Supporting information

Specific Phospholipid Modulation by Muscarinic Signaling in a Rat Lesion Model of Alzheimer's Disease

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Table S1. Relative intensity of representative lipid species which have been found to be modified in the NBM and cortex of vehicle (aCSF) and 192IgG-saporin treated rats, in both positive and negative ion mode.

			% Intensity[1]			
			NBM		Cortex	
Assignment	**Cal m/z**	**Exp m/z**	aCSF	**SAP**	aCSF	**SAP**
Positive ion mode						
PE (14:1/20:4) + H^+	710.4755	710.4893	15.21 ± 0.70	**11.87 ± 1.38***	9.97 ± 0.62	**10.67 ± 0.67**
SM (d18:1/18:0) + H^+	731.6061	731.6070	7.11 ± 1.13	**4.15 ± 0.80***	4.19 ± 0.44	**3.50 ± 0.53**
SM (d18:1/16:0) + K^+	741.5313	741.5311	0.96 ± 0.14	**10.63 ± 3.33****	0.55 ± 0.13	**0.78 ± 0.11**
PC (32:0)+Na^+/PC (34:3) + H^+	756.5514	756.5520	15.75 ± 1.23	**21.94 ± 2.46***	16.94 ± 1.02	**17.41 ± 1.31**
PC (34:2)+Na^+/PC (36:5) + H^+	780.5514	780.5521	0.85 ± 0.09	**2.02 ± 0.27****	1.10 ± 0.08	**1.07 ± 0.08**
PC (34:1)+Na^+/PC (36:4) + H^+	782.5670	782.5673	32.75 ± 1.65	**46.10 ± 3.86****	32.31 ± 1.64	**30.9 ± 1.29**
PC (34:2) + K^+	796.5253	796.5255	3.27 ± 0.17	**5.2 ± 0.30*****	4.20 ± 0.15	**4.16 ± 0.07**
PC (34:1) + K^+	798.5410	798.5405	100 ± 0	**100 ± 0**	100 ± 0	**100 ± 0**
PC (36:4)+Na^+/PC (38:7) + H^+	804.5514	804.5510	2.79 ± 0.31	**7.69 ± 1.52***	3.63 ± 0.31	**4.3 ± 0.24**
PC (36:1)+Na^+/PC (38:4) + H^+	810.5983	810.5984	14.62 ± 0.30	**21.64 ± 1.53****	10.62 ± 0.48	**8.95 ± 0.63***
PC (36:4) + K^+	820.5253	820.5249	10.32 ± 1.01	**18.61 ± 2.71***	13.17 ± 0.99	**15.63 ± 0.48***
PC (38:4)+Na^+/PC (40:7) + H^+	832.5827	832.5826	3.00 ± 0.30	**7.98 ± 1.40****	2.82 ± 0.25	**3.11 ± 0.21**
PC (38:4)+K^+/PC (42:9) + H^+	848.5566	848.5560	10.23 ± 0.93	**18.27 ± 2.15****	9.71 ± 0.80	**10.75 ± 0.38**
PC (40:6) + Na^+	856.5827	856.5820	0.88 ± 0.17	**2.25 ± 0.40****	0.75 ± 0.11	**1.14 ± 0.09***
Negative ion mode						
SM (d18:1/15:0) - H^-	687.5447	687.5443	1.26 ± 0.24	**6.44 ± 1.50****	0.94 ± 0.23	**1.52 ± 0.28**
PS (18:0/18:1) - H^-	788.5447	788.5447	11.04 ± 2.16	**3.95 ± 0.99****	8.54 ± 1.28	**6.30 ± 0.85**
PE (40:4)/PC (18:0/20:4) - H^-	794.5705	794.5705	0.82 ± 0.13	**1.35 ± 0.17***	0.83 ± 0.08	**0.98 ± 0.14**
PG (22:6/22:6) - H^-	865.5025	865.5033	0.06 ± 0.01	**0.86 ± 0.24****	0.04 ± 0.01	**0.16 ± 0.05***

[1] The maximal peak is the most intense peak of the lipid spectrum, in this case PC (34:1) in positive and PI (20:4/18:0) in negative ion modes, which are set at 100%. Data are mean ± S.E.M values of aCSF (n = 8) and 192IgG-saporin-treated (n = 8) rats. *p < 0.05, **p < 0.01 and ***p < 0.001 when compared to aCSF group. PC: phosphatidylcholine; SM: sphingomyelin; PS: phosphatidylserine; PE: phosphatidylethanolamine; PG: phosphoglycerol; PI: phosphatidylinositol; Cal: calculated; Exp: experimental.

Table S2. [^{35}S]GTPγS basal and carbachol-induced (100 μM) binding in cortical, hippocampal and several brain regions of vehicle (aCSF) and 192IgG-saporin-treated rats.

	Basal binding (nCi/g t.e.)		**Carbachol stimulation (% Over basal)**	
Brain region	aCSF	SAP	**aCSF**	**SAP**
Cerebral cortex				
Cingulate	304 ± 26	306 ± 20	**52 ± 12.1**	**48 ± 6.7**
Ectorhinal	352 ± 34	290 ± 29	**66 ± 12.5**	**58 ± 8.0**
Entorhinal	342 ± 35	335 ± 36	**76 ± 10.5**	**93 ± 11.6**
Perirhinal	338 ± 49	309 ± 29	**35 ± 5.8**	**43 ± 3.5**
Somatosensory	355 ± 28	333 ± 20	**31 ± 7.0**	**54 ± 10.6**
Motor	311 ± 33	319 ± 20	**40 ± 13.3**	**66 ± 6.5**
Hippocampus				
CA1				
Oriens	320 ± 40	269 ± 32	**26 ± 9.4**	**40 ± 10.4**
Pyramidal	527 ± 64	457 ± 50	**40 ± 11.5**	**30 ± 6.2**
Radiatum	363 ± 33	323 ± 36	**45 ± 11.5**	**42 ± 9.0**
CA3				
Oriens	317 ± 26	303 ± 38	**26 ± 8.0**	**48 ± 6.6**
Pyramidal	477 ± 36	453 ± 42	**11 ± 5.6**	**32 ± 4.2***
Radiatum	328 ± 21	281 ± 32	**28 ± 9.5**	**40 ± 13.0**
Dentate gyrus				
Granular	467 ± 29	452 ± 50	**24 ± 6.1**	**48 ± 9.7***
Molecular	315 ± 49	278 ± 45	**34 ± 14.7**	**34 ± 9.5**
Mesencephalon				
Periaqueductal gray	496 ± 44	469 ± 45	**51 ± 15.0**	**47 ± 8.5**
Substantia nigra	456 ± 52	399 ± 38	**31 ± 10.5**	**37 ± 5.4**
Basal ganglia/cholinergic forebrain				
Globus pallidus	335 ± 43	347 ± 36	**46 ± 13.2**	**49 ± 12.5**
Striatum	339 ± 26	345 ± 21	**74 ± 15.4**	**62 ± 11.1**
NBM	535 ± 49	398 ± 31	**43 ± 9.2**	**11 ± 7.5***
Horiz. diagonal band	310 ± 25	327 ± 31	**121 ± 20.7**	**115 ± 17.6**
Vertical diagonal band	330 ± 25	399 ± 42	**135 ± 24.1**	**113 ± 23.2**
Medial septum	267 ± 24	284 ± 32	**152 ± 24.4**	**139 ± 23.7**

Data are mean ± S.E. M values of aCSF (n = 9) and SAP (n = 11) treated rats.
***** $p < 0.05$, when compared to aCSF group.

Table S3. [^{35}S]GTPγS basal and carbachol-induced (100 μM) binding in the different amygdaloid nuclei and several brain regions of vehicle (aCSF) and 192IgG-saporin-treated rats.

	Basal binding (nCi/g t.e.)		**Carbachol stimulation (% Over basal)**	
Brain region	aCSF	SAP	**aCSF**	**SAP**
Telencephalon				
Amygdaloid nuclei				
Anterior	421 ± 33	402 ± 29	**59 ± 9.1**	**47 ± 13.6**
Basolateral	487 ± 47	390 ± 44	**43 ± 6.6**	**48 ± 10.9**
Central	710 ± 91	525 ± 55	**27 ± 9.2**	**52 ± 7.8**
Lateral	483 ± 50	425 ± 43	**41 ± 9.3**	**36 ± 7.2**
Medial	720 ± 94	621 ± 69	**39 ± 8.5**	**34 ± 4.7**
Ventral subiculum	345 ± 29	294 ± 18	**37 ± 4.8**	**47 ± 6.7**
Rhinencephalon				
Lat olfactory tract	379 ± 45	324 ± 37	**82 ± 13.1**	**47 ± 11.5**
Midbrain				
AV thalamic nucleus	425 ± 32	397 ± 41	**232 ± 17.4**	**251 ± 28.6**
Gray sup colliculus	341 ± 27	362 ± 36	**197 ± 15.4**	**211 ± 19.9**
Rhomboencephalon				
Dorsal raphe	703 ± 103	590 ± 74	**19 ± 4.9**	**24 ± 4.7**
Locus coeruleus	220 ± 36	132 ± 16	**26 ± 11.9**	**78 ± 11.9**

Data are mean ± S.E. M values of aCSF (n = 9) and SAP (n = 11) treated rats.

Table S4. [^{35}S]GTPγS basal and carbachol-induced (100 μM) binding in different regions of grey matter from SHAM-operated rats.

	Basal binding (nCi/g t.e.)	**Carbachol stimulation (% Over basal)**		Basal binding (nCi/g t.e.)	**Carbachol stimulation (% Over basal)**
Brain region			**Brain region**		
Telencephalon			**Cerebral cortex**		
Amygdala			Cingulate	354 ± 30	**43 ± 12.7**
Anterior	403 ± 33	**37 ± 14.4**	Ectorhinal	337 ± 56	**36 ± 6.5**
Basolateral	387 ± 41	**53 ± 10.5**	Entorhinal	308 ± 30	**47 ± 7.1**
Central	536 ± 53	**32 ± 8.39**	Perirhinal	309 ± 43	**36 ± 13.5**
Lateral	373 ± 53	**78 ± 16.9**	Piriform	270 ± 31	**61 ± 9.8**
Medial	672 ± 84	**34 ± 11.7**	Somatosensory	368 ± 28	**34 ± 4.9**
Hippocampus			Motor	353 ± 37	**34 ± 14.8**
CA1			**Basal ganglia**		
Oriens	252 ± 25	**27 ± 12.0**	Globus pallidus	275 ± 25	**54 ± 12.2**
Pyramidal	366 ± 44	**55 ± 15.6**	Striatum	330 ± 37	**85 ± 14.0**
Radiatum	279 ± 19	**42 ± 9.8**	*Diencephalon*		
CA3			NBM	492 ± 51	**47 ± 12.3**
Oriens	280 ± 44	**21 ± 6.8**	Horiz diag band	356 ± 29	**115 ± 19.5**
Pyramidal	419 ± 52	**9 ± 6.2**	Vertical diag band	366 ± 28	**112 ± 25.6**
Radiatum	264 ± 38	**40 ± 19.8**	Medial septum	280 ± 50	**150 ± 41.2**
Dentate gyrus			*Rhinencephalon*		
Granular	437 ± 32	**25 ± 1.2**	Lat olfactory tract	356 ± 27	**41 ± 9.8**
Molecular	263 ± 45	**44 ± 9.2**	*Rhomboencephalon*		
Polimorphic	290 ± 39	**28 ± 15.7**	Dorsal raphe	533 ± 99	**42 ± 9.4**
Vent subic	301 ± 35	**49 ± 11.4**	Locus coeruleus	197 ± 30	**52 ± 6.0**
Brainstem			*Mesencephalon*		
Spinal trig N	178 ± 25	**87 ± 19.8**	Periaqueduc gray	450 ± 82	**47 ± 10.3**
			Substantia nigra	361 ± 56	**32 ± 14.9**

Data are mean ± S.E. M values of SHAM-operated (n = 6) rats.

Table S5. [^{3}H]-pirenzepine and [^{3}H]-oxotremorine binding in different brain regions of vehicle (aCSF) and 192IgG-saporin-treated rats.

[^{3}H]-pirenzepine binding (fmol/mg t.e.)			[^{3}H]-oxotremorine binding (fmol/mg t.e.)		
Brain region	aCSF	**SAP**	**Brain region**	aCSF	**SAP**
Amygdala			**Amygdala**		
Anterior	7.3 ± 0.8	**3.6 ± 0.9***	Anterior	21.2 ± 2.7	**31.1 ± 1.9**
Basolateral	70.2 ± 5.9	**74.5 ± 4.8**	Basolateral	5.2 ± 1.7	**5.1 ± 1.8**
Central	30.5 ± 3.6	**37.6 ± 6.6**	Lateral	5.4 ± 1.1	**5.3 ± 1.6**
Lateral	66.1 ± 6.2	**66.1 ± 4.8**	**Hippocampus**		
Hippocampus			CA1		
CA1	111.3 ± 10	**131.8 ± 8.8**	Pyramidal	6.2 ± 1.5	**7.5 ± 2.2**
Oriens	114.8 ± 10	**126.1 ± 4.2**	CA3		
Pyramidal	127.9 ± 9.7	**134.9 ± 7.9**	Pyramidal	11.0 ± 2.3	**11.3 ± 2.6**
Radiatum	120.6 ± 8.6	**136.2 ± 7.7**	Dentate gyrus		
CA3	58.2 ± 6.6	**64.1 ± 2.5**	Granular	3.6 ± 1.5	**7.1 ± 1.7**
Oriens	43.4 ± 3.4	**58.3 ± 2.3****	**Midbrain**		
Pyramidal	43.4 ± 4.5	**53.5 ± 4.1**	AV thalamic nucleus	66.3 ± 4.4	**62.1 ± 2.7**
Radiatum	49.5 ± 3.6	**58.2 ± 3.6**	Gray sup colliculus	57.8 ± 2.9	**72.9 ± 4.5***
Dentate gyrus	108.1 ± 4.5	**129.3 ± 5.0***	Opt sup colliculus	28.8 ± 5.3	**31.3 ± 6.2**
Granular	34.4 ± 2.5	**46.4 ± 2.0***	**Cerebral cortex**		
Molecular	106.3 ± 8.8	**114.9 ± 7.1**	Cingulate	11.2 ± 2.3	**17.9 ± 1.6***
Polimorphic	74.9 ± 5.5	**79.7 ± 4.2**	Ectorhinal	10.0 ± 2.1	**14.6 ± 1.4**
Cerebral cortex			Perirhinal	6.9 ± 2.3	**10.7 ± 1.2**
Cingulate	38.2 ± 1.0	**36.1 ± 2.1**	Motor		
Ectorhinal	58.5 ± 5.5	**54.6 ± 2.9**	Layer I-II	19.6 ± 2.6	**26.5 ± 2.1**
Entorhinal	51.4 ± 4.6	**47.6 ± 3.7**	Layer V-VI	12.7 ± 3.0	**21.8 ± 2.4***
Perirhinal	55.2 ± 4.7	**54.6 ± 3.0**	Somatosensory		
Motor	39.5 ± 3.1	**36.9 ± 1.3**	Layer I-II	22.9 ± 1.1	**29.7 ± 2.5**
Somatosensory	43.7 ± 2.6	**43.9 ± 2.6**	Layer V-VI	12.2 ± 2.0	**20.3 ± 2.5***
Basal ganglia/cholinergic forebrain			**Basal ganglia/cholinergic forebrain**		
Striatum	62.1 ± 6.1	**63.8 ± 3.2**	Striatum	8.2 ± 1.9	**10.0 ± 1.5**
Globus pallidus	5.0 ± 0.7	**3.3 ± 0.5**	Medial septum	30.1 ± 1.7	**31.2 ± 3.6**
NBM	5.4 ± 1.4	**4.5 ± 0.7**	NBM	7.2 ± 0.6	**3.1 ± 0.4*****

Data are mean ± S.E. M values of aCSF (n = 7) and SAP (n = 9) treated rats.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared to aCSF group.

Table S6. Absolute intensity of lipid species in organotypic cultures after treatments in both positive and negative ion mode.

			Intensity (u.a.)			
Assignment	**Cal *m/z***	**Exp *m/z***	**VEHICLE**	**CARBACHOL**	**CAR+SCOP**	**CAR+PIR**
Positive ion mode						
LPC (16:0) + H^+	496.3398	496.3379	$1.71 \cdot 10^9$ ± 224629	**$4.7 \cdot 10^9$ ± $6 \cdot 10^5$*a**	$3.1 \cdot 10^9$ ± $1 \cdot 10^5$	**4.25E+09 ± $6 \cdot 10^5$*b**
LPC (16:1) + H^+	494.3241	494.3270	3809. ± 461	**11696.00 ± 2506*a**	5400.00 ± 1336	**7250.00 ± 2101**
LPC (18:0) + H^+	524.3744	524.3711	910345 ± 166700	**$5.52 \cdot 10^9$ ± $5 \cdot 10^5$*a**	$4. \cdot 10^9$ ± $2 \cdot 10^5$	**$5.2 \cdot 10^9$ ± $6 \cdot 10^5$*b**
LPC (O-18:0) + H^+	508.3761	508.3788	6580 ± 864	**18654 ± 2098**a**	10133 ± 840	**15956 ± 1373*b**
LPC (O-18:1) + H+	506.3594	506.3605	$8.84 \cdot 10^4$ ± 13851	**$1.39 \cdot 10^9$ ± $2 \cdot 10^5$*a**	$9.5 \cdot 10^5$ ± $1 \cdot 10^5$	**$1.4 \cdot 10^9$ ± $1 \cdot 10^5$*b**
PC (36:4) + H^+	782.5694	782.5727	$9.29 \cdot 10^9$ ± $1 \cdot 10^9$	**$5.89 \cdot 10^9$ ± $3 \cdot 10^5$*a**	$7.1 \cdot 10^9$ ± $2 \cdot 10^5$	**$5.8 \cdot 10^9$ ± $2 \cdot 10^5$**b**
PC (O-36:4) + H^+	768.5902	768.5943	173714 ± 7706	**54424 ± 9721*a**	146795 ± 6801	**69580 ± 12002*b**
PC (36:5) + Na^+	802.5357	802.5401	44361 ± 1268	**23056 ± 3204**	35767 ± 6284	**14701 ± 3088**b**
PC (38:5) + H^+	808.5851	808.5861	761567 ± 42629	**526654 ± 21651*a**	700222 ± 63349	**568492 ± 48686**
PC (38:6) + H^+	806.5694	806.5742	764787 ± 81976	**349308 ± 23788**a**	481258 ± 32195	**349138 ± 4729*b**
PC(38:7) + H^+	804.5565	804.5538	597052 ± 100617	**270280 ± 26512*a**	402153 ± 59147	**248105 ± 40459*b**
PC(O-38:7) + H^+	790.5745	790.5795	63971 ± 5613	**26141 ± 4941*a**	64351 ± 9306	**30403 ± 4465**
PC(40:7) + Na^+ /PC (36:4) + H^+	832.5851	832.5851	288228 ± 24796*	**197092 ± 19490**	282757 ± 21872	**185810 ± 35558**
Negative ion mode						
LPC (O-18:0) -CH_3	508.3409	508.3418	3997 ± 1106	**18226 ± 1697*a**	11564 ± 1505	**20301 ± 3041**b**

The maximal peak in negative ion mode, PI (20:4/18:0), changed with treatments. Data are mean ± S.E.M values of absolute intensity of VEHICLE (n = 6), CARBACHOL (n = 4), CAR+SCOP: Carbachol+Scopolamine (n = 4), and CAR+PIR: Carbachol+Pirenzepine (n = 4) of absolute intensity. *****p < 0.05, ******p < 0.01. **a*** when compared VEHICLE *vs* CARBACHOL. **b*** when compared VEHICLE *vs* CAR+PYR. PC: phosphatidylcholine; LPC: phosphatidylcholine; Cal: calculated; Exp: experimental.