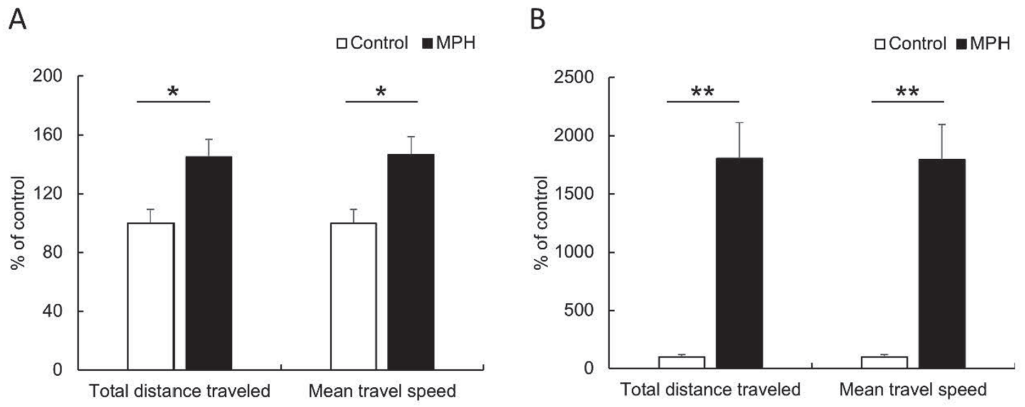

Fig. S1. Effects of MPH treatment on locomotor activity in sire. On day 20 during a total of 21 days of MPH administration, a spontaneous locomotor activity test was carried out on male mice before (A) and just after (B) the administration of MPH. A video tracking system was used to analyze the total distance traveled and the mean travel speed in the activity chambers during a 20-min period. Data from control and MPH-treated male mice are shown as mean ± S.E.M. and were compared using Student's *t*-test (*p < 0.05 and **p < 0.01; control group, n = 10; MPH group, n = 10).

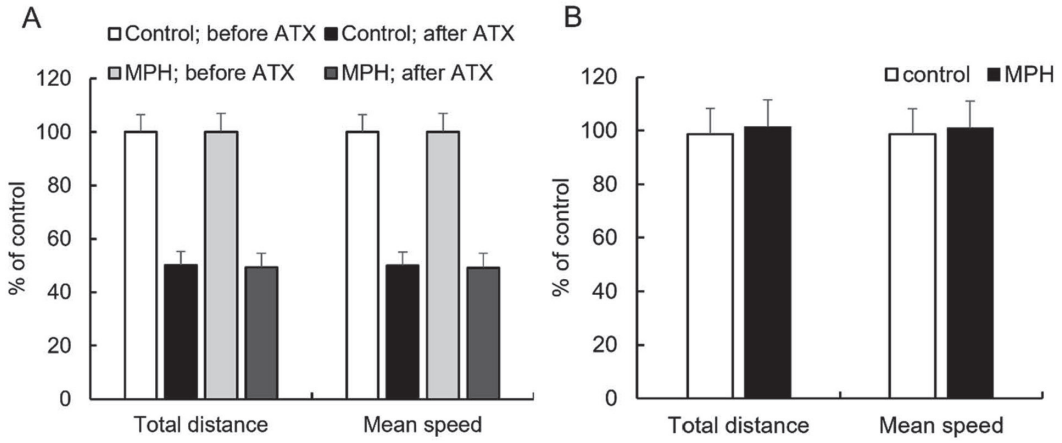

Fig. S2. Effects of atomoxetine (ATX) on locomotor activity in the offspring. The day after the spontaneous locomotor activity test, half of the mice were subjected to a therapeutic experiment with ATX (3 mg/kg, *s.c.*). Thirty mins after ATX administration, mice were again subjected to the spontaneous locomotor activity test. (A) The total distance traveled and the mean travel speed in the activity chambers during a 30-min period were measured. Data are shown as mean ± S.E.M. (B) Differences in data before and after ATX administration were calculated and compared for the control and MPH groups, respectively. Data are shown as mean ± S.E.M. and analyzed with mixed ANOVA with group × sex model and litter as a random factor (control group, n = 27 (M15/F12) and MPH group, n = 24 (M12/F12)). (M, male; F, female)

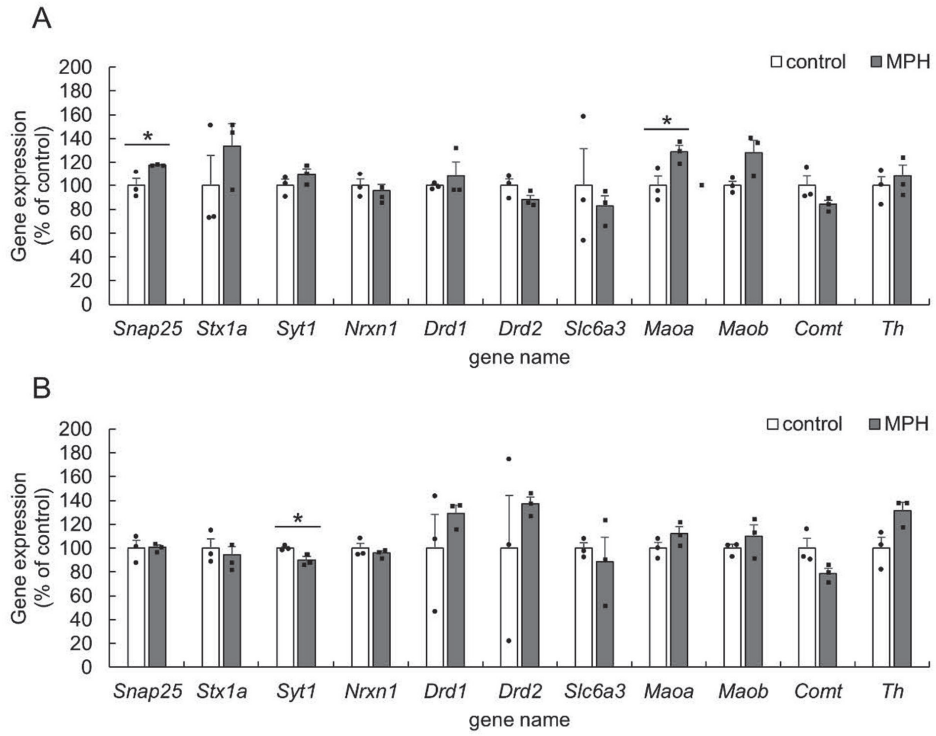

Fig. S3. Gene expression of ADHD-related genes in the striatum and the frontal cortex of male offspring from MPH-administered sire. Brain samples from offspring were obtained at 6 weeks of age. (A) shows results for the striatum and (B) for the frontal cortex. Expressions of genes involved in exocytosis and reportedly associated with ADHD (*Snap25, Stx1a, Syt1, Nrxn1*) and candidate genes for ADHD pathogenesis (*Drd1, Drd2, Slc6a3, Maoa, Maob, Comt, Th*) were determined by RT-qPCR. Data from control and MPH groups are shown as mean ± S.E.M. and were compared using Student's *t*-test (*$p < 0.05$; control group, n = 3 and MPH group, n = 3).

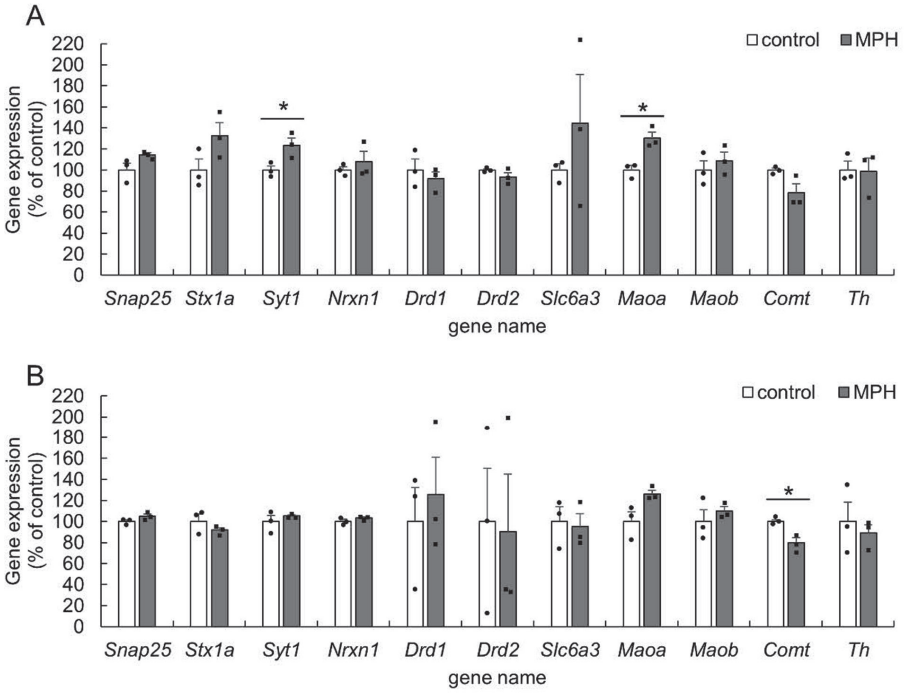

Fig. S4. Gene expression of ADHD-related genes in the striatum and the frontal cortex of female offspring from MPH-administered sire. Brain samples from offspring were obtained at 6 weeks of age. (A) shows results for the striatum and (B) for the frontal cortex. Expressions of genes involved in exocytosis and reportedly associated with ADHD (*Snap25, Stx1a, Syt1, Nrxn1*) and candidate genes for ADHD pathogenesis (*Drd1, Drd2, Slc6a3, Maoa, Maob, Comt, Th*) were determined by RT-qPCR. Data from control and MPH groups are shown as mean ± S.E.M. and were compared using Student's *t*-test (*$p < 0.05$; control group, n = 3 and MPH group, n = 3).

Table S1. Details of the mice used in this study.

Control							MPH						
Parent number	Number of offspring	Offspring number	Spontaneous locomotor activity and Elevated plus-maze tests	Therapeutic experiments	PCR	RNA-seq	Parent number	Number of offspring	Offspring number	Spontaneous locomotor activity and Elevated plus-maze tests	Therapeutic experiments	PCR	RNA-seq
Ⅰ	15	F1	✓		✓		Ⅰ	13	F1	✓			
		F2	✓						F2	✓	✓		
		F3	✓	✓					M1	✓		✓	
		M1	✓						M2	✓			
		M2	✓	✓					M3	✓	✓		
		M3	✓	✓					M4	✓	✓		
Ⅱ	15	F1	✓	✓			Ⅱ	17	F1	✓		✓	✓
		M1	✓						F2	✓			
		M2	✓		✓				F3	✓	✓		
		M3	✓						F4	✓	✓		
		M4	✓	✓					M1	✓			
		M5	✓	✓					M2	✓	✓		
Ⅲ	17	F1	✓		✓	✓	Ⅲ	13	F1	✓		✓	
		F2	✓						F2	✓			
		F3	✓	✓					F3	✓	✓		
		M1	✓						M1	✓			
		M2	✓	✓					M2	✓	✓		
		M3	✓	✓					M3	✓	✓		
Ⅳ	15	F1	✓				Ⅳ	13	F1	✓			
		F2	✓	✓					F2	✓	✓		
		M1	✓		✓	✓			F3	✓	✓		
		M2	✓						M1	✓			
		M3	✓	✓					M2	✓		✓	✓
		M4	✓	✓					M3	✓	✓		
Ⅴ	15	F1	✓				Ⅴ	14	F1	✓			
		F2	✓						F2	✓			
		F3	✓	✓					F3	✓	✓		
		F4	✓	✓					M1	✓		✓	
		M1	✓						M2	✓	✓		
		M2	✓	✓					M3	✓	✓		
Ⅵ	16	F1	✓		✓	✓	Ⅵ	14	F1	✓		✓	✓
		F2	✓						F2	✓	✓		
		F3	✓	✓					F3	✓	✓		
		M1	✓						M1	✓			
		M2	✓	✓					M2	✓			
		M3	✓	✓					M3	✓	✓		
Ⅶ	13	F1	✓				Ⅶ	13	F1	✓			
		F2	✓	✓					F2	✓			
		F3	✓	✓					F3	✓	✓		
		M1	✓						M1	✓			
		M2	✓						M2	✓	✓		
		M3	✓	✓					M3	✓	✓		
Ⅷ	14	F1	✓				Ⅷ	11	F1	✓			
		F2	✓	✓					F2	✓	✓		
		F3	✓	✓					F3	✓	✓		
		M1	✓		✓				M1	✓			
		M2	✓						M2	✓			
		M3	✓	✓					M3	✓	✓		
Ⅸ	8	F1	✓										
		F2	✓	✓									
		M1	✓										
		M2	✓										
		M3	✓	✓									
		M4	✓	✓									

Ten pairs of parent mice were mated in each group, with nine dams giving birth in the control group and eight in the MPH group. The number of offspring born to each dam is also shown. When offspring were 6 weeks old, 6 mice from each dam were randomly selected for spontaneous locomotor activity and elevated plus-maze tests (control group, n = 54 (M30/F24) and MPH group, n = 48 (M24/F24)). Half of the offspring were subjected to therapeutic experiments with ATX (control group, n = 27 (M15/F12) and MPH group, n = 24 (M12/F12)). Offspring from each dam that were not subjected to therapeutic experiments were subjected to RT-qPCR (control group, n = 6 (M3/F3) and MPH group, n = 6 (M3/F3)) and RNA-seq (control group, n = 3 (M1/F2) and MPH group, n = 3 (M1/F2)). (M, male; F, female)

Table S2. The average numbers of offspring and the survival rates of neonates.

	Control sires (n=9)	MPH-treated sires (n=8)	*p*-value
Average numbers of offspring	13.5 ± 0.53	14.2 ± 0.86	0.512
Average numbers of survivors	13. 5± 0.53	14.2 ± 0.86	0.512
Survival rate on P4 (%)	100 ± 0	100 ± 0	

The number of offspring (both living and stillborn) was counted at P1. The survival rate on P4 was calculated by dividing the number of offspring alive at P4 by the number of offspring at P1. Data from the control and MPH groups are shown as mean ± S.E.M. and were compared using Student's *t*-test (control group, n = 9 and MPH group, n = 8).

Table S3. Fold changes and p-values of genes associated with exocytosis.

Gene symbol	Gene name	FC	*p*-value
Genes associated with exocytosis			
Lin7a	lin-7 homolog A (C. elegans)	1.211	<0.01
Nrxn1	neurexin I	1.246	<0.01
Rims1	regulating synaptic membrane exocytosis 1	1.295	<0.01
Snap25	synaptosomal-associated protein 25	1.236	<0.01
Stx1a	syntaxin 1A (brain)	1.484	0.05
Syn1	synapsin I	1.303	<0.01
Syn2	synapsin II	1.208	0.01
Syt1	synaptotagmin I	1.235	<0.01

List of exocytosis-related genes in Reactome gene set of "neuronal system". Fold change (FC) and *p*-value for each gene are shown (control group, n = 3 (M1/F2) and MPH group, n = 3 (M1/F2)). (M, male; F, female)