAAAGTCAGC	
LHX2	Forward	GACTTGGTTTATCACCTCAACTGC	241
	Reverse	AGGGAAGACCCAGCGGATTA	
EMX2	Forward	GGCTCACAGTCTCAGTCTTACGGA	240
	Reverse	TGCTCTGTGCTGTCCATTTTTGTAG	
CAMK2	Forward	AGGACGAAGACACCAAAGTGC	244
	Reverse	TGGATGTGAGGGTTCAGGATG	
Adrbk2	Forward	CCTAGATGAATATGGGCACGTGAG	227
	Reverse	GCTTGTCTTTGGTTTTATGCTGC	
PKHD1	Forward	CAACTACACCTGCCCCAAAGATT	187
	Reverse	TGACCCTGTTTCCTCTCTTGCC	
ACTIN	Forward	GCCTTCCTTCTTGGGTAT	97
	Reverse	GGCATAGAGGTCTTTACGG	

qRT-PCR = quantitative reverse-transcription polymerase chain reaction.

olfactory receptor genes were tested; of these, 64 (11.2%) OR genes changed expression, including 50 genes that were upregulated and 14 genes that were downregulated.

Validation

Because the pathway of axon guidance, GO cellular component of cell projection, and GO biological process of cell projection assembly were enriched and they are axon-related, a total 37 differentially expressed olfactory auxiliary olfactory genes passed our screening criteria for involvement in olfaction. Eleven of these genes belong to the DS1, DS2, and DS3 subgroup in GOSdb, whose relation to the olfaction function has been supported by the literature via in vitro, mouse ortholog knockout or OMIM database studies. The 11 genes were as follows: *ADCY4*, *PKHD1*, *MYO1C*, *GNAS*, *PKD1*, *BBS1*, *RPGRIP1*, *FYN*, *HTT*, *LHX2*, *EMX2*, *CAMK2*, and *Adrbk2*. Additionally, the differentially expressed genes calcium/calmodulin-dependent protein kinase II (*CAMK2*) and adrenergic receptor kinase, beta 2 (*Adrbk2*) were also identified as important auxiliary olfactory genes, because they belong to the pathway of olfactory transduction. Finally, we chose these 13 genes to quantify by real-time RT-PCR using samples from the WK2 and Ctrl groups. Primers and probes are listed in Table 9 (Life Invitrogen, Shanghai).

Of these 13 genes, 10 of 11 downregulated genes were found to be expressed at a significantly lower level in WK2 compared to Ctrl. Of the 2 upregulated genes, 1 was found to be expressed to a significantly greater extent in WK2 (Fig. 3).

Discussion

This study shows a global view of genes regulated by glucocorticoids, especially olfactory-related genes that have been preliminarily identified by Keydar et.al.[21] These results support a new concept: that the direct effect of glucocorticoids on growth of olfactory receptor neurons (ORNs) axon function may play a major role in affecting olfactory function, at least in an undamaged olfactory system.

Mounting evidence suggests that glucocorticoids affect cell function via both the well-known genomic effects and non-genomic mechanisms.[22] The glucocorticoid receptor (GR), a transcription factor that regulates gene expression either positively or negatively, mediates most effects of glucocorticoids via genomic effects, either by binding to palindromic glucocorticoid response elements (GREs) in the promoters of target genes or by interacting with other transcription factors, such as activator protein-1 (AP-1) or nuclear factor-κB (NF-κB), affecting their gene expression activating ability.[23] Robinson et al.[11] have confirmed the expression of GR mRNA and protein in the OM, which suggests that corticosteroid medications may have direct effects on the cells of the OM.

Studies by Fong et al.[12] and Nishimura et al.[13] showed that olfactory Na^+K^+-ATPase is regulated by aldosterone, a steroid analogue. Our previous study showed that DEX enhanced both the mRNA expression of CNG channels and the production of cAMP with a dose-dependent effect, including the gene effect and non-gene effect.[14] These data provide some evidence to support the speculation that steroids might have some direct effect on the olfactory transduction in addition to their well-known anti-inflammatory activity. However, the fact that glucocorticoid receptors exist in the OE and adding steroids may change expression of some genes does not necessarily translate to improved function. Furthermore, because steroids

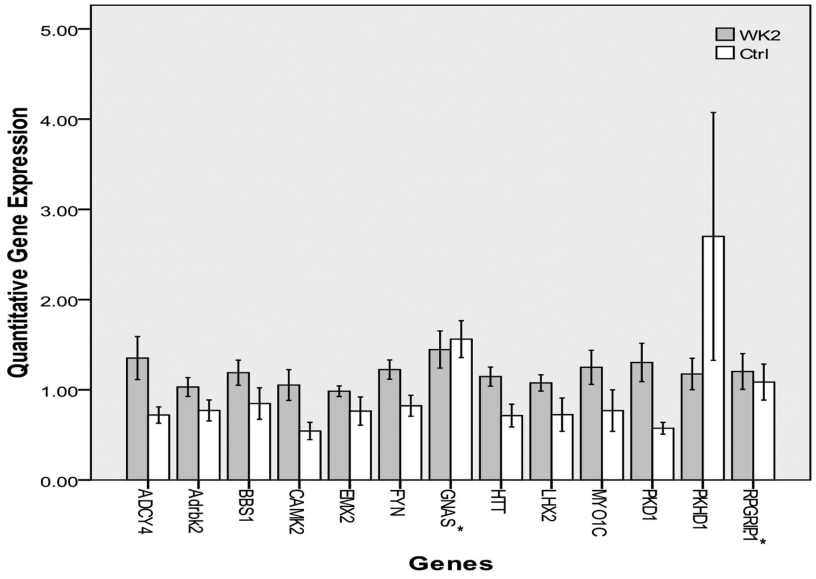

FIGURE 3. Validation of the microarray data results by qRT-PCR test of 13 genes of interest. The differentially expressed genes CAMK2 and Adrbk2 were identified as important auxiliary olfactory genes because they belong to the pathway of olfactory transduction. The other auxiliary olfactory genes were selected from 37 enriched and axon-related genes because they were identified in the GOSdb (subgroups DS1, DS2, and DS3), a key list of genes involved in olfactory function. The asterisk (*) represents the gene that did not show differential expression between the DEX group and control group during validation by qRT-PCR. Ctrl = control; DEX = dexamethasone; DS1/DS2/DS3 = subgroups of GOSdb; GOSdb = General Olfactory Sensitivity Database.; qRT-PCR = real-time quantitative polymerase chain reaction; WK2 = 2-week DEX treatment.

always have a wide effect on the host, including desired therapeutic effects and adverse reactions, maybe it is difficult to draw definitive conclusions based on expression changes.

Glucocorticoids have been shown a significant effect on nervous system, such as cell proliferation, neurotransmitter synthesis, survival, and differentiation of neurons.[24] However, whether damage to the neurons can be mitigated or reversed by the administration of glucocorticoids is still controversial, with both practical and theoretical implications. For example, an animal experiment by Kratskin et al.[16] found that high levels of systemic steroids can actually potentiate damage to olfactory receptor cells. On a clinical level, the long-term use of synthetic glucocorticoids increases the risk of mood depression and has myriad additional negative side effects.[25] New understanding of the molecular mechanisms may allow targeted therapy of these pathways, avoiding detrimental side effects.

Data mining of GOSdb may be helpful to understand which genes are important for the maintenance of olfactory function at a systems level, although KEGG Pathway and GO terms are currently not well annotated. However, there is no doubt that olfactory perception relies on an elaborate interplay of numerous genes, in addition to the classical olfactory transduction genes, which suggest that mechanisms of olfactory dysfunction are more complicated than what we have imagined. It also hints that it is hard to solve all the potential problems related to olfactory dysfunction via a single-drug treatment.

We concede that the differently expressed genes may reflect changes in the respiratory epithelium as well as the olfactory epithelium. However, most olfactory auxiliary genes identified by GOSdb are expressed only in olfactory sensory neurons (OSNs). On the other hand, tissue-specific gene analysis may be better than cell-based analysis, because tissue-specific gene analysis can reflect these surrounding milieu, which is known to be essential to keep the OSNs functional. For instance, axon guidance of OSNs might rely on signals from the surrounding environment.

There are still several limitations of this study. We only analyzed a single dose of DEX, so we cannot comment on dose dependence. Further, we did not evaluate the effect of changes of OR genes and olfactory transduction–related genes on actual olfactory function. We also did not test gene expression in a damaged olfactory system (eg, postviral insult or trauma), something that will remain for future work. These studies provide the foundation for such investigations. We note that microarrays measure mRNA abundance rather than gene expression from a strict perspective. When a change in mRNA abundance is observed, whether this is due to a change in the rate of transcription or the rate of RNA degradation cannot be known from the methods we used. Thus, this issue remains to be answered definitively.

Conclusion

Systemic steroids exert a considerable effect on murine OM gene expression in an intact system, with pathway effects on axon guidance and growth, in addition to inflammation. Dissection of these pathways in future experiments may offer the hope of targeted therapy for olfactory disorders that reduces or eliminates the side effects of steroids. Our results shed some light into the mechanisms of the positive effects steroids have in the olfactory system.

References

1. Patel RM, Pinto JM. Olfaction: anatomy, physiology, and disease. *Clin Anat*. 2014;27:54–60.
2. Leopold D. A perplexing olfactory loss. *Arch Otolaryngol Head Neck Surg*. 2000;126:803.
3. Hummel T, Rissom K, Reden J, Hähner A, Weidenbecher M, Hüttenbrink K. Effects of olfactory training in patients with olfactory loss. *Laryngoscope*. 2009;119:496–499.
4. Damm M, Pikart LK, Reimann H, et al. Olfactory training is helpful in postinfectious olfactory loss: a randomized, controlled, multicenter study. *Laryngoscope*. 2014;124:826–831.
5. Schriever VA, Lehmann S, Prange J, Hummel T. Preventing olfactory deterioration: olfactory training may be of help in older people. *J Am Geriatr Soc*. 2014;62:384–386.
6. Heilmann S, Huettenbrink KB, Hummel T. Local and systemic administration of corticosteroids in the treatment of olfactory loss. *Am J Rhinol*. 2004;18:29–33.
7. Stevens MH. Steroid-dependent anosmia. *Laryngoscope*. 2001;111:200–203.
8. Landis BN, Konnerth CG, Hummel T. A study on the frequency of olfactory dysfunction. *Laryngoscope*. 2004;114:1764–1769.
9. Duncan HJ, Seiden AM. Long-term follow-up of olfactory loss secondary to head trauma and upper respiratory tract infection. *Arch Otolaryngol Head Neck Surg*. 1995;121:1183–1187.
10. Reden J, Mueller A, Mueller C, et al. Recovery of olfactory function following closed head injury or infections of the upper respiratory tract. *Arch Otolaryngol Head Neck Surg*. 2006;132:265–269.
11. Robinson AM, Kern RC, Foster JD, Fong KJ, Pitovski DZ. Expression of glucocorticoid receptor mRNA and protein in the olfactory mucosa: physiologic and pathophysiologic implications. *Laryngoscope*. 1998;108(8 Pt 1):1238–1242.
12. Fong KJ, Kern RC, Foster JD, Zhao JC, Pitovski DZ. Olfactory secretion and sodium, potassium-adenosine triphosphatase: regulation by corticosteroids. *Laryngoscope*. 1999;109:383–388.
13. Nishimura T, Teranishi S, Kawashima A, Ishimaru T, Miwa T, Furukawa M. Glucocorticoid enhances Na(+)/K(+) ATPase mRNA expression in rat olfactory mucosa during regeneration: a possible mechanism for recovery from olfactory disturbance. *Chem Senses*. 2002;27:13–21.
14. Wei Y, Zhang C, Miao X, et al. Effects of glucocorticoid on cyclic nucleotide-gated channels of olfactory receptor neurons. *J Otolaryngol Head Neck Surg*. 2009;38:90–95.
15. Ribarac-Stepic N, Djurica S, Zakula Z, Koricanac G, Milosevic DP. [Molecular basis of glucocorticoid action]. *Srp Arh Celok Lek*. 2005;133(Suppl 1):61–66. [Serbian]
16. Kratskin IL, Kimura Y, Hastings L, Doty RL. Chronic dexamethasone treatment potentiates insult to olfactory receptor cells produced by 3-methylindole. *Brain Res*. 1999;847:240–246.
17. Genter MB, Van Veldhoven PP, Jegga AG, et al. Microarray-based discovery of highly expressed olfactory mucosal genes: potential roles in the various functions of the olfactory system. *Physiol Genomics*. 2003;16:67–81.
18. Shu J, Wu C, Wu Y, et al. Induction of pluripotency in mouse somatic cells with lineage specifiers. *Cell*. 2013;153:963–975.
19. Yang X, Dong X, Jia C, Wang Y. Profiling of genes associated with the murine model of oxygen-induced retinopathy. *Mol Vis*. 2013;19:775–788.
20. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4:44–57.
21. Keydar I, Ben-Asher E, Feldmesser E, et al. General Olfactory Sensitivity Database (GOSdb): candidate genes and their genomic variations. *Hum Mutat*. 2013;34:32–41.
22. Haller J, Mikics É, Makara GB. The effects of non-genomic glucocorticoid mechanisms on bodily functions and the central neural system. A critical evaluation of findings. *Front Neuroendocrinol*. 2008;29:273–291.
23. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids—new mechanisms for old drugs. *N Engl J Med*. 2005;353:1711–1723.
24. Glick RD, Medary I, Aronson DC, Scotto KW, Swendeman SL, La Quaglia MP. The effects of serum depletion and dexamethasone on growth and differentiation of human neuroblastoma cell lines. *J Pediatr Surg*. 2000;35:465–472.
25. Celano CM, Freudenreich O, Fernandez-Robles C, Stern TA, Caro MA, Huffman JC. Depressogenic effects of medications: a review. *Dialogues Clin Neurosci*. 2011;13:109–125.