Supplemental Information for Sittig et al.

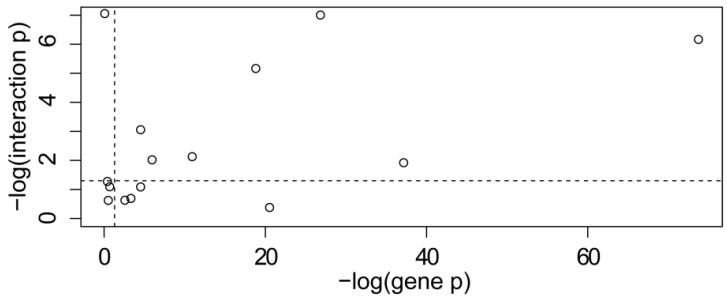

Figure S1. Main and interactive effects of *Cacna1c* and *Tcf7l2* null alleles. Related to Table 1. Phenotypic variance explained by null allele genotype (+/- vs. +/+) (x axis) and the interaction between genotype x genetic background (y-axis) for the 15 measured phenotypes. Dotted lines represent p=0.05.

Table S1. Maternal inbred mouse strains and numbers of phenotyped F_1 offspring. Related to Experimental Procedures.

Strain	**Short ID**	**JAX Stock**	***Cacna1c* +/+ M**	***Cacna1c* +/+ F**	***Cacna1c* +/- M**	***Cacna1c* +/- F**	***Tcf7l2* +/+ M**	***Tcf7l2* +/+ F**	***Tcf7l2* +/- M**	***Tcf7l2* +/- F**
129S1/SvImJ	129	002448	5	6	5	7	5	6	5	6
A/J	AJ	000646	7	6	6	6	4	3	4	6
AKR/J	AKR	000648	5	6	5	5	2	4	1	4
BALB/cByJ	BALB	001026	6	6	6	6	5	4	4	5
BTBR T+ Itpr3tf/J	BTBR	002282	6	5	6	7	4	4	5	6
BUB/BnJ	BUB	000653	5	4	5	5	5	7	5	5
C3H/HeJ	C3H	000659	5	5	5	6	4	4	5	7
C57BL/6J	B6	000664	16	17	15	15	9	8	9	9
C57BLKS/J	BKS	000662	6	6	6	6	6	5	5	5
C57L/J	57L	000668	-	-	-	-	5	7	5	4
C58/J	C58	000669	5	5	6	6	4	6	4	4
CBA/J	CBA	000656	8	6	5	5	4	5	6	4
DBA/2J	DBA	000671	5	6	6	5	5	5	5	5
DDY/JclSidSeyFrkJ	DDY	002243	7	6	5	6	5	4	5	5
FVB/NJ	FVB	001800	6	5	6	6	7	5	7	4
I/LnJ	ILN	000674	6	4	6	6	6	6	9	4
KK/HlJ	KK	002106	5	6	6	6	5	4	3	6
LP/J	LP	000676	5	6	5	5	6	3	2	6
LG/J	LG	000675	7	6	6	6	-	-	-	-
MA/MyJ	MA	000677	6	6	6	6	5	6	5	5
MRL/MpJ	MRL	000486	6	6	6	6	5	7	5	5
NOD/ShiLtJ	NOD	001976	6	7	6	6	6	5	7	6
NON/ShiLtJ	NON	002423	4	5	6	5	5	4	8	7
NZB/BlNJ	NZB	000684	6	6	5	6	4	5	9	8
NZW/LacJ	NZW	001058	5	6	5	5	6	4	5	4
PL/J	PL	000680	5	5	6	5	6	5	4	6
RIIIS/J	RII	000683	6	6	6	6	8	5	4	6
SEA/GnJ	SEA	000644	5	6	6	6	7	9	7	5
SJL/J	SJL	000686	6	6	6	6	4	6	4	6
SM/J	SM	000687	6	6	6	5	6	4	5	5
SWR/J	SWR	000689	6	6	3	6	4	4	4	5

Table S2. Phenotyping schedule. Related to Experimental Procedures.

Week of Testing	Test	Phenotype	Construct
***Cacna1c* cohort**			
1	Light-dark box	Percent time in the light box	Anxiety-like behavior
2	Open field test + methamphetamine sensitivity	Exploratory activity	Exploratory activity
		Duration in center	Anxiety-like behavior
		Methamphetamine sensitivity	Methamphetamine sensitivity
3	Forced Swim test	Duration of Immobility	Depression-like behavior
4	Sensorimotor gating	Acoustic startle response	Acoustic startle response
***Tcf7l2* cohort**			
1	Body weight	Body weight	Body weight
	Open field test	Exploratory activity	Exploratory activity
		Duration in center	Anxiety-like behavior
2	Fear conditioning	Contextual fear learning	Contextual fear learning
		Cued fear learning	Cued fear learning
3	Sensorimotor gating	Acoustic startle response	Acoustic startle response
		Prepulse inhibition	Sensorimotor gating
5	Fasted glucose	Fasted blood glucose levels	Fasted blood glucose levels
6	Baseline glucose	Baseline blood glucose levels	Baseline blood glucose levels

Table S3. Related to Table 1 and Figure 2. See attached excel spreadsheet.

Supplemental Experimental Procedures

Null alleles and genotyping

Cacna1c +/- mice were developed by Deltagen (San Mateo, CA) and were obtained from the Jackson Laboratory (B6.129P2-Cacna1c^{tm1Dgen}/J, Strain 005783, RRID: MGI:3606691). Genotyping was performed by PCR amplification of genomic DNA followed by gel electrophoresis to distinguish the presence of a common 400 bp product and knockout-specific 650 bp product. The PCR primers were: a common sense primer (5' TCTCTCCCACCTCGCACGCCGAATC 3'), a wild-type specific anti-sense primer (5'-CACGACTGGCCTCTACTGCTCTTGAC-3') and a knockout-specific anti-sense primer (5'-GACGAGTTCTTCTGAGGGGATCGATC-3').

A null mutation in *Tcf7l2* was engineered using a zinc finger nuclease (ZFN) construct targeting a constitutive exon encoding the high mobility group box DNA-binding domain (Savic et al. 2011). The ZFN construct was prepared courtesy of Dr. Marcelo Nobrega as previously described (Savic et al. 2011) and microinjections were performed at the University of Chicago Transgenics/ES Cell Technology Mouse Core Facility. We obtained a mutant line on a C57BL/6J background with a 10 bp frameshift-inducing deletion. The founder male was backcrossed to C57BL/6J for one generation before generating F_1 crosses. Genotyping was performed by PCR amplification of genomic DNA followed by gel electrophoresis to distinguish the presence of the shorter allele in heterozygotes. The PCR primers flanking the ZFN targeted region were: (5′-AGCAAAGCATCGAGTCTCATTT-3′, 5′-AAGCAGGTAGTAAGCCATCCTCT-3′).

Phenotyping

Light/dark Box

Light/dark boxes were white plastic testing chambers (40 x 40 x 30 cm) containing a black plastic box insert (20 x 20 x 30 cm). The light side (opposite the black insert) was open at the top and was illuminated with bright fluorescent light (~500 lux). The black insert had a removable lid that blocked all light from entering. A small doorway located on the insert wall facing the light side allowed mice to freely pass between the light and dark side of the chamber. Mice were placed in the light side of the chamber and their behavior was video recorded for 5 min. Noldus Ethovision XT v. 5.1 software (Noldus Information Technology, Lessburg, VA, USA) was used to record and automatically score behavior.

Open Field Test

Activity was measured using automated chambers (40 x 40 x 30 cm) as described previously (Bryant et al. 2009; Sittig et al. 2014). Activity chambers (Versamax, AccuScan Instruments, Columbus, OH, USA) were housed within sound-attenuating chambers with dim 80 lux overhead lighting and a rear wall fan masking background noise. Mice were placed in chambers and allowed to freely explore for 30 min before being removed from the chambers. Positional data were automatically converted into the distance traveled by the software, which monitored infrared beam breaks within the chamber. The center region was defined as the inner 20 x 20 cm of the chamber. Exploratory activity was total distance travelled during the test.

Open Field Test + Methamphetamine Sensitivity

Methamphetamine sensitivity was assessed in a 3-day test that was given at the same time each day (Bryant et al. 2009). The equipment and behavioral monitoring software were the same as for the Open Field Test (above). Mice were transported from the vivarium to the testing room and were allowed at least 30 min to habituate in their home cages. On the first and second days of testing, mice were removed from their home cages, weighed, and placed in individual holding cages with clean bedding. Mice then received an i.p. injection of sterile 0.9% saline solution and were immediately placed in individual activity chambers where locomotor activity was monitored for 30 min. On the third day of testing, mice received i.p. injections of 2 mg/kg methamphetamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) dissolved in sterile 0.9% saline solution. This dose has been used to assess the acute locomotor response to methamphetamine (Bryant et al. 2009), and in particular, that of *Cacna1c* +/- mice (Dao et al. 2010). All injections were administered in a volume of 10 ml/kg body weight. Following the appropriate treatment mice were immediately placed in the activity chambers and their locomotor activity was monitored for 30 min. Exploratory activity and duration in center phenotypes were assessed on day 1. The methamphetamine sensitivity phenotype was exploratory activity on day 3 minus the exploratory activity on day 2.

Fear Conditioning

Fear conditioning was carried out in a three day paradigm identical to that described previously (Ponder et al. 2007). Chambers (29 cm x 19 cm x 25 cm with metal walls, clear plastic front and back walls, and stainless steel bars on the floor) were manufactured by Med Associates (St. Albans, VT, USA). Illumination inside the chamber was ~3 lux and background noise was provided by a fan. Behavior was recorded with digital video and FreezeFrame software by Actimetrics (Evanston, IL, USA) was used to analyze freezing. A 5-min trial occurred on each day of testing at the same time each day. On day 1, mice were conditioned to associate a test chamber and a tone with a shock by being placed into the chamber and being exposed 180 s later to two conditioned

stimuli consisting of an 85 dB, 3 kHz tone. The tone persisted for 30 s and co-terminated with the unconditioned stimulus, a 2 s, 0.5 mA foot shock. On day 2, mice were re-exposed to the same context but no tones or shocks were given. On day 3, mice were exposed to the conditioned stimulus in an altered context: the metal shock grid, chamber door and one wall were covered with white plastic; yellow film was placed over the overhead light; 0.1% acetic acid solution was used to clean the grid between mice; the fan was partially obstructed to change noise level; the holding cages contained no bedding; and the experimenter was a different individual who wore gloves of a different material. The fear phenotypes consisted of two measurements of immobility: average proportion of time spent freezing in the 30-180 s interval on day 2, and average proportion of time spent freezing to the tone during the 180-210 s and 240-270 s intervals on day 3.

Forced Swim Test

White polyethylene buckets (15.2 cm depth x 20.3 cm diameter) were filled with 25° C water to 3.8 cm below the top of the bucket. Mice were placed in the water for 6 min. The water was maintained at 23-25°C (Porsolt et al. 1977)(Lucki et al. 2001). The last 4 min of the trial was used to score duration of immobility which was defined as the number of seconds spent with a mobility threshold of <2% movement between frames. Behavior was recorded and automatically scored by Noldus Ethovision XT v. 5.1 software (Noldus Information Technology, Lessburg, VA, USA).

Sensorimotor Gating

The apparatus, testing procedures, and data analysis were conducted as previously described (Samocha et al. 2010; Sittig et al. 2016). Animals were placed in a 5 cm diameter Plexiglas cylinder on a platform contained within a lighted, ventilated chamber (San Diego Instruments, San Diego, CA, USA). The cylinder was connected to a piezoelectric accelerometer which measured the mouse's startle responses. Mice experienced 5 min of 70 dB white noise followed by 62 trials which occurred with the 70 dB noise in the background. Testing consisted of pulse-alone trials (40 ms, 120 dB burst); no-stimulus trials; and prepulse trials (20 ms prepulse, 3, 6, or 12 dB above background noise) followed 100 ms later by a 40 ms, 120 db pulse. Trials were arranged into four blocks. Blocks 1 and 4 were pulse alone trials; blocks 2 and 3 contained pseudo-random combinations of pulse alone, no stimulus, and each type of prepulse trial (3, 6, and 12 dB). Responses were recorded for 65 ms after the beginning of the 120 dB stimulus. The inter-trial interval was 9-20 s (average 15 s) throughout the test. The prepulse inhibition (PPI) phenotype was defined as the difference of the average startle amplitude during the 6-db prepulse trials to the average startle amplitude during the pulse-alone trials, normalized by the pulse-alone amplitude: Prepulse inhibition = (SApulse – SAprepulse) / SApulse. SApulse (acoustic startle response) was the average startle amplitude measured in the pulse-alone trials in testing blocks 2 and 3.

Physiological Phenotypes

Blood glucose levels were measured with a Contour TS glucometer (Bayer, Pittsburgh, PA, USA) between 0900-1600 h. Fasting glucose levels were measured between 0900-1000 h after lab chow had been removed from the cage for 16 h overnight.

Supplemental References

Bryant, C.D. et al., 2009. A role for casein kinase 1 epsilon in the locomotor stimulant response to methamphetamine. *Psychopharmacology*, 203, pp.703–711.

Dao, D.T. et al., 2010. Mood disorder susceptibility gene CACNA1C modifies mood-related behaviors in mice and interacts with sex to influence behavior in mice and diagnosis in humans. *Biological psychiatry*, 68(9), pp.801–10.

Lucki, I., Dalvi, A. & Mayorga, A.J., 2001. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. *Psychopharmacology*, 155(3), pp.315–22.

Ponder, C.A. et al., 2007. Selection for contextual fear conditioning affects anxiety-like behaviors and gene expression. *Genes, brain, and behavior*, 6(8), pp.736–49.

Porsolt, R.D., Bertin, A. & Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. *Archives internationales de pharmacodynamie et de thérapie*, 229(2), pp.327–36.

Samocha, K.E. et al., 2010. Fine mapping of QTL for prepulse inhibition in LG/J and SM/J mice using F2 and advanced intercross lines. *Genes, Brain and Behavior*, 9(7), pp.759–767.

Savic, D. et al., 2011. Alterations in TCF7L2 expression define its role as a key regulator of glucose metabolism. *Genome research*, 21(9), pp.1417–25.

Sittig, L.J. et al., 2016. Integration of genome-wide association and extant brain expression QTL identifies candidate genes influencing prepulse inhibition in inbred F1 mice. *Genes, brain, and behavior*, 15(2), pp.260–70.

Sittig, L.J. et al., 2014. Phenotypic instability between the near isogenic substrains BALB/cJ and BALB/cByJ. *Mammalian genome : official journal of the International Mammalian Genome Society*, 25(11-12), pp.564–72.