Supporting Information

Garratt et al. 10.1073/pnas.1322282111

SI Materials and Methods

Experimental Procedures. After a 2-wk habituation period in experimental cages (53 × 35 × 18 cm), during which males were singly housed, males of each genotype (*Sod1*$^{+/+}$, *Sod1*$^{+/-}$, *Sod1*$^{-/-}$) were randomly allocated to two experimental groups that had their social environment manipulated in one of two ways over a 3-wk period: one group of males were exposed to the presence and odor of male and female con-specifics to induce investment in territory defense (termed "territorial"; *Sod1*$^{-/-}$ $n = 6$; *Sod1*$^{+/-}$ $n = 8$; *Sod1*$^{+/+}$ $n = 8$), but the other were not (controls; *Sod1*$^{-/-}$ $n = 7$; *Sod1*$^{+/-}$ $n = 10$; *Sod1*$^{+/+}$ $n = 8$). These manipulations were based on a previously published approach (1). Territorial males were exposed four times a week for a 2-h period to either the presence of a male or female (sex was alternated at each exposure); animals were presented behind a mesh barrier that inhibited direct contact. All disturbances associated with these manipulations (e.g., by the experimenter opening cages to add animals) were mimicked in control animals, but keeping the space behind the mesh barrier partition empty. Twice weekly territorial males also received a small handful of female substrate and nesting material in their cage. Control males had an equal amount of their own substrate and nesting material added to their cage. Finally, each male's cage contained four small painted metal tiles, which preliminary experiments revealed males would readily scent-mark. Three times per week territorial males had their tiles swapped with a separate set of tiles that had been kept in the cage of a CBA male, thus simulating the presence of intruder scent-marks within their territory. The tiles of control males were rotated randomly within their own cage to control for the disturbance.

Mitochondrial Bioenergetics. Preparation of permeabilized livers were conducted at 4 °C as described elsewhere (2). After permeabilization, the samples were blotted and weighed using a Sartorius CP2P Electronic Micro Precision Balance (Sartorius). All measurements were performed at 37 °C using an Oxygraph-2K respirometer (Oroboros Instruments). The different respiration rates measured are presented with the abbreviations of the complexes involved followed by the state of respiration (complex-STATE) and a typical graph of oxygen consumption (in pmol·s^{-1}·mg^{-1} of permeabilized liver) is presented in Fig. 4*A*. Malate (2 mM) and Palmitoyl Carnitine (50 μM) were injected into the oxygraph chambers and the samples were transferred shortly thereafter. This process allowed the measurement of the Leak state during β-oxidation (ETF+CI-Leak) by providing electrons to the electron transport flavoprotein (ETF) and to complex I (CI). This state is a resting state of nonphoshorylating respiration when oxygen flux is maintained mainly to compensate for the proton leak. Injection of excess ADP (5 mM) allowed it to reach a state of OXPHOS for ETF (ETF+CI-OXPHOS). This reaction enables measurement of the maximum oxygen flux when the transport of electrons from ETF is coupled to the production of ATP. Subsequent injection of cytochrome *c* from equine heart (15 μM) allowed evaluation of the functional integrity of the outer mitochondrial membrane (ETF+CIc-OXPHOS): if less than a 5% increase in oxygen consumption was observed, the sample had an intact outer mitochondrial membrane and was therefore used for the rest of the experiment. Additional O_2 fluxes were then determined by sequentially injecting different compounds: glutamate and pyruvate (10 mM each) to monitor the maximum contribution of complex I during the OXPHOS state (CIc+ETF-OXPHOS); succinate (10 mM) to monitor maximum OXPHOS state (CIc+CII+ETF-OXPHOS) with convergent electron flow from complex I, complex II and ETF; FCCP (optimum concentration reached between 1.00 and 1.25 μM) to stimulate uncoupled respiration for complex I, complex II, and ETF as a measure of electron transport system (ETS) capacity (CIc+CII+ETF-ETS); rotenone (1 μM), which is an inhibitor of complex I, allowed us to measure the oxygen flux of electron input through complex II in the uncoupled state (CII-ETS); and antimycin A (2.5 μM), inhibitor of complex III to measure residual oxygen consumption. The inhibition of complex III by antimycin A allowed us to measure the residual oxygen consumption because of residual oxidative side reactions occurring in permeabilized livers and to correct all of the O_2 fluxes. Finally, complex IV (CIV) activity was measured after inhibition of complexes I and III, by injecting *N*,*N*,*N*′,*N*′-tetramethyl-p-phenylenediamine (TMPD) and ascorbate (0.5 μM and 2 mM respectively) into the chambers. Because of autoxidation of TMPD, ascorbate, and cytochrome *c*, chemical backgrounds were evaluated at the end of each experiment after inhibition of CIV and were subtracted from the activity. All measurements were expressed as means of respiration rates expressed in picomole of oxygen consumed per second per milligram of permeabilized livers ± SEM or in picomole of oxygen consumed per unit of citrate synthase. Citrate synthase activity was determined by following the increase in absorbance because of the reduction of DTNB at 412 nm.

1. Garratt M, et al. (2012) Tissue-dependent changes in oxidative damage with male reproductive effort in house mice. *Funct Ecol* 26(2):423–433.

2. Pichaud N, Garratt M, Ballard JWO, Brooks RC (2013) Physiological adaptations to reproduction. II. Mitochondrial adjustments in livers of lactating mice. *J Exp Biol* 216(Pt 15): 2889–2895.

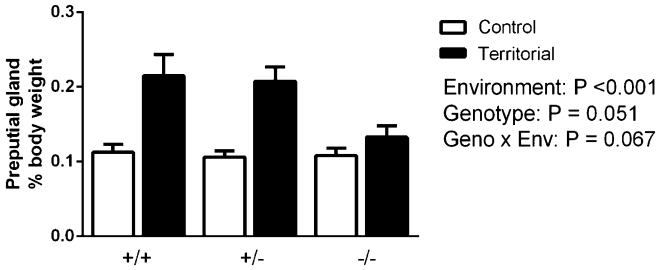

Fig. S1. Differences between genotypes in preputial gland mass when expressed as a percentage of body mass. The genotype by environment interaction is a consequence of a difference between genotypes in preputial gland mass in the territorial environment ($F_{2,20} = 4.05$, $P = 0.033$), but not in the control environment ($F_{2,20} = 0.20$, $P = 0.82$).

Table S1. Multivariate analysis of variance (MANOVA) test exploring the effects of SOD1 deficiency, investment in sexual signaling, and the interaction between these treatments on measures of oxidative stress in the liver and gastrocnemius muscle

	Multivariate tests		
	Genotype	Environment	Geno × Env
Wilks' λ	0.21	0.53	0.42
F	3.85	2.93	1.80
df	14, 46	7, 23	14, 46
P	<0.001	0.024	0.068

With data from individuals where we had managed to assess all seven markers of oxidative stress ($n = 41$), we first conducted a multivariate test to examine whether there was an overall effect of genotype and environment on oxidative stress. In SPSS, v21, each of the seven markers of oxidative stress [liver protein thiols, liver malonaldhye (MDA), liver aconitase, liver glutathione oxidation (GSSH), liver glutathione concentration (GSH), gastrocnemius muscle aconitase, gastrocnemius muscle MDA] were included as dependent variables in a MANOVA; genotype (Geno), environment (Env), and batch were included as fixed effects in a full factorial model. Wilks' λ multivariate test results are reported, which gives an exact *F*-test statistic. To explore the influence of genotype and environment on each oxidative stress marker individually, we then used general linear mixed models, with genotype and environment added as fixed effects, and batch as a random effect. See also Table S2.

Table S2. Univariate tests exploring the effects of SOD1 deficiency, investment in sexual signaling, and the interaction between these treatments on measures of oxidative stress in the liver and gastrocnemius muscle

	Univariate tests		
Source and dependent variable	Df	*F*	*P*
Genotype			
GSH (liver)	2, 41	3.69	0.034
GSSH (liver)	2, 41	5.96	0.005
Aconitase (liver)	2, 40	14.72	<0.001
Protein thiols (liver)	2, 40	1.35	0.27
MDA (liver)	2, 36	1.19	0.32
Aconitase (gastrocnemius muscle)	2, 41	1.28	0.29
MDA (gastrocnemius muscle)	2, 37	2.81	0.073
Environment			
GSH (liver)	1, 41	2.29	0.14
GSSH (liver)	1, 41	0.57	0.45
Aconitase (liver)	1, 40	0.27	0.61
Protein thiols (liver)	1, 40	0.27	0.61
MDA (liver)	1, 37	2.44	0.13
Aconitase (gastrocnemius muscle)	1, 41	0.11	0.74
MDA (gastrocnemius muscle)	1, 38	4.11	0.050
Genotype × Environment			
GSH (liver)	2, 41	1.19	0.31
GSSH (liver)	2, 41	0.12	0.89
Aconitase (liver)	2, 40	2.04	0.14
Protein thiols (liver)	2, 40	2.95	0.064
MDA (liver)	2, 36	0.14	0.87
Aconitase (gastrocnemius muscle)	2, 41	0.91	0.41
MDA (gastrocnemius muscle)	2, 37	2.61	0.055

Table S3. Effect of genotype and environment on body mass, oxygen consumption (O_2 mL/h), and heat production (KCAL/h)

	Genotype			Environment			Genotype × Environment			Body mass		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Body mass	2, 40	5.48	0.008	1, 40	11.57	0.002	2, 40	2.89	0.067	—	—	—
Metabolic rate (including body mass)	2, 35	3.63	0.037	1, 35.1	1.21	0.28	2, 35.1	0.79	0.46	1, 35	0.09	0.77
Metabolic rate (body mass removed)	2, 39	3.47	0.041	1, 39.1	1.53	0.22	2, 39.1	0.9	0.42	—	—	—
Heat (including body mass)	2, 35	3.74	0.034	1, 35.2	1	0.32	2, 35.1	0.92	0.41	1, 35	0.04	0.95
Heat (body mass removed)	2, 36.1	4.45	0.019	1, 36.1	1.43	0.24	2, 36.1	1.19	0.32	—	—	—

Respiration parameters are presented both with body mass added as a covariate and without. Body mass was dropped from the final model in both cases as it did not predict either variable.

Table S4. Effect of genotype and environment on measures of bioenergetic respiratory capacity

	Genotype			Environment			Genotype × Environment		
Variable	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Respiration per gram of permeabilized tissue									
ETF+CI LEAK	**40.10**	**10.62**	**<0.001**	40.08	1.84	0.180	40.07	0.51	0.610
ETF+CI OXPHOS	**40.01**	**10.85**	**<0.001**	40.06	1.36	0.250	40.05	0.54	0.590
CIc+ETF OXPHOS	**40.01**	**2.95**	**0.064**	**40.02**	**5.59**	**0.023**	40.02	0.37	0.690
CIc+CII+ETF OXPHOS	**40.01**	**4.38**	**0.019**	**40.03**	**7.50**	**0.009**	40.03	0.56	0.570
CIc+CII+ETF ETS	**40.01**	**2.77**	**0.075**	**40.04**	**5.21**	**0.028**	40.04	0.86	0.430
CII-ETS	40.03	0.40	0.670	40.21	2.26	0.140	**40.19**	**2.76**	**0.075**
CIV	41.00	0.98	0.390	41.00	0.52	0.480	41.00	0.45	0.640
Citrate synthase activity	**40.05**	**8.25**	**0.001**	40.32	0.01	0.96	40.29	0.69	0.51
Respiration/citrate synthase activity									
ETF+CI LEAK	40.01	0.98	0.38	40.09	0.05	0.83	40.08	0.26	0.78
ETF+CI OXPHOS	40.01	1.02	0.37	40.07	0.01	0.93	40.06	0.19	0.83
CIc+ETF OXPHOS	40.01	0.26	0.77	40.02	2.16	0.15	40.02	0.5	0.61
CIc+CII+ETF OXPHOS	40.01	0.97	0.39	40.05	1.87	0.18	40.04	0.62	0.54
CIc+CII+ETF ETS	40.01	1.05	0.36	40.05	1.12	0.3	40.04	0.65	0.53
CII-ETS	**40.02**	**3.23**	**0.050**	40.16	1.26	0.27	**40.14**	**2.89**	**0.067**
CIV	40.04	1.19	0.31	40.26	<0.01	0.99	40.23	0.1	0.91
Ratio of complexes									
ETF+CI OXPHOS/CII-ETS	**40.03**	**10.19**	**<0.001**	40.18	0.03	0.87	**40.17**	**3.18**	**0.052**
CIc+ETF OXPHOS/CII-ETS	**40.01**	**3.63**	**0.036**	**40.03**	**2.9**	**0.098**	40.03	1.13	0.33
CIc+ETF OXPHOS/CIc+CII+ETF OXPHOS	40.01	0.98	0.39	40.04	0.75	0.39	40.04	0.03	0.97
CII-ETS/CIc+CII+ETF OXPHOS	**40.02**	**3.36**	**0.045**	40.14	0.17	0.68	40.13	0.95	0.37

Test statistics are derived from general linear mixed models on individual bioenergetic parameters. Boldface entries denote results with a value of $P < 0.1$.

Other Supporting Information Files

Dataset S1 (DOC)