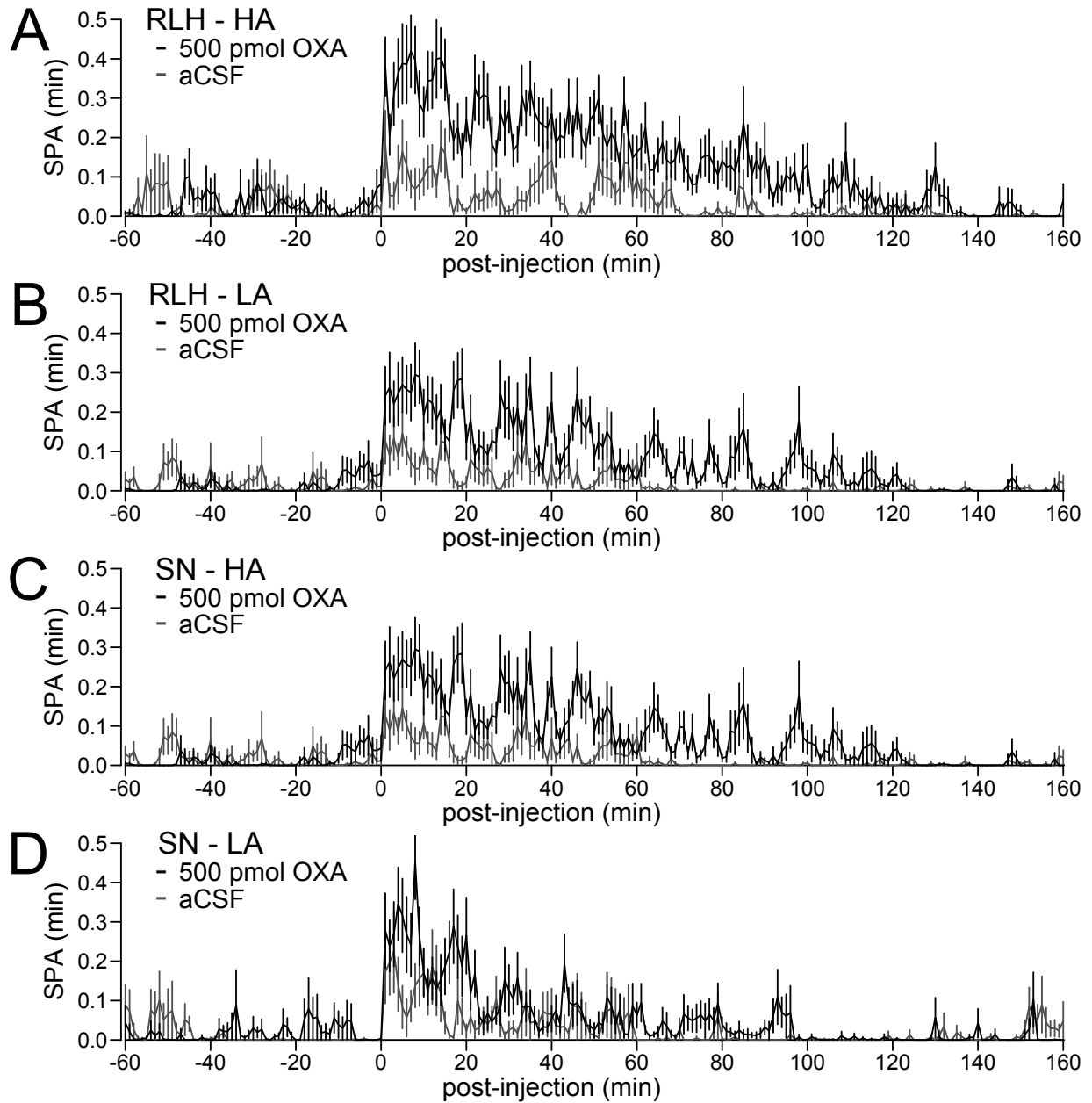


Supplementary Figure 1. Adaptation to the spontaneous physical activity (SPA) chamber for 24 h recording of intrinsic SPA (SPA_{INT}) in Sprague-Dawley rats. SPA_{INT} for three consecutive 24 h periods was measured in Sprague-Dawley rats (n = 8). A repeated measures ANOVA shows there is a significant effect of day ($F_{2,14} = 19.32$, $P = 0.001$), but pairwise analyses show higher SPA_{INT} only during the first 24 h of recording with no differences between 24h SPA_{INT} recorded on the second and third day (SPA_{INT} day 1 vs. day 2, $P = 0.0039$; SPA_{INT} day 1 vs. day 3, $P = 0.0039$, SPA_{INT} day 2 vs. day 3, $P = 0.526$). These data show that, under our experimental conditions, 24 h adaptation is sufficient for SPA_{INT} behavioral measures.



Supplementary Figure 2. Time-course of ambulatory spontaneous physical activity (SPA) before and after orexin-A (OXA) injections. Time course of ambulatory SPA before and after injection of either aCSF (vehicle) or 500 pmol OXA in either (A, B) rostral lateral hypothalamus (RLH) or (C, D) substantial nigra pars compacta (SN) from high activity (HA) and low activity (LA) rats. Note return of activity to baseline 2 h after orexin-A injection. Data is shown in summary form in Figure 2 from main text. Y-axis, mean \pm sem.

Supplementary Table 1A. Effect of selection criteria on body weight comparisons between high activity (HA) and low activity (LA) rats

SPA \geq (min / 24 h)	HA	SPA \leq (min / 24 h)	LA	t	df	p-value
	Body Weight (g, mean \pm SEM)		Body Weight (g, mean \pm SEM)			
100	289.052 \pm 3.64	60	282.5 \pm 9.176	0.664	12.027	0.519
		70	285.786 \pm 7.418	0.395	19.858	0.697
		80	285.56 \pm 5.968	0.5	43.655	0.62
		90	282.256 \pm 5.35	1.05	74.904	0.297
		100	283.068 \pm 4.73	1.003	121.118	0.318
110	287.775 \pm 4.758	60	282.5 \pm 9.176	0.51	14.356	0.618
		70	285.786 \pm 7.418	0.226	25.107	0.823
		80	285.56 \pm 5.968	0.29	56.391	0.773
		90	282.256 \pm 5.35	0.771	90.976	0.443
		100	283.068 \pm 4.73	0.702	127.011	0.484
120	291.804 \pm 5.201	60	282.5 \pm 9.176	0.882	15.45	0.391
		70	285.786 \pm 7.418	0.664	27.36	0.512
		80	285.56 \pm 5.968	0.789	59.356	0.433
		90	282.256 \pm 5.35	1.28	88.899	0.204
		100	283.068 \pm 4.73	1.243	111.364	0.217
130	291.095 \pm 6.079	60	282.5 \pm 9.176	0.781	17.878	0.445
		70	285.786 \pm 7.418	0.554	31.783	0.584
		80	285.56 \pm 5.968	0.65	61.124	0.518
		90	282.256 \pm 5.35	1.091	78.377	0.278
		100	283.068 \pm 4.73	1.042	83.921	0.3
140	289.091 \pm 7.482	60	282.5 \pm 9.176	0.557	22.188	0.583
		70	285.786 \pm 7.418	0.314	37.248	0.755
		80	285.56 \pm 5.968	0.369	55.641	0.714
		90	282.256 \pm 5.35	0.743	59.902	0.46
		100	283.068 \pm 4.73	0.68	57.608	0.499
150	294 \pm 9.134	60	282.5 \pm 9.176	0.888	25.448	0.383
		70	285.786 \pm 7.418	0.698	34.899	0.49
		80	285.56 \pm 5.968	0.774	38.382	0.444
		90	282.256 \pm 5.35	1.109	37.156	0.274
		100	283.068 \pm 4.73	1.063	34.442	0.295
160	297.889 \pm 10.263	60	282.5 \pm 9.176	1.118	24.939	0.274
		70	285.786 \pm 7.418	0.956	29.038	0.347
		80	285.56 \pm 5.968	1.038	28.16	0.308
		90	282.256 \pm 5.35	1.351	26.617	0.188
		100	283.068 \pm 4.73	1.312	24.663	0.202
170	304.636 \pm 12.288	60	282.5 \pm 9.176	1.443	18.032	0.166
		70	285.786 \pm 7.418	1.313	16.89	0.207
		80	285.56 \pm 5.968	1.396	14.928	0.183
		90	282.256 \pm 5.35	1.67	14.018	0.117
		100	283.068 \pm 4.73	1.638	13.133	0.125

Supplementary Table 1B. Effect of selection criteria on fat mass comparisons between high activity (HA) and low activity (LA) rats.

HA		LA		t	df	p-value
SPA \geq (min / 24 h)	Fat Mass (g, mean \pm SEM)	SPA \leq (min / 24 h)	Fat Mass (g, mean \pm SEM)			
100	28.498 \pm 0.47	60	27.564 \pm 1.701	0.529	10.421	0.608
		70	28.892 \pm 1.509	-0.249	15.624	0.806
		80	28.824 \pm 1.081	-0.277	33.615	0.784
		90	28.49 \pm 0.799	0.008	65.682	0.993
		100	28.85 \pm 0.676	-0.428	111.479	0.67
110	28.553 \pm 0.574	60	27.564 \pm 1.701	0.551	11.147	0.593
		70	28.892 \pm 1.509	-0.21	16.968	0.836
		80	28.824 \pm 1.081	-0.222	38.38	0.826
		90	28.49 \pm 0.799	0.064	76.325	0.949
		100	28.85 \pm 0.676	-0.335	120.027	0.738
120	29.004 \pm 0.585	60	27.564 \pm 1.701	0.801	11.227	0.44
		70	28.892 \pm 1.509	0.069	17.107	0.945
		80	28.824 \pm 1.081	0.146	38.642	0.884
		90	28.49 \pm 0.799	0.519	74.799	0.605
		100	28.85 \pm 0.676	0.172	111.444	0.864
130	28.607 \pm 0.694	60	27.564 \pm 1.701	0.568	12.173	0.58
		70	28.892 \pm 1.509	-0.171	18.817	0.866
		80	28.824 \pm 1.081	-0.169	43.522	0.867
		90	28.49 \pm 0.799	0.111	76.584	0.912
		100	28.85 \pm 0.676	-0.251	95.176	0.803
140	28.006 \pm 0.78	60	27.564 \pm 1.701	0.236	13.021	0.817
		70	28.892 \pm 1.509	-0.521	20.288	0.608
		80	28.824 \pm 1.081	-0.613	46.107	0.543
		90	28.49 \pm 0.799	-0.433	69.73	0.666
		100	28.85 \pm 0.676	-0.817	74.868	0.416
150	28.444 \pm 1.027	60	27.564 \pm 1.701	0.443	15.89	0.664
		70	28.892 \pm 1.509	-0.245	24.698	0.808
		80	28.824 \pm 1.081	-0.255	45.997	0.8
		90	28.49 \pm 0.799	-0.035	46.77	0.972
		100	28.85 \pm 0.676	-0.33	42.226	0.743
160	29.022 \pm 1.082	60	27.564 \pm 1.701	0.723	16.339	0.48
		70	28.892 \pm 1.509	0.07	24.792	0.944
		80	28.824 \pm 1.081	0.129	39.799	0.898
		90	28.49 \pm 0.799	0.396	35.822	0.695
		100	28.85 \pm 0.676	0.135	31.48	0.894
170	29.226 \pm 1.39	60	27.564 \pm 1.701	0.757	17.864	0.459
		70	28.892 \pm 1.509	0.163	22.945	0.872
		80	28.824 \pm 1.081	0.228	22.358	0.822
		90	28.49 \pm 0.799	0.459	17.202	0.652
		100	28.85 \pm 0.676	0.243	15.151	0.811

Supplementary Table 1C. Effect of selection criteria on lean mass comparisons between high activity (HA) and low activity (LA) rats.

	HA		LA		t	df	p-value
	SPA \geq (min / 24 h)	Lean Mass (g, mean \pm SEM)	SPA \leq (min / 24 h)	Lean Mass (g, mean \pm SEM)			
100	232.645 \pm 2.859	60	228.691 \pm 6.558	0.553	12.701	0.59	
		70	231.01 \pm 5.294	0.272	21.435	0.788	
		80	230.096 \pm 4.121	0.508	49.717	0.614	
		90	228.232 \pm 3.866	0.918	81.114	0.361	
		100	228.165 \pm 3.5	0.991	126.46	0.323	
110	231.847 \pm 3.692	60	228.691 \pm 6.558	0.419	15.41	0.681	
		70	231.01 \pm 5.294	0.13	27.515	0.898	
		80	230.096 \pm 4.121	0.316	63.884	0.753	
		90	228.232 \pm 3.866	0.676	95.712	0.501	
		100	228.165 \pm 3.5	0.724	127.789	0.471	
120	235.23 \pm 4.042	60	228.691 \pm 6.558	0.849	16.741	0.408	
		70	231.01 \pm 5.294	0.634	30.156	0.531	
		80	230.096 \pm 4.121	0.889	65.817	0.377	
		90	228.232 \pm 3.866	1.251	91.198	0.214	
		100	228.165 \pm 3.5	1.321	109.841	0.189	
130	234.927 \pm 4.701	60	228.691 \pm 6.558	0.773	19.497	0.449	
		70	231.01 \pm 5.294	0.553	34.742	0.584	
		80	230.096 \pm 4.121	0.773	63.833	0.442	
		90	228.232 \pm 3.866	1.1	77.138	0.275	
		100	228.165 \pm 3.5	1.154	81.375	0.252	
140	233.208 \pm 5.79	60	228.691 \pm 6.558	0.516	24.34	0.61	
		70	231.01 \pm 5.294	0.28	39.656	0.781	
		80	230.096 \pm 4.121	0.438	54.114	0.663	
		90	228.232 \pm 3.866	0.715	57.298	0.478	
		100	228.165 \pm 3.5	0.745	55.566	0.459	
150	237.609 \pm 6.97	60	228.691 \pm 6.558	0.932	26.818	0.36	
		70	231.01 \pm 5.294	0.754	34.995	0.456	
		80	230.096 \pm 4.121	0.928	36.031	0.36	
		90	228.232 \pm 3.866	1.176	35.662	0.247	
		100	228.165 \pm 3.5	1.211	33.682	0.234	
160	240.988 \pm 7.873	60	228.691 \pm 6.558	1.2	25.545	0.241	
		70	231.01 \pm 5.294	1.052	28.286	0.302	
		80	230.096 \pm 4.121	1.226	26.197	0.231	
		90	228.232 \pm 3.866	1.454	25.522	0.158	
		100	228.165 \pm 3.5	1.488	24.108	0.15	
170	243.818 \pm 9.938	60	228.691 \pm 6.558	1.27	17.02	0.221	
		70	231.01 \pm 5.294	1.137	15.518	0.273	
		80	230.096 \pm 4.121	1.275	13.567	0.224	
		90	228.232 \pm 3.866	1.462	13.176	0.167	
		100	228.165 \pm 3.5	1.486	12.601	0.162	

Supplementary Table 1. Differences in body weight (A), fat mass (B) or lean mass (C) between HA and LA rats selected using different values of ambulatory SPA_{INT}. HA and LA rats were defined using increments of 10 minutes over a range of ambulatory SPA_{INT} recorded for 24 h. For HA rats, the range was 100 to 170 min. For LA rats, the range was 60 to 100 min. Using these criteria, the minimum number of HA or LA rats in each comparison was 10. Pairwise comparisons were done using a Welch T-test and p-values are shown uncorrected for multiple comparisons. t, t-test statistic, df, degrees of freedom).

Parameter	Phenotype	IDC RECORDING SESSIONS			$F_{HA/LA}$	F_{TIME}	$F_{[HA/LA * TIME]}$
		1	2	3			
Body Weight (g)	HA	384.85 ± 8.196	388.21 ± 7.473	389.43 ± 8.04	0.057	0.081	0.989
	LA	385.5 ± 11.024	389.1 ± 12.418	391.5 ± 11.95			
Un-corrected EE (kcal / h)	HA	3.2 ± 0.057	3.17 ± 0.055	3.19 ± 0.055	0.795	0.992	0.996
	LA	3.15 ± 0.109	3.15 ± 0.122	3.16 ± 0.113			
Food Intake (g)	HA	29.17 ± 1.317	26.48 ± 1.033	26.94 ± 0.486	0.366	0.237	0.873
	LA	30.27 ± 1.964	29.28 ± 1.570	28.57 ± 1.281			

Supplementary Table 2. Body weight, uncorrected energy expenditure (EE) and food intake (g) in high activity (HA, N = 14) and low activity (LA, N = 10) rats selected based on 24 h ambulatory SPA_{INT} recorded in indirect calorimetry (IDC) cages. Values shown are mean ± SEM. Each IDC session correspond to 24 h of recording and sessions were consecutive. Data was analyzed with a 2-way repeated measures ANOVA and no significant differences were detected in any variable. P-values are shown in the last three columns from right to left. Terms of the ANOVA are: $F_{HA/LA}$, main effect for differences between HA and LA rats; F_{TIME} , main effect for differences between IDC recording sessions and $F_{[HA/LA * TIME]}$ term for interaction between HA/LA phenotype and IDC recording session.

	HA (n = 11)	LA (n = 7)	t	df	p-value
Fat Mass (g)	44.027 ± 2.479	47.575 ± 26.39	-0.911	16	0.3756
Lean Mass (g)	287.449 ± 26.394	310.67 ± 11.50	-0.6702	16	0.5123

Supplementary Table 3. Fat and lean mass recorded at the end of IDC sessions for a subset of high activity (HA) and low activity (LA) rats selected based on 24 h ambulatory spontaneous physical activity recorded in indirect calorimetry (IDC) cages. Analysis for group differences between HA and LA rats with a t-test did not detect significant differences in either fat or lean mass. Values shown are mean ± SEM. t, t-test statistic, df, degrees of freedom).