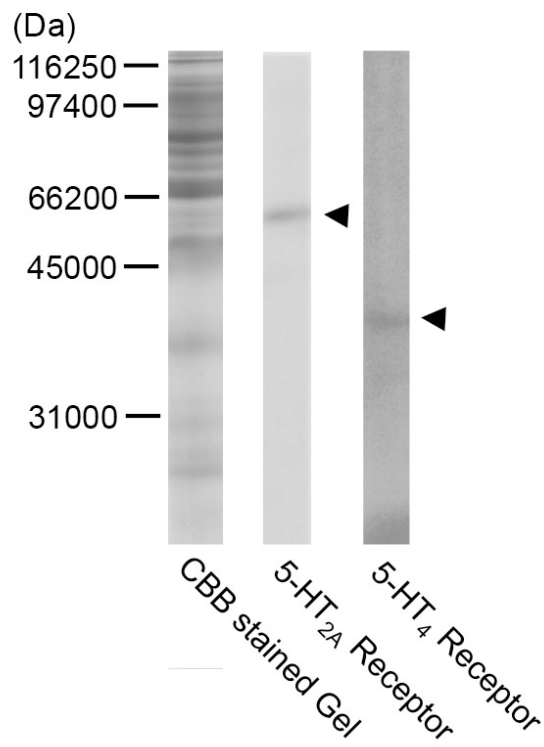
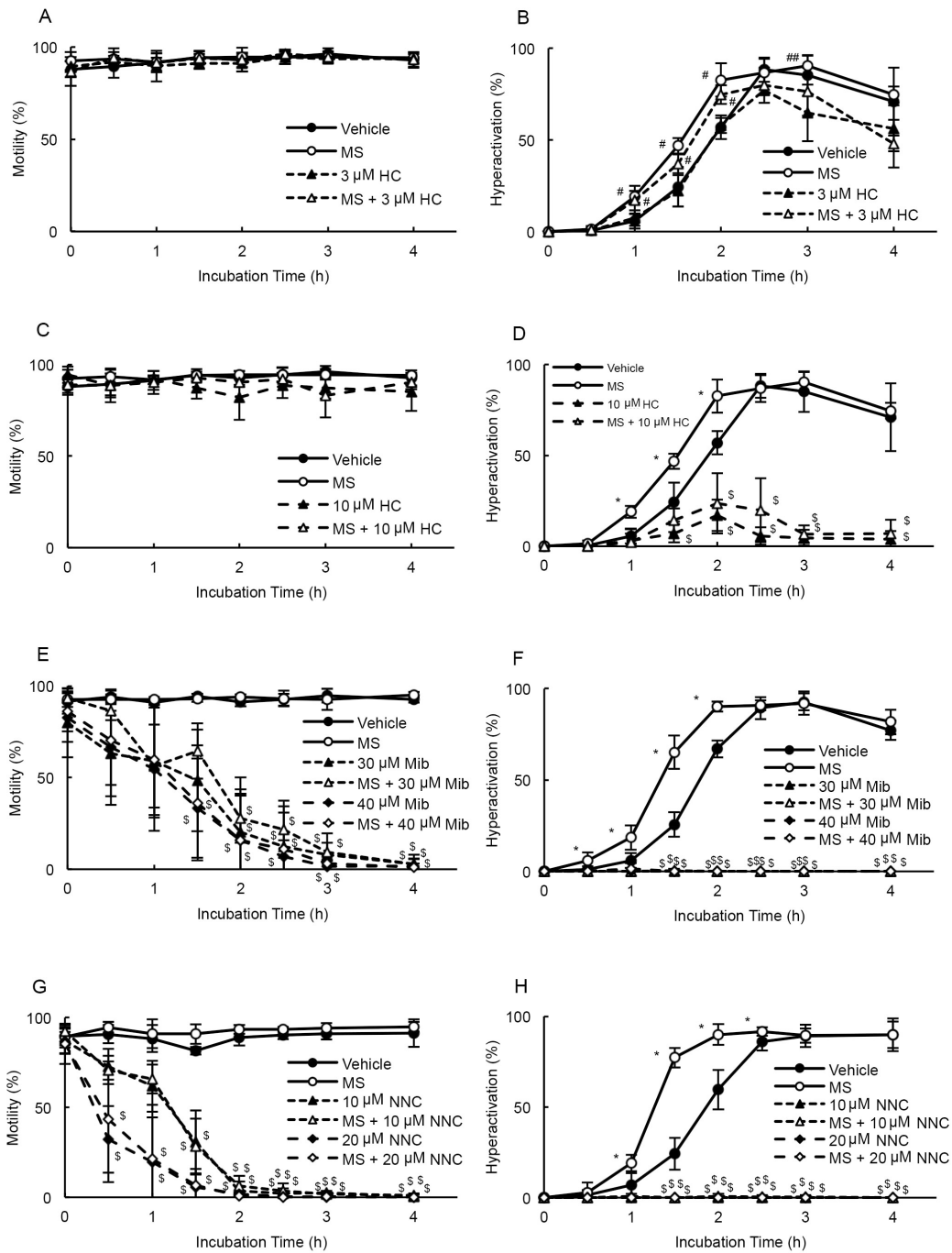


Supplementary Fig. 1. Effects of 5-HT receptor agonists on hamster sperm hyperactivation. Percentages of motility and hyperactivation were determined after 2 h of culture when sperm were cultured for 4 h with 17 nM or 100 nM sumatriptan (A), 100 fM MS (B), 100 μ M mCPBG (C), 10 pM MT (D), 7.3 nM WAY (E), 0.13 nM LP12 (F), 0.75 nM TCB2 (G), 2 μ M BW723C86 (H), and 0.3 nM MK212 (I). Data represent the mean \pm standard deviation (SD). (A) (Vehicle) the medium with 0.1% (v/v) pure water as vehicle; (respective concentrations of sumatriptan) the medium with indicated concentrations of sumatriptan and vehicle. (B) (Vehicle) same as above; (MS) the medium with 100 fM MS and vehicle. (C) (Vehicle) same as above; (mCPBG) the medium with 100 μ M mCPBG and vehicle. (D) (Vehicle) medium with 0.1% (v/v) ethanol as vehicle; (MT) medium with 10 pM MT and vehicle. (E) (Vehicle) medium with 0.1% (v/v) pure water as vehicle; (WAY) medium with 7.3 nM WAY and vehicle. (F) (Vehicle) same as above; (LP12) medium with 0.13 nM LP12 and vehicle. (G) (Vehicle) same as above; (TCB2) medium with 0.75 nM TCB2 and vehicle. (H) (Vehicle) medium with 0.1% (v/v) dimethyl sulfoxide as vehicle; (BW723C86) medium with 2 μ M BW723C86 and vehicle. (I) (Vehicle) medium with 0.1% (v/v) pure water as vehicle; (MK212) medium with 0.3 nM MK212 and vehicle. * indicates significant differences compared with “Vehicle” ($P < 0.05$). MS, α -methylserotonin maleate; MT, 5-methoxytryptamine; mCPBG, 1-(3-chlorophenyl) biguanide hydrochloride.

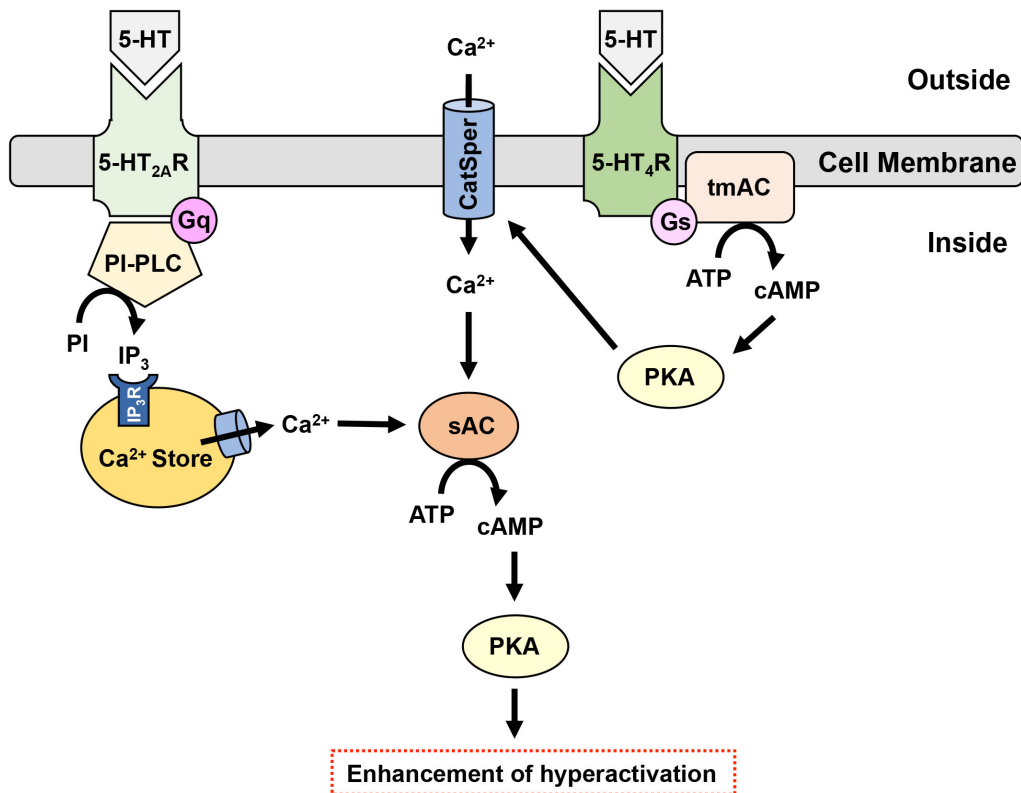


Supplementary Fig. 2. Detection of 5-HT_{2A} and 5-HT₄ receptors from hamster sperm. Left lane shows CBB stained gel. Middle lane shows western blotting against the anti-5-HT_{2A} receptor antibody. Right lane shows western blotting against anti-5-HT₄ receptor antibody. The numbers on the left side show molecular weight markers. Sperm protein extracts were applied at 10 μ l in each lane. Arrow indicates the antibody reaction.



Supplementary Fig. 3. Suppression of MS-enhanced hyperactivation by CatSper inhibitors. Percentages of motility (A, C, E, and G) and hyperactivation (B, D, F, and H) were determined after sperm were cultured for 4 h with 100 fM MS and inhibitors, including 3 μ M HC (A and B), 10 μ M HC (C and D), 30 and 40 μ M Mib (E and F), and 10 and 20 μ M NNC (G and H). Data represent the mean \pm standard deviation (SD). **(A and B)** (Vehicle) medium with 0.1% (v/v)

pure water and 0.1% (v/v) dimethyl sulfoxide as vehicle; (MS) medium with 100 fM MS and vehicle; (3 μ M HC) medium with 3 μ M HC and vehicle; (MS + 3 μ M HC) medium with 100 fM MS, 3 μ M HC, and vehicle. **(C and D)** (Vehicle) same as above; (MS) medium with 100 fM MS and vehicle; (10 μ M HC) medium with 10 μ M HC and vehicle; (MS + 10 μ M HC) medium with 100 fM MS, 10 μ M HC, and vehicle. **(E and F)** (Vehicle) same as above; (MS) medium with 100 fM MS and vehicle; (30 μ M Mib) medium with 30 μ M Mib and vehicle; (MS + 30 μ M Mib) medium with 100 fM MS, 30 μ M Mib, and vehicle; (40 μ M Mib) medium with 40 μ M Mib and vehicle; (MS + 40 μ M Mib) medium with 100 fM MS, 40 μ M Mib, and vehicle. **(G and H)** (Vehicle) same as above; (MS) medium with 100 fM MS and vehicle; (10 μ M NNC) medium with 10 μ M NNC and vehicle; (MS + 10 μ M NNC) medium with 100 fM MS, 10 μ M NNC, and vehicle; (20 μ M NNC) medium with 20 μ M NNC and vehicle; (MS + 20 μ M NNC) medium with 100 fM MS, 20 μ M NNC, and vehicle. * indicates significant differences compared with “Vehicle,” “inhibitors,” and “MS + inhibitors” ($P < 0.05$). # indicates significant differences compared with “Vehicle” and “inhibitors” ($P < 0.05$). ## indicates significant differences compared with “inhibitors” ($P < 0.05$). \$ indicates significant differences compared with “Vehicle” and “MS” ($P < 0.05$). MS, α -methylserotonin maleate; HC, 2,4-dithenoyl-1,2,5-oxadiazole n2-oxide; Mib, mibefradil.



Supplementary Fig. 4. Hypothesis regarding regulatory mechanisms of 5-HT-enhanced hyperactivation in hamster sperm. 5-HT, 5-hydroxytryptamine; 5-HT_{2A}R, 5-HT_{2A} receptor; 5-HT₄R, 5-HT₄ receptor; Gq, Gq-protein; Gs, Gs-protein; tmAC, transmembrane adenylate cyclase; sAC, soluble adenylate cyclase; PI, phosphatidylinositol; PI-PLC, phosphatidylinositol-phospholipase C; IP₃, inositol 1,4,5-trisphosphate; IP₃R, inositol 1,4,5-trisphosphate receptor; PKA, protein kinase A.

	5-HT ₁ Receptor			5-HT ₂ Receptor		5-HT ₃ Receptor	
	Vehicle	17 nM Sumatriptan	100 nM Sumatriptan	Vehicle	MS	Vehicle	mCPBG
VSL (µm/sec)	110.73 ± 12.46	104.82 ± 16.56	90.28 ± 18.96	104.90 ± 18.57	83.45 ± 6.22*	115.85 ± 39.66	85.16 ± 17.29
VCL (µm/sec)	447.59 ± 104.29	400.38 ± 53.59	549.33 ± 94.50	498.89 ± 89.79	462.56 ± 44.30	512.13 ± 38.24	391.47 ± 62.71*
VAP (µm/sec)	163.11 ± 12.16	176.04 ± 6.14	178.58 ± 23.42	155.91 ± 15.98	132.44 ± 13.65*	176.05 ± 24.89	135.47 ± 34.99
LIN	0.26 ± 0.09	0.30 ± 0.08	0.19 ± 0.08	0.23 ± 0.08	0.19 ± 0.03	0.24 ± 0.09	0.25 ± 0.07
STR	0.60 ± 0.07	0.62 ± 0.09	0.52 ± 0.15	0.67 ± 0.07	0.64 ± 0.07	0.65 ± 0.14	0.65 ± 0.03
ALH (µm)	11.50 ± 1.80	10.33 ± 0.75	11.11 ± 0.78	10.94 ± 1.55	10.03 ± 0.94	12.18 ± 0.76	9.39 ± 0.74*
BCF (Hz)	7.34 ± 3.15	9.91 ± 1.65	7.41 ± 2.62	7.08 ± 3.94	5.98 ± 1.61	7.01 ± 1.75	7.94 ± 1.22
WOB	0.39 ± 0.10	0.47 ± 0.06	0.35 ± 0.06	0.34 ± 0.09	0.31 ± 0.03	0.35 ± 0.07	0.38 ± 0.12

	5-HT ₄ Receptor		5-HT ₆ Receptor		5-HT ₇ Receptor	
	Vehicle	MT	Vehicle	WAY208466	Vehicle	LP12
VSL (µm/sec)	98.06 ± 13.24	94.60 ± 14.92	114.66 ± 28.42	102.94 ± 26.26	80.91 ± 17.19	90.91 ± 26.39
VCL (µm/sec)	437.32 ± 17.68	373.94 ± 92.14	492.85 ± 142.35	464.14 ± 91.87	479.68 ± 170.69	585.77 ± 90.05
VAP (µm/sec)	143.07 ± 15.47	131.59 ± 22.68	164.97 ± 39.79	169.98 ± 39.95	140.03 ± 27.62	145.30 ± 38.68
LIN	0.24 ± 0.05	0.27 ± 0.07	0.0026 ± 0.06	0.24 ± 0.05	0.19 ± 0.06	0.16 ± 0.03
STR	0.69 ± 0.06	0.72 ± 0.07	0.70 ± 0.06	0.62 ± 0.09	0.59 ± 0.03	0.64 ± 0.07
ALH (µm)	10.53 ± 0.68	9.49 ± 0.94*	11.21 ± 2.88	11.25 ± 2.06	10.22 ± 3.28	12.35 ± 1.80
BCF (Hz)	7.72 ± 3.00	9.45 ± 3.89	6.62 ± 1.77	6.74 ± 0.41	4.48 ± 1.18	3.49 ± 1.37
WOB	0.34 ± 0.07	0.37 ± 0.07	0.36 ± 0.07	0.38 ± 0.05	0.32 ± 0.08	0.25 ± 0.05

	5-HT _{2A} Receptor		5-HT _{2B} Receptor		5-HT _{2C} Receptor	
	Vehicle	TCB2	Vehicle	BW723C86	Vehicle	MK212
VSL (µm/sec)	120.62 ± 14.38	80.47 ± 20.12*	128.74 ± 37.81	148.62 ± 34.69	121.64 ± 22.16	109.13 ± 21.69
VCL (µm/sec)	350.12 ± 34.71	276.68 ± 70.44	410.98 ± 112.59	421.54 ± 115.61	386.79 ± 68.93	344.52 ± 60.71
VAP (µm/sec)	179.49 ± 20.84	142.07 ± 32.29	215.71 ± 52.05	225.38 ± 52.92	215.23 ± 46.85	179.78 ± 19.41
LIN	0.36 ± 0.06	0.36 ± 0.07	0.32 ± 0.04	0.37 ± 0.07	0.32 ± 0.03	0.33 ± 0.06
STR	0.67 ± 0.08	0.62 ± 0.05	0.61 ± 0.12	0.67 ± 0.06	0.57 ± 0.07	0.63 ± 0.06
ALH (µm)	8.46 ± 1.03	7.79 ± 2.07	9.71 ± 1.97	8.91 ± 2.89	9.20 ± 0.73	8.92 ± 8.92
BCF (Hz)	7.83 ± 1.40	7.91 ± 1.58	8.12 ± 0.92	9.15 ± 1.67	7.98 ± 1.97	8.68 ± 0.98
WOB	0.53 ± 0.05	0.55 ± 0.05	0.54 ± 0.05	0.55 ± 0.07	0.56 ± 0.03	0.53 ± 0.07

Supplementary Table 1

Effects of 5-HT receptor agonists on motility assay of hamster sperm. Each value was indicated at 2 h culture when sperm were cultured for 4 h with 17 nM or 100 nM sumatriptan, 100 fM MS, 100 µM mCPBG, 10 pM MT, 7.3 nM WAY, 0.13 nM LP12, 0.75 nM TCB2, 2 µM BW723C86 and 0.3 nM MK212. Data represent the mean ± SD. (5-HT₁ Receptor) (Vehicle) the medium with 0.1% (v/v) pure water as vehicle; (respective concentrations of sumatriptan) the medium with indicated concentrations of sumatriptan and vehicle. (5-HT₂ Receptor) (Vehicle) same as above; (MS) the medium with 100 fM MS and vehicle. (5-HT₃ Receptor) (Vehicle) same as above; (mCPBG) the medium with 100 µM mCPBG and vehicle. (5-HT₄ Receptor) (Vehicle) medium with 0.1% (v/v) ethanol as vehicle; (MT) medium with 10 pM MT and vehicle. (5-HT₆ Receptor) (Vehicle) medium with 0.1% (v/v) pure water as vehicle; (WAY) medium with 7.3 nM WAY and vehicle. (5-HT₇ Receptor) (Vehicle) same as above; (LP12) medium with 0.13 nM LP12 and vehicle. (5-HT_{2A} Receptor) (Vehicle) same as above; (TCB2) medium with 0.75 nM TCB2 and vehicle. (5-HT_{2B} Receptor) (Vehicle) medium with 0.1% (v/v) dimethyl sulfoxide as vehicle; (BW723C86) medium with 2 µM BW723C86 and vehicle. (5-HT_{2C} Receptor) (Vehicle) medium with 0.1% (v/v) pure water as vehicle; (MK212) medium with 0.3 nM MK212 and vehicle. * indicates significant differences compared with "Vehicle" (P < 0.05).