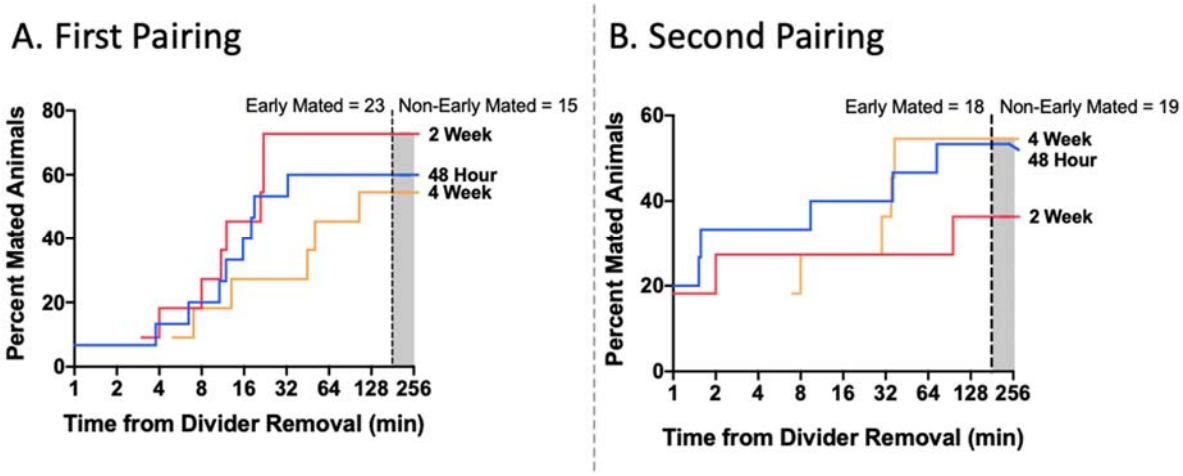
Supplemental Materials



Supplemental Figure 1. Mating latency comparison across 3 separation groups. A) There were no significant group differences in latency to mate with the first partner ($p = 0.505$). **B)** There were no significant group differences in latency to mate with the second partner ($p = 0.653$).

Experiment	Partner	Measurement	Statistical Test	Comparison	° of freedom, error	F or T	p	*	Group Size	Fig.	Notes
Partner preference	1	Prop. Partner huddle	RM-ANOVA	Time (RM)	1, 36	1.726	0.197		n = 37	2A	no data for 2242, long term PPT
		Short Term	one way T-test	relative to 50%	37	2.495	0.017	*	n = 38		
		Long Term	one way T-test	relative to 50%	36	4.667	0.000	***	n = 37		
		Group differences prop. Partner huddle	RM-ANOVA	Time (RM)	1, 34	1.444	0.238		48 hr = 15, 2 wk = 11, 4 wk = 11	not shown	2242 missing for long term test
				Time x group	2, 34	2.19	0.127				
				Group	2, 34	0.091	0.913				
		48hr, short term	one way T-test	relative to 50%	16	1.428	0.174				
		48hr, long term	one way T-test	relative to 50%	15	4.24	0.001	**			
		2 wk, short term	one way T-test	relative to 50%	10	0.77	0.459				
		2 wk, long term	one way T-test	relative to 50%	10	3.261	0.009	**			
		4 wk, short term	one way T-test	relative to 50%	10	2.163	0.056				
		4 wk, long term	one way T-test	relative to 50%	10	1.009	0.337				
		Short vs long term, partner 1	partner time	pearson, spearman		0.335, 0.341	0.043, 0.039	*	n = 37		
			partner huddle	pearson, spearman		0.347, 0.417	0.036, 0.010	*	n = 37		
			percent partner huddle	pearson, spearman		0.290, 0.387	0.081, 0.018	*	n = 37		
			novel time	pearson, spearman		0.413, 0.423	0.011, 0.009	*	n = 37		
			novel huddle	pearson, spearman		0.294, 0.353	0.077, 0.032	*	n = 37		
		Ndist/Pdist corr with Pctpartner huddle	Short term	pearson, spearman		0.782, 0.827	6.7e-9, 1.55e-10	***	n = 38	2D	
		Ndist/Pdist corr with Pctpartner huddle	Long term	pearson, spearman		0.760, 0.790	1.55e-10, 4.73e-8	***	n = 37		
Huddle time	1	Partner huddle time	paired t-test	Time (RM)	36.000	0.192	0.849		n = 37	2B	no data for 2242, long term PPT
Huddle time	1	Novel huddle time	paired t-test	Time (RM)	36.000	2.347	0.025	*	n = 37		
Avg. distance from tethered	1	Distance when in	RM-ANOVA	Time (RM)	1, 36	4.090	0.051		n = 37	2C	no data for 2242, long
				Tethered animal (RM)		24.252	0.000	***			
				Time x tethered		1.534	0.224				
			48 hr; Paired t-test short term		14	-3.899	0.002	**	n = 15		
			48 hr; Paired t-test long term		14	-3.727	0.002	**			

partner		chamber	2 wk; Paired t-test short term		10	-2.765	0.020	*	n = 11		term PPT
			2 wk; Paired t-test long term		10	-3.615	0.005	***			
			4 wk; Paired t-test short term		10	-3.367	0.007	***	n = 11		
			4 wk; Paired t-test long term		10	-2.334	0.042	*			
Distance from tethered	1	PminusN distance	RM-ANOVA	Time (RM)	1, 36	1.534	0.224		n = 37	not shown	no data for 2242, long term PPT
			RM-ANOVA	Time (RM)	1, 34	1.224	0.276				
				Group	2, 34	0.545	0.585				
				Time x group	2, 34	1.276	0.292				
Total distance	1	Total distance traveled by group	RM-ANOVA	Time (RM)	1, 34	13.993	0.001	**			
				Group	2, 34	3.031	0.061				
				Time x group	2, 34	1.159	0.326				
		Total distance	short term vs long term	pearson, spearman		0.370, 0.284	0.026, 0.093	**			
Huddle time	2	48hr, 2nd partner; Partner huddle time	RM-ANOVA	Time (RM)	1, 12	3.392	0.090		n = 13	2B	
Huddle time	2	48hr, 2nd partner; Novel huddle time	RM-ANOVA	Time (RM)	1, 12	2.106	0.172		n = 13		
Huddle time	2	2wk, 2nd partner; Partner huddle time	RM-ANOVA	Time (RM)	1, 9	2.754	0.131		n = 10	2E	
Huddle time	2	2wk, 2nd partner; Novel huddle time	RM-ANOVA	Time (RM)	1, 9	6.424	0.032	*	n = 10		
Huddle time	2	4wk, 2nd partner; Partner huddle time	RM-ANOVA	Time (RM)	1, 10	0.322	0.577		n = 11	2H	
Huddle time	2	4wk, 2nd partner; Novel huddle time	RM-ANOVA	Time (RM)	1, 10	0.343	0.571		n = 11		
Avg. distance from tethered partner	2	48hr: Distance when in chamber	RM-ANOVA	Time (RM)	1, 12	6.405	0.026	*	n = 13 - 15	2C	excludes 2262, 2267 from long-term
				Tethered animal (RM)		5.007	0.045	*			
				Time x tethered		1.669	0.221				
			Paired t-test short term		14	-3.727	0.002	**			
			Paired t-test long term		12	-1.371	0.196				
Avg. distance from tethered	2	2wk: Distance when in chamber	RM-ANOVA	Time (RM)	1, 9	2.636	0.139		n = 10 - 11	2F	excludes 1872
				Tethered animal (RM)		8.734	0.016	*			
				Time x tethered		3.290	0.103				

partner			Paired t-test short term		10	-3.615	0.005	**			
			Paired t-test long term		9	-0.623	0.549				
Avg. distance from tethered partner	2	4 wk: Distance when in chamber	RM-ANOVA	Time (RM)	1, 10	1.655	0.227		n = 11	2I	excludes 2242
				Tethered animal (RM)		19.06	0.001				
				Time x tethered		1.872	0.201				
			Paired t-test short term		10	-2.334	0.042	*			
			Paired t-test long term		10	-4.1	0.002	**			
Partner preference	2	Proportion partner huddle time	RM-ANOVA	Time (RM)	1, 31	4.991	0.033	*		3A,D,G	excludes 1872, 2242, 2262, 2267
				Time x group	2, 31	3.263	0.052				
				Group	2, 31	0.342	0.712				
		48hr, short term	one way T-test	relative to 50%	14	2.584	0.022	*	n = 15	3A	excludes 1872
		48hr, long term	one way T-test	relative to 50%	12	1.119	0.285		n = 13		
		48hr	Paired t-test short vs long term	Percent huddle	12	2.031	0.065				
		2 wk, short term	one way T-test	relative to 50%	10	3.833	0.003	**	n = 11	3D	
		2wk, long term	one way T-test	relative to 50%	9	0.194	0.850		n = 10		
		2 wk	Paired t-test short vs long term	Percent huddle	9	3.297	0.009	**			
		4wk, short term	one way T-test	relative to 50%	10	1.985	0.075		n = 11	3G	
		4 wk, long term	one way T-test	relative to 50%	10	2.901	0.016	*			
		4 wk	Paired t-test short vs long term	Percent huddle	10	-0.553	0.592				
Partner preference	2	Short vs long term, partner 2	partner time	pearson, spearman		0.557, 0.593	0.001, 0.0003	**	n = 33	not shown	excludes 1872, 2262, 2267, 2245, no data for 1850, 1867, 1924
			partner huddle	pearson, spearman		0.660, 0.732	0.00003, 0.000001	**			
			percent partner huddle	pearson, spearman		0.535, 0.560	0.001, 0.001	**			
			novel time	pearson, spearman		0.594, 0.520	0.000268, 0.002	**			
			novel huddle	pearson, spearman		0.614, 0.374	0.000147, 0.032	**			
Total distance	2	Total distance traveled by group	RM-ANOVA	Time (RM)	1, 30	1.209	0.280		n = 33	Not shown	4-week separated group traveled less than 2 week or 48 hour; excludes 1872, 2262, 2267, 2245, no data for 1850, 1867, 1924
				Group	2, 30	10.252	0.000	***			
				Time x group	2, 30	0.212	0.810				
		Total distance traveled	short term vs long term	pearson, spearman		0.215, 0.331	0.229, 0.060				

Mating latency 1st partner - survival analysis	48 hr vs 2 wk vs 4wk	Latency to mate	Kaplan Meyer with Log Rank for overall comparison		Chi sq = 1.367	df = 2	0.505			S1A	
Mating latency 2nd partner - survival analysis	48 hr vs 2 wk vs 4wk	Latency to mate	Kaplan Meyer with Log Rank for overall comparison		Chi sq = 0.852	df = 2	0.653			S1B	excludes 2242
Mating latency 1st vs 2nd partner	1st vs 2nd	Latency to mate	Kaplan Meyer with Log Rank for overall comparison		Chi sq = 0.565	df = 1	0.452			4C	excludes 2242
Proportion mated within 3 hours	1st vs 2nd		Fisher exact		F = 0.313		0.187			4A, B	
Mating latency 1st vs 2nd partner	Correlation	Latency to mate		pearson, spearman		0.193, 0.176	0.253, 0.296			not shown	non-maters assigned 10800; 9 animals (24%) did not mate early with either partner
Avg. distance from tethered partner for early and late mating animals	1	Distance when in chamber	RM-ANOVA	Time (RM)	1, 35	0.220	0.642		Early mating = 23; Late mating = 14	Not shown	
				Time x latency	1, 35	0.683	0.414				
				Latency (early vs non-early)	1, 35	0.029	0.866				
Avg. distance from tethered novel for early and late mating animals	1	Distance when in chamber	RM-ANOVA	Time (RM)	1, 35	3.818	0.059		Early mating = 23; Late mating = 14		
				Time x latency	1, 35	0.007	0.933				
				Latency (early vs non-early)	1, 35	0.507	0.481				
Avg. distance from tethered partner minus novel for early and late mating animals	1	Distance when in chamber	RM-ANOVA	Time (RM)	1, 35	1.122	0.297		Early mating = 23; Late mating = 14		
				Time x latency	1, 35	0.287	0.595				
				Latency (early vs non-early)	1, 35	0.097	0.757				
Partner preference	1	Proportion partner huddle time	RM-ANOVA	Time (RM)	1, 35	1.21	0.279		Early mating = 23; Late mating = 14	4D, E	2242 only had data for short term
				Time x latency	1, 35	0.458	0.503				
				Latency (early vs non-early)	1, 35	0.002	0.969				
		Early maters, short term	one way T-test	relative to 50%	22	1.752	0.094				
		Late maters, short term	one way T-test	relative to 50%	14	1.766	0.099				
		Early maters, long term	one way T-test	relative to 50%	22	4.13	0.000	**			
		Late maters, long term	one way T-test	relative to 50%	13	2.291	0.039	*			
		Proportion partner huddle time	RM-ANOVA	Time (RM)	1, 32	4.533	0.041	*	Early = 16, Late = 18		
				Time x latency		1.296	0.263				
				Latency (early vs non-early)		9.077	0.005	**			

Partner preference	2	Early maters, short term	one way T-test	relative to 50%	17	10.302	0.998	****		4F, G	Excluded 1872, 2262, 2267 from long term test
		Late maters, short term	one way T-test	relative to 50%	18	1.048	0.309				
		Early maters, long term	one way T-test	relative to 50%	15	3.832	0.002	**			
		Late maters, long term	one way T-test	relative to 50%	17	0.501	0.623				
Huddle time	1 vs 2	Partner 2 huddle time	ANOVA	Group	1, 30	1.06	0.359		48 hr = 14, 2 wk = 10, 4 wk = 9	5A	
		Parnter 1 huddle time	ANOVA	Group	1, 30	2.455	0.103				
Partner preference	1 vs 2	48 hr group	one way T-test	relative to 50%	13	-0.025	0.981		n = 14		
		2wk group	one way T-test	relative to 50%	9	0.871	0.406		n = 10		
		4wk group	one way T-test	relative to 50%	8	10.658	0.000	***	n = 9		
Avg. distance from tethered partner	1 vs 2	Distance when in chamber	RM-ANOVA	Group	2, 30	2.281	0.120		48 hr = 14, 2 wk = 10, 4 wk = 9	5B	excluded 2245, no data for 1850, 1867, 1942, 2242
				Tethered animal (RM)	1, 30	17.062	0.000	***			
				Group x tethered	2, 30	6.480	0.005	**			
			48 hr: Paired t-test		13	-0.578	0.573				
			2 wk: Paired t-test		9	-1.064	0.315				
			4 wk: Paired t-test		8	-7.953	0.000	***			
Total Distance by group	1 vs 2	Total distance by group	ANOVA	Group	2, 30	0.002	0.998		48 hr = 14, 2 wk = 10, 4 wk = 9	Not shown	excluded 2245, no data for 1850, 1867, 1942, 2242

Supplemental Table 2 Excluded Animals.

Animal	Separation Condition	Test Excluded From	Reason for Exclusion
1872	2 Week	P2 Long term	Death of Partner
1850	4 Week	P1 vs P2	Death of Partner
1867	4 Week	P1 vs P2	Death of Partner
1924	2 Week	P1 vs P2	Death of Partner
2242	48 Hour	P1 long term, P2 all	Death of Partner
2245	48 Hour	P1 vs P2	Escaped Apparatus
2262	48 Hour	P2 long term	Camera shift so box was out of frame
2267	48 Hour	P2 long term	Camera shift so box was out of frame

P1 = partner 1, P2 = partner 2