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ORIGINAL ARTICLE

Olfactory bulb proteins linked to olfactory memory in C57BL/6J mice

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Abstract Information on systematic analysis of olfactory memory-related proteins is poor. In this study, the odor discrimination task to investigate olfactory recognition memory of adult male C57BL/6J mice was used. Subsequently, olfactory bulbs (OBs) were taken, proteins extracted, and run on two-dimensional gel electrophoresis with in-gel-protein digestion, followed by mass spectrometry and quantification of differentially expressed proteins. Dual specificity mitogen-activated protein kinase kinase 1 (MEK1), dihydropyrimidinase-related protein 1 (DRP1), and fascin are related with Lemon odor memory. Microtubule-associated protein RP/EB family member 3 is related to Rose odor memory. Hypoxanthine-guanine phosphoribosyltransferase is related with both Lemon and Rose odors memory. MEK1 and DRP1 levels were increased, while microtubule-associated protein RP/EB family member 3, fascin and hypoxanthine-guanine phosphoribosyltransferase levels were decreased during olfactory memory. In summary, neurogenesis, signal transduction, cytoskeleton, and nucleotide metabolism are involved in olfactory memory formation and storage of C57BL/6J mice.

Keywords Olfactory bulb · Olfactory memory · C57BL/6J mice · Olfactory discrimination task

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Introduction

In mammals, olfaction is involved in a series of processes including mating success, predator–prey balance, food preferences, orientation, social interactions, and mother care. Olfactory learning and memory (OM) is highly conserved in the animal kingdom and even humans and is essential for survival. Although neuroanatomy and physiology of OM has been intensively studied, knowledge on the neurochemistry of OM, however, is still limited.

The rationale to study pathways and cascades involved in OM formation is warranted by the medical implication: OM dysfunction not only represents pathophysiology but is also an early indicator for various known neurodegenerative disorders. The major goal of this study was to link OM to olfactory bulb protein levels in C57BL/6J mice, a strain widely used in cognitive research.

For the evaluation of OM the Olfactory Discrimination Task (ODT) (Schellinck et al. 2001) was selected because it is well established in several mouse models (Brown and Wong 2007; Schellinck et al. 2004; Wong and Brown 2007), well accepted, mainly olfactory bulb-dependent (Forestell et al. 2001; Schellinck et al. 2001), robust, and mice may preserve the OM for at least 6 months. In this task, mice were trained to distinguish between two odors, a reward-related and a reward-unrelated. Two control groups were used to rule out impact of odors and reward per se. Mice underwent four training days and were tested on day

5- at least 24 h after the last training, to be considered as long-term memory. Six hours following the probe trial, mice were killed and the olfactory bulb extirpated. The parameter "ratio of time spent digging and nosing in the target area/time spent digging and nosing in target area + digging and nosing outside the target area" was used for the evaluation of OM.

The olfactory bulb was chosen as the area of interest because it is an important area for coding and processing of odor molecule information (Gao and Strowbridge 2009; Mori et al. 1999; Sánchez-Andrade et al. 2005) and a key region involved in protein synthesis-dependent consolidation of OM (Juch et al. 2009).

Although a series of proteins have been linked to memory formation, information on proteins linked to olfactory memory formation is still missing. Herein, we applied a gel-based proteomic approach to study differential olfactory bulb protein levels between experimental and control groups using stringent statistical analysis and show that five proteins from several protein pathways and cascades are involved in OM formation in the mouse.

Materials and methods

Animals

Male, adult C57BL/6J mice, of age 10–14 weeks, were used. All mice were bred and maintained in polycarbonate cages Type II (207 × 140 × 265 mm, Fa. Ehret, Austria) and filled with autoclaved wood chips (Ligncell select, Fa. Rettenmaier, Austria) in the Core unit of Biomedical Research, Division of Laboratory Animal Science and Genetics, Medical University of Vienna, Himberg, Austria. Animals were housed under standardized conditions (Patil et al. 2008). Each subject was assigned to one of four conditions: in the L+/R− condition, sugar was included in the odor pots containing Lemon (Linalool, Sigma-Aldrich, St. Louis, MO) and not in those containing Rose (Phenylacetate, Sigma-Aldrich, St. Louis, MO). In the L−/R+ condition, sugar was present in odor pots containing Rose (Phenylacetate, Sigma-Aldrich, St. Louis, MO), and was absent in odor pots containing Lemon (Linalool, Sigma-Aldrich, St. Louis, MO). In the L−/R− condition sugar never was presented, either in Rose, or in Lemon odor pots. In the NO condition, mice did not receive any training at all; they were not allowed to smell any odors and not given sugar reward. All experiments on animals were approved by the local animal committee and authorities (BMWF-66.009/0247-C/GT/2007). All efforts were made to minimize animal suffering and the number of animals used. Housing and maintenance of animals were in compliance with European and national regulations.

Behavioral studies

Odorants and odor pots

Two odorants, Phenylacetate (Rose) and Linalool (Lemon), both purchased from Sigma-Aldrich, St. Louis, MO, were diluted with 1, 2-Propanediol (Sigma-Aldrich, St. Louis, MO) to a concentration of 15%. To avoid any degradation of odor strength, odors were aliquoted in 1.5 mL Eppendorf tubes and stored at −80°C for long-term storage or −20°C for short-term storage. During training, 0.1 mL of each odorant was dripped on ashless filter paper (Whatman no. 1441-070, grade 41 asshless, 70 mm in diameter), which was placed in each odor pot. The odor pots were constructed out of Petri dishes, having 10 holes drilled in the tops of the upper dish lid making sure to leave an area of 3 cm in diameter to place a 1 × 1 mm sugar cube on top. This allowed subjects to smell the odors, yet prevented any physical contact with them. The Petri dishes and their lids were fixed by tape to prevent mice from opening the odor pot. Petri dishes with sugar cubes on top were buried at least 2 cm deep in embedding. All odor pots were then covered with woodchip bedding (Lignocell select, Fa. Rettenmaier, Austria).

Sugar

Sugar (Saccharose, Wiener Zucker Würfelzucker, Agrana, Austria) cubes cut into small pieces using a razor blade was used as a reward. Sugar pieces were about 1 mm in diameter.

Olfactory discrimination task (conditioned odor preference task)

The conditioned odor preference task described in this protocol was developed by Schellinck et al. (2001) and slightly modified according to our project aims. The aim of this task was to investigate the ability of C57BL/6J mice to discriminate between two odors and to remember conditioned odor. Three days prior to training, each mouse was reduced to 85–90% of its ad libitum weight through food restriction. During this period, each mouse was weighed daily at the same time to avoid fluctuations and fed accordingly. Food restriction was continued throughout the training phase. On the test day, after the end of the test, mice returned to food ad libitum feeding.

24 h before the first training session, mice were fed with a 1 × 1 mm sugar cube additionally to food in order to be introduced to the sugar smell and taste.

Training: The odors are presented separately. Training took place every day between 9 a.m. and 1 p.m. (during light phase) in two separate rooms, one for the Rose and

one for the Lemon odor. A neutral room was used to house mice and to keep them during the inter-trial intervals (approximately 10 min). The training phase lasted 4 days, making mice finding the sugar and digging for reward. For the training, clear polyethylene cages Type III (265 × 150 × 420 mm, Fa. Ehret, Austria)—bigger than their home cages—were filled up to 2 cm height with woodchip bedding. Odor pots were placed randomly in the cage. On the first day of training and on the first reinforced trial (of the rewarded groups) the sugar pieces were placed on top of the bedding, then just slightly covered, but buried completely.

Each trial consisted of 10 min free exploration time; at the end of each trial mice were returned to the home cages and given 10–15 min time to recover. Each mouse received four Rose trials and four Lemon trials per day. The order of odor presentation was randomized across training days. Three hours after training, mice were fed their daily food ration. When switching the odors, the cages were washed with hot water and the bedding as well as the filter paper was replaced in each odor pot.

Testing (discrimination task, odor preference): Both odors were presented simultaneously. After completing 4 days of odor training, the final odor preference test was performed in a novel (4th) room to prevent any influence of contextual cues or odor association. Testing took place under regular (white) light, during day phase. The odor preference test apparatus consisted of a 69 × 20 × 20 cm box, divided by two walls into three compartments of equal size (23 × 20 × 20 cm) and made of clear 3 mm acrylic. Two openings (6 × 5.5 cm) located at floor level in each dividing wall allowed mice to move from the center into each end. The apparatus was prepared for a habituation trial by covering the floor of the three compartments with about 2 cm of bedding. A neutral odor pot (no odors or sugar) was placed in each end compartment, buried in the Lignocell bedding. Mice were placed in the middle compartment and given 1 min to explore. After habituation, mice were returned to the home cage for a recovery time of 5 min.

Prior to discrimination testing, the apparatus was rotated 180° to prevent any influence of extra-maze visual cues and the neutral pots were exchanged to odor pots. The Lignocell bedding from the habituation trial was not replaced until after the discrimination test. Odor pots containing Lemon and Rose odors were prepared without sugar, and placed centrally in each of the end chambers. Mice were placed in the central chamber, given 3 min of time. The time spent digging in the odor pots was measured with a separate stopwatch for each odor. Digging was operationally defined as when a mouse noses in the bedding and digs with its paws right above the pot. At the end of each test trial beddings and filter paper were discarded and the apparatus was washed with warm water with emphasis on cleaning the corners and openings between compartments. A JVC hard disk camera fixed above the apparatus was used for video analysis.

Protein studies

Six hours after the olfactory discrimination task, mice were anesthetized with CO_2 and killed by neck dislocation. The olfactory bulbs were rapidly dissected as shown in Fig. 1 and immediately frozen in liquid nitrogen and stored at −80°C until used for analysis. The freezing chain was never interrupted.

Sample preparation

The olfactory bulb was separated from the whole brain sample by a scalpel. Then tissue was powderized and resuspended in 1.2 mL of sample buffer consisting of 7 M urea (Merck, Darmstadt, Germany), 2 M thiourea (Sigma-Aldrich, St. Louis, MO, USA), 4% w/v CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate) (Sigma), 20 mM Tris, 65 mM DTT (1,4-dithioerythritol; Merck), 1 mM EDTA (Merck), protease inhibitors complete (Roche, Basel, Switzerland) and 1 mM phenylmethylsulfonyl chloride (PMSF). The suspension was sonicated for approximately 30 s. After homogenization, samples were left at 21 ± 1°C for 1 h and centrifuged at 15,000×*g* for 1 h at 12°C. The supernatant was transferred into Ultrafree-4 centrifugal filter unit (Millipore, Bedford, MA, USA) with a cut-off molecular weight of 10,000 Da (Millipore, Bedford, MA, USA) at 3,000×*g* at

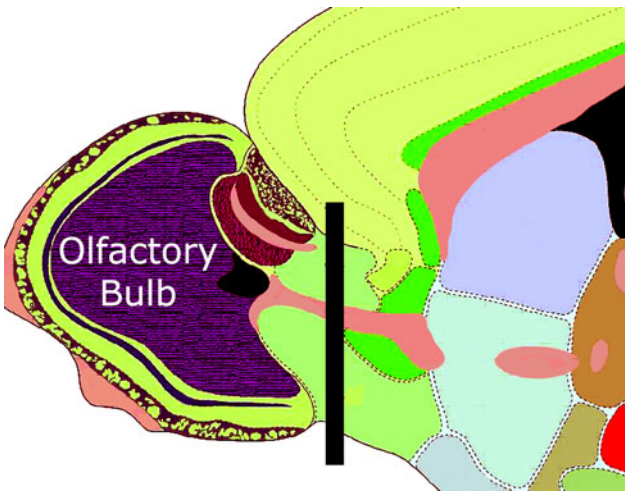

Fig. 1 The olfactory bulb was cut as indicated by the *black vertical bar*

12°C until the eluted volume was about 4 mL and the remaining volume reached 100–200 μL, for desalting and concentrating proteins. The protein content of the supernatant was determined by the Bradford protein assay system.

Two-dimensional gel electrophoresis (2-DE)

2-DE was performed as reported previously (Ahmed et al. 2009; Zheng et al. 2009). Samples prepared from individual mouse ($n = 8$–15 per group) were subjected to 2-DE. 700 μg of protein were applied on immobilized pH 3–10 nonlinear gradient strips at their basic and acidic ends. Focusing was started at 200 V and voltage was gradually increased to 8,000 V over 31 h and then kept constant for further 3 h (approximately 150,000 V h totally). After the first dimension, strips (18 cm) were equilibrated for 15 min in the buffer containing 6 M urea, 30% v/v glycerol, 2% w/v SDS, 50 mM pH 8.8 Tris–HCl, 1% w/v DTT, and then for 15 min in the same buffer containing 4% w/v iodoacetamide instead of DTT. After equilibration, strips were loaded on 10–16% gradient sodium dodecylsulfate polyacrylamide gels for second-dimensional separation. Gels (180 × 200 × 1.5 mm) were run at 40 mA per gel. Immediately after the second dimension run, gels were fixed for 18 h in 50% methanol, containing 10% acetic acid; the gels were then stained with Colloidal Coomassie Blue (Novex, San Diego, CA, USA) for 12 h on a rocking shaker. Molecular masses were determined by running standard protein markers (Biorad Laboratories, Hercules, CA, USA) covering the range 10–250 kDa. p*I* values 3–10 were used as given by the supplier of the immobilized pH gradient strips (Amersham Bioscience, Uppsala, Sweden). Excess dye was washed out from the gels with distilled water and the gels were scanned with Image-Scanner (Amersham Bioscience).

Quantification of protein levels

Protein spots from each gel of each group were outlined (first automatically and then manually) and quantified using the Proteomweaver software (Definiens, Munich, Germany). The percentage of the volume of the spots representing a certain protein was determined in comparison with the total proteins present in the 2-DE gel (Diao et al. 2008). The software used also revealed that spots evaluated did not contain other proteins. Only those proteins (spots) differently expressed between (1) L+/R− group and L−/R− group and between L+/R− group and NO group, but not between L+/R− group and L−/R+ group and between L−/R− group and NO group (2) L−/R+ group and L−/R− group and between L−/R+ group and NO group, but not between L+/R− group and L−/R+ group and between L−/R− group and NO group were further studied.

In-gel digestion with trypsin and chymotrypsin

Gel pieces of interest were cut into small pieces to increase surface and placed into a 0.6 mL tube. They were initially washed with 50 mM ammonium bicarbonate and then two times with 50% 50 mM ammonium bicarbonate/50% acetonitrile for 30 min with occasional vortexing. The washing solution was discarded at the end of each step. 100 μL of 100% acetonitrile was added to the tubes to cover the gel pieces completely and incubated for at least 5 min. The gel pieces were dried completely in a Speedvac Concentrator 5301 (Eppendorf, Germany). Reduction of cysteine residues was carried out with a 10-mM dithiothreitol solution in 0.1 M ammonium bicarbonate pH 8.6 for 60 min at 56°C. The same volume of a 55 mM solution of iodoacetamide in 0.1 M ammonium bicarbonate buffer pH 8.6 was added and incubated in darkness for 45 min at 25°C to alkylate cysteine residues. The reduction/alkylation solutions were replaced by 50 mM ammonium bicarbonate buffer for 10 min. Gel pieces were washed and dried in acetonitrile followed by Speedvac concentrator.

The dried gel pieces were re-swollen with 12.5 ng/μL trypsin (Promega, Mannheim, Germany) solution buffered in 25 mM ammonium bicarbonate. Subsequently, they were incubated for 16 h (overnight) at 37°C. The supernatant was transferred to new 0.6 mL tubes, and the gel pieces were extracted again with 50 μL of 0.5% formic acid/20% acetonitrile for 15 min in a sonication bath. This step was performed two times. Samples in extraction buffer were pooled in a 0.6 mL tube and evaporated in a Speedvac concentrator. The volume was reduced to approximately 10 μL and then 10 μL HPLC grade water (Fluka, Germany) was added for nano-LC-ESI-MS/MS analysis. The protocol for chymotrypsin digestion is similar as the protocol for trypsin, incubating gel pieces for 3 h at 30°C (Kang et al. 2009).

Analysis of peptides by nano-LC-ESI-(CID/ETD)-MS/MS (High capacity ion trap, HCT)

A total of 10 μL of extracted peptides were analyzed by ESI-MS/MS using an HCT instrument. The HPLC used was a biocompatible Ultimate 3000 system (Dionex Corporation, Sunnyvale, CA) equipped with a Pep-Map100 C-18 trap column (300 μm × 5 mm) and PepMap100 C-18 analytic column (75 μm × 150 mm). The gradient consisted of [A] 0.1% formic acid in water, [B] 0.08% formic acid in acetonitrile: 4–30% B from 0 to 105 min,

80% B from 105 to 110 min, and 4% B from 110 to 125 min. The flow rate was 300 nL/min from 0 to 12 min, 75 nL/min from 12 to 105 min, and 300 nL/min from 105 to 125 min. An HCT ultra PTM discovery system (Bruker Daltonics, Bremen, Germany) was used to record peptide spectra over the mass range of *m/z* 350–1,500, and MS/MS spectra in information-dependent data acquisition over the mass range of *m/z* 100–2,800. Repeatedly, MS spectra were recorded followed by three data-dependent CID MS/MS spectra and three ETD MS/MS spectra generated from three highest intensity precursor ions. The voltage between ion spray tip and spray shield was set to 1,400 V. Drying nitrogen gas was heated to 170°C and the flow rate was 10 L/min. The collision energy was set automatically according to the mass and charge state of the peptides chosen for fragmentation. +1, +2, +3 charged peptides were chosen for MS/MS experiments due to their good fragmentation characteristics. MS/MS spectra were interpreted and peak lists were generated by DataAnalysis 4.0 (Bruker Daltonics, Bremen, Germany). Searches were done by using the MASCOT v2.2 (Matrix Science, London, U.K.) against latest UniProtKB database for protein identification. Searching parameters were set as follows. MASCOT: enzyme selected as used with two maximum missing cleavage sites, species limited to mouse, a mass tolerance of 0.2 Da for peptide tolerance, 0.2 Da for MS/MS tolerance, fixed modification of carbamidomethyl (C), and variable modification of methionine oxidation. Positive protein identifications were based on a significant MOWSE scores. After protein identification, an error-tolerant search was done to detect unspecific cleavage and unassigned modifications. Protein identification and modification information returned from Mascot were manually inspected and filtered to obtain confirmed protein identification and modification lists of CID MS/MS and ETD MS/MS (Kang et al. 2009).

Data analysis

The time spent on digging in each odor pot is the primary measure of this task. The time spent digging in the CS+ is compared with the time spent digging in the CS− using a repeated-measure one-way analysis of variance (ANOVA). The time spent in each chamber during habituation can be analyzed to show whether there was a chamber preference, with time in each chamber. Statistical analyses were carried out with the statistical computing environment R version 2.0.1 (Murphy and Segal 1997, http://www.R-project.org) using ANOVA for protein levels. Proteins with significant effects were selected by adjusting the resulting *P* values for multiple testing using Post hoc correction. In any instance $P < 0.05$ was accepted as statistically significant.

Results

C57BL/6J mice showed olfactory discrimination, recognition ability, and conditioned odor preference

In the experimental groups, mean time for digging in the CS+ odor pot were 27.7 and 24.2 s, while in the CS− odor pot 1.6 and 0 s. The preference for CS+ odor was 96 and 100%, respectively (Fig. 2; Tables 1, 2). Control groups did not show any preference for any odors. The difference between digging time of the experimental group and control group was statistically significant ($P < 0.001$). No statistically significant differences were observed between two experimental groups or the control groups. The preference of C57/BL6J mice for digging in the previously rewarded odor pot suggested that conditioned odor preference has occurred.

Two-dimensional gel electrophoresis images and analysis

Proteomweaver software analysis of 612 protein spots clearly separated on 2D gel images from C57BL/6J mice olfactory bulbs (OBs) was carried out. Only five spots were significantly different (Tables 3, 4). These proteins were dual specificity mitogen-activated protein kinase kinase 1, dihydropyrimidinase-related protein 1, microtubule-associated protein RP/EB family member 3, fascin, and hypoxanthine-guanine phosphoribosyltransferase.

A representative gel is shown in Fig. 3 indicating UniProtKB accession numbers.

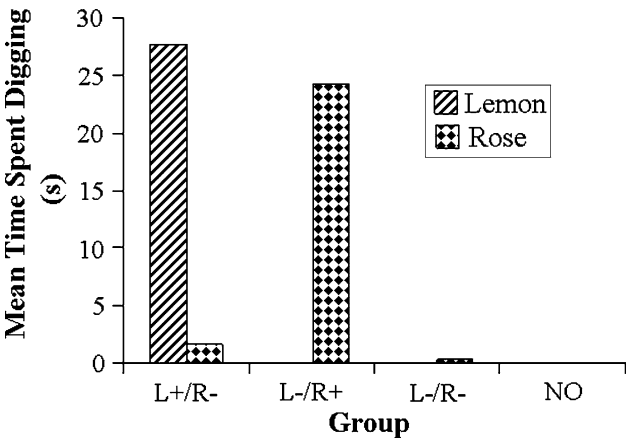

Fig. 2 Mean time spent digging in Lemon and Rose during the tests for the experimental groups, i.e., those for whom Lemon was paired with sugar (group L+/R−) or Rose was paired with sugar (group L−/R+) in training, and control groups, i.e., those for whom neither Lemon nor Rose was paired with sugar (group L−/R−), or who received no odors or sugar (group NO) in training

Table 1 Time spent digging in Lemon and Rose during the test phase for individual animals

Experimental group[a]	Lemon	Rose	Prefer. for CS+[b]	Control group	Lemon	Rose
L+/R− ($n = 11$)	4	0	1	L−/R− ($n = 9$)	0	0
	1	0	1		0	0
	56	0	1		0	0
	5	0	1		0	0
	22	5	0.81481		0	3
	28	0	1		0	0
	44	9	0.83019		0	0
	14	0	1		0	0
	35	4	0.89744		0	0
	58	0	1			
	38	0	1			
L−/R+ ($n = 12$)	0	60	1	NO ($n = 8$)	0	0
	0	14	1		0	0
	0	2	1		0	0
	0	21	1		0	0
	0	86	1		0	0
	0	29	1		0	0
	0	2	1		0	0
	0	16	1		0	0
	0	15	1			
	0	4	1			
	0	16	1			
	0	26	1			

[a] Group treatments were as follows: group L+/R− received Lemon paired with sugar and Rose alone; group L−/R+ received Rose paired with sugar and Lemon alone; group L−/R− received Lemon and Rose alone; group NO was not exposed to any odors or sugar in training

[b] Proportion of total digging time/time spent digging in CS+ in the tests

Table 2 Statistical analysis of the mean time spent digging in CS+ odor during the tests for each group

F value	*P* value	A versus B	A versus C	A versus D	B versus C	B versus D	C versus D
730.01	<0.001	ns	<0.001	<0.001	<0.001	<0.001	ns

A represents the group L+/R−, B represents the group L−/R+, C represents the group L−/R−, D represents the group NO

ns not significant

Table 3 Olfactory bulb proteins with different levels between the experimental group and control group

Acc. No.	Protein name	A	B	C	D
P97427	Dihydropyrimidinase-related protein 1	0.273 ± 0.118	0.229 ± 0.057	0.139 ± 0.036	0.173 ± 0.045
Q61553	Fascin	0.118 ± 0.037	0.134 ± 0.031	0.176 ± 0.023	0.157 ± 0.024
P31938	Dual specificity mitogen-activated protein kinase kinase 1	0.429 ± 0.067	0.385 ± 0.064	0.354 ± 0.035	0.299 ± 0.035
Q64531	Hypoxanthine-guanine phosphoribosyltransferase	0.205 ± 0.053	0.213 ± 0.094	0.319 ± 0.074	0.335 ± 0.07
Q6PER3	Microtubule-associated protein RP/EB family member 3	0.101 ± 0.033	0.093 ± 0.045	0.144 ± 0.034	0.194 ± 0.055

A represents the group L+/R−, B represents the group L−/R+, C represents the group L−/R−, D represents the group NO. Relative protein expression resulting from software-assisted quantification is given (Mean ± SD)

Table 4 Statistical analysis of olfactory bulb protein expression in L+/R− (A), L−/R+ (B), L−/R− (C), NO (D) mouse groups

Acc. No.	Protein name	*F* value	*P* value	A versus B	A versus C	A versus D	B versus C	B versus D	C versus D
P31938	Dual specificity mitogen-activated protein kinase kinase 1	9.749	<0.001	ns	0.021	<0.001	ns	0.009	ns
P97427	Dihydropyrimidinase-related protein 1	6.392	<0.01	ns	0.001	0.037	ns	ns	ns
Q6PER3	Microtubule-associated protein RP/EB family member 3	10.209	<0.001	ns	ns	<0.001	0.045	<0.001	ns
Q61553	Fascin	7.37	<0.001	ns	<0.001	0.040	0.021	ns	ns
Q64531	Hypoxanthine-guanine phosphoribosyltransferase	8.869	<0.001	ns	0.005	0.002	0.014	0.005	ns

Acc. No.	Protein name	Main effect (group) (*P*)	Post hoc analysis (group comparison) (*P*)					
			A versus B	A versus C	A versus D	B versus C	B versus D	C versus D
P31938	Dual specificity mitogen-activated protein kinase kinase 1	<0.001	ns	0.021	<0.001	ns	0.009	ns
P97427	Dihydropyrimidinase-related protein 1	<0.01	ns	0.001	0.037	ns	ns	ns
Q6PER3	Microtubule-associated protein RP/EB family member 3	<0.001	ns	ns	<0.001	0.045	<0.001	ns
Q61553	Fascin	<0.001	ns	<0.001	0.040	0.021	ns	ns
Q64531	Hypoxanthine-guanine phosphoribosyltransferase	<0.001	ns	0.005	0.002	0.014	0.005	ns

ns not significant

Mass spectrometric identification of differentially expressed spots

Five proteins could be unambiguously identified by mass spectrometry. Identification information is given in Table 5 listing accession number, protein name, total scores, numbers of matching peptides, sequence coverage, and the protease used for digestion. In addition, MS/MS peptides with ion score and mass errors in Da are provided.

Discussion

The major outcome of this study is the presence of preference of C57BL/6J mice for digging in the previously rewarded odor pot representing conditioned odor preference and that olfactory bulb levels of five proteins were linked to OM formation and for storage.

A large series of kinases including MAP kinases have been shown to be involved in memory formation. Herein, we show for the first time involvement of dual specificity mitogen-activated protein kinase kinase 1 (short names MAP kinase kinase 1, MAPKK1 or MEK1) in OM. MEK1 has been shown to play a major role in long-term synaptic plasticity and memory processes (Kelleher et al. 2004) including fear conditioning in mice (Shalin et al. 2004). This finding is of pivotal interest because recently a potent MEK1 inhibitor was published (Daouti et al. 2010) that would now allow testing the biological relevance of MEK1 modulation and indeed, these pharmacological studies are planned in our laboratory.

Dihydropyrimidinase-related protein 1 (DRP-1; collapsing-response mediator protein 1) is a member of collapsing response mediator proteins that are involved in cytoskeleton modification, neuronal differentiation, and axonal guidance (Deo et al. 2004) by serving in semaphorin 3A signaling that modulates neurite outgrowth and axonal repulsion. DRP-2 and DRP-4 could be linked to behavioral sensitization (Iwazaki et al. 2007), but DRP-1 was not associated with any form of memory so far and we propose a role in OM formation/storage.

A cytoskeleton protein, microtubule-associated protein RP/EB family member 3 was proposed to be involved in microtubule polymerization in spindle function by stabilizing microtubules, anchoring them at centrosomes, and possibly playing a role in cell migration. This protein was, however, already observed in a synaptic terminal preparation (Munton et al. 2007) and may therefore serve a role in synaptic plasticity. MAP2 itself is a neuron-specific cytoskeletal protein important for genesis and maintenance of dendrites (Garner et al. 1988; Rioux et al. 2004). The current study unequivocally assigns a role of this cytoskeleton element in OM.

Table 5 Protein identification of mouse olfactory bulb proteins with different levels between the experimental group and control group by nano-ESI-LC-MS/MS

Acc No.	Protein name	Total score	Match peptides	Seq. Cov. %	Enzyme	MS/MS peptides (ion score/mass error, Da)	Theor. MW	Theor. pI
P31938	Dual specificity mitogen-activated protein kinase kinase 1	1,216	43	81%	Trypsin	6 K.PTPIQLNPAPDGSAVNGTSSAETNLEALQK.K 2 Phospho (ST); 3 Dioxidation (P) 35 (38/−0.0637)	43788	6.24
						36 K.KLEELELDEQQR.K 47 (107/−0.1230)		
						36 K.KLEELELDEQQRK.R 48 (38/−0.1466)		
						37 K.LEELELDEQQR.K 47 (89/−0.1361)		
						48 R.KRLEAFLTQK.Q 57 (45/−0.0682)		
						49 K.RLEAFLTQK.Q 57 (45/−0.1019)		
						50 R.LEAFLTQK.Q 57 (53/−0.0742)		
						60 K.VGELKDDDFEK.I 70 (68/−0.0694)		
						71 K.ISELGAGNGGVVFK.V 84 (114/−0.0636)		
						71 K.ISELGAGNGGVVFK.V Deamidated (NQ) 84 (102/−0.0564)		
						85 K.VSHKPSGLVMAR.K 96 (54/−0.0685)		
						85 K.VSHKPSGLVMAR.K Oxidation (M) 96 (33/−0.0586)		
						97 R.KLIHLEIKPAIR.N 108 (63/−0.1420)		
						98 K.LIHLEIKPAIR.N 108 (45/0.0006)		
						161 R.IPEQILGK.V 168 (41/−0.0444)		
						*169 K.VSIAVIKGLTYLR.E Carbamidomethyl (K) [+57.02] 181 (35/0.0054)		
						190 R.DVKPSNILVNSR.G 201 (47/−0.0857)		
						206 K.LCDFGVSGQLIDSMANSFVGTR.S 227 (106/−0.1882)		
						206 K.LCDFGVSGQLIDSMANSFVGTR.S Deamidated (NQ) 227 (58/−0.0952)		
						206 K.LCDFGVSGQLIDSMANSFVGTR.S Oxidation (M) 227 (87/−0.1142)		
						228 R.SYMSPER.L 234 (32/−0.0718)		
						*235 R.LQGTHYSVQSDIWSMGLSLVEMAVGR.Y 2 Oxidation (M) 260 (32/−0.0556)		
						261 R.YPIPPPDAK.E 269 (46/−0.0426)		
						*270 K.ELELLFGCHVEGDAAETPPRPR.T 291 (65/−0.1948)		
						325 K.LPSGVFSLEFQDFVNK.C 340 (115/−0.1164)		
						354 K.QLMVHAFIK.R 362 (42/0.0139)		
						354 K.QLMVHAFIK.R Oxidation (M) 362 (37/0.0101)		
					Chymotrypsin	55 L.TQKQKVGELKDDDFEKISEL.G 74 (40/−0.0674)		
						64 L.KDDDFEKISELGAGNGGVVF.K 83 (70/0.0816)		
						*84 F.KVSHKPSGLVM.A 94 (48/−0.0367)		
						167 L.GKVSIAVIKGLTY.L 179 (60/0.0537)		
						188 M.HRDVKPSNIL.V 197 (41/−0.0859)		
						210 F.GVSGQLIDSMANSF.V 223 (78/−0.0359)		
						210 F.GVSGQLIDSMANSF.V Oxidation (M) 223 (57/0.0023)		
						*230 Y.MSPERLQGTHY.S 240 (41/−0.0135)		
						*230 Y.MSPERLQGTHY.S Oxidation (M) 240 (44/−0.0061)		
						*252 L.SLVEMAVGRYPIPPPDAKEL.E 271 (57/0.0762)		

Table 5 continued

Acc No.	Protein name	Total score	Match peptides	Seq. Cov. %	Enzyme	MS/MS peptides (ion score/mass error, Da)	Theor. MW	Theor. p*I*
P31938						*252 L.SLVEMAVGRYPIPPPDAKEL.E Oxidation (M) 271 (72/0.0716)		
						257 M.AVGRYPIPPPDAKEL.E 271 (45/−0.0259)		
						257 M.AVGRYPIPPPDAKELELL.F 274 (56/0.0755)		
						*275 L.FGCHVEGDAAETPPRPRTPGRPL.S 297 (42/0.0152)		
						276 F.GCHVEGDAAETPPRPRTPGRPL.S 297 (52/−0.0637)		
						301 Y.GMDSRPPMAIF.E 311 (36/0.0613)		
						301 Y.GMDSRPPMAIF.E Oxidation (M) 311 (48/0.0607)		
						301 Y.GMDSRPPMAIF.E 2 Oxidation (M) 311 (42/0.0958)		
						315 L.DYIVNEPPPKLPSGVF.S 330 (59/−0.0598)		
						331 F.SLEFQDF.V 337 (47/−0.0347)		
						338 F.VNKCLIKNPAERADL.K 352 (48/−0.0020)		
						343 L.IKNPAERADL.K 352 (36/−0.0609)		
						*343 L.IKNPAERADLKQL.M 355 (61/0.0432)		
						*353 L.KQLMVHAF.I Oxidation (M) 360 (34/−0.0086)		
						361 F.IKRSDAEEVDFAGW.L 374 (60/0.0542)		
						375 W.LCSTIGLNQPSTPTHAASI.- Phospho (ST) 393 (73/0.0327)		
P97427	Dihydropyrimidinase-related protein 1	2034	67	83%	Trypsin	7 K.KSIPHITSDR.L 16 (60/0.0241)	62471	6.63
						8 K.SIPHITSDR.L 16 (34/−0.0461)		
						*24 R.IINDDQSFYADVYLEDGLIK.Q 43 (36/0.1913)		
						44 K.QIGENLIVPGGVK.T 56 (43/−0.0094)		
						64 R.MVIPGGIDVNTYLQK.P 78 (59/−0.1223)		
						*64 R.MVIPGGIDVNTYLQK.P Oxidation (M) 78 (52/−0.0306)		
						79 K.PSQGMTSADDFFQGTK.A 94 (101/-0.1779)		
						79 K.PSQGMTSADDFFQGTK.A Oxidation (M) 94 (50/-0.1966)		
						95 K.AALAGGTTMIIDHVVPEPGSSLLTSFEK.W 122 (38/−0.1766)		
						95 K.AALAGGTTMIIDHVVPEPGSSLLTSFEK.W Oxidation (M) 122 (53/−0.1887)		
						150 R.EELEVLVQDK.G 159 (62/−0.0173)		
						160 K.GVNSFQVYMAYK.D 171 (73/−0.0622)		
						160 K.GVNSFQVYMAYK.D Oxidation (M) 171 (76/−0.0799)		
						*190 K.GLGAVILVHAENGDLIAQEQK.R Deamidated (NQ) 210 (35/0.0113)		
						239 R.AIAIAGR.I 245 (39/−0.0362)		
						246 R.INCPVYITK.V 254 (71/−0.0760)		
						259 K.SAADIIALAR.K 268 (91/−0.0132)		
						270 K.KGPLVFGEPIAASLGTDGTHYWSK.N 293 (57/−0.0319)		
						*271 K.GPLVFGEPIAASLGTDGTHYWSK.N 293 (33/−0.1011)		
						346 K.DNFTLIPEGVNGIEER.M 361 (53/−0.0974)		
						346 K.DNFTLIPEGVNGIEER.M Deamidated (NQ) 361 (71/−0.1232)		

Table 5 continued

Acc No.	Protein name	Total score	Match peptides	Seq. Cov. %	Enzyme	MS/MS peptides (ion score/mass error, Da)	Theor. MW	Theor. pI
						362 R.MTVVWDK.A 368 (34/−0.0575)		
						362 R.MTVVWDK.A Oxidation (M) 368 (38/−0.0409)		
						375 K.MDENQFVAVTSTNAAK.I 390 (88/−0.1085)		
						375 K.MDENQFVAVTSTNAAK.I Deamidated (NQ) 390 (88/−0.1385)		
						375 K.MDENQFVAVTSTNAAK.I 2 Deamidated (NQ); Methyl (DE) 390 (67/−0.0885)		
						375 K.MDENQFVAVTSTNAAK.I Oxidation (M) 390 (117/−0.1170)		
						391 K.IFNLYPR.K 397 (43/−0.0730)		
						401 R.IAVGSDADVVIWDPDK.M 416 (123/−0.0986)		
						*401 R.IAVGSDADVVIWDPDKMK.T Oxidation (M) 418 (45/−0.0011)		
						427 K.STVEYNIFEGMECHGSPLVVISQGK.I 451 (41/−0.1689)		
						427 K.STVEYNIFEGMECHGSPLVVISQGK.I 451 (39/−0.1357)		
						452 K.IVFEDGNISVSK.G 463 (82/−0.0638)		
						472 R.KPFPEHLYQR.V 481 (61/−0.0742)		
						488 K.VFGLHSVSR.G 496 (60/−0.0466)		
						497 R.GMYDGPVYEVPATPK.H 511 (75/−0.1358)		
						497 R.GMYDGPVYEVPATPK.H Oxidation (M) 511 (103/−0.0929)		
						533 R.NLHQSNFSLSGAQIDDNNPR.R 552 (39/−0.1760)		
						533 R.NLHQSNFSLSGAQIDDNNPR.R Deamidated (NQ) 552 (53/−0.1220)		
					Chymotrypsin	*1 -.MSHQGKKSIPHITSDRLL.I Met-loss + Acetyl (Protein N-term M) 18 (60/0.0211)		
						18 L.LIRGGRIINDDQSFY.A 32 (44/0.0455)		
						19 L.IRGGRIINDDQSFY.A 32 (39/0.0404)		
						37 Y.LEDGLIKQIGENL.I 49 (52/0.0399)		
						50 L.IVPGGVKTIEANGRMVIPGGIDVNTY.L 75 (32/−0.0249)		
						65 M.VIPGGIDVNTY.L 75 (43/0.0357)		
						*98 L.AGGTTMIIDHVVPEPGSSLL.T 117 (34/−0.0496)		
						*98 L.AGGTTMIIDHVVPEPGSSLL.T Oxidation (M) 117 (32/−0.0363)		
						104 M.IIDHVVPEPGSSLL.T 117 (60/0.0461)		
						*118 L.TSFEKWHEAADTKSCCDY.S 135 (41/0.0153)		
						124 W.HEAADTKSCCDY.S 135 (55/0.1450)		
						136 Y.SLHVDITSW.Y 144 (54/−0.0059)		
						145 W.YDGVREELEVL.V 155 (45/0.0831)		
						156 L.VQDKGVNSF.Q 164 (63/−0.0119)		
						156 L.VQDKGVNSFQVY.M 167 (57/0.0580)		
						188 F.LKGLGAVIL.V 196 (45/0.0438)		
						*189 L.KGLGAVIL.V 196 (45/0.0317)		
						226 L.SRPEELEAEAVF.R 237 (59/0.0155)		
						238 F.RAIAIAGRINCPVY.I 251 (56/0.0468)		
						257 M.SKSAADIIAL.A 266 (60/0.0254)		

Table 5 continued

Acc No.	Protein name	Total score	Match peptides	Seq. Cov. %	Enzyme	MS/MS peptides (ion score/mass error, Da)	Theor. MW	Theor. pI
P97427						274 L.VFGEPIAASL.G 283 (60/−0.0059)		
						274 L.VFGEPIAASLGTDGTHY.W 290 (101/−0.0066)		
						276 F.GEPIAASLGTDGTHY.W 290 (32/0.0050)		
						276 F.GEPIAASLGTDGTHYW.S 291 (97/−0.0063)		
						302 F.VTSPPLSPDPTTPDYL.T 317 (42/0.0328)		
						321 L.LACGDLQVTGSGHCPY.S 336 (91/0.0125)		
						322 L.ACGDLQVTGSGHCPY.S 336 (125/0.0289)		
						*337 Y.STAQKAVGKDNF.T 348 (47/0.0617)		
						349 F.TLIPEGVNGIEERM.T 362 (41/0.0316)		
						349 F.TLIPEGVNGIEERMTVVW.D Oxidation (M) 366 (44/−0.0043)		
						367 W.DKAVATGKMDENQF.V 380 (62/0.0071)		
						367 W.DKAVATGKMDENQF.V Oxidation (M) 380 (66/0.0324)		
						381 F.VAVTSTNAAKIF.N 392 (48/0.0530)		
						432 Y.NIFEGMECHGSPL.V 444 (57/0.0453)		
						432 Y.NIFEGMECHGSPL.V Oxidation (M) 444 (41/0.0445)		
						445 L.VVISQGKIVF.E 454 (51/0.0189)		
						445 L.VVISQGKIVFEDGNISVSKGM.G 465 (88/0.0201)		
						445 L.VVISQGKIVFEDGNISVSKGMGRF.I Oxidation (M) 468 (56/−0.0006)		
						455 F.EDGNISVSKGM.G 465 (42/0.0893)		
						455 F.EDGNISVSKGMGRF.I Oxidation (M) 468 (31/0.0226)		
						*469 F.IPRKPFPEHLY.Q 479 (31/0.0465)		
						490 F.GLHSVSRGMY.D 499 (34/0.0844)		
Q6PER3	Microtubule-associated protein RP/EB family member 3	954	35	88%	Trypsin	2 M.AVNVYSTSVTSENLSR.H Acetyl (N-term) 17 (100/−0.1670)	32231	5.33
						18 R.HDMLAWVNDSLHLNYTK.I 34 (97/0.0100)		
						*18 R.HDMLAWVNDSLHLNYTK.I Oxidation (M) 34 (89/−0.0585)		
						*67 K.LEHEYIHNFK.V 76 (52/−0.0873)		
						77 K.VLQAAFK.K 83 (47/−0.0678)		
						101 K.FQDNFEFIQWFK.K 112 (83/−0.0399)		
						113 K.KFFDANYDGK.D 122 (58/0.0214)		
						114 K.FFDANYDGK.D 122 (50/−0.0048)		
						114 K.FFDANYDGKDYNPLLAR.Q 130 (58/−0.0864)		
						123 K.DYNPLLAR.Q 130 (37/0.0532)		
						131 R.QGQDVAPPPNPGDQIFNK.S 148 (55/−0.1790)		
						151 K.KLIGTAVPQR.T 160 (74/−0.0209)		
						152 K.LIGTAVPQR.T 160 (60/−0.0482)		
						175 R.LSNVAPPCILR.K 185 (39/−0.1007)		
						193 R.NGGHEADAQILELNQQLLDLK.L Deamidated (NQ) 213 (89/−0.1342)		

Table 5 continued

Acc No.	Protein name	Total score	Match peptides	Seq. Cov. %	Enzyme	MS/MS peptides (ion score/mass error, Da)	Theor. MW	Theor. pI
Q6PER3						193 R.NGGHEADAQILELNQQLLDLK.L 2 Deamidated (NQ) 213 (68/−0.0760)		
						222 K.ERDFYFSK.L 229 (38/−0.0291)		
						224 R.DFYFSK.L 229 (38/−0.0607)		
					Chymotrypsin	7 Y.STSVTSENL.S 15 (33/0.0238)		
						24 W.VNDSLHL.N 30 (33/0.0155)		
						24 W.VNDSLHLNY.T 32 (72/0.0607)		
						31 L.NYTKIEQLCSGAAY.C 44 (89/−0.0144)		
						33 Y.TKIEQL.C 38 (34/−0.0474)		
						33 Y.TKIEQLCSGAAY.C 44 (65/0.0289)		
						49 M.DMLFPGCVHL.R Oxidation (M) 58 (34/0.0571)		
						*64 F.QAKLEHEYIHNF.K 75 (39/0.0286)		
						76 F.KVLQAAF.K 82 (47/0.0324)		
						*83 F.KKMGVDKIIPVEKL.V 96 (32/−0.0153)		
						*83 F.KKMGVDKIIPVEKL.V Oxidation (M) 96 (43/0.1670)		
						*86 M.GVDKIIPVEKL.V 96 (47/0.0843)		
						128 L.LARQGQDVAPPPNPGDQIF.N 146 (82/0.0371)		
						129 L.ARQGQDVAPPPNPGDQIF.N 146 (47/0.0583)		
						*153 L.IGTAVPQRTSPTGPKNM.Q 169 (32/0.0980)		
						176 L.SNVAPPCIL.R 184 (52/0.0135)		
						*215 L.TVDGLEKERDFY.F 226 (55/0.0288)		
						*227 Y.FSKLRDIEL.I 235 (52/0.0805)		
						236 L.ICQEHESENSPVISGIIGILY.A 256 (40/0.0028)		
						257 Y.ATEEGFAPPEDDEIEEHQQEDQDEY.- 281 (47/−0.1130)		
Q61553	Fascin	1000	33	56%	Trypsin	23 K.YLTAEAFGFK.V 32 (42/−0.0211)	55215	6.44
						33 K.VNASASSLK.K 41 (48/−0.0436)		
						44 K.QIWTLEQPPDEAGSAAVCLR.S 63 (39/−0.0099)		
						*69 R.YLAADKDGNVTCER.E 82 (76/−0.1070)		
						*91 R.FLVVAHDDGR.W 100 (54/−0.0699)		
						101 R.WSLQSEAHR.R 109 (65/0.0094)		
						111 R.YFGGTEDR.L 118 (58/0.0291)		
						119 R.LSCFAQSVSPAEK.W 131 (66/−0.1355)		
						159 R.PADEIAVDR.D 167 (74/−0.0260)		
						*186 R.YSVQTSDHR.F 194 (53/−0.0529)		
						202 R.LVARPEPATGFTLEFR.S 217 (63/0.0433)		
						248 K.VGKDELFALEQSCAQVVLQAANER.N 271 (53/0.1839)		
						314 K.YWTLTATGGVQSTASTK.N 330 (114/−0.1524)		
						380 K.LINRPIIVFR.G 389 (32/−0.0115)		
						390 R.GEHGFIGCR.K 398 (36/−0.1323)		

Table 5 continued

Acc No.	Protein name	Total score	Match peptides	Seq. Cov. %	Enzyme	MS/MS peptides (ion score/mass error, Da)	Theor. MW	Theor. pI
Q61553						399 R.KVTGTLDANR.S 408 (53/−0.0483)		
						400 K.VTGTLDANR.S 408 (49/−0.1001)		
						469 R.YLKGDHAGVLK.A 479 (50/−0.0164)		
						480 K.ACAETIDPASLWEY.- 493 (46/−0.1195)		
					Chymotrypsin	17 L.ISCGNKYLTAEAF.G Phospho (ST) 29 (36/0.0980)		
						30 F.GFKVNASASSL.K 40 (52/0.0199)		
						47 W.TLEQPPDEAGSAAVCL.R 62 (96/0.0062)		
						112 Y.FGGTEDRLSCF.A 122 (61/0.0578)		
						*156 L.SARPADEIAVDRDVPW.G 171 (60/0.0891)		
						187 Y.SVQTSDHRFL.R 196 (41/0.0057)		
						203 L.VARPEPATGF.T 212 (41/0.0581)		
						255 F.ALEQSCAQVVL.Q 265 (54/−0.0155)		
						316 W.TLTATGGVQSTASTKNASCY.F 335 (89/−0.0280)		
						355 F.VTAKKNGQLAASVETAGDSELF.L 376 (56/0.0313)		
						364 L.AASVETAGDSELF.L 376 (39/0.0515)		
						*424 Y.NIKDSTGKYW.T 433 (66/0.0315)		
						479 L.KACAETIDPASLW.E 491 (57/0.0637)		
						479 L.KACAETIDPASLWEY.- 493 (68/0.0326)		
Q64531	Hypoxanthine-guanine phosphoribosyltransferase	1370	41	86%	Trypsin	35 K.VFIPHGLIMDR.T 45 (55/−0.0342)	24294	5.74
						*35 K.VFIPHGLIMDR.T Oxidation (M) 45 (48/−0.0551)		
						*52 R.DVMKEMGGHHIVALCVLK.G 2 Oxidation (M) 69 (35/−0.1271)		
						*56 K.EMGGHHIVALCVLK.G 69 (79/−0.0467)		
						*56 K.EMGGHHIVALCVLK.G Oxidation (M) 69 (45/−0.0940)		
						74 K.FFADLLDYIK.A 83 (82/−0.0670)		
						92 R.SIPMTVDFIR.L 101 (49/−0.0249)		
						92 R.SIPMTVDFIR.L Oxidation (M) 101 (44/−0.0912)		
						104 K.SYCNDQSTGDIK.V 115 (100/−0.1291)		
						*104 K.SYCNDQSTGDIK.V 2 Deamidated (NQ); Methyl (DE) 115 (32/−0.1044)		
						116 K.VIGGDDLSTLTGK.N 128 (101/−0.0502)		
						129 K.NVLIVEDIIDTGK.T 141 (121/−0.0423)		
						129 K.NVLIVEDIIDTGK.T Methyl (DE) 141 (68/−0.0496)		
						142 K.TMQTLLSLVK.Q 151 (66/−0.0409)		
						142 K.TMQTLLSLVK.Q Oxidation (M) 151 (81/−0.1118)		
						160 K.VASLLVK.R 166 (45/−0.0494)		
						171 R.SVGYRPDFVGFEIPDK.F 186 (51/−0.1300)		
						187 K.FVVGYALDYNEYFR.D 200 (108/−0.1142)		
						201 R.DLNHVCVISETGK.A 213 (67/−0.0954)		
						201 R.DLNHVCVISETGK.A Deamidated (NQ) 213 (47/−0.0662)		

Table 5 continued

Acc No.	Protein name	Total score	Match peptides	Seq. Cov. %	Enzyme	MS/MS peptides (ion score/mass error, Da)	Theor. MW	Theor. pI
Q64531					Chymotrypsin	23 F.CIPNHYAEDLEKVF.I 36 (43/−0.0017)		
						29 Y.AEDLEKVF.I 36 (32/0.0376)		
						29 Y.AEDLEKVFIPHGL.I 41 (47/0.0654)		
						*37 F.IPHGLIMDRTERL.A Oxidation (M) 49 (35/−0.0573)		
						*42 L.IMDRTERLARDVM.K Oxidation (M) 54 (33/0.0394)		
						*42 L.IMDRTERLARDVM.K 2 Oxidation (M) 54 (45/−0.0345)		
						*50 L.ARDVMKEMGGHHIVAL.C 2 Oxidation (M) 65 (42/−0.0249)		
						*55 M.KEMGGHHIVAL.C Oxidation (M) 65 (48/0.0416)		
						*86 L.NRNSDRSIPM.T 95 (36/0.0148)		
						86 L.NRNSDRSIPMTVDF.I Oxidation (M) 99 (39/−0.0515)		
						96 M.TVDFIRL.K 102 (46/0.0164)		
						*96 M.TVDFIRLKSY.C 105 (44/0.1023)		
						*100 F.IRLKSY.C 105 (32/−0.0044)		
						103 L.KSYCNDQSTGDIKVIGGDDLSTL.T 125 (35/−0.0530)		
						106 Y.CNDQSTGDIKVIGGDDLSTL.T 125 (57/−0.0383)		
						126 L.TGKNVLIVEDIIDTGKTM.Q 143 (69/−0.1698)		
						126 L.TGKNVLIVEDIIDTGKTM.Q Oxidation (M) 143 (123/−0.0707)		
						126 L.TGKNVLIVEDIIDTGKTMQTL.L Oxidation (M) 146 (60/−0.1085)		
						132 L.IVEDIIDTGKTM.Q 143 (90/0.0165)		
						132 L.IVEDIIDTGKTM.Q Oxidation (M) 143 (100/0.0092)		
						132 L.IVEDIIDTGKTMQTL.L Oxidation (M) 146 (39/−0.0587)		
						*132 L.IVEDIIDTGKTMQTLL.S 147 (34/−0.1325)		
						132 L.IVEDIIDTGKTMQTLL.S Oxidation (M) 147 (88/0.0120)		
						147 L.LSLVKQY.S 153 (37/0.0283)		
						148 L.SLVKQY.S 153 (38/−0.0261)		
						*154 Y.SPKMVKVASLL.V 164 (47/0.0753)		
						154 Y.SPKMVKVASLL.V Oxidation (M) 164 (48/−0.0189)		
						175 Y.RPDFVGF.E 181 (42/−0.0195)		
						175 Y.RPDFVGFEIPDKF.V 187 (49/0.0292)		
						182 F.EIPDKFVVGY.A 191 (38/−0.0107)		
						192 Y.ALDYNEY.F 198 (34/−0.0484)		
						199 Y.FRDLNHVCVISETGKAKY.K 216 (65/−0.0800)		
						*200 F.RDLNHVCVISETGKAKY.K 216 (48/0.0127)		

Note: Most peptide sequences were from CID sources. The peptide sequences with asterisk represent those from ETD source

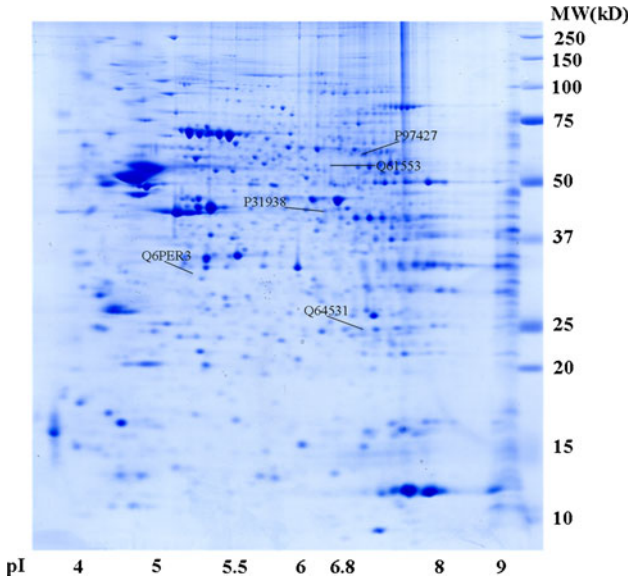

Fig. 3 Two-dimensional map of C57BL/6J mouse olfactory bulb proteins Spots from four groups were quantified and statistically analyzed using ANOVA. The five spots that were significantly different among experimental group and control group were identified using nano-LC-ESI-MS/MS

Another cytoskeleton element, fascin, can be herewith assigned a role in OM formation. Fascin can organize actin bundling and indeed, actin bundling is involved in dendritic spine formation as well as in vesicle trafficking, thus representing neurotransmission. Formation and functions of postsynaptic mushroom-shaped structures, dendritic spines rely on actin cytoskeleton remodeling (Korobova and Svitkina 2010).

Hypoxanthine-guanine phosphoribosyltransferase (HPRT) is an enzyme from purine metabolism catalyzing the reactions

$$\text{IMP+diphosphate} = \text{hypoxanthine} + \text{5-phospho-alpha-D-ribose 1-diphosphate.}$$

$$\text{GMP+diphosphate} = \text{guanine} + \text{5-phospho-alpha-D-ribose 1-diphosphate}$$

known to serve IMP biosynthesis. Impairment of purine metabolism by deficient HPRT results in dopaminergic defects (Boer et al. 2001; Ceballos-Picot et al. 2009; Lewers et al. 2008). A role for olfactory bulb HPRT in formation of OM may be therefore based upon the dopaminergic system, a key system for memory formation per se.

Taken together, a role for five proteins in olfactory memory formation was proposed. These findings contribute to the understanding of memory formation by protein pathways and form the basis for further functional studies of these proteins in learning and memory by, e.g., genetic or pharmacological modification of protein levels. The question whether these proteins are participating in the generation of other forms of memories remains open.

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