Data Supplement

Behavioural and molecular characterisation of the Dlg2 haploinsufficiency rat model of genetic risk for psychiatric disorder

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Running title

SUPPLEMENTAL DATA – SEX EFFECTS IN THE CHARACTERISATION OF THE DLG2 +/- RAT

Supplementary Results

S1 Example Western blot
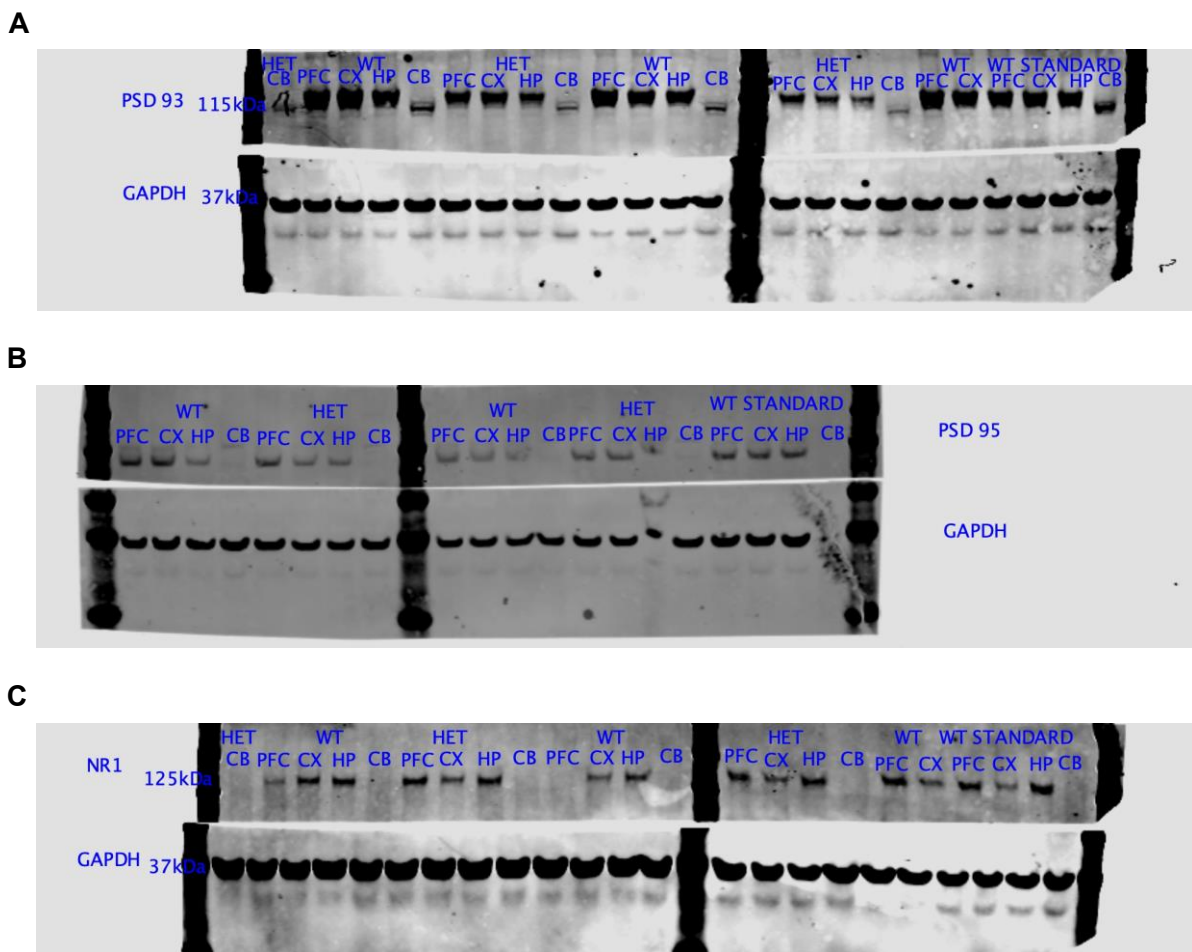
A

B

C

Figure S1: Sample blots showing the same samples and brain regions for PSD93 (A), PSD95 (B) and NR1 (C). Lack of bands for NR1 cerebellum shown.

S2 Sex effects in the characterisation of the *Dlg2+/-* rat

The use of male-only cohorts when investigating psychiatric risk with rodent models is prevalent in the literature but this may result in conclusions that overlook sex differences in how these risk factors operate. Thus, the current experimental work used both male and female animals to allow sex differences to be investigated – however, as noted in the main text, no sex by genotype interactions were observed for any of the outcome measures considered. Thus, the main text report focused on genotype effects regardless of sex, while here we report the full results of the analyses including sex as a variable (oestrus stage was included as a variable initially – but had no effects so was not reported here). These support the conclusion that the current results are consistent with the *Dlg2+/-* manipulation being similar (or similarly absent) in male and female rats.

S2.1 Behaviour on anxiety tests

<u>S2.1.1 Elevated Plus Maze</u>

As shown in Figure S1 sex had no effect on any measures recorded in the EPM including time in closed and open arms (Figure S2A), head dips (Figure S2B), stretch-attend postures (Figure S2C), grooming (Figure S2D), distance travelled (Figure S2E), velocity (Figure S2G) or defecation (Figure S2F). All main effects and interactions (along with Bayes factors providing evidence for the null effects) in the EPM assays can be seen in Table S1.
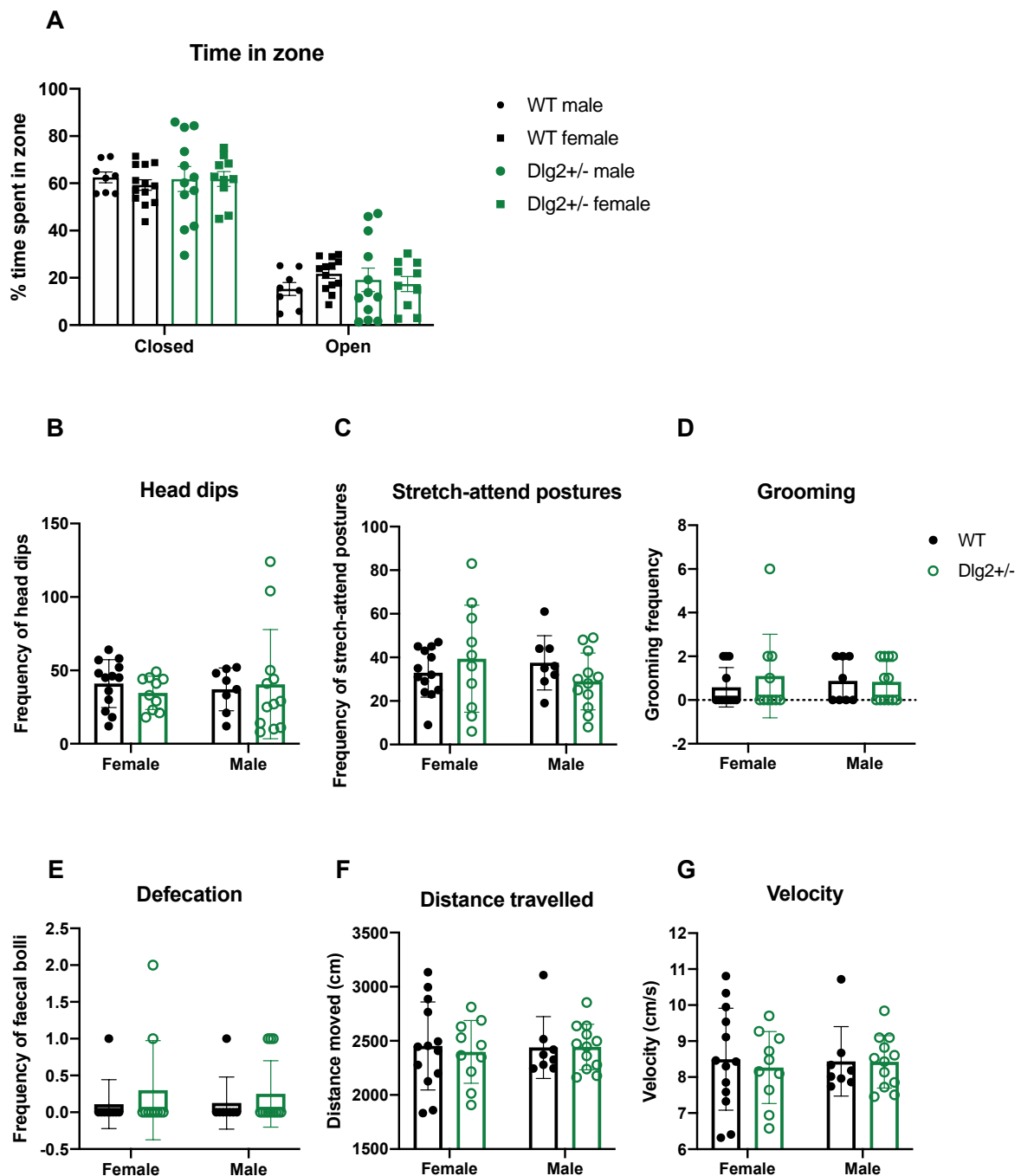
Figure S2: Effect of *Dlg2* heterozygous knockout and sex on anxiety-related behaviour in the EPM. Mean ± SEM with data points representing individuals **A)** time in zone **B)** head dips **C)** stretch-attend postures **D)** grooming **E)** defecation **F)** distance moved **G)** velocity.
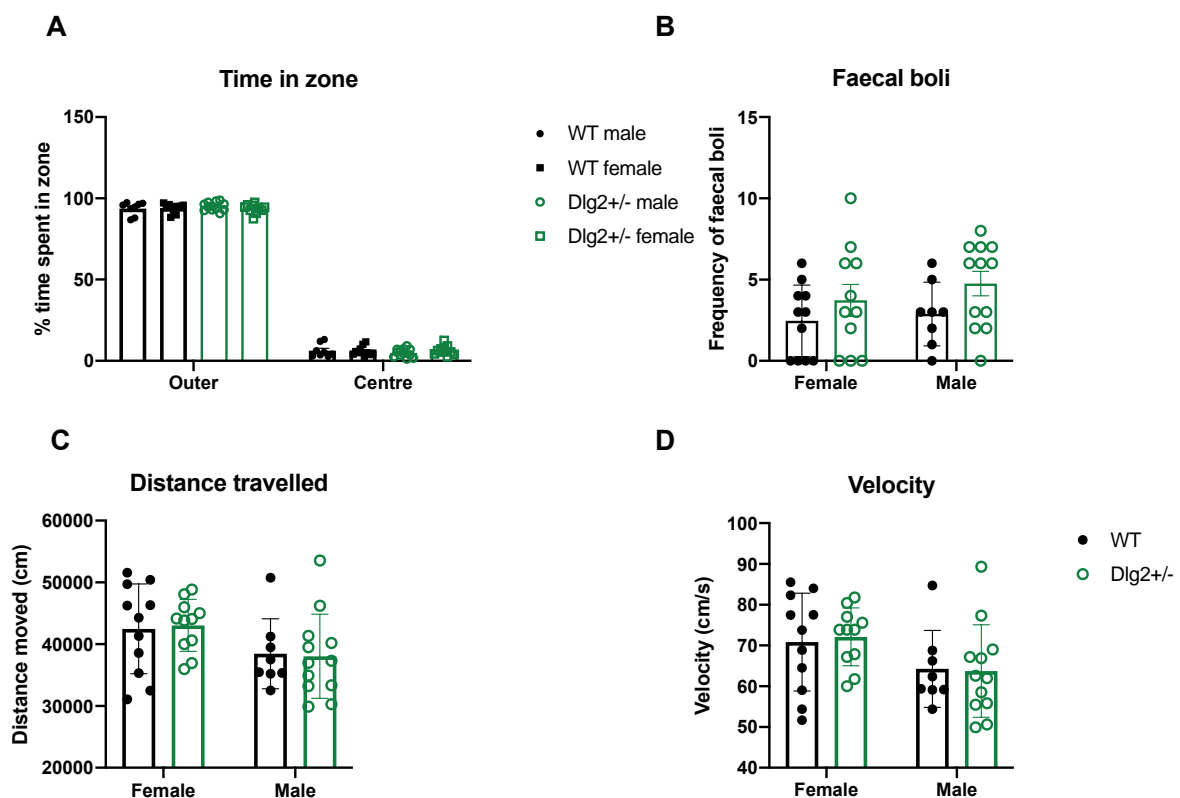
Table S1: Repeated measures ANOVA and Bayesian ANOVA inferential statistics for analyses including sex as a factor on EPM measures.

Analysis	Effect	F	p	n^2_p	BF$_{exclusion}$
Time in zone	Zone main effect	(1.347, 52.518) = 158.103	< 0.001	0.802	0.000
	Genotype main effect	(1,39) = 1.275	0.266	0.032	11.197
	Sex main effect	(1,39) = 1.497	0.228	0.037	10.610
	Genotype × sex	(1,39) = 0.261	0.612	0.007	40.913
	Genotype × zone	(1.347,52.518) = 0.052	0.887	0.001	17.680
	Zone × sex	(1.347,52.518) = 0.277	0.670	0.007	14.764
	Zone × genotype × sex	(1.347,52.518) = 0.843	0.395	0.021	539.677
Head dips	Genotype main effect	(1,33) = 0.034	0.854	0.001	4.266
	Sex main effect	(1,33) = 0.054	0.818	0.002	4.222
	Genotype × sex	(1,33) = 0.107	0.746	0.003	9.677
Stretch attend postures	Genotype main effect	(1,33) = 0.190	0.666	0.006	3.528
	Sex main effect	(1,33) = 0.088	0.769	0.003	3.578
	Genotype × sex	(1,33) = 1.493	0.230	0.043	5.343
Grooming	Genotype main effect	(1,33) = 0.002	0.961	0.000	3.816
	Sex main effect	(1,33) = 0.001	0.970	0.000	3.825
	Genotype × sex	(1,33) = 0.748	0.393	0.022	7.897
Distance travelled	Genotype main effect	(1,33) = 0.082	0.776	0.002	4.182
	Sex main effect	(1,33) = 0.092	0.764	0.003	4.170

	Genotype × sex	(1,33) = 0.003	0.995	0.000	10.242
Velocity	Genotype main effect	(1,33) = 0.082	0.776	0.002	4.151
	Sex main effect	(1,33) = 0.092	0.763	0.003	4.139
	Genotype × sex	(1,33) = 0.003	0.995	0.000	9.743
Defecation	Genotype main effect	(1,39) = 1.505	0.227	0.039	2.471
	Sex main effect	(1,39) = 0.00000460	0.995	0.002	3.970
	Genotype × sex	(1,39) = 0.120	0.731	0.008	5.940

S2.1.2 Open Field

Sex also had no effect on open-field hyperactivity and anxiety measures as shown in Figure S2, including time in centre and peripheral zones (Figure S3A), velocity (Figure S3B), distance travelled (Figure S3C) and defecation (Figure S3D). Inferential statistics including sex for these measures can be found in Table S2, which shows significant sex effects for distance travelled and velocity in the open field yet no sex × genotype interactions.

A

Time in zone

B

Faecal boli

- ● WT male
- ■ WT female
- ○ Dlg2+/- male
- ▫ Dlg2+/- female

C

Distance travelled

D

Velocity

- ● WT
- ○ Dlg2+/-

Figure S3: Effect of *Dlg2* heterozygous knockout and sex on open-field measures A) time in zone **B)** defection **C)** distance travelled and **D)** velocity. Mean ± SEM with data points representing individuals.
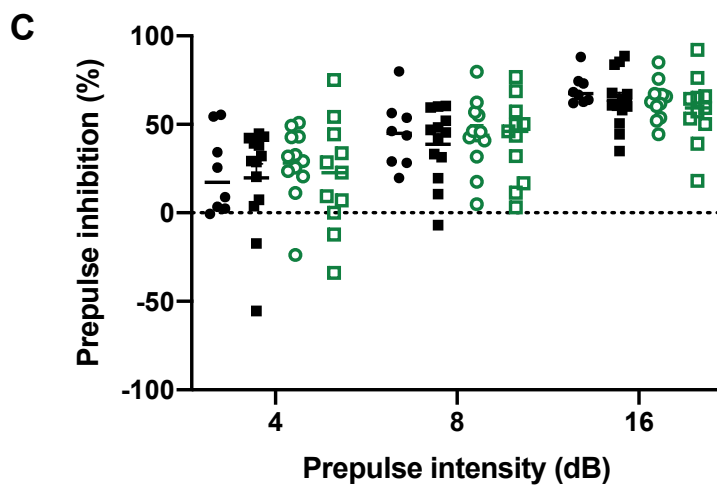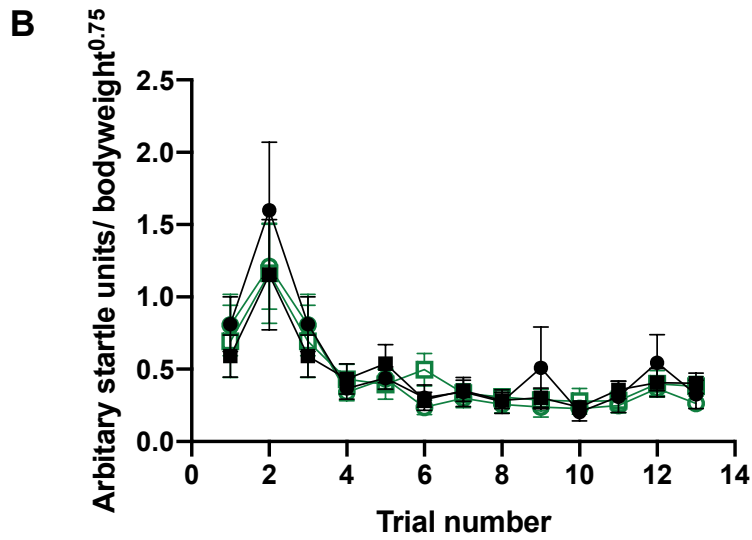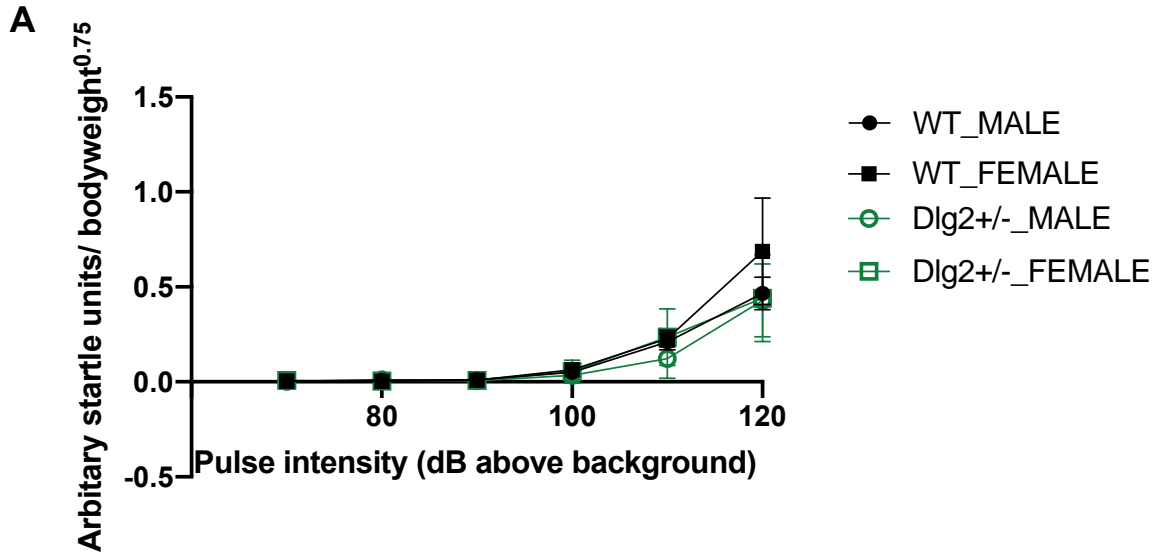
Table S2: Repeated measures ANOVA and Bayesian ANOVA inferential statistics for sex effects and interactions on open field measures.

Analysis	Effect	F	p	n^2_p	BF$_{exclusion}$
Time in zone	Zone main effect	(1,38) = 9100.383	< 0.001	0.996	0.000
	Genotype main effect	(1,38) = 0.697	0.409	0.018	7.292
	Sex main effect	(1,38) = 0.222	0.641	0.006	7.169
	Genotype × sex	(1,38) = 0.095	0.759	0.003	19.332
	Zone × genotype	(1,38) = 0.232	0.633	0.000	6.064
	Zone × sex	(1,38) = 0.362	0.551	0.000	4.372
	Zone × genotype × sex	(1,38) = 0.973	0.330	0.000	42.124
Velocity	Genotype main effect	(1,38) = 0.013	0.908	0.000	3.753
	Sex main effect	(1,38) = 5.481	0.025	0.126	0.453
	Genotype × sex	(1,38) = 0.083	0.775	0.002	3.462
Distance travelled	Genotype main effect	(1,38) = 0.002	0.961	0.000	3.551
	Sex main effect	(1,38) = 5.526	0.024	0.127	0.434
	Genotype × sex	(1,38) = 0.061	0.806	0.002	3.853
Defecation	Genotype main effect	(1,38) = 3.769	0.060	0.090	0.880
	Sex main effect	(1,38) = 0.792	0.379	0.020	2.601
	Genotype × sex	(1,38) = 0.138	0.712	0.004	3.300

S2.2 Sensorimotor gating

Rats of different sexes performed comparably on tests of startle to increasing auditory stimuli (Figure S4A), habituation of startle response at 105 dB (Figure S4B) and 120 dB (Figure S4C) and pre-pulse inhibition at 105 dB (Figure S4D) and 120 dB (Figure S4E). All main effects and interactions (along with Bayes factors providing evidence for the null effects) in the EPM assays can be seen in Table S3.

Figure S4: Effect of *Dlg2* heterozygosity and sex on acoustic startle response and pre-pulse inhibition. A) Mean ± SEM weight-adjusted ASR to 70-120 dB pulses above background.
 B) Habituation of startle response through increasing pulse trials. Mean ± SEM ASR shown at 120 dB and **C)** Mean ± SEM with data points representing individuals PPI by a 4, 8 and 16 dB (above background) pre-pulse on 120 dB pulse.

Table S3: Repeated measures ANOVA and Bayesian ANOVA inferential statistics for sex effects and interactions on sensorimotor gating measures.

| Analysis	Effect	F	p	$n^2{}_p$	BF$_{exclusion}$
Weight-adjusted ASR to 70-120 dB pulses above background	Pulse main effect	(1.089, 43.542) = 29.705	< 0.001	0.426	< 0.0001
	Genotype main effect	(1, 40) = 0.872	0.356	0.021	8.964
	Sex main effect	(1, 40) = 0.858	0.360	0.021	9.573
	Genotype × sex	(1, 40) = 0.056	0.815	0.001	38.490
	Genotype × pulse	(1.089, 43.542) = 0.590	0.460	0.015	22.407
	Sex × pulse	(1.089, 43.542) = 0.450	0.522	0.011	37.906
	Genotype × sex × pulse	(1.089, 43.542) = 0.485	0.505	0.012	9759.370
Habituation of startle response (105 dB)	Trial main effect	(1.573, 62.934) = 6.058	0.007	0.132	< 0.0001
	Sex main effect	(1, 40) = 2.127	0.153	0.050	4.607
	Genotype main effect	(1, 40) = 1.497	0.228	0.036	6.416
	Genotype × sex	(1, 40) = 0.056	0.813	0.001	18.698
	Trial × genotype	(1.573, 62.934) = 0.519	0.555	0.013	127.064
	Trial × sex	(1.573, 62.934) = 0.681	0.476	0.017	102.095

	Trial × sex × genotype	(1.573, 62.934) = 1.247	0.288	0.030	63776.307
Habituation of startle response (120 dB)	Trial main effect	(2.130, 85.190) = 13.127	< 0.001	0.247	< 0.0001
	Sex main effect	(1, 40) = 0.012	0.913	0.000	10.694
	Genotype main effect	(1, 40) = 0.347	0.559	0.009	10.545
	Genotype × sex	(1, 40) = 0.072	0.790	0.002	32.699
	Trial × genotype	(2.130, 85.190) = 0.498	0.621	0.012	643.328
	Trial × sex	(2.130, 85.190) = 1.160	0.320	0.028	174.990
	Trial × sex × genotype	(2.130, 85.190) = 0.543	0.594	0.013	5964000
PPI 105 dB	Pre-pulse main effect	(1.380, 55.210) = 42.203	< 0.001	0.513	< 0.0001
	Sex main effect	(1, 40) = 1.495	0.229	0.036	6.523
	Genotype main effect	(1, 40) = 0.696	0.409	0.017	3.437
	Genotype × sex	(1, 40) = 1.106	0.299	0.027	8.717
	Pre-pulse × genotype	(1.380, 55.210) = 0.314	0.650	0.008	3.713
	Pre-pulse × sex	(1.380, 55.210) = 0.104	0.827	0.003	7.207
	Pre-pulse × genotype × sex	(1.380, 55.210) = 1.952	0.163	0.047	87.336
PPI 120 dB	Pre-pulse main effect	(2, 80) = 83.401	< 0.001	0.676	< 0.0001
	Sex main effect	(1, 40) = 0.902	0.348	0.022	4.447
	Genotype main effect	(1, 40) = 0.011	0.917	0.000	5.482

	Genotype × sex	(1, 40) = 0.0001929	0.989	0.000	9.240
	Pre-pulse × sex	(2, 80) = 0.030	0.970	0.001	10.064
	Pre-pulse × genotype	(2, 80) = 1.097	0.339	0.027	5.712
	Pre-pulse × genotype × sex	(2, 80) = 0.177	0.838	0.004	206.171

S2.3 Social preference

There were no sex effects on exploration in the social preference task as seen for raw exploration time (Figure S5A) and discrimination ratio (Figure S5B). All main effects and interactions (along with Bayes factors providing evidence for the null effects) in the EPM assays can be seen in Table S4.
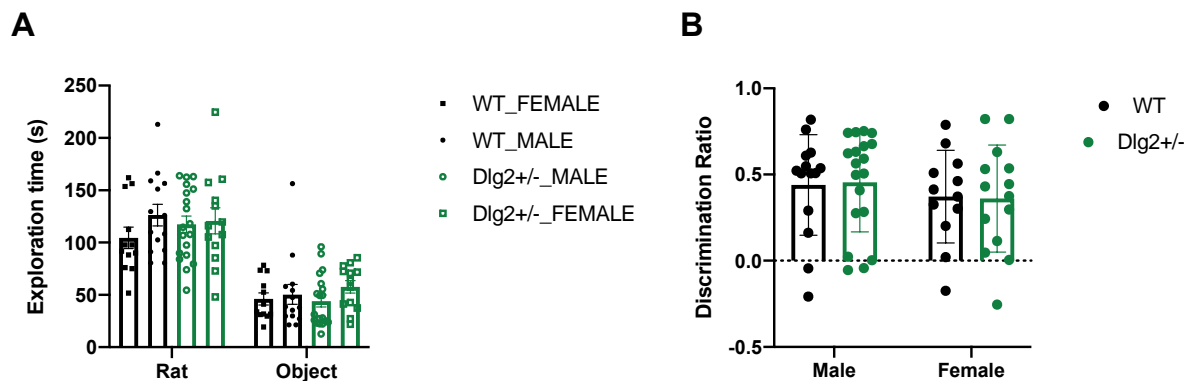

Figure S5: Effect of *Dlg2* heterozygosity and sex on the social preference task. Mean and SEM ± with data points representing individuals **A)** raw exploration and **B)** d2 discrimination ratios.

Table S4: Repeated measures ANOVA and Bayesian ANOVA inferential statistics for sex effects and interactions on social preference raw exploration and d2 scores.

Analysis	Effect	F	p	$n^2{}_p$	BF$_{exclusion}$
Raw exploration time (s)	Item main effect	$(1, 50) = 76.012$	< 0.001	0.603	0.000
	Genotype main effect	$(1, 50) = 0.014$	0.907	0.000279	7.986
	Sex main effect	$(1, 50) = 0.108$	0.744	0.002	6.845
	Genotype × item	$(1, 50) = 0.479$	0.492	0.009	7.678
	Genotype × sex	$(1, 50) = 1.737$	0.193	0.034	9.851
	Item × sex	$(1, 50) = 0.391$	0.535	0.008	3.923
	Item × genotype × sex	$(1, 50) = 0.003$	0.957	0.0000577	46.918
Discrimination ratio	Genotype main effect	$(1, 50) = 0.850$	0.361	0.017	4.973
	Sex main effect	$(1, 50) = 0.894$	0.349	0.018	2.451
	Genotype × sex	$(1, 50) = 0.337$	0.564	0.007	8.268

S2.4 PCP-induced locomotion

In the pre-injection habituation period females habituated faster than males (significant time bin × sex interaction $F_{(2, 98)} = 3.903$), $p = 0.023$, $n^2_p = 0.074$) and females travelled less distance overall than males (sex main effect: $F_{(1, 49)} = 8.302$), $p = 0.006$, $n^2_p = 0.145$) however sex × genotype and sex × genotype × time bin interactions were non-significant as shown in Table S5. There were no sex effects in response to 5 mg/kg PCP with all sex effects and interactions non-significant as shown in Table S6.
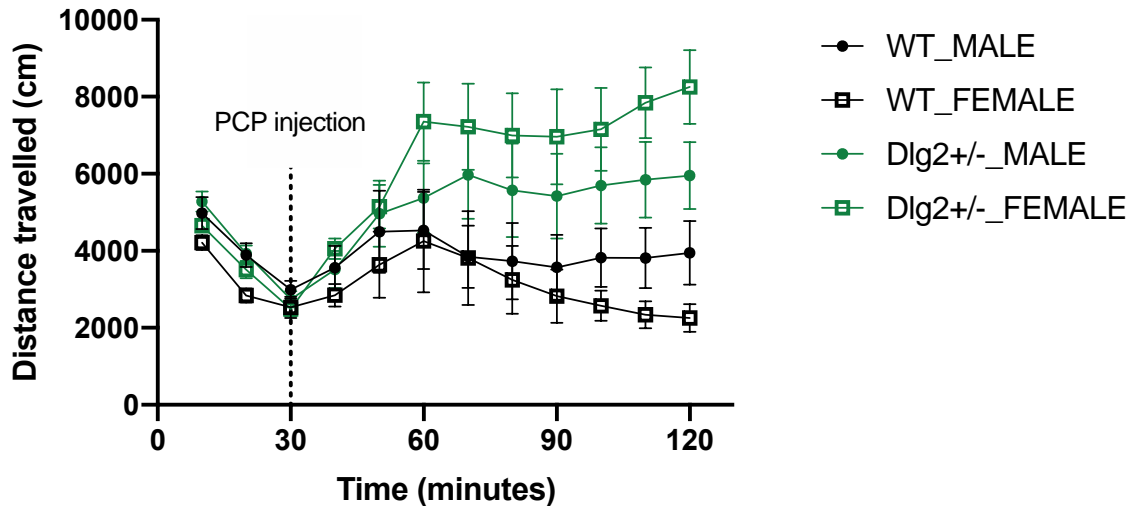
Figure S6: Locomotor activity in response to PCP injection in male and female *Dlg2$^{+/-}$* and wild-type rats. Mean ± SEM distance travelled is plotted in 10-minute bins. The dotted line at 30 minutes denotes when the PCP injection occurred.

Table S5: Sex effects and interactions from repeated measures ANOVA and Bayesian repeated measures ANOVA of distance moved over the 30-minute pre-injection habituation period.

Effect	F	p	n^2_p	BF$_{exclusion}$
Time bin main effect	$(2, 98) =$ 227.098	< 0.001	0.823	< 0.001
Sex main effect	$(1, 49) =$ 8.302	0.006	0.145	0.064
Genotype main effect	$(1, 49) =$ 0.452	0.505	0.009	2.165
Genotype × sex	$(2, 98) =$ 1.077	0.304	0.022	1.596
Time bin × sex	$(2, 98) =$ 3.903	0.023	0.074	0.153
Time bin × genotype	$(2, 98) =$ 2.516	0.086	0.049	1.243
Time bin × sex × genotype	$(2, 98) =$ 0.298	0.743	0.006	4.156

Table S6: Sex effects and interactions from repeated measures ANOVA and Bayesian repeated measures ANOVA of distance moved over the 90-minute post-injection period.

Effect	F	p	n^2_p	BF$_{exclusion}$
Time bin main effect	(2.829, 141.460) = 4.044	0.010	0.075	< 0.001
Sex main effect	(1, 50) = 0.183	0.670	0.004	3.328
Genotype main effect	(1, 50) = 9.873	0.003	0.165	< 0.001
Genotype × sex	(2.829, 141.460) = 2.124	0.151	0.041	1.691
Time bin × sex	(2.829, 141.460) = 0.714	0.537	0.014	57.096
Time bin × genotype	(2.829, 141.460) = 5.125	0.003	0.093	< 0.001
Time bin × sex × genotype	(2.829, 141.460) = 0.786	0.497	0.015	305.148