Table S1: qPCR 1 primer sequences, design and efficiencies. WTSP = White throated sparrow (*Zonotrichia albicollis*); ZF = Zebra finch (*Taeniopygia guttata*).

Gene	Primer	Sequence	Reference	Genome	Product length	Efficiency
FSHR	FWD REV	ACGCCACCGAGCTGAGATTTGT TCAACAAGTCCTGTGAAAGCTCCCT	Designed by CMBB	WTSP	73	1.996
RPL4	FWD REV	GTCTAAAGGCCACCGCATTGA CGCTGGGAGGCGTAAACCTT	Designed by CMBB	WTSP	150	1.996
PPIA	FWD REV	TCCCGAAGACAGCAGAAAACT CCATTGTGGCGTGTGAAGTC	Designed by CMBB	WTSP	130	1.954
GR	FWD REV	TGAAGAGCCAGTCCCTGTTCGAG CAACCACATCATGCATAGAGTCCAGCA	Rosvall et al. 2016b	see ref	see ref	1.985
MR	FWD REV	AAGAGTCGGCCAAACATCCTTGTTCT AAGAAACGGGTGGTCCTAAAATCCCAG	Rosvall et al. 2016b	see ref	see ref	1.998
GnIHR	FWD REV	TGGCCCTTTGACAACATCACGTGCAA ACAGCAATGGCGACCAGCGTGAAA	Rosvall et al. 2016b	see ref	see ref	2.034
LHR	FWD REV	TCTCAGAGCGACTCCCTG, TCCGTCCTCAATGTGCAAC	Burgeon-Burns et al. 2014	see ref	see ref	1.994
StAR	FWD REV	TGGGCCAGCACATGCTAGTTAAGCAA AGCAGGGCTTCCTTCAGGAACCAAGTAT	Rosvall et al. 2016a	see ref	see ref	1.983
P450	FWD REV	GACCGCGAGAAGATGCTGAAA TCTCCTTGATGGTGGCCTTGAG	Rosvall et al. 2016a	see ref	see ref	2.045
CYP17	FWD REV	CATCAACCTCTGGTCTGTGCAC AAGCGGCCAGGATTGAACT	Rosvall et al. 2016a	see ref	see ref	1.93
3βHSD	FWD REV	ATGAGCTACGCTGACCTGAA CAGCAGCAGCGAGAAGTAATAG	Rosvall et al. 2016a	see ref	see ref	2.016

Table S2: Change in baseline testosterone over the course of experiment. SE = standard error. Interaction (in italic font) was not significant and was taken out of the final model.

Linear mixed model

Fixed variable	Estimate	SE	t	p
Sampling round (after vs before)	0.401	0.077	5.219	**<0.001**
Long-term treatment (disturbance)	-0.373	0.183	-2.038	**0.049**
Sampling round x Long-term treatment (disturbance)	*-0.195*	*0.150*	*-1.301*	*0.210*

Sample size	
Group	**n**
Before long-term treatment	24
After long-term treatment	33

Table S3: Effect of disturbance treatment on change in testosterone in response to handling. SE = standard error.

Linear mixed model

Fixed variable	Estimate	SE	t	p
Acute stressor (handling)	-0.702	0.207	-3.395	**0.003**
Long-term treatment (disturbance)	-0.714	0.251	-2.846	**0.008**
Acute stressor (handling) x long-term treatment (disturbance)	0.669	0.296	2.265	**0.034**

Sample size

Group	n
Long-term control, unhandled baseline	16
Long-term control, acute handling stress	14
Long-term disturbance, unhandled baseline	17
Long-term disturbance, acute handling stress	12

Table S4: Effect of disturbance treatment on testosterone increase in response to GnRH injection. SE = standard error. Interaction (in italic font) was not significant and was taken out of the final model.

Linear mixed model

Fixed variable	Estimate	SE	t	p
GnRH injection	1.941	0.214	9.077	**<0.001**
Long-term treatment (disturbance)	0.016	0.266	0.059	0.953
GnRH injection x Long-term treament (disturbance)	*0.016*	*0.435*	*0.037*	*0.971*

Sample size

Group	n
Long-term control, pre-injection	14
Long-term control, post-injection	16
Long-term disturbance, pre-injection	12
Long-term disturbance, post-injection	18

Table S5: Relationship between baseline testosterone and testes mass. SE = standard error. Interactions that were not significant (in italic font) were taken out of the final model.

Linear model

Variable	Estimate	SE	t	p
	Baseline testosterone			
Testes mass	7.120	7.500	0.949	0.352
Long-term treatment (disturbance)	-0.513	0.255	-2.010	0.056
Time of sampling	0.004	0.002	2.692	**0.013**
Testes mass x Long-term treatment (disturbance)	*22.128*	*14.623*	*1.513*	*0.144*
	Post-handling testosterone			
Testes mass	12.393	4.367	2.838	**0.010**
Long-term treatment (disturbance)	0.051	0.153	0.333	0.742
Testes mass x Long-term treatment (disturbance)	*1.311*	*8.935*	*0.147*	*0.885*
	GnRH-induced testosterone			
Testes mass	34.627	11.072	3.127	**0.004**
Long-term treatment (disturbance)	-0.189	0.364	-0.518	0.608
Testes mass x Long-term treatment (disturbance)	*23.579*	*22.439*	*1.051*	*0.302*

Sample size

Group	n
Long-term control	13
Long-term disturbance	14
Long-term control	13
Long-term disturbance	12
Long-term control	12
Long-term disturbance	14

Table S6. Significantly differentially expressed genes in response to chronic disturbance treatment (excel file available separately).

Click here to Download Table S6

Table S7. Significantly differentially expressed genes in response to acute handling treatment (excel file available separately).

Click here to Download Table S7

Table S8. Correlation coefficients between genes involved in steroidogenesis. Coefficients are below the diagonal, p-values are above the diagonal.

Gene	*LHR*	*StAR*	*P450*	*CYP17*	*3βHSD*
LHR		0.004	0.251	0.024	0.438
StAR	0.48		0.002	0.017	0.121
P450	0.20	0.50		0.001	0.001
CYP17	0.38	0.40	0.54		0.301
3βHSD	0.14	0.27	0.56	0.18	

Table S9. Variance and loadings of principal components for genes involved in testosterone synthesis

	PC1	PC2	PC3	PC4	PC5
Proportion of variance explained	0.498	0.209	0.138	0.103	0.052
Standard deviation	1.578	1.021	0.831	0.719	0.508
Genes			*Loadings*		
LHR	-0.377	0.601	-0.437	0.474	-0.285
StAR	-0.486	0.234	-0.260	-0.741	0.304
P450	-0.520	-0.370	0.241	-0.154	-0.715
CYP17	-0.461	0.195	0.707	0.285	0.411
3βHSD	-0.372	-0.640	0.429	0.349	0.382

Table S10. Variance and loadings of principal components for genes involved in testosterone synthesis from animals in the control treatment only.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**
Proportion of variance explained	0.411	0.213	0.195	0.124	0.057
Standard deviation	1.434	1.033	0.987	0.787	0.532
Genes			*Loadings*		
LHR	-0.486	0.612			0.619
StAR	-0.536	0.240	0.422	-0.342	-0.600
P450	-0.394	-0.657		-0.541	0.337
CYP17	-0.351		-0.841	0.166	-0.376
3βHSD	-0.445	-0.370	0.317	0.750	

Table S11. Effect of acute handling treatment on steroidogenesis gene expression in birds from the long-term control treatment (acute handling treatment n=8; unhandled controls n=9). Positive coefficient indicates higher gene expression in unhandled birds. SE = standard error

Gene	n	Coefficient	SE	t	p value	adjusted p value
LHR	17	0.202	0.142	1.424	0.175	0.438
StAR	17	0.132	0.170	0.777	0.449	0.562
P450	17	0.117	0.200	0.583	0.568	0.568
CYP17	17	0.163	0.077	2.102	0.053	0.265
3βHSD	17	0.133	0.131	1.015	0.326	0.544

Table S12. Effect of chronic and acute handling treatment of *GR*, *MR*, and *GnIHR* (sample sizes: chronic disturbance & acute handling n=9; chronic disturbance & unhandled control n=9; chronic control & acute handling n=9; chronic control & unhandled control n=8). SE = standard error. Interactions that were not significant (in italic font) were taken out of the final model.

Fixed effect	coefficient	SE	t	p value	adjusted p value
			GR		
Chronic treatment (disturbance)	-0.057	0.060	-0.956	0.346	0.5192
Acute treatment (unhandled)	0.137	0.060	2.282	0.029	0.0879
Chronic x acute treatment	*0.006*	*0.122*	*0.050*	*0.960*	*na*
			MR		
Chronic treatment (disturbance)	-0.067	0.066	-1.022	0.315	0.5192
Acute treatment (unhandled)	0.037	0.066	0.556	0.582	0.5822
Chronic x acute treatment	*-0.127*	*0.132*	*-0.963*	*0.343*	*na*
			GnIHR		
Chronic treatment (disturbance)	0.058	0.140	0.413	0.682	0.6822
Acute treatment (unhandled)	-0.262	0.140	-1.875	0.070	0.1049
Chronic x acute treatment	*0.418*	*0.274*	*1.524*	*0.138*	*na*

Figure S1: Effect of chronic disturbance and acute handling stressor on the expression of genes involved in steroidogenesis; A) *LHR*, B) *StAR*, C) *P450*, D) *CYP17*, E) *3βHSD*. Shown are group means (open circles) +/- standard error; points represent individual measurements.

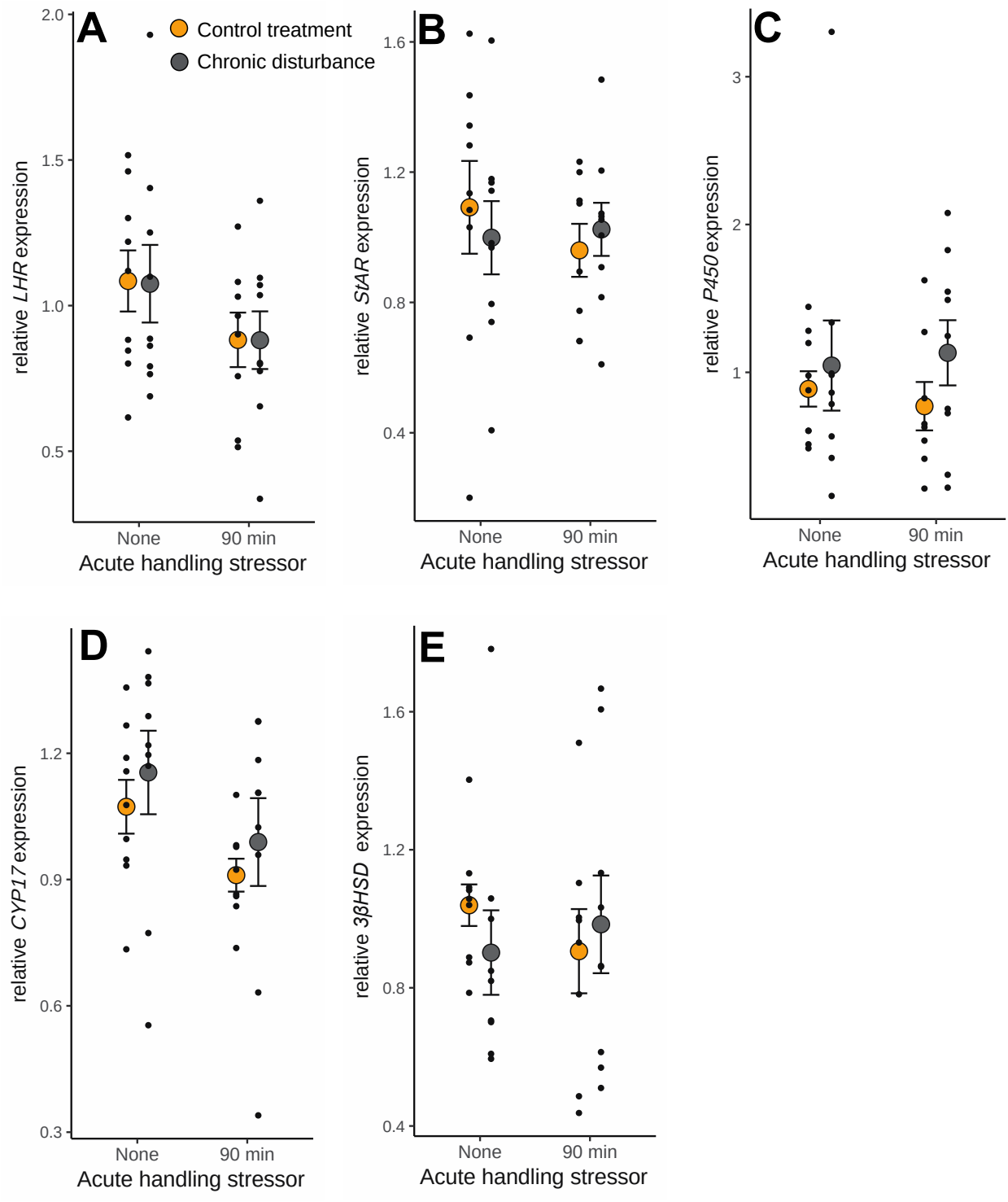
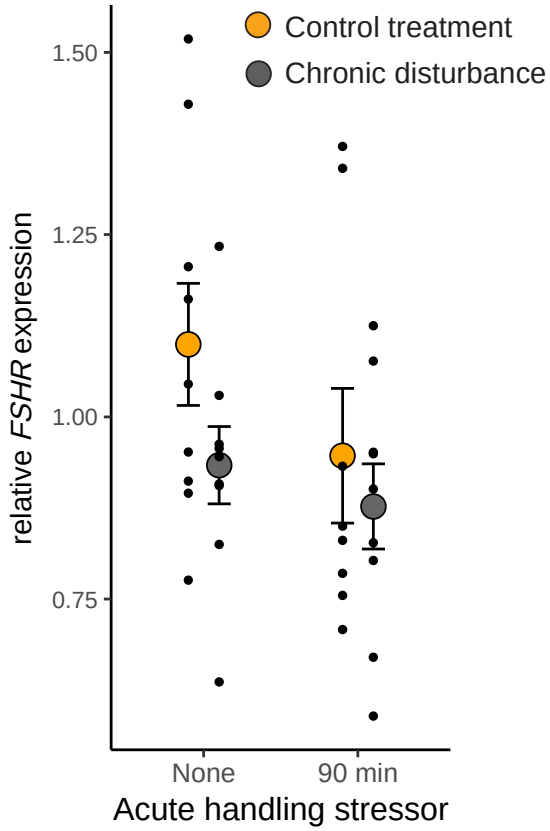


Figure S2: Effect of chronic disturbance and acute handling stressor on the expression of *FSHR*. Shown are group means (open circles) +/- standard error; points represent individual measurements.



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