Supplementary information

Moderate voluntary exercise attenuates the metabolic syndrome in melanocortin-4 receptor-deficient rats showing central dopaminergic dysregulation

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Figure S1

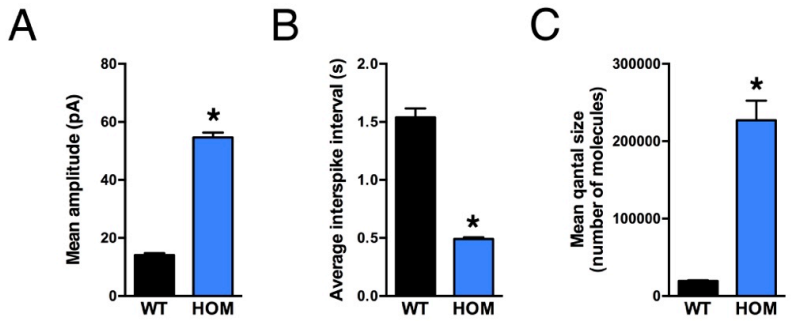

Figure S1 | Local depolarization-induced catecholamine quantal release in chromaffin cells obtained from adrenal glands of sedentary HOM rats and WT controls. Chromaffin cells obtained from adrenal glands of sedentary WT and HOM rats were stimulated, 48 h post plating, with 80 mM KCl and 6 mM Ca^{2+} for 6 sec and amperometric spikes after stimulation of cells were measured. (**A**) Mean peak amplitude, (**B**) mean interspike interval (lower values indicate increased frequency of quantal release), and (**C**) mean catecholamine quantal size from the recorded cells (n=423-472 quanta in WT cells; n=1440-1505 quanta in HOM cells). * $p < 0.001$.

Figure S2

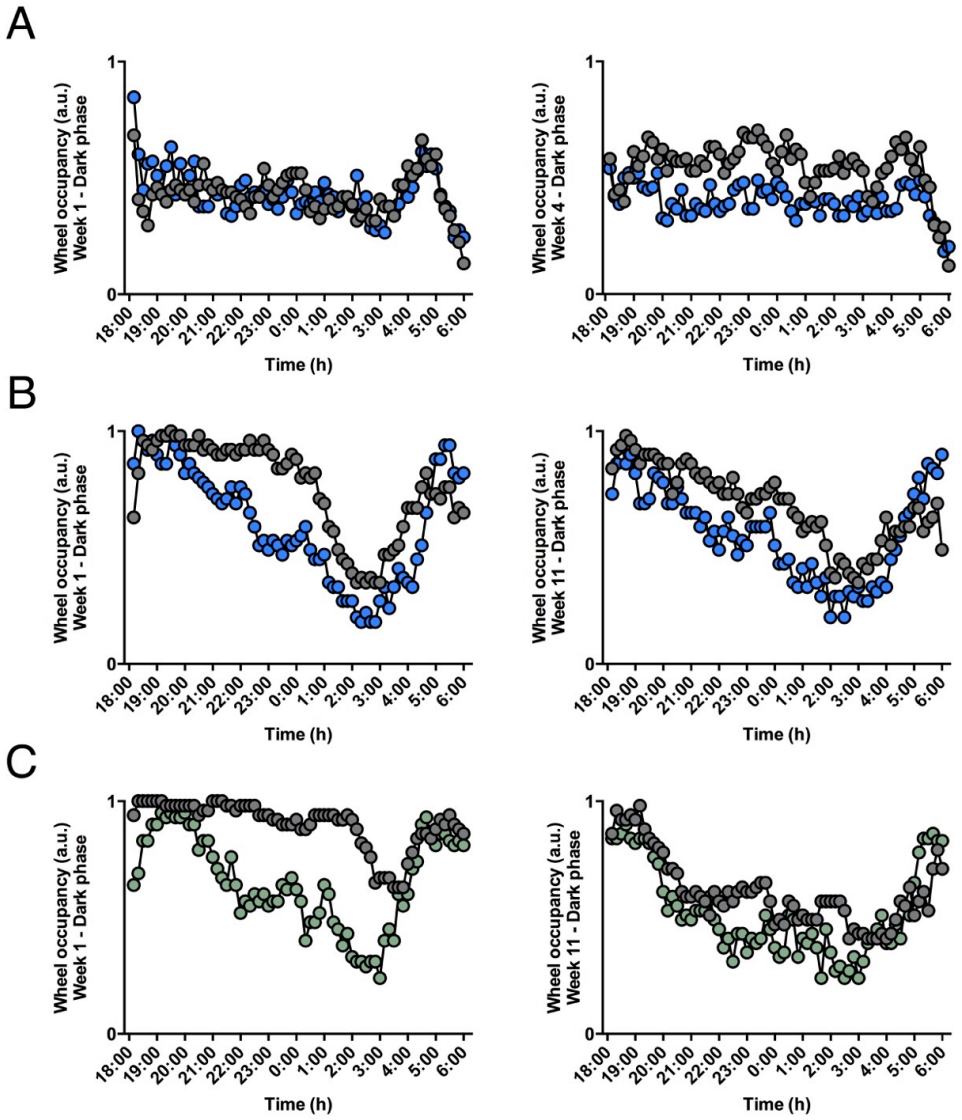

Figure S2 | Voluntary running-wheel occupancy. Dark-phase running-wheel occupancy of (**A**) WT control and HOM rats during week 1 (*left*) and week 4 (*right*) of voluntary wheel running (n = 14 rats/group), (**B**) WT control and loxTB^{Mc4r} mice during week 1 (*left*) and week 11 (*right*) of voluntary wheel running (n = 6-7 mice/group), and (**C**) WT control and WT^{BWM} mice during week 1 (*left*) and week 11 (*right*) of wheel running (n = 6-7 mice/group).

Figure S3

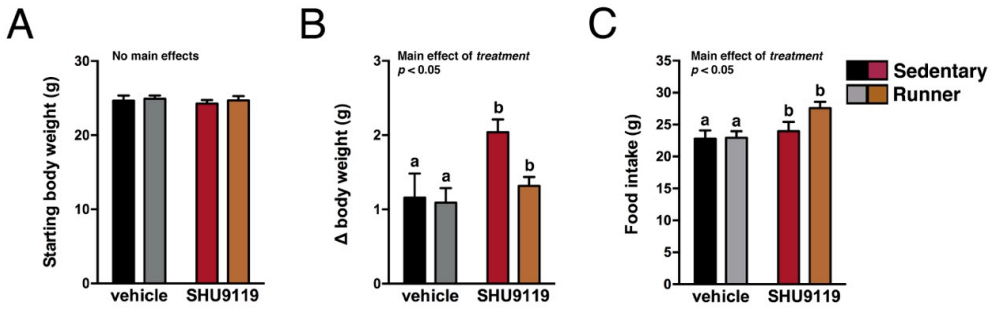

Figure S3 | Effects of ICV SHU9119 on body weight gain and food intake. (**A**) Starting body weight, (**B**) body weight gain, and (**C**) cumulative food intake during 7 d of ICV SHU9119 (5 ng/day) administration (n = 6-7 mice/group). [a,b] $p < 0.05$, effect of *treatment*.

Figure S4

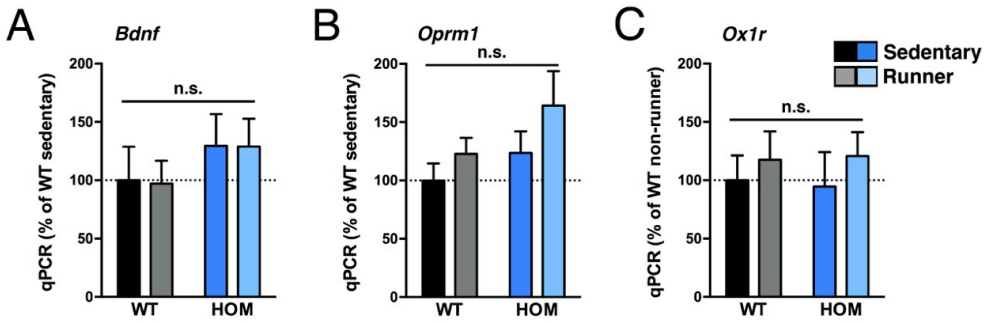

Figure S4 | Gene expression in VTA of HOM wheel-runners. (**A**) *Bdnf*, (**B**) *Oprm1*, and (**C**) *Ox1r* expression in the ventral tegmental area of WT and HOM littermate rats without (sedentary) or with (runner) free access to running wheels for 5 wk (n = 14/group). All data are represented relative to WT sedentary controls. n.s., not significant.

Figure S5

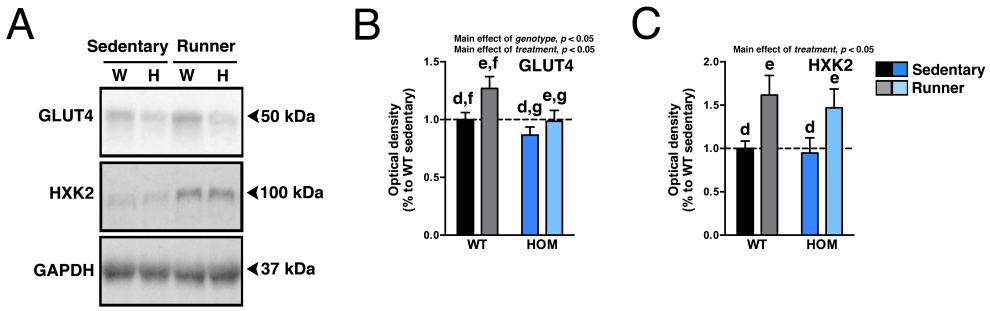

Figure S5 | Effects of VWR on muscle GLUT4 and HXK2 levels. (**A**) Representative western blots and immunoblot analyses of (**B**) GLUT4 and (**C**) HXK2 in triceps muscle of WT (W) and HOM (H) rats without (sedentary) or with (runner) free access to running wheels for 5 wk (n = 5-6/group). Different letters indicate significant difference as following: [d,e] $p < 0.05$, effect of *treatment*; [f,g] $p < 0.05$, effect of *genotype*.

Table S1

Gene	Accession #	Sense sequence	Antisense sequence	Tissue analyzed
ObRb	NM_012596	5′-AATTGGAGCAGTCCAGCCTA-3′	5′-TTTCCCACATCTTGTGACCA-3′	VTA, MBH
Drd1a	NM_012546	5′CGAGGCTCCATCTCCAAGGACTG-3′	5′-CGGTGTCATAGTCCAATATGACCG-3′	VTA
Drd2	NM_012547	5′-ACCTCCCTTAAGACGATGAGCCGC-3′	5′-CAGCCAGCAGATGATGAACACACC-3′	VTA
Th	NM_012740	5′-TCTTCCTACGGAGAGCTCCTG-3′	5′-GCGCTGGATACGAGAGGCA-3′	VTA
Slc6a3	NM_012694	5′-CCTGCTTCCTCCTGTATGTGG-3′	5′-AGGCTGCAGAACTTGTAGGT-3′	VTA
Oprk1	NM_017167	5′-GCCATCCCTGTTATCATCACCG-3′	5′-GTAGATGTTGGTTGCGGTCTTCATC-3′	VTA
Bdnf	NM_012513	5′-GCGGCAGATAAAAAGACTGC-3′	5′-GCAGCCTTCCTTGGTGTAAC-3′	VTA
Oprm1	NM_013071	5′-TAGTGCGCCTCTTCGGAAAC-3′	5′-GTCTGCCAGAGCCAAGGTTGA-3′	VTA
Ox1r	NM_013064	5′-CTCTGGTCTGCCTGGCTGTGTG-3′	5′-AAGGCATGGCCGAAGAGCCATGA-3′	VTA
Pomc	NM_139326	5′-AGTGCCAGGAC TCACCACG-3′	5′-AAGCGGTCCCAGCGGAAG-3′	MBH
Agrp	NM_033650	5′-GCAGAAGGCAGAAGCTTTGGC-3′	5′-CCCAAGCAGGACTCGTGCAG-3′	MBH
Npy	NM_012614	5′-TACTCCGCTCTGCGACACTACATC-3′	5′-CACATGGAAGGGTCTTCAAGCC-3′	MBH
Il6	NM_012589	5′-GACAAAGCCAGAGTCATTCAGA-3′	5′-GTCTTGGTCCTTAGCCACTCC-3′	MBH
Scd1	NM_139192	5′-ATTCAATCTCGGGAGAACATCCTG-3′	5′-GCCTTAGAAACTTTTTTCCGGTCG-3′	Liver
Rplp0 (*36B4*)	NM_022402	5′- ATCCCTGACGCACCGCCGTG-3′	5′- GCGCATCATGGTGTTCTTGC-3′	Reference gene
Mrpl32	NM_001106116	5′- CAGACGCACCATCGAAGTTA-3′	5′- AGCCACAAAGGACGTGTTTC-3′	Reference gene

ObRb, leptin receptor B; *Drd1a*, dopamine D1a receptor D1A; *Drd2*, dopamine D2 receptor; *Th*, tyrosine hydroxylase; *Slc6a3*, solute carrier family 6 (neurotransmitter transporter), member 3; *Oprk1*, opioid receptor, kappa 1; *Bdnf*, brain-derived neurotrophic factor; *Oprm1*, opioid receptor, mu 1; *Oxr1*, orexin receptor 1; *Pomc*, proopiomelanocortin; *Agrp*, agouti-related protein; *Npy*, neuropeptide Y; *IL-6*, interleukin-6; *Scd1*, stearoyl coenzyme-A desaturase 1; *RplP0*, ribosomal protein, large, PO; *Mrpl32*, mitochondrial ribosomal protein L32; VTA, ventral tegmental area; MBH, mediobasal hypothalamus.

Table S1. Forward and reverse primer sequences for qPCR analyses

Table S2

Measurement	Graph	Main effects/interactions	F value	*p* value
VWR, starting body weight HOM rats	Fig. 2B	main effect: *genotype*	$F_{(1,52)} = 289.22$	< 0.001
VWR, daily distance HOM rats	Fig. 2C	*genotype* x *treatment*	$F_{(3,78)} = 4.40$	< 0.01
VWR, starting body weight loxTB^{Mc4r} mice	Fig. 2E	main effect: *genotype*	$F_{(1,24)} = 77.19$	< 0.001
VWR, daily distance loxTB^{Mc4r} mice	Fig. 2F	main effect: *genotype*	$F_{(1,12)} = 18.58$	< 0.001
		main effect: *time*	$F_{(10,120)} = 5.99$	< 0.001
VWR, daily distance ICV SHU9119 mice	Fig. 2J	main effect: *drug*	$F_{(1,9)} = 6.19$	< 0.05
		main effect: *time*	$F_{(6,54)} = 25.33$	< 0.001
VWR, body weight evolution	Fig. 3A	*time* x *genotype* x *treatment*	$F_{(4,208)} = 9.79$	< 0.001
VWR, Δ body weight	Fig. 3B	*genotype* x *treatment*	$F_{(1,52)} = 11.36$	< 0.005
VWR, Δ fat mass	Fig. 3C	*genotype* x *treatment*	$F_{(1,52)} = 22.58$	< 0.001
VWR, Δ lean mass	Fig. 3D	main effect: *treatment*	$F_{(1,52)} = 11.24$	< 0.005
VWR, wild-type rats food intake/week	Fig. 3E	*time* x *treatment*	$F_{(3,78)} = 14.30$	< 0.001
VWR, HOM rats food intake/week	Fig. 3F	*time* x *treatment*	$F_{(3,78)} = 5.01$	< 0.005
VWR, cumulative food intake	Fig. 3G	*genotype* x *treatment*	$F_{(1,52)} = 12.47$	< 0.001
MBH *ObRb* mRNA expression	Fig. 3H	*genotype* x *treatment*	$F_{(1,16)} = 5.05$	< 0.05
MBH *Pomc* mRNA expression	Fig. 3I	*genotype* x *treatment*	$F_{(1,17)} = 7.93$	< 0.05
MBH *Agrp* mRNA expression	Fig. 3J	*genotype* x *treatment*	$F_{(1,17)} = 6.29$	< 0.05
MBH *Npy* mRNA expression	Fig. 3K	No main effects		
MBH *Il6* mRNA expression	Fig. 3L	main effect: *treatment*	$F_{(1,17)} = 5.90$	< 0.05
ipGTT, body weight	Fig. 4A	*genotype* x *treatment*	$F_{(1,48)} = 6.29$	< 0.05
ipGTT, fasting glucose	Fig. 4B	main effect: *treatment*	$F_{(1,48)} = 8.54$	< 0.01
ipGTT, glucose excursions	Fig. 4C	*time* x *genotype* x *treatment*	$F_{(5,240)} = 2.29$	< 0.05
ipGTT, glucose AUC	Fig. 4C, *insert*	main effect: *genotype*	$F_{(1,48)} = 109.51$	< 0.001
		main effect: *treatment*	$F_{(1,48)} = 15.82$	< 0.001
ipGTT, plasma insulin	Fig. 4D	*time* x *genotype* x *treatment*	$F_{(2,96)} = 9.43$	< 0.001
ipGTT, fasting HOMA-IR	Fig. 4E	*genotype* x *treatment*	$F_{(1,47)} = 9.14$	< 0.005
VTA *ObRb* mRNA expression	Fig. 5A	*genotype* x *treatment*	$F_{(1,20)} = 5.58$	< 0.05
VTA *Drd1* mRNA expression	Fig. 5B	*genotype* x *treatment*	$F_{(1,21)} = 5.26$	< 0.05
VTA *Drd2* mRNA expression	Fig. 5C	*genotype* x *treatment*	$F_{(1,21)} = 5.26$	< 0.05
VTA *Th* mRNA expression	Fig. 5D	*genotype* x *treatment*	$F_{(1,21)} = 15.53$	< 0.001
VTA *Slc6a3* mRNA expression	Fig. 5E	*genotype* x *treatment*	$F_{(1,18)} = 6.63$	< 0.05
VTA *Oprk1* mRNA expression	Fig. 5F	*genotype* x *treatment*	$F_{(1,20)} = 19.11$	< 0.001
NAc D1R protein expression	Fig. 6C	No main effects		
NAc D2R protein expression	Fig. 6D	No main effects		
NAc DARPP32 protein expression	Fig. 6E	No main effects		
NAc DARPP32-T34 protein expression	Fig. 6F	*genotype* x *treatment*	$F_{(1,8)} = 7.69$	< 0.05
NAc DARPP32-T75 protein expression	Fig. 6G	main effect: *treatment*	$F_{(1,13)} = 48.33$	< 0.001
NAc TH protein expression	Fig. 6H	*genotype* x *treatment*	$F_{(1,25)} = 7.46$	< 0.05
NAc DAT protein expression	Fig. 6I	main effect: *treatment*	$F_{(1,13)} = 7.21$	< 0.05
SHU9119, starting body weight	Fig. S3A	No main effects		
SHU9119, Δ body weight	Fig. S3B	main effect: *drug*	$F_{(1,19)} = 6.27$	< 0.05
SHU9119, food intake	Fig. S3C	main effect: *drug*	$F_{(1,16)} = 5.71$	< 0.05
Plasma leptin	Fig. S4A	*genotype* x *treatment*	$F_{(1,24)} = 12.97$	< 0.005
Plasma insulin	Fig. S4C	*genotype* x *treatment*	$F_{(1,23)} = 4.37$	< 0.05
Plasma triglycerides	Fig. S4D	main effect: *genotype*	$F_{(1,24)} = 12.59$	< 0.005
		main effect: *treatment*	$F_{(1,24)} = 6.09$	< 0.05
Plasma cholesterol	Fig. S4E	*genotype* x *treatment*	$F_{(1,24)} = 4.75$	< 0.05
Liver lobe weights	Fig. S4F	main effect: *genotype*	$F_{(1,24)} = 60.95$	< 0.001
		main effect: *treatment*	$F_{(1,24)} = 5.08$	< 0.05
Liver triglycerides	Fig. S4G	*genotype* x *treatment*	$F_{(1,50)} = 6.90$	< 0.05
Liver *Scd1* mRNA expression	Fig. S4H	main effect: *genotype*	$F_{(1,50)} = 56.41$	< 0.001
		main effect: *treatment*	$F_{(1,50)} = 8.02$	< 0.01
Muscle GLUT4	Fig. S4J	main effect: *genotype*	$F_{(1,20)} = 5.78$	< 0.05
		main effect: *treatment*	$F_{(1,20)} = 5.20$	< 0.05
Muscle HXKII	Fig. S4K	main effect: *treatment*	$F_{(1,20)} = 6.59$	< 0.05
VTA *Bdnf* mRNA expression	Fig. S5A	No main effects		
VTA *Oprm1* mRNA expression	Fig. S5B	No main effects		
VTA *Oxr1* mRNA expression	Fig. S5C	No main effects		

VWR, voluntary wheel running; ICV, intracerebroventricular; *ObRb*, leptin receptor B; *Pomc*, proopiomelanocortin; *Agrp*, agouti-related protein; *Npy*, neuropeptide Y; *IL-6*, interleukin-6; ipGTT, intraperitoneal glucose tolerance test; AUC, area under the curve; HOMA-IR, Homeostatic model assessment of insulin resistance; NAc, nucleus accumbens; VTA, ventral tegmental area; MBH, mediobasal hypothalamus; D1R, *Drd1a*, dopamine D1a receptor D1A; D2R, *Drd2*, dopamine D2 receptor; *Th*, tyrosine hydroxylase; *Slc6a3*, solute carrier family 6 (neurotransmitter transporter), member 3; *Oprk1*, opioid receptor, kappa 1; *Scd1*, stearoyl coenzyme-A desaturase 1; GLUT4, glucose transporter 4; HXKII, hexokinase 2; *Bdnf*, brain-derived neurotrophic factor; *Oprm1*, opioid receptor, mu 1; *Oxr1*, orexin receptor 1.

Table S2. Statistical information