

Grissom et al – Executive function and maternal diet- Supplemental Figure Captions

Supporting Information 1. Pavlovian Conditioned Approach (PCA) measures in male offspring. **a-d** illustrate measures of sign-tracking, while **e-h** illustrate goal tracking (see **Figure 1**). **a**) LP males spent more time in contact with the sign while it was illuminated (Mann-Whitney $U=51$, $p=0.008$). **b**) LP males contacted the sign before the goal on more trials (out of 25 total trials) than controls (this data also depicted in **Figure 1**). **c**) LP males contacted the sign on more trials than control (Mann-Whitney $U=60.5$, $p=0.02$). **d**) LP males tended to make more approaches to the sign during each trial (Mann-Whitney $U=74$, $p=0.09$). **e**) There were no group differences in the latency to contact the sign from the onset of the sign-illuminated period. **f**) HF males tended to spend less time in contact with the goal compared to controls (Mann-Whitney $U=97$, $p=0.06$). **g**) LP males tended to contact the goal before the sign on fewer trials than controls (Mann-Whitney $U=77$, $p=0.11$). **h**) There were no group differences in the number of trials (out of 25 total) the goal was contacted. **i**) HF males tended to made more unique approaches to the goal during each trial compared to controls (Mann-Whitney $U=7$, $p=0.07$). **j**) There were no group differences in the latency to contact the goal from the onset of the sign-illuminated period. **k-p**) Because the behaviors were detected by beam breaks at the front (magazine or goal-proximal) or back (9-hole array or sign-proximal) of the chamber, differences in activity in these areas during the period when the sign is not illuminated (CS-) between groups might indicate nonspecific behavior. We selected the 8 second periods immediately preceding the cue presentation for each of the 25 trials presented in a-j, and analyzed the measures leading to the strongest effects during the CS+ period. **k**) There were not differences in the duration of time spent at the rear of the chamber (one way ANOVA $F(2,45)=0.999$, $p=0.37$. A t-test comparing CTL and LP offspring was also not significant. **l**) there were no group differences in the number of trials where the rear of the chamber was approached during an 8-second CS- period. **m**) There were no differences in the number of approaches to the rear of the chamber during the CS-. **n**) There were no differences in the duration of time spent at the front of the chamber during the CS-. **o**) There were no differences in the number of “trials” in which the front of the chamber was approached before the rear of the chamber during the CS-. **p**) There were no differences in the number of approaches to the front of the chamber during the CS-. * $p\leq 0.05$ vs. control group, + $p\leq 0.11$ vs control. n: male: CTL=15, LP=14, HF=19. Figures depict box-and-whisker plots of the full response range.

Supporting Information 2. PCA measures in female offspring. **a-d** illustrate measures of sign-tracking, while **e-h** illustrate goal tracking (see **Figure 1**). **a**) LP females contacted the sign before the goal on more trials (out of 25 total) than controls (this data is also depicted in **Figure 1**). **b**) There were no group differences in the latency to contact the sign. **c**) There were no group differences in the total number of trials the sign was contacted. **d**) There were no group differences in the average number of contacts with

the sign during individual trials. **e)** LP females contacted the goal before the sign on significantly fewer trials than controls ($t(27)=2.34$, $p=0.02$). **f)** There were no group differences in the latency to contact the goal while the sign was illuminated. **g)** There were no group differences in the total number of trials that the goal was contacted. **h)** There were not group differences in the number of contacts with the goal within an individual trial. **i-l)** As in males (Supplemental Information 1), we wanted to investigate whether the approaches to the rear of the chamber were specific to the CS+ period. Thus, we selected the 8 second periods immediately preceding the cue presentation for each of the 25 trials presented in a-h, and analyzed the measures leading to the strongest effects during the CS+ period. **i)** There were no significant differences in the number of “trials” in which the rear of the chamber was approached before the front of the chamber. **j)** There were no significant differences in the number of “trials” the rear of the chamber was approached during the CS-. **k)** There was a significant difference in the number of “trials” in which the front of the chamber was approached “before” the rear of the chamber (CTL vs LP, Mann-Whitney $U=34.5$, $p=0.001$). **l)** There was also a significant difference in the number of approaches to the front of the chamber overall during the CS- (LP vs CTL Mann-Whitney $U=42.5$, $p=0.005$). In conjunction, the data in k and l are consistent with a reduction in goal approaches in all groups during the CS- (compare “l” to “g”) which was particularly strong in the LP animals. This suggests that as in the males, all animals were aware of the CS+/CS- distinction as reflected in differences in goal approach during these periods, and tendencies towards sign approach in the LPs only during the CS+. * $p \leq 0.05$ vs. control group. **n:** female: CTL=14, LP=15, HF=17 in all figures. Figures depict box-and-whisker plots of the full response range.

Supporting Information 3. Performance on the 5-choice serial reaction time test (5-CSRTT) rapidly reaches an asymptote at the time of acquisition. As noted in the text, the performance of both humans and animal models of neurodevelopmental vulnerability on the 5-CSRTT are susceptible to improvements due to overtraining, and a loss of the ability to distinguish the vulnerable populations. As a result, we closely monitored the performance of animals from the beginning of the 5-CSRTT to first acquisition, defined as greater than 50% correct performance and greater than 20 correct trials for two consecutive days. The day of acquisition is identified as Day 0 in this figure, and data depicted in Figure 2 of the main manuscript are from Day 0. This figure depicts only percent correct performance for clarity. Though each animal acquired the task at their own pace, such that Day 0 occurred on a different date depending on the individual animal (see Figure 2b), the acquisition curve is remarkably similar between groups, with day 0 forming a clear inflection point. Performance before Day 0 (-2 and -1) was significantly different than Days 0-3 (main effect of day, $F(5, 230)=96.9$, $p<0.0001$). In addition, on days 0-3, there was a clear main effect of maternal diet ($F(2,46)=5.89$, $p=0.005$) indicating that offspring of both maternal low-protein and

high-fat diets were significantly impaired at the task on Days 0-3. These deficits became harder to observe over time, as training was extended further (Supporting Information 4). Thus, the day of acquisition is a time of high levels of performance for all animals, but has not exposed the animals to overtraining conditions, allowing the detection of executive function deficits.

Supporting Information 4. 5-choice serial reaction time test (5-CSRTT) performance after acquisition of the task in males. After animals demonstrate acquisition of the task on training settings (10s stimulus duration with a fixed 5s ITI), animals progressed through a series of increasingly challenging settings, including shortening the stimulus duration and creating a variable-duration ITI. It is not uncommon for animals to continue to improve on the task despite the settings becoming increasingly challenging. However, reaction time demonstrated a continued vulnerability in these animals even as performance indices normalized. All data is taken from the day each animal met performance criteria on that setting of the task (universally 1-2 days additional training). **a)** Both LP and HF males performed significantly fewer trials correctly early in 5-CSRTT training, but as training progressed they were able to perform similarly to controls (see **Figure 2** for statistics). **b)** The reaction time to make a correct response revealed a more persistent vulnerability in LP and HF male offspring. Compared to controls, reaction times remained elevated in both LP and HF over the first three training sessions (repeated measures ANOVA $F(2,45)=3.97$, $p=0.02$). **c)** Impulsive premature responses were high in HF males early in training (see **Figure 2**). However, introduction of a variable 3-7s ITI caused a uptick in impulsive responding in both LP and HF compared to control (LP: $t(27)=2.4$, $p=0.02$; HF $t(27)=1.8$, $p=0.07$). This suggests that HF and LP animals were more reliant on a “timing” strategy to appropriately inhibit premature responding. **d)** Incorrect responses were high in HF males early in training, but as training progressed they were able to accurately target their responses to a similar degree as controls. **e)** Inattentive omitted trials were elevated in LP males early in training, but as training progressed this group difference was eliminated. * $p\leq 0.05$ vs. control group. n: male: CTL=15, LP=14, HF=19. Figures depict mean \pm SEM.

Supporting Information 5. 5-choice serial reaction time test (5-CSRTT) performance after acquisition of the task in females. As in males, deficits apparent early in training recovered with extended experience with the task. **a)** Both LP and HF females performed significantly fewer correct trials than controls in early training (see **Figure 2**) but as training progressed group differences were mitigated. **b)** Reaction times to respond correctly were elevated in both LP and HF females relative to controls in early training ($F(2,43)=3.9$, $p=0.02$). However, reaction times recovered more rapidly than in males and were no longer significantly different by the second 5-CSRTT setting. **c)** Premature responses were not different between groups at any point of training. **d)** Incorrect responses were high in HF females early in training, but as training progressed they were able to accurately target their responses to a similar degree as controls. **e)**

Inattentive omitted trials were elevated in LP males early in training, but as training progressed this group difference was eliminated. **f**) Task acquisition curve for learning the 5-CSRTT on the earliest settings. LP females acquired the task more rapidly than controls ($X^2=5.7$, $p=0.01$). There were no differences in acquisition between HF and controls. * $p\leq 0.05$ vs. control group. n: female: CTL=14, LP=15, HF=17. Figures depict mean \pm SEM.

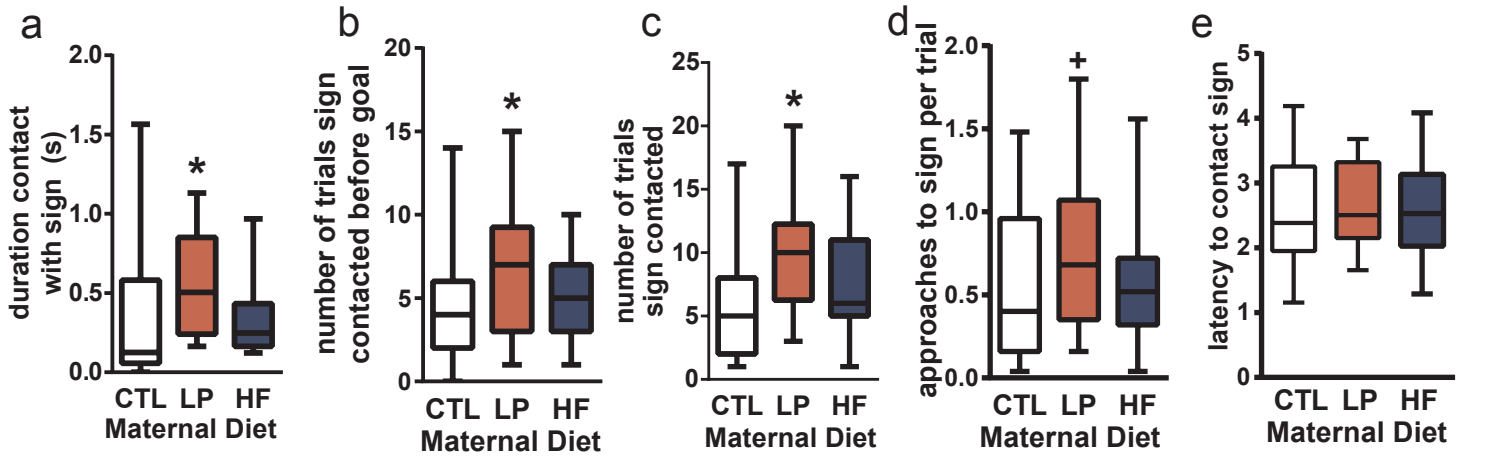
Supporting Information 6. Acute shortening of the stimulus duration in the 5-CSRTT recapitulates inattention deficits in male low-protein offspring and impulsive deficits in female high-fat offspring. **A**) Schematic of schedules. Animals were highly trained on the 5-CSRTT with a two-second stimulus duration, and were tested for one day with trials of a 1 second stimulus duration. All trials included a 2-second limited hold period following stimulus presentation allowing sufficient time for responses. **B-E**) Performance of male animals of the three maternal diet conditions on the 1 second stimulus trials. **B**) Percent correct performance. Though performance was decreased in all groups by stimulus shortening, low-protein male offspring were significantly affected (maternal diet x duration interaction, $F(2,45)=3.6$, $p=0.03$). **C**) Low-protein male offspring demonstrated a significant return of inattention due to acute stimulus shortening (maternal diet x duration interaction, $F(2,45)=6.7$, $p=0.003$). **D**) Acute stimulus shortening lead to a non-significant increase in premature responses in control and high-fat male offspring, but not low-protein (main effect maternal diet $F(2,45)=2.6$, $p=0.08$). **E**) Acute stimulus shortening increased incorrect responses in all groups, indicating that all animals found this trial duration challenging (main effect duration, $F(1,45)=100.3$, $p<0.0001$). **F-I**) Performance of female animals of the three maternal diet conditions on the 1 second stimulus trials. **F**) Percent correct trials. Female animals of all conditions performed fewer correct trials due to stimulus shortening (main effect duration $F(1,43)=110.2$, $p<0.0001$). **G**) Female animals of all conditions omitted a greater proportion of trials due to stimulus shortening (main effect duration $F(1,43)=77.9$, $p<0.0001$). **H**) Female offspring of a high-fat diet were particularly susceptible to increased premature response rates due to stimulus shortening (maternal diet x duration interaction, $F(2, 43)=6.3$, $p=0.004$). **I**) All animals demonstrated an increased rate of incorrect responses due to stimulus shortening (main effect duration $F(1,43)=23.1$, $p<0.0001$).

Supporting Information 7. Full correlation tables comparing gene expression levels to behavioral indices. R values indicating direction and strength of correlation are in bold and listed in the row containing the gene name, while p values indicating confidence in findings are italicized and listed beneath the corresponding r value. Correlations reaching or approaching significance (all relationships where $p\leq 0.1$) are in black, while nonsignificant relationships have been colored gray for clarity. Gene expression was calculated as $2^{-\Delta C_T}$ values from the geometric mean of GAPDH and ACTB expression. **a**) Description of behavioral indices used for correlation calculations. Behaviors where higher numbers

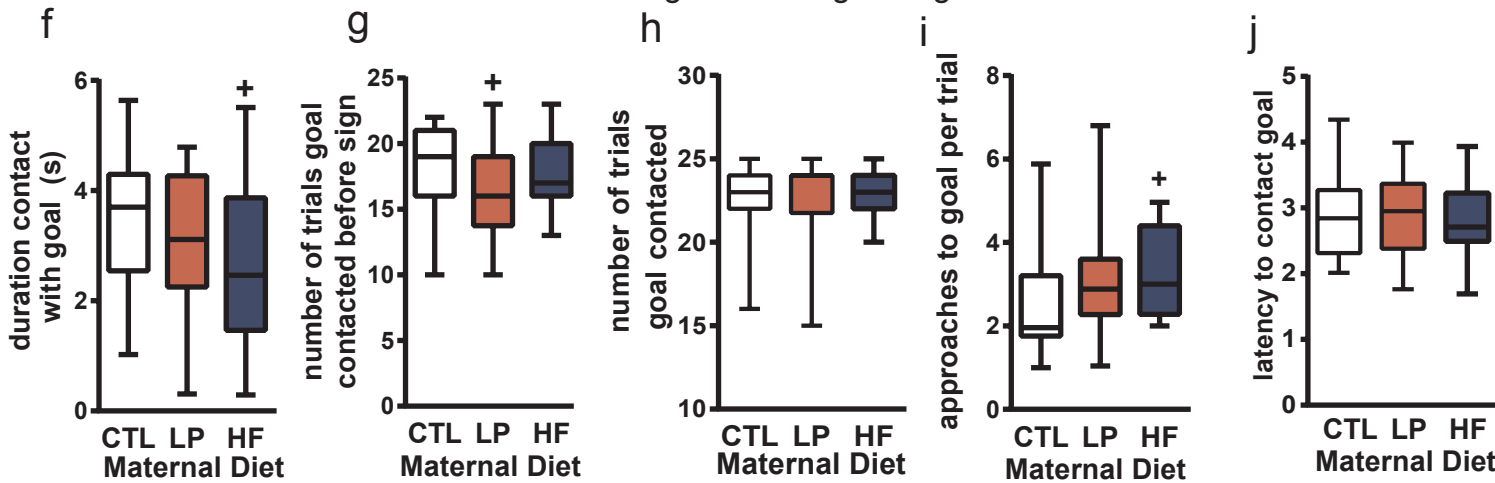
indicated better performance (fixed ratio, progressive ratio, % correct responses) were multiplied by -1 to better illustrate the association between overexpression of genes and poor performance in the operant chamber. **b)** Correlation table comparing expression of 18 target genes and performance on key behavioral indices across all animals in the experiment. The full correlation table is also depicted as a heatmap in **Figure 3c**.

Supporting Information 8. Correlation tables comparing gene expression and behavioral indices, separating HF and LP animals. Layout of tables is as described in **SI5**. **a)** Correlation table calculated from values obtained from LP and control animals only, depicting the specific genes associated with LP behavioral deficits. Portions of this data are depicted as a heat map in Figure 3e. **b)** Correlation table comparing gene expression and behavioral indices, calculated from HF and control animals only, depicting the specific genes associated with HF behavioral deficits.

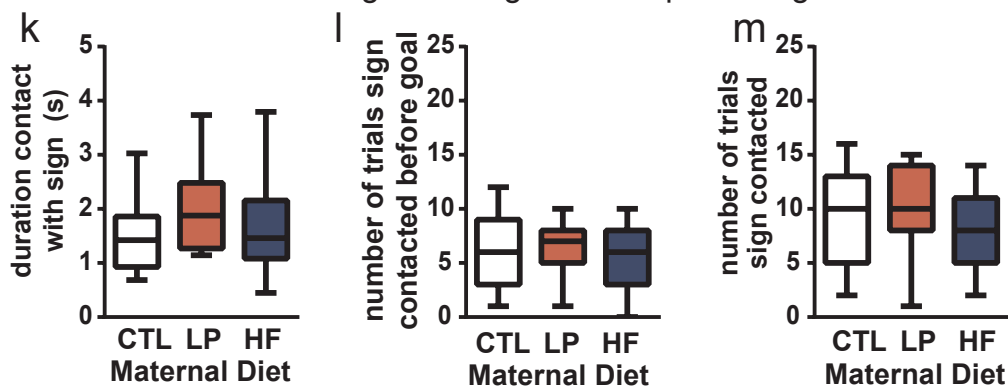
♂ males - measures of sign tracking during the 8s cue



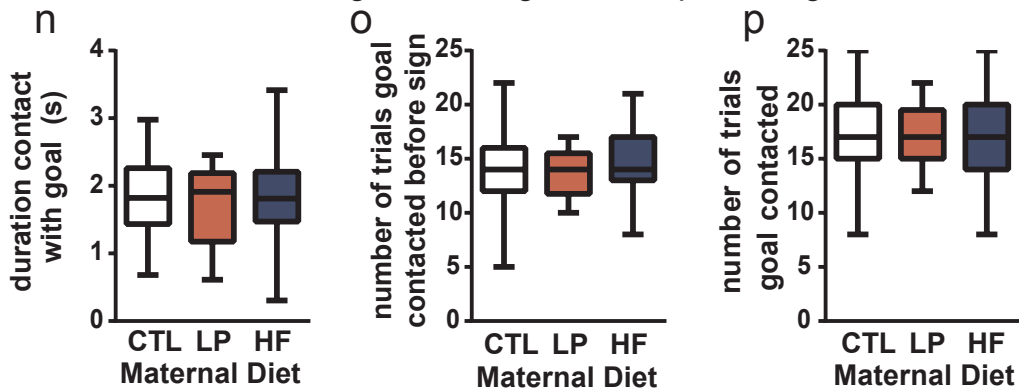
measures of goal tracking during the 8s cue



measures of sign tracking in the 8s preceding the cue

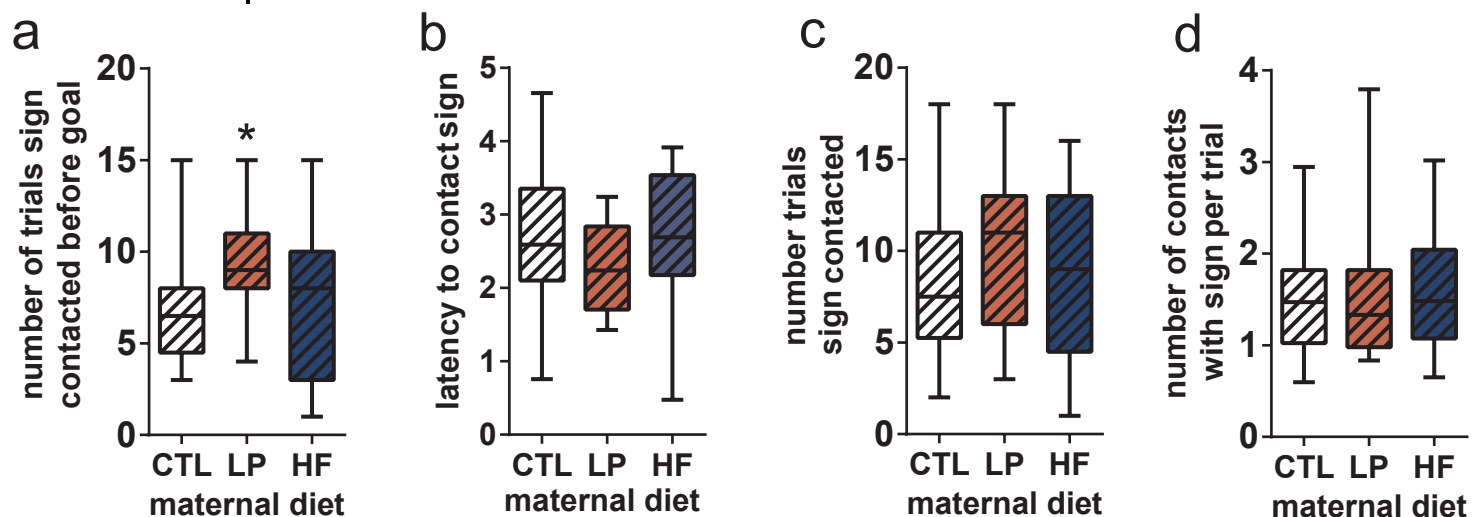


measures of goal tracking in the 8s preceding the cue

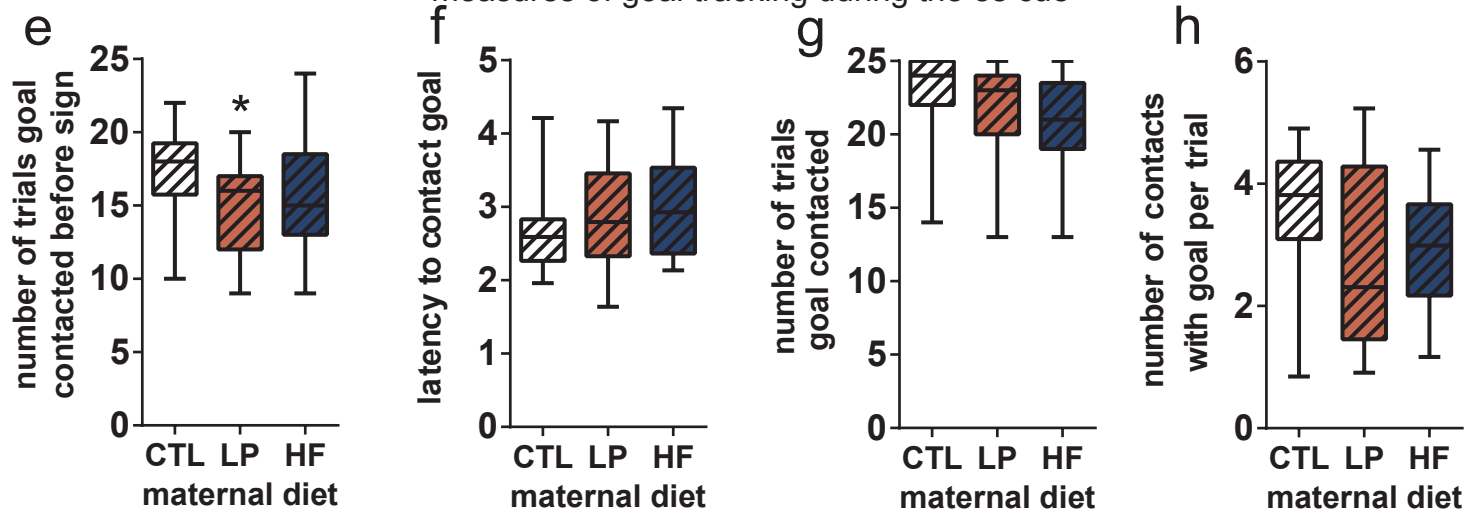


♀ females

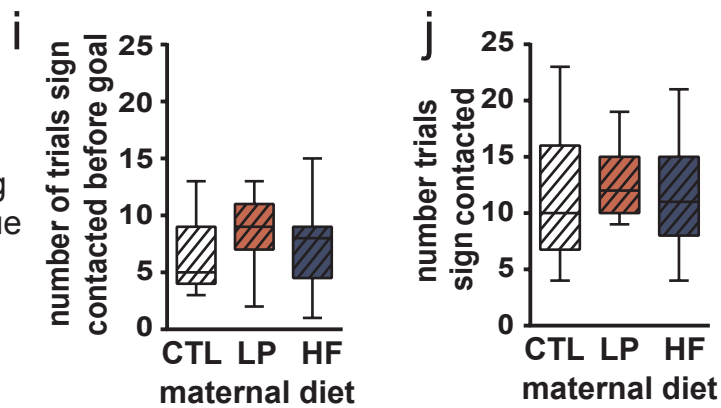
measures of sign tracking during the 8s cue



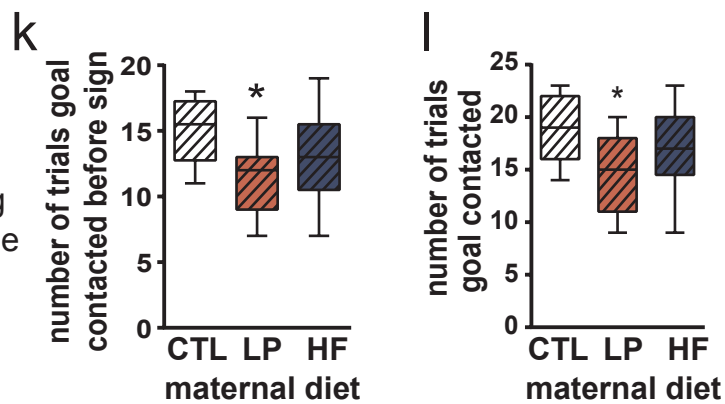
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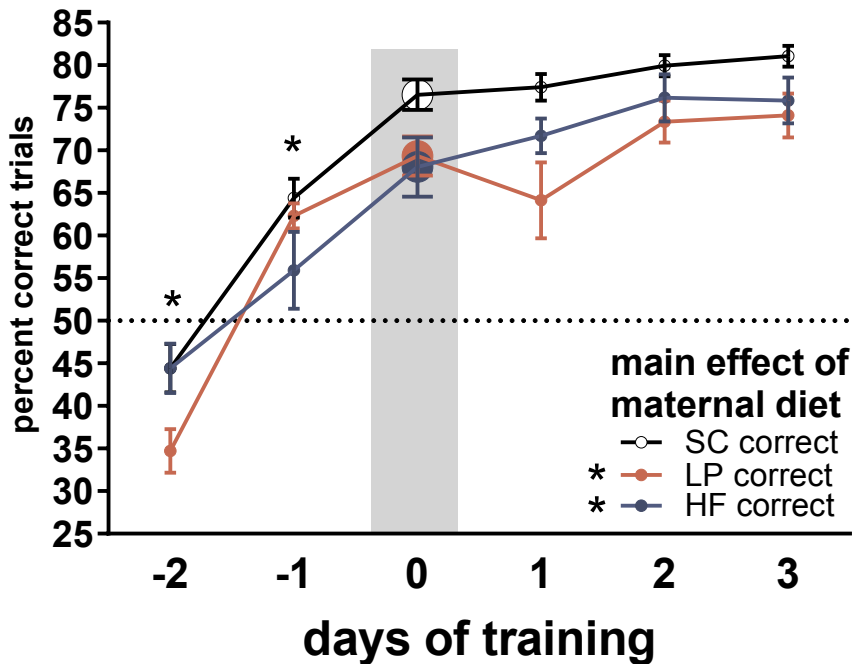


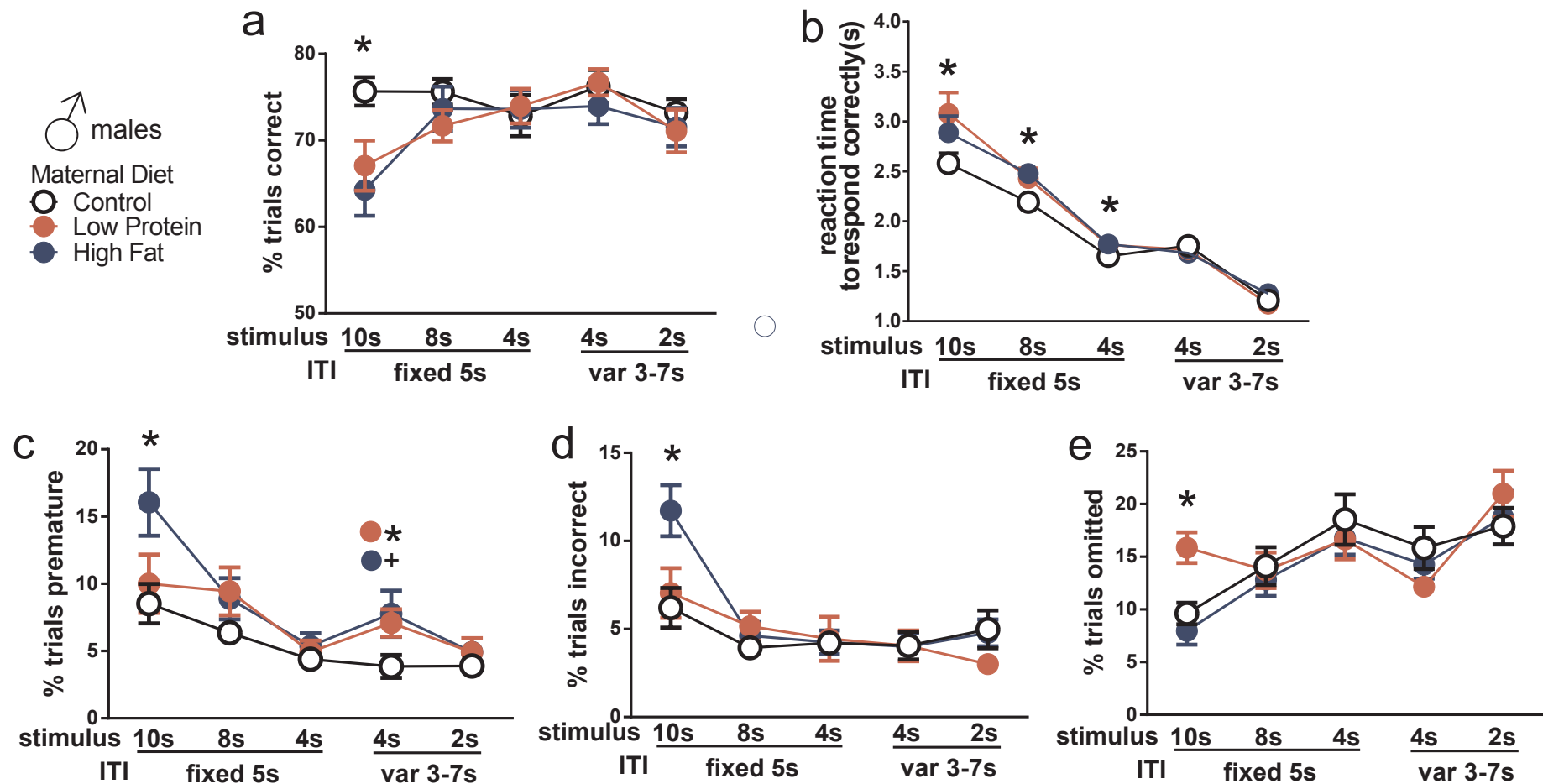
measures of sign tracking in the 8s preceding the cue

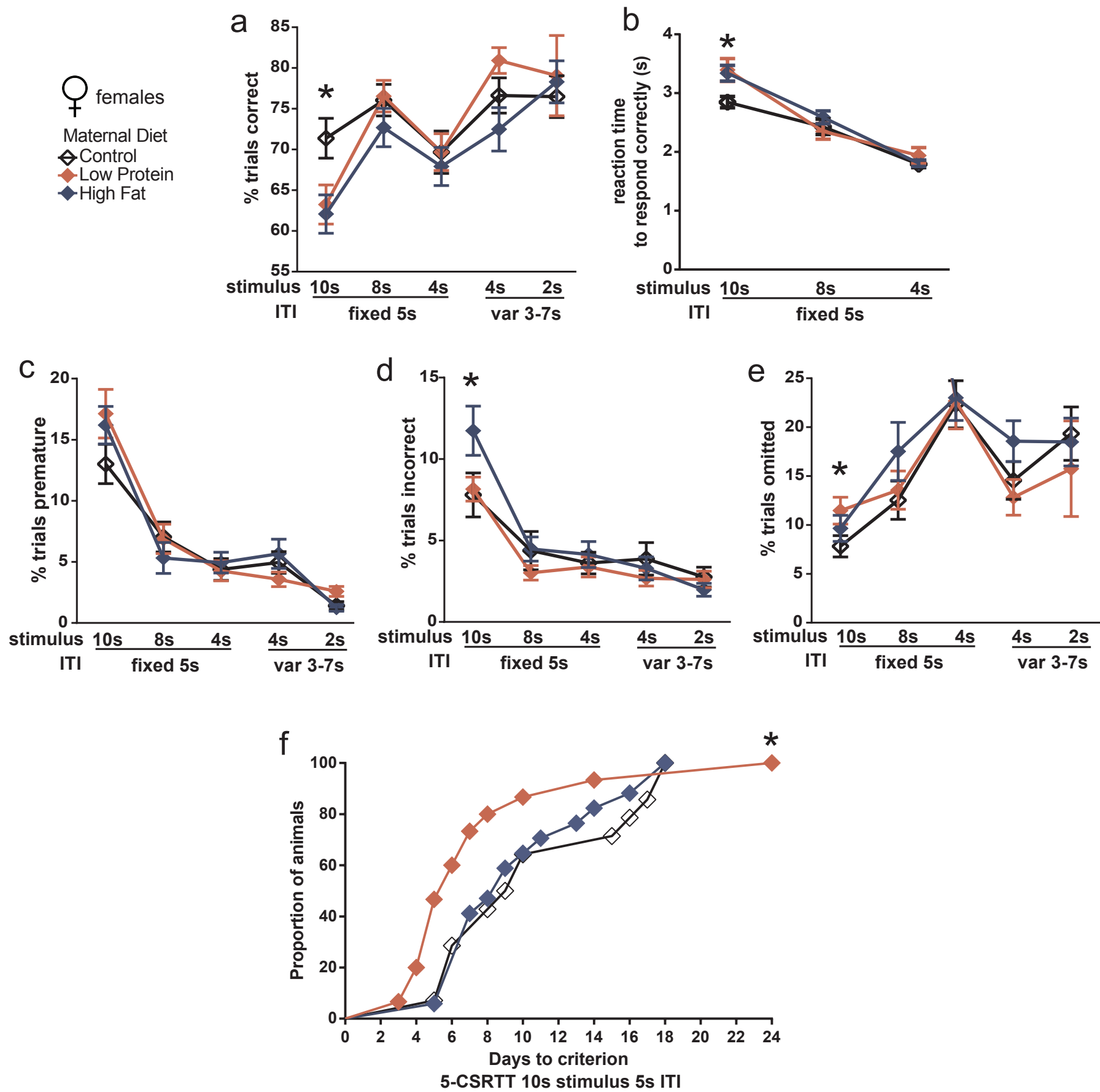


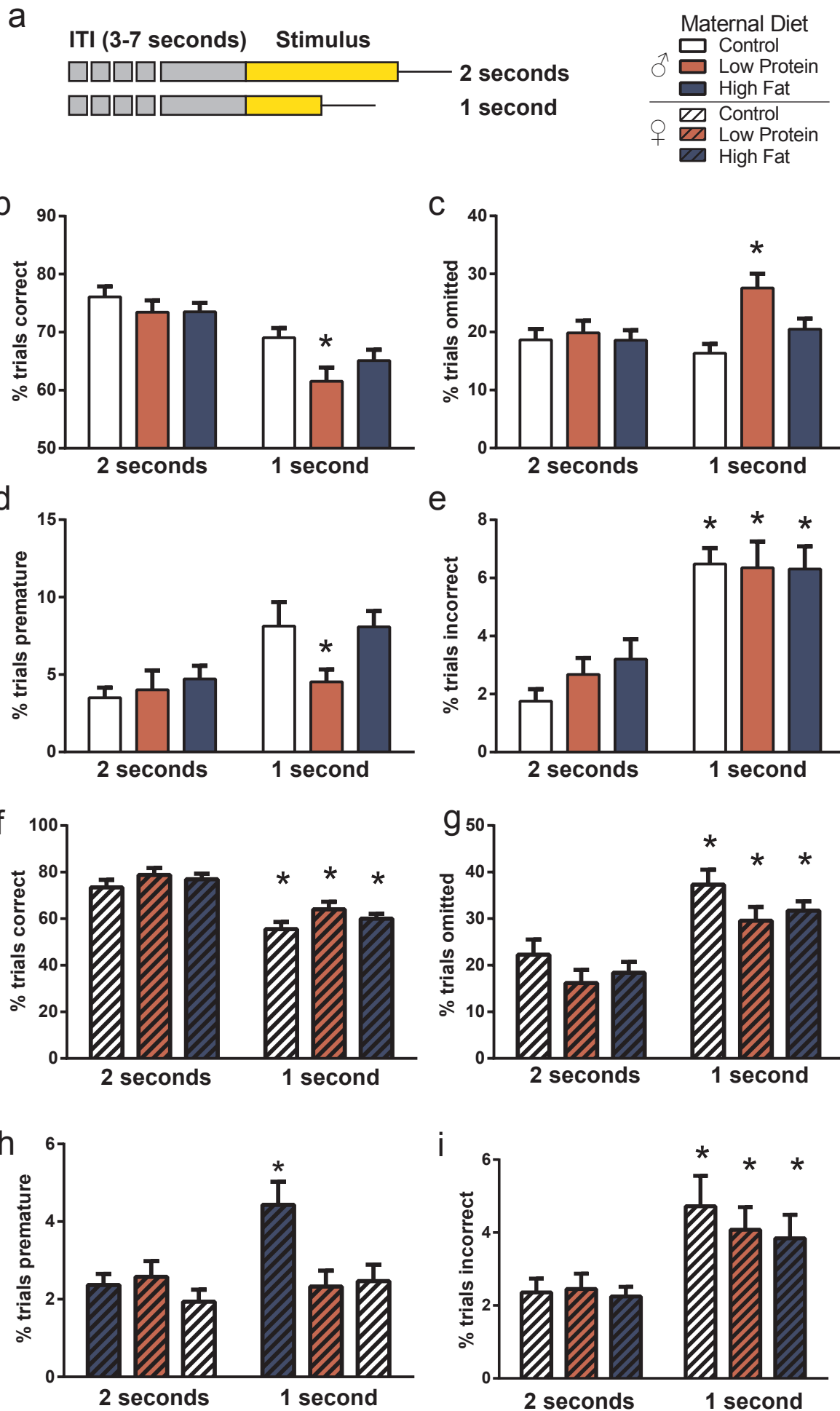
measures of goal tracking in the 8s preceding the cue











- a**
- sign track number of trials sign approached before goal
 - goal track number of trials goal approached before sign
 - 1(fixed ratio) -1 * trials completed on day 3 of fixed ratio training
 - 1(breakpoint) -1 * last trial completed in progressive ratio testing
 - 1(%correct) -1 * percentage of correct trials on first 5-CSRTT schedule (10s stim 5s ITI)
 - reaction time reaction time for correct responses on first 5-CSRTT schedule
 - %omitted percentage omitted trials on first 5-CSRTT schedule
 - %premature percentage premature trials on first 5-CSRTT schedule

b

all animals	sign track	goal track	-1(fixed ratio)	-1(breakpoint)	-1(%correct)	reaction time	%omitted	%premature
CNR1	0.267	-0.130	-0.290	0.186	0.249	0.397	0.055	0.219
<i>p val</i>	0.07	0.38	0.05	0.21	0.09	0.01	0.71	0.14
COMT	-0.135	-0.228	0.010	0.250	0.409	0.382	0.313	0.189
<i>p val</i>	0.36	0.12	0.95	0.09	0.00	0.01	0.03	0.20
DNMT1	0.097	-0.042	0.014	0.276	0.281	0.448	0.167	0.249
<i>p val</i>	0.51	0.78	0.93	0.06	0.05	0.00	0.26	0.09
DRD1	-0.110	-0.110	0.246	-0.006	0.306	-0.017	0.177	0.048
<i>p val</i>	0.46	0.46	0.09	0.97	0.03	0.91	0.23	0.75
DRD2	-0.140	-0.040	0.295	-0.045	0.103	-0.068	0.206	-0.113
<i>p val</i>	0.34	0.79	0.04	0.76	0.49	0.65	0.16	0.45
EHMT2	0.076	0.032	-0.093	-0.092	0.163	0.099	0.267	0.042
<i>p val</i>	0.61	0.83	0.53	0.53	0.27	0.50	0.07	0.77
GAD1	-0.135	0.046	0.151	-0.064	0.058	0.031	0.219	-0.118
<i>p val</i>	0.36	0.75	0.31	0.67	0.70	0.84	0.14	0.42
GADD45B	-0.124	-0.152	-0.056	0.324	0.263	-0.093	0.012	0.322
<i>p val</i>	0.40	0.30	0.71	0.02	0.07	0.53	0.93	0.03
HDAC2	-0.044	-0.106	-0.194	0.200	0.385	0.248	0.266	0.128
<i>p val</i>	0.77	0.47	0.19	0.17	0.01	0.09	0.07	0.38
HDAC5	-0.101	-0.123	0.026	0.119	0.183	0.025	-0.017	0.225
<i>p val</i>	0.49	0.41	0.86	0.42	0.21	0.87	0.91	0.12
MECP2	-0.028	0.054	0.115	0.089	0.176	0.256	0.283	0.032
<i>p val</i>	0.85	0.71	0.43	0.55	0.23	0.08	0.05	0.83
OPRD1	0.193	-0.231	-0.045	0.339	0.293	0.419	0.047	0.307
<i>p val</i>	0.19	0.11	0.76	0.02	0.04	0.00	0.75	0.03
OPRK1	-0.114	-0.095	0.183	-0.017	0.312	0.003	0.204	0.042
<i>p val</i>	0.44	0.52	0.21	0.91	0.03	0.98	0.16	0.78
OPRM1	-0.031	-0.342	0.145	-0.038	0.343	-0.002	0.094	0.137
<i>p val</i>	0.84	0.02	0.32	0.80	0.02	0.99	0.52	0.35
PDYN	-0.213	-0.047	0.188	-0.028	0.265	-0.066	0.117	0.080
<i>p val</i>	0.15	0.75	0.20	0.85	0.07	0.66	0.43	0.59
PENK	-0.169	-0.008	0.287	-0.004	0.186	-0.058	0.195	-0.028
<i>p val</i>	0.25	0.96	0.05	0.98	0.21	0.69	0.18	0.85
PPP1R1B	-0.135	-0.081	0.307	-0.001	0.210	-0.064	0.169	-0.022
<i>p val</i>	0.36	0.58	0.03	0.99	0.15	0.66	0.25	0.88
SETD7	-0.159	-0.082	0.100	0.253	0.157	0.169	-0.004	0.305
<i>p val</i>	0.28	0.58	0.50	0.08	0.29	0.25	0.98	0.03

Supplemental Information 8

a

LP and CTL	sign track	goal track	-1(fixed ratio)	-1(breakpoint)	-1(%correct)	reaction time	%omitted	%premature
CNR1	0.310	-0.160	-0.345	0.164	0.346	0.455	0.328	0.130
<i>p val</i>	0.10	0.40	0.06	0.39	0.06	0.01	0.08	0.49
COMT	-0.106	-0.298	-0.098	0.293	0.547	0.567	0.567	0.328
<i>p val</i>	0.58	0.11	0.61	0.12	0.00	0.00	0.00	0.08
DNMT1	0.207	-0.077	0.000	0.050	0.261	0.517	0.481	0.001
<i>p val</i>	0.27	0.68	1.00	0.79	0.16	0.00	0.01	0.99
DRD1	-0.173	-0.116	0.204	0.082	0.365	0.000	0.144	0.134
<i>p val</i>	0.36	0.54	0.28	0.67	0.05	1.00	0.45	0.48
DRD2	-0.196	-0.068	0.298	0.125	0.189	-0.037	0.121	0.006
<i>p val</i>	0.30	0.72	0.11	0.51	0.32	0.85	0.52	0.98
EHMT2	0.173	0.036	-0.043	-0.150	0.280	0.242	0.414	0.070
<i>p val</i>	0.36	0.85	0.82	0.43	0.13	0.20	0.02	0.71
GAD1	-0.199	0.006	0.151	0.064	0.130	0.101	0.245	-0.141
<i>p val</i>	0.29	0.97	0.43	0.74	0.49	0.60	0.19	0.46
GADD45B	-0.129	-0.112	-0.075	0.213	0.289	-0.012	0.179	0.201
<i>p val</i>	0.50	0.56	0.69	0.26	0.12	0.95	0.34	0.29
HDAC2	-0.040	-0.193	-0.260	0.285	0.433	0.428	0.441	0.120
<i>p val</i>	0.83	0.31	0.17	0.13	0.02	0.02	0.01	0.53
HDAC5	-0.004	-0.128	-0.025	0.010	0.107	0.281	0.260	0.139
<i>p val</i>	0.98	0.50	0.89	0.96	0.57	0.13	0.17	0.46
MECP2	0.117	-0.005	0.148	-0.142	0.246	0.396	0.578	-0.104
<i>p val</i>	0.54	0.98	0.44	0.45	0.19	0.03	0.00	0.58
OPRD1	0.174	-0.243	-0.199	0.198	0.138	0.459	0.315	0.043
<i>p val</i>	0.36	0.20	0.29	0.29	0.47	0.01	0.09	0.82
OPRK1	-0.192	-0.120	0.166	0.081	0.362	0.046	0.229	0.104
<i>p val</i>	0.31	0.53	0.38	0.67	0.05	0.81	0.22	0.58
OPRM1	-0.078	-0.380	0.122	0.035	0.312	0.038	0.133	0.109
<i>p val</i>	0.68	0.04	0.52	0.86	0.09	0.84	0.48	0.57
PDYN	-0.250	-0.043	0.157	0.023	0.347	-0.076	0.073	0.202
<i>p val</i>	0.18	0.82	0.41	0.90	0.06	0.69	0.70	0.28
PENK	-0.223	-0.014	0.283	0.116	0.260	-0.074	0.100	0.087
<i>p val</i>	0.24	0.94	0.13	0.54	0.16	0.70	0.60	0.65
PPP1R1B	-0.198	-0.089	0.287	0.148	0.279	-0.049	0.112	0.085
<i>p val</i>	0.29	0.64	0.12	0.44	0.14	0.80	0.56	0.66
SETD7	-0.175	-0.081	0.030	0.144	0.148	0.381	0.341	0.051
<i>p val</i>	0.36	0.67	0.87	0.45	0.43	0.04	0.07	0.79

b

HF and CTL	sign track	goal track	-1(fixed ratio)	-1(breakpoint)	-1(%correct)	reaction time	%omitted	%premature
CNR1	0.408	-0.038	-0.262	0.270	0.347	0.377	-0.044	0.221
<i>p val</i>	0.02	0.83	0.13	0.12	0.04	0.03	0.80	0.21
COMT	-0.066	-0.143	0.054	0.307	0.296	0.194	0.053	0.137
<i>p val</i>	0.71	0.42	0.76	0.08	0.09	0.27	0.77	0.44
DNMT1	0.073	0.129	0.118	0.335	0.527	0.463	0.218	0.332
<i>p val</i>	0.68	0.47	0.51	0.05	0.00	0.01	0.22	0.05
DRD1	0.052	-0.450	0.284	-0.034	0.159	0.099	0.054	0.045
<i>p val</i>	0.77	0.01	0.10	0.85	0.37	0.58	0.76	0.80
DRD2	-0.206	-0.266	0.293	-0.219	-0.225	-0.140	0.033	-0.143
<i>p val</i>	0.24	0.13	0.09	0.21	0.20	0.43	0.85	0.42
EHMT2	0.224	0.002	-0.051	0.001	0.162	0.017	0.150	0.056
<i>p val</i>	0.20	0.99	0.77	1.00	0.36	0.92	0.40	0.75
GAD1	-0.189	-0.104	0.136	-0.185	-0.140	-0.090	0.041	-0.060
<i>p val</i>	0.29	0.56	0.44	0.29	0.43	0.61	0.82	0.73
GADD45B	0.018	-0.260	-0.122	0.390	0.306	-0.062	-0.107	0.432
<i>p val</i>	0.92	0.14	0.49	0.02	0.08	0.73	0.55	0.01
HDAC2	0.090	-0.002	-0.261	0.159	0.517	0.208	0.175	0.159
<i>p val</i>	0.61	0.99	0.14	0.37	0.00	0.24	0.32	0.37
HDAC5	-0.095	-0.177	0.131	0.248	0.364	0.001	-0.022	0.246
<i>p val</i>	0.59	0.32	0.46	0.16	0.03	1.00	0.90	0.16
MECP2	-0.016	0.055	0.102	0.171	0.219	0.233	0.240	0.049
<i>p val</i>	0.93	0.76	0.57	0.33	0.21	0.19	0.17	0.78
OPRD1	0.354	-0.175	0.070	0.455	0.598	0.410	0.015	0.390
<i>p val</i>	0.04	0.32	0.70	0.01	0.00	0.02	0.93	0.02
OPRK1	0.085	-0.395	0.105	-0.122	0.095	-0.003	0.007	0.010
<i>p val</i>	0.63	0.02	0.55	0.49	0.59	0.99	0.97	0.96
OPRM1	0.133	-0.540	0.119	-0.031	0.330	0.087	0.034	0.134
<i>p val</i>	0.45	0.00	0.50	0.86	0.06	0.63	0.85	0.45
PDYN	-0.126	-0.432	0.206	-0.016	-0.065	0.016	-0.039	0.012
<i>p val</i>	0.48	0.01	0.24	0.93	0.71	0.93	0.83	0.94
PENK	-0.201	-0.306	0.328	-0.091	-0.147	-0.032	0.047	-0.026
<i>p val</i>	0.25	0.08	0.06	0.61	0.41	0.86	0.79	0.88
PPP1R1B	-0.111	-0.439	0.345	-0.105	-0.013	-0.048	-0.037	0.001
<i>p val</i>	0.53	0.01	0.05	0.55	0.94	0.79	0.84	0.99
SETD7	-0.039	-0.054	0.122	0.270	0.241	0.079	-0.080	0.349
<i>p val</i>	0.83	0.76	0.49	0.12	0.17	0.66	0.65	0.04