Materials and methods

Animals

The zQ175 and WT animals were divided into two groups. Each group was examined with two radioligands at 6 and 9 months of age. Animals in Group 1 were examined with ^{11}C-raclopride and ^{18}F-MNI-659 and animals in Group 2 were examined with ^{11}C-NNC 112 and ^{11}C-MDL 100907. After the last imaging session, tail biopsy was taken to confirm genotype of each animal. In Group 1: one animal originally marked as WT came back as heterozygous and was therefore moved to that group. Another animal from Group 1 came back as homozygous and the results from that particular animal were excluded from the analysis since the aim was to image WT and heterozygous zQ175 mice. Number of animals included in the analysis is shown in Supplementary Table 3. The animals were housed at the animal department of Karolinska University Hospital in a temperature (± 21ºC) and humidity (± 40%) controlled environment on a 12h light/dark cycle (lights on 7:00 AM) with access to food and water ad libitum. Animals were allowed at least one week to habituate to their new environment in the animal department before the start of the imaging sessions. All experiments were conducted during the light phase of the 12 h light/dark cycle (lights on 7:00AM).

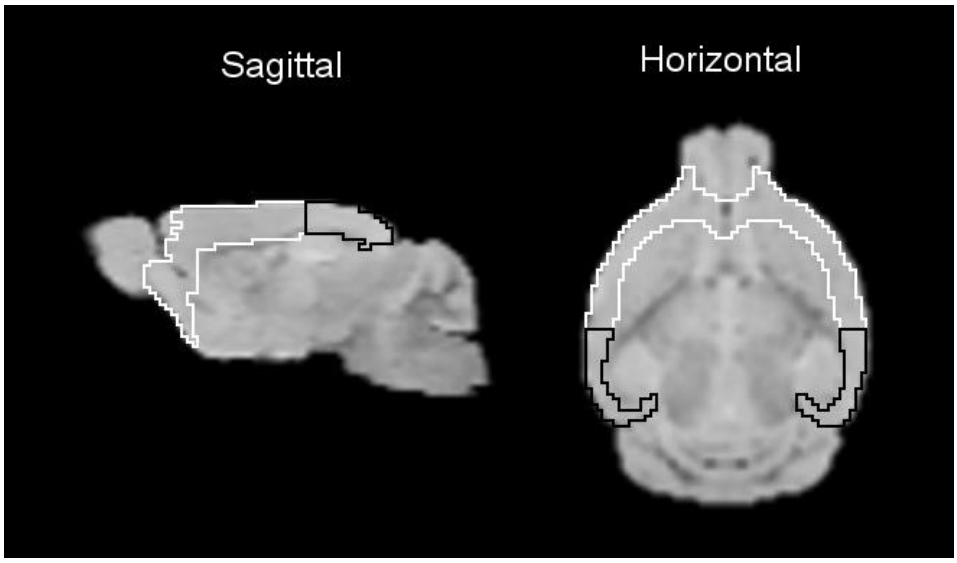
Brain tissue

Brain tissue harvested from ten months old mice (Q175 KI heterozygous, n=8; wild types, n=8) were sectioned at coronal projections (14 μm) on a cryostat at -20°C and mounted onto glass

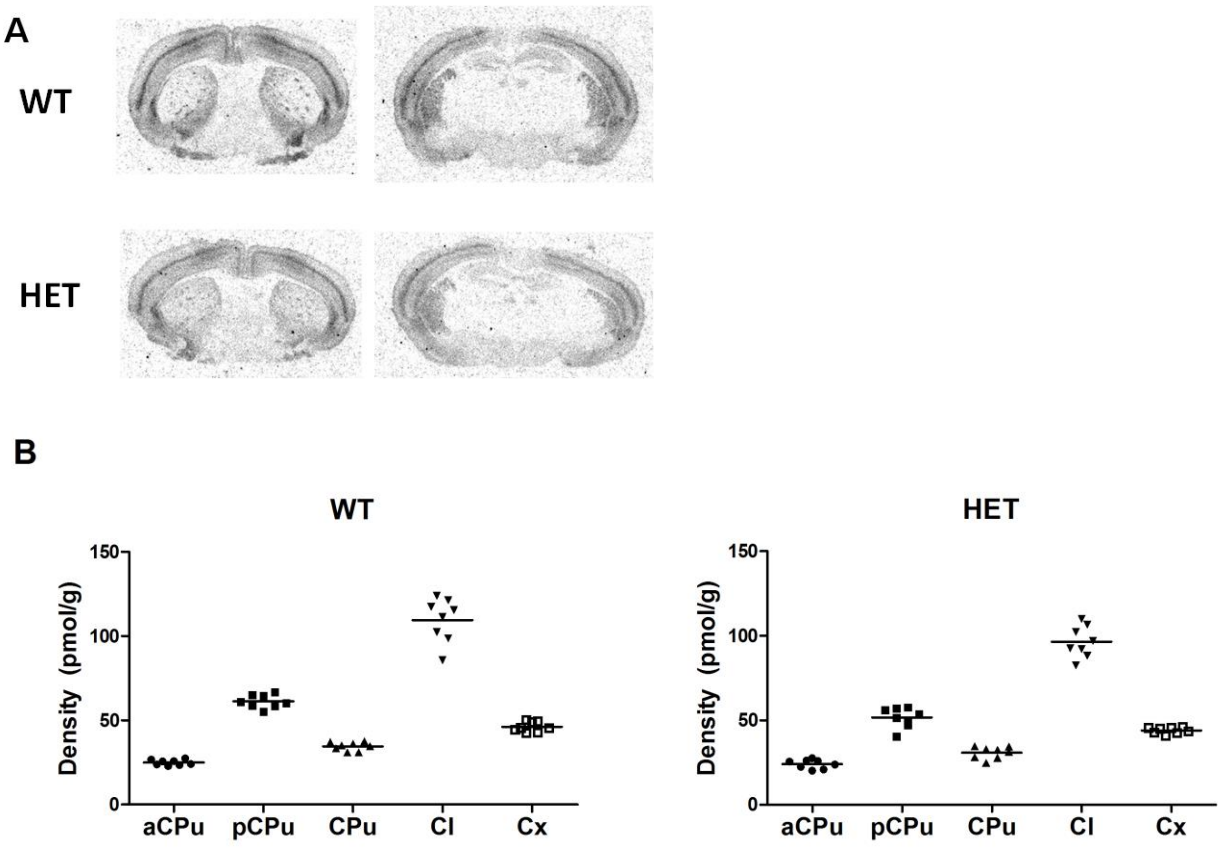
slides (SuperFrost Plus, Menzel GmbH, Braunschweigh, Germany). The sections were kept at -20°C until use.

In vitro Autoradiography

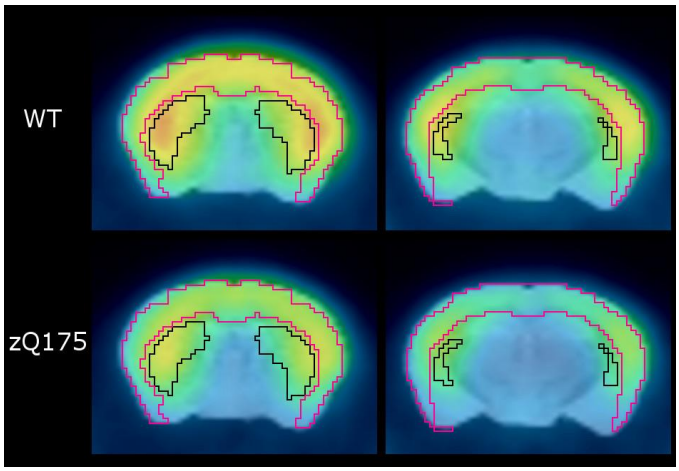
Binding to the 5-HT_{2A} receptor was studied using ^{3}H-MDL 100907 as a radioligand. Sections were pre-incubated for 15 minutes in 50 mM Tris-HCl buffer, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 2mM $CaCl_2$ and 1 mM $MgCl_2$. Incubations were carried out for 1 h in 50 mM Tris-HCl buffer, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 2mM $CaCl_2$, 1 mM $MgCl_2$ and 0.4 nM ^{3}H-MDL100907 (specific radioactivity, 81 Ci/mmol). Non-specific binding was determined in adjacent sections in the presence of 10 µM ketanserin. Sections were washed for 2 x 5 min in cold (4 ºC) 50 mM Tris-HCl buffer, pH 7.4, followed by a brief dip in distilled water. Radioactivity was detected and quantified with a phosphor imager (scanner: Fuji BAS-5000 image reader; imaging plates: BAS-TR2025, Fujifilm, Tokyo, Japan). The measured photostimulated luminescence (PSL) /mm2 values were transformed into radioactivity units based on intensity values obtained using tritium standards (Microscales, American Radiolabeled Chemicals Inc.). Regional specific binding was calculated by subtracting nonspecific binding, defined in the presence of 10 µM ketanserin, from the total ^{3}H-MDL100907 binding.



Supplemental Figure 1. The ROI template for the cortex was modified to separate the rostral and caudal part at approximately Bregma – 3mm. The white ROI represents the rostral cortex and the black ROI represents the caudal cortex.



Supplemental Figure 2. (A) Autoradiogram of ^{3}H-MDL 100907 in 10 months old WT and Het zQ175 mice at two different levels of the caudate-putamen (anterior and posterior caudate-putamen). (B) Quantification of the regional specific binding of ^{3}H-MDL 100907 in WT and het zQ175. Group differences in binding density were statistically significant (P<0.05) for pCPu, CPu and Cl, and trend-level statistically significant (P<0.10) for Cx. Abbreviations: aCPu; anterior caudate-putamen, pCPu: posterior caudate-putamen, Cl; claustrum, Cx; Cortex.



Supplemental Figure 3. The ROI template in PMOD shown at two different levels of the caudate-putamen (anterior (left) and posterior (right) caudate-putamen). In black, the ROI for the striatum is shown and in pink the ROI for the rostral cortex.

Supplemental Table 1. Average injected radioactivity (RA), injected mass and weight of the WT and zQ175 animals during the different PET measurements. Values are expressed as Mean ± S.D.

Radioligand	Age	Injected RA (MBq)		Specific Radioactivity (GBq/μmol)		Injected mass (μg)		Weight (g)	
		WT	zQ175	WT	zQ175	WT	zQ175	WT	zQ175
^{11}C-raclopride	6M	12.6 ± 0.8	12.3 ± 1.7	636 ± 297	561 ± 196	0.026 ± 0.072	0.022 ± 0.054	30.2 ± 1.2	28.1 ± 1.9
	9M	12.5 ± 0.7	12.6 ± 0.9	876 ± 308	652 ± 119	0.0054 ± 0.0016	0.0069 ± 0.0014	30.7 ± 1.2	27.0 ± 1.7
^{18}F-MNI-659	6M	12.0 ± 2.5	12.5 ± 1.4	132 ± 64	116 ± 67	0.069 ± 0.054	0.076 ± 0.038	29.6 ± 1.7	28.5 ± 1.5
	9M	11.8 ± 1.1	12.3 ± 0.9	128 ± 42	118 ± 49	0.054 ± 0.016	0.066 ± 0.030	30.1 ± 0.9	26.5 ± 1.6
^{11}C-NNC 112	6M	12.6 ± 0.8	12.9 ± 1.0	664 ± 171	786 ± 534	0.0066 ± 0.0019	0.0083 ± 0.0073	29.1 ± 2.2	29.1 ± 1.6
	9M	11.9 ± 1.8	12.8 ± 0.8	289 ± 190	324 ± 175	0.037 ± 0.054	0.029 ± 0.035	30.3 ± 1.7	27.6 ± 1.0
^{11}C-MDL 100907	6M	12.3 ± 0.9	12.7 ± 0.7	1079 ± 708	1188 ± 582	0.0059 ± 0.0034	0.0049 ± 0.0021	29.3 ± 2.0	29.1 ± 1.4
	9M	12.7 ± 0.5	12.2 ± 1.0	1137 ± 744	1269 ± 803	0.0067 ± 0.0051	0.0047 ± 0.0021	29.8 ± 2.1	27.3 ± 1.0

Supplemental Table 2. Binding potential values for each radioligand. Only animals that had both PET measurements (at 6 and 9M of age) are included in the analysis. Values are expressed as Mean ± S.D.

Radioligand	Region	WT		zQ175		Repeated measurement ANOVA		
		6M	9M	6M	9M	Strain	Age	Interaction
^{11}C-raclopride	Striatum	2.63 ± 0.27	2.32 ± 0.19	1.58 ± 0.20	1.31 ± 0.12	$p < 0.0001$	$p < 0.0001$	$p > 0.05$
^{18}F-MNI-659	Striatum	1.44 ± 0.46	1.38 ± 0.50	0.69 ± 0.32	0.81 ± 0.27	$p < 0.0001$	$p > 0.05$	$p > 0.05$
^{11}C-NNC 112	Striatum	17.3 ± 4.57	16.2 ± 4.15	12.5 ± 2.34	10.6 ± 3.16	$p < 0.0001$	$p > 0.05$	$p > 0.05$
	Rostral Cortex	2.52 ± 0.46	2.07 ± 0.35	1.96 ± 0.30	1.60 ± 0.25	$p < 0.0001$	$p < 0.0001$	$p > 0.05$
	Caudal Cortex	0.78 ± 0.14	0.67 ± 0.10	0.74 ± 0.15	0.58 ± 0.10	$p > 0.05$	$p < 0.0001$	$p > 0.05$
	Hippocampus	1.47 ± 0.27	1.36 ± 0.22	1.40 ± 0.15	1.12 ± 0.25	$p < 0.05$	$p < 0.0001$	$p > 0.05$
^{11}C-MDL 100907	Striatum	2.12 ± 0.30	1.88 ± 0.27	1.88 ± 0.23	1.51 ± 0.20	$p < 0.01$	$p < 0.0001$	$p > 0.05$
	Rostral Cortex	1.85 ± 0.36	1.52 ± 0.24	1.70 ± 0.25	1.34 ± 0.22	$p > 0.05$	$p < 0.0001$	$p > 0.05$
	Caudal Cortex	0.77 ± 0.13	0.66 ± 0.12	0.70 ± 0.09	0.54 ± 0.08	$p < 0.05$	$p < 0.0001$	$p > 0.05$
	Hippocampus	0.84 ± 0.18	0.67 ± 0.09	0.71 ± 0.09	0.54 ± 0.08	$p < 0.01$	$p < 0.0001$	$p > 0.05$

Supplemental Table 3. Nr of WT and zQ175 animals imaged at 6 and 9 months with each of the radioligands. Both the total number of animals imaged and total number of animals in the final analysis is shown in the table. Only animals that were imaged at the two time points were included in the final analysis. The average ± S.D CAG repeats for the zQ175 animals are displayed for the animals included in the final analysis.

Radioligand	Age	Genotype/CAG repeat	Nr of animals imaged	Nr of animals in final analysis
^{11}C-raclopride	6M	WT	14	14
	9M	WT	14	14
	6M	zQ175/204 ± 5	15	14
	9M	zQ175/204 ± 5	14	14
^{18}F-MNI-659	6M	WT	14	14
	9M	WT	14	14
	6M	zQ175/204 ± 5	15	14
	9M	zQ175/204 ± 5	14	14
^{11}C-NNC 112	6M	WT	19	15
	9M	WT	15	15
	6M	zQ175/201 ± 5	15	13
	9M	zQ175/201 ± 5	13	13
^{11}C-MDL 100907	6M	WT	17	14
	9M	WT	14	14
	6M	zQ175/201 ± 5	15	12
	9M	zQ175/201 ± 5	12	12