



Supplemental Figure 1. MR and GR mRNA expression in the hippocampus is unaltered in aged 24-27 months C57BL6J mice (n=14) compared to 6 months controls (n=10). Hybridization signal within hippocampal subregions was assessed by computer-assisted grain counting using the KS300 image analysis system (Imaging Associates, UK). Silver grains were counted in a fixed circular area under brightfield illumination over individual hippocampal cells (except in the dentate gyrus where cells are closely packed and more than one cell is counted within the fixed area). The threshold, sensitivity and pixel size was adjusted to allow accurate and optimal detection of the number of silver grains to avoid under or overestimation. For each animal 9-12 cells/subregion/section were assessed and background, counted over areas of white matter, was subtracted. Representative autoradiograph images of MR and GR expression in brain sections at the level of the anterior hippocampus from a young control C57BL/6J mouse and of photo-microscopic images (x400) showing mRNA expression (silver grains) over CA3 pyramidal cells.

Supplementary material: Tables

Table S1. Unaltered affective behaviour in CamIHK-HSD1 transgenic mice

Young (a) and old (b) CamIHK-HSD1 (Tg) mice and their wildtype (wt) littermate controls were behaviourally assessed in the elevated-plus maze and open field tests.

Male Tg mice and wildtype mice, young (6-9m) and aged (18m) were accustomed to the behavioural room to minimise stress. Mice were tested for affective behaviours in the open field and elevated plus maze (Holmes et al., 2006). Tests were captured by a computer tracking program (Limelight, Actimetrics, Wilmette, IL) to allow full analysis. For the open field test, mice were placed in a box (60x60 cm) marked off in 25 squares. The outer row of squares adjacent to the walls of the field are considered less anxiogenic than the inner squares. For a 5 minute period the number of crossings, time and distance (movement of all four legs into a new square) into each square was noted, together with other ethological parameters such as time spent grooming, number of rearings and fecal boli. Total movement in the maze reflects general activity and the movement in inner zone is correlated to anxiety state of the mouse. For the elevated-plus maze, mice were placed on the central region of the elevated plus maze (two intercrossing arms of white plastic approx 90cm long and 5 cm wide, two opposite arms are enclosed and two are open and the maze is elevated approx 1 metre from the floor), and movement around the maze for 5 minutes was assessed. The open arms are considered a more anxiogenic environment and so crossing, movement and time spent on these arms compared to total movement is a reflection of anxiety state.

Parameters reflecting anxiety, general activity and ethological behaviours were unchanged between genotypes. N=8 per group.

Table S1. Unaltered affective behaviour in CamIIK-HSD1 transgenic mice

(a) young mice

Measure	Wt	Tg	
Elevated plus maze			
Closed arm time (s)	193.7±21.4	210.5±9.4	
Open arm time(s)	51.9±15.1	37.5±9.1	
Grooming episodes	2.25±0.3	2.0±0.4	
Stretch attend.	10.7±2.1	12.4±2.0	.
Immobile (s)	10.4±4.1	18.2±7.4	
Open field			
Inner time (s)	34.9±6.9	39.9±9.9	
Centre time (s)	0.964±0.5	1.6±0.3	
Total distance (cm)	476±40	381±58	.
Rearing no.	12.4±3.7	10.1±2.9	.
Immobile (s)	35.1±6.8	61.0±24.2	.

(b) old mice

Measure	Wt	Tg	
Elevated plus maze			
Closed arm time (s)	182.3±22.2	202.0±40.0	
Open arm time(s)	76.1±25.7	59.8±30.2	
Grooming episodes	1.71±0.42	1.17±0.6	
Stretch attend.	10.0±1.74	8.5±3.24	.
Immobile (s)	18.9±8.6	98.3±47.2	
Open field			
Inner time (s)	39.8±19.9	63.1±22.8	
Centre time (s)	4.4±2.5	3.8±1.22	
Total distance (cm)	497±102.6	596±95	.
Rearing no.	1.8±0.8	3.0±0.82	.
Immobile (s)	93.6±30.4	79.7±0.76	.

Table S2. Plasma corticosterone and its response to stress are unaltered in CamIHK-HSD1 transgenic mice

Plasma corticosterone levels in CamIHK-HSD1 (Tg) mice and wildtype (wt) littermate controls at diurnal nadir (basal am), diurnal peak (basal pm) and 10 or 90 min following restraint stress. N=8 per group. There were significant effects of time of day ($F=35.6$, $p<0.0001$) and of stress ($F=24.3$, $p<0.0001$) but no effect of genotype ($F=2.0$, ns) on basal or stress responses ($F=0.2$, ns) or interaction between genotype and time of day ($F=0.5$, ns).

Condition	wt (nmol/l)	Tg (nmol/l)
Basal AM	42.8±7.9	48.5±13
Basal PM	159.4±15.8	195.6±30.4
Stress 10 min	348.7±52.0	270.6±40.8
Stress 90 min	276.9±54.1	294.3±57.6