Supporting Information

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Materials and Methods

Mice. Dominant-negative disrupted in schizophrenia 1 (DN-DISC1) mice express a putative dominant-negative C-terminal truncated *DISC1* under the control of the alpha calcium/calmodulin-dependent kinase II (αCaMKII) promoter. To enhance the phenotype of these mice, we examined homozygotes. However, when using PCR (even semiquantitative PCR) to genotype transgenic mice, we can reliably distinguish only whether the mouse has the transgene (i.e., is either heterozygous or homozygous) or does not (i.e., is wild type). To have a homogeneous population to study with full certainty, rather than a possible mix of heterozygous and homozygous mice, we created a homozygous line by systematic breeding of heterozygotes and testing their homozygosity by breeding with wild-type mice (all offspring must be transgenic). Once the homozygous line is generated, the only way to maintain homozygosity is by homozygous inbreeding. In adopting this approach of homozygous inbreeding, we do not have littermate controls. Thus, as controls for the present experiments we used wild types obtained by breeding heterozygotes with wild types in the same mouse room where we maintain the homozygous population (WT) and C57BL/6N mice from Charles River (C57). We acknowledge that theoretically there may be a risk of non–*DISC1*-relevant mutations accumulating in the inbred homozygous line and contributing to the phenotype; however, we believe that this risk is minimal in the present study because of our conducting two sets of experiments (Fig. S1).

Mice were housed three or four to a cage under a 12-h light/dark cycle (lights on from 7:00 A.M to 7:00 P.M) and weighed 25–35 g. Except for the social interaction and odor detection tasks, food deprivation began 5 d before the start of each experiment and continued throughout the protocol; mice were maintained at 90% of their ad libitum weights by limiting access to a single daily meal. Behavioral training and testing were completed in the light cycle between 9:00 A.M and 5:00 P.M. All experimental procedures were approved by the Johns Hopkins Institutional Animal Care and Use Committee.

Apparatus. For all behavioral experiments except for social interaction and odor novelty, mice were trained in eight chambers with aluminum front and back walls, clear polycarbonate sides, and a floor made of stainless steel rods (Med Associates). Each chamber was fitted with a liquid dispenser into which 50 μL of liquid could be delivered and with a vacuum attached to the bottom of the food cup so that the reward could be suctioned off when desired.

An infrared photocell placed inside the food cup monitored time spent and number of entries into the food cup. In the nose-poke discrimination and reversal learning, two nose-poke devices (Med Associates) were available on the left and right sides of the food cup. Each nose-poke device contained an illuminated yellow stimulus LED located at the rear of the recessed hole and a photo beam sensor to monitor nose-poke entries. In reinforcer devaluation and reward hedonics with effort assessments, retractable ultrasensitive mouse levers (Med Associates) were available at the two nose-poke locations used in reversal learning. Ambient illumination for the chamber was provided by a 28-V, 100-mA house light mounted on the inside wall of the sound-attenuating chamber. In reward hedonics with effort and progressive ratio assessments, custom-designed consummatory chambers were used and included a custom lickometer, which used fiber optics to introduce a light beam through the fluid–air interface of a fluid bolus. Licks were detected as disturbances in the amplified light surface at the interface when the fluid was contacted, permitting time-stamping of individual licks. We previously conducted an extensive set of parametric studies to validate the use of this apparatus for detecting licking behavior and its microstructure in mice (1). Individual licks were time-stamped and subsequently analyzed for the microstructure of licking. An IBM-compatible computer equipped with Med-PC software (Med Associates) controlled and recorded all stimuli and responses.

Nose-Poke Discrimination and Reversal Training. Reversal learning was conducted with experimentally naive DN-DISC1 mice ($n = 14$) and control mice ($n = 5$ WT mice and $n = 8$ C57 mice). All mice first received a single food cup training session each day for a total of 3 d. During the session mice received 60 deliveries of 50-μL 10% (wt/vol) sucrose solution on a random-time (RT) 30-s schedule.

Mice then received single daily 40-min discrete trial nose-poke training sessions. Each session began with the illumination of a house light for a 2-min period (baseline). Responses to each nose-poke were recorded but were not reinforced with sucrose delivery. After the baseline period, the house light was switched off, and the nose-pokes became illuminated (discrimination trial). This illumination signified the start of the trial, where each response (fixed ratio 1; FR-1) to the rewarded nose-poke (e.g., left nose-poke) resulted in sucrose delivery, whereas responses on the other nose-poke (e.g., right nose-poke) were nonrewarded. Nose-poke locations were counterbalanced across groups. After a 2-min period the nose-poke lights were switched off, and the house light was illuminated for a 2-min intertrial interval (ITI) period. In total mice received 10 discrimination trials separated by 2-min ITI periods. Once each session was complete, the percentage of correct responses for each trial was calculated (rewarded nose-poke responses/rewarded + nonrewarded nose-poke responses) × 100, and the average was calculated across the session. As each mouse acquired the training criterion under a particular FR schedule (>85% correct responses for two consecutive sessions and zero trial omissions), the response-reinforcement schedule was altered to FR-5, FR-10, and finally FR-15. On the day after they achieved the training criterion under the FR-15 schedule, mice underwent a reversal test session in which the instrumental nose-poke contingencies were reversed. One control C57 mouse was excluded because of a failure to acquire the pretest criterion. In the reversal training stage, during each discrete discrimination trial each response to the formerly incorrect and nonrewarded nose-poke was rewarded (i.e., was correct) under an FR-1 schedule, and responses to the formerly correct nose-poke were not rewarded. Each mouse received two reversal sessions separated by a 24-h interval.

Lever Training and Reinforcer Devaluation. Reinforcer devaluation was conducted with naive DN-DISC1 mice ($n = 10$) and control mice ($n = 7$ WT mice and $n = 11$ C57 mice). Food cup training was similar to the nose-poke discrimination experiment except that in one session the reward was 50 μL of 6% (wt/vol) polycose solution and in the other session was 50 μL of 5% (wt/vol) sucrose solution. The order of the two sessions was counterbalanced across mice.

Mice then received two instrumental training sessions per day (separated by ∼2 h); one with only the left lever present and one with only the right lever present, with the order of the two sessions alternating daily. For half of the mice in each group, left lever responses resulted in delivery of sucrose, and responses on

the right lever produced delivery of polycose. The remaining mice were assigned the opposite response–outcome contingencies. For the first 2 d mice were given 30-min sessions in which each response was reinforced. For the remaining 8 d the duration of the session was reduced to 20 min, and reward was delivered on a random ratio (RR) 5 schedule (i.e., on average, every five responses resulted in reward delivery) on days 3 and 4; an RR10 schedule on days 5–8; and an RR15 schedule on days 9 and 10. Thus, mice were given a total of 10 sessions of instrumental training on each lever. Two wild-type control mice were excluded at this stage because they failed to acquire responses on at least one lever.

The next day, mice received sensory-specific satiety treatment in which each mouse was prefed with one of the two reward substances for a 2-h period. Each mouse was placed in a separate home cage, with a cube filled with 5 mL of either sucrose or polycose, fully counterbalanced across the prior response–outcome contingencies. The experimenter closely monitored and recorded reward consumption during this phase. As the solution was consumed, it was replaced in 2-mL increments to ensure continuous reward availability for each mouse.

Immediately after the satiety treatment, the mice were given a 10-min extinction test session in the experimental chamber during which responses were not reinforced with reward delivery. Unlike the training sessions, both levers were available in this test session. After the extinction and reward choice test (see below), mice received a retraining session each day for a total of 3 d (under an RR15 schedule), followed the next day by prefeeding with the other reward substance and a subsequent second 10-min extinction test. This schedule served to control for any inherent preferences for the reinforcers that might complicate test interpretation. To the extent that responding was controlled by the current value of the reward anticipated after each of the two responses (left and right lever presses), mice preferentially would perform the response that previously had been reinforced with the reward that had not been prefed (i.e., the maintained response).

Finally, 24 h after the completion of each extinction test, the effectiveness of the prefeeding devaluation treatment in altering reward preference (via satiation) was assessed (reward choice test). Mice were prefed for 2 h with a particular reinforcer, as before the extinction test. After prefeeding, mice were given access to two cubes (in the home cage), one containing 5 mL of the prefed reward and other containing 5 mL of the other reward. Mice were given 30 min to consume each reward, with the expectation that consumption would be greater for the nondevalued, maintained reward.

Reward Hedonics and Effort. Modulation of the hedonics of reward value by effort was conducted with DN-DISC1 mice ($n = 17$) and control mice ($n = 23$ C57 mice). Mice received food cup training, similar to reinforcer devaluation, except that the rewards were 50 μL of 2% (wt/vol) polycose solution and 50 μL of 1.5% (wt/vol) sucrose solution. After food cup training, all mice received single daily 10-min baseline consumption sessions for 4 d in the automated consummatory chamber. For the first session half of the mice consumed the polycose reinforcer, and the remainder consumed the sucrose reinforcer (counterbalanced across mice). On the second day the alternate reinforcer was consumed in the chamber. This order was repeated in sessions 3 and 4, respectively.

Mice then were divided into two groups based on their mean level of consumption for each reinforcer. For half of the DN-DISC1 and control mice, the high-effort lever was subsequently associated with sucrose reinforcer, and the low-effort lever was subsequently associated with the polycose reinforcer. For the remaining mice, the response–outcome contingencies were reversed. Mice then received two instrumental training sessions each day, one on each lever. For half of the mice, a single response (FR-1) to the left lever resulted in delivery of sucrose reinforcer for a 10-s period and the retraction of the lever for 20 s, whereas right-lever responses (FR-1) led to delivery of the polycose reinforcer. The session was completed when 25 reinforcer deliveries had been made or after 45 min had passed. The order of the training sessions was reversed each day. After four sessions of training, the FR schedule was increased to FR-5 (for two sessions); FR-10 (for two sessions), and FR-15 (for eight sessions) on the high-effort lever. The low-effort lever remained at FR-1 for the duration of behavioral training (i.e., 16 sessions). Two DN-DISC1 mice and one C57 control mouse were excluded because of poor training performance. On completion of training, mice received a single-access consumption test session in the automated consummatory chamber, with access to either the low- or hight-effort reinforcer (counterbalanced across groups). Licking microstructure analysis was then conducted on intake data collected from the consummatory chamber.

Progressive Ratio Testing. Progressive ratio testing was conducted with DN-DISC1 mice ($n = 8$) and C57 mice ($n = 7$). Mice initially received a single 20-min consumption session each day for 4 d. This session served to habituate the mice to both the apparatus and the food reward [10% (wt/vol) sucrose]. During each consumption session the photo beam lickometer recorded each lick made by the mouse. At the start of the session, 50 μL of the tested reinforcer was available in the food well; additional 50-μL deliveries were made approximately every 40 licks as mice consumed the liquid, so that mice had continuous access to the sucrose reward throughout the consumption session. The experimenter carefully monitored the amount of liquid in each food well; under very rare conditions when the food well appeared empty, the experimenter manually stimulated reward delivery via the Med-PC software. After these acclimatization sessions, mice were tested on a progressive ratio. The session commenced with the delivery of 50 μL of the tested reinforcer. On contact with the sucrose, mice were allowed 10 s to consume the reward. Any remaining reward was removed by brief deployment of the vacuum. At this stage mice were reinforced for licking the empty food well under an arithmetically increasing fixed-response schedule (i.e., 40, 42, 44, 46, 48, 50 licks). Once mice attained the schedule (e.g., 40 licks to the empty food well), 50 μL of sucrose was made available followed by a similar 10-s consumption period before reward removal. Mice then were required to lick at the empty well according to the corresponding lick schedule (e.g., 42 licks of the empty food well). This process was repeated until the 3-h session was complete or until the period between individual licks exceeded 5 min (i.e., breakpoint (2)).

Social Interaction Test. The social interaction test was conducted with DN-DISC1 mice ($n = 13$) and WT mice ($n = 9$) at the Johns Hopkins School of Medicine Behavioral Core. We used the three-chamber apparatus to assess sociability and preference for social novelty (3). The test consisted of three subsequent 10-min phases: (*i*) Habituation: free exploration of the three chambers; (*ii*) Sociability: a stranger mouse was inserted into an enclosure in one of the side chambers (normal mice prefer the chamber with a mouse over the empty chamber); (*iii*) Social novelty: the by-now familiar mouse was left in place, and a different stranger mouse was inserted into an enclosure in the second side chamber (normal mice prefer the chamber with the unfamiliar mouse over the chamber with the familiar mouse). Interaction was quantified by counting how many times the mice sniffed the enclosure. The stranger mice were young adult male C57 mice.

Odor Detection. For odor detection, experientially naive DN-DISC1 mice ($n = 15$) and WT mice ($n = 20$) were used. Mice were habituated to a square open field (40.6 × 40.6 cm; Accuscan) that contained odorless empty cubes placed in the center of the right

and left sidewalls (4). Each mouse received two 30-min sessions separated by a 24-h interval. On the third day, one of the empty odor cubes was filled with conspecific cage bedding (odor cube) from the home cage of the to-be-tested mouse; the other odorless cube remained empty (empty cube). The placement of each cube was fully counterbalanced with respect to sidewall location for all groups of mice. Finally, mice received a single 10-min test in which the amount of time (in seconds) under each cube was used to determine odor detection.

Assessment of Oxidative Stress and the Nuclear GAPDH Cascade. The prefrontal cortex and the striatum were dissected from sequential coronal sections. The prefrontal cortex block consisted of the dorsal (cortical) portion of the most anterior coronal section (excluding the olfactory bulbs), thus including the orbitofrontal cortex and medial prefrontal cortex and additional cortical regions.

For a general biochemical measurement of oxidative stress, we used a carbonyl assay: We used a dot blot procedure with the OxyBlot Protein Oxidation Detection Kit (S7150; Millipore), which immunodetects carbonyl groups that arise from oxidized proteins, as described in ref. 5. In addition, we used a histological assay of oxidative stress: We looked at 8-oxo-dG staining (a marker for oxidative damage to the DNA). Immunohistochemical staining was performed with anti–8-oxo-dG antibodies (Trevigen 4354-MC-050) and DAPI in four control and four DN-DISC1 mice. We counted the number of 8-oxo-dG–positive and –negative cells in approximately four sections per mouse in one frame on the right and one on the left prelimbic prefrontal cortex and lateral orbitofrontal cortex, resulting in ~32 percentages per group. The activation of the nuclear GAPDH cascade was measured with the levels of GAPDH–seven in absentia homolog 1 (Siah1) protein interaction: We performed coimmunoprecipitation by precipitation with anti-Siah antibodies (SC-5506; Santa Cruz) and immunoblot with anti-GAPDH antibodies (Biogenesis catalog no. 4699–9555) following a published protocol (6). As we have reported (6), when the augmentation of the GAPDH–Siah binding occurs under oxidative stress, Siah is stabilized. Thus, the coimmunoprecipitation data are to be normalized only with the GAPDH intensities, not with the Siah intensities.

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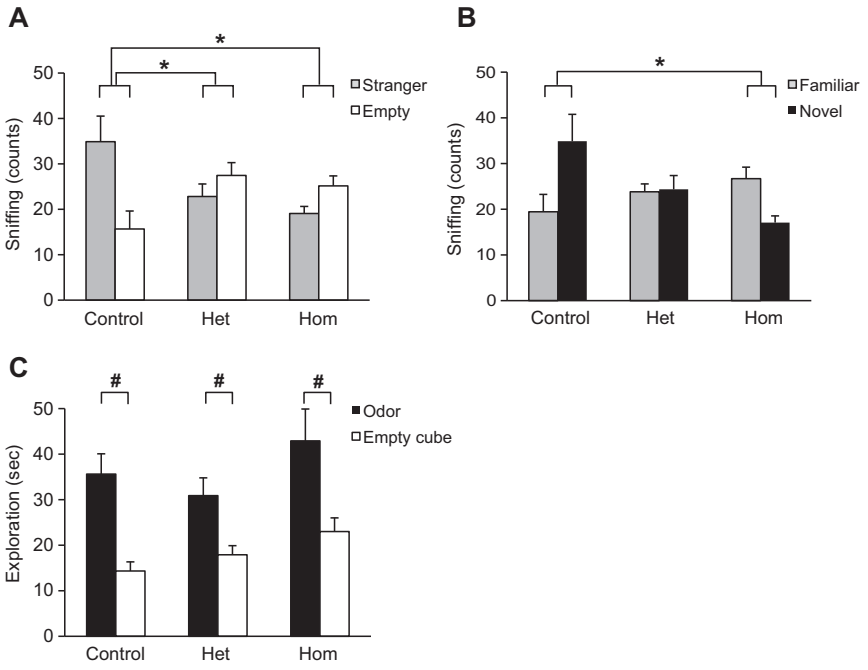

Fig. S1. Transgene dose-dependent behavioral changes in social interaction tests. (*A*) Sociability: Heterozygous (het) DN-DISC1 mice ($n = 11$) showed an intermediate phenotype between wild-type ($n = 9$) and the homozygous (hom) DN-DISC1 mice ($n = 13$). (*B*) Social novelty: Heterozygous DN-DISC1 mice ($n = 11$) showed an intermediate phenotype between wild-type ($n = 9$) and the homozygous DN-DISC1 mice ($n = 13$). (*C*) Social odor detection: Wild-type ($n = 20$), heterozygous ($n = 15$), and homozygous ($n = 15$) mice all showed a preference for the cube containing familiar cage bedding relative to an empty odor cube. $^{*}P < 0.05$, Group x Chamber interaction; $^{\#}P < 0.001$, odor detection effect. Error bars indicate SEM.

Table S1. Comparison of WT mice from our colony and C57 control groups across experimental behavioral assays

Behavioral assay	WT	C57	Statistics
Reversal training 1	$n = 5$	$n = 7$	Group × Block$F(4,40) = 0.82$, $P = 0.51$
Final block, percent correct ± SEM	41.1 ± 7.05	35.35 ± 17.06	
Reinforcer devaluation	$n = 5$	$n = 11$	Group × Response$F(1,14) = 1.19$, $P = 0.29$
Devalued responses/min ± SEM	13.14 ± 3.18	4.34 ± 0.67	
Maintained responses/min± SEM	18.42 ± 3.31	6.85 ± 1.24	
Odor detection	$n = 20$	$n = 8$	Group × Cube$F(1,28) = 1.74$, $P = 0.19$
Duration odor cube ± SEM, in seconds	35.62 ± 4.45	58.38 ± 7.00	
Duration empty cube ± SEM, in seconds	14.34 ± 1.62	28.23 ± 2.50	

Wild-type mice from our colony (WT) and C57 mice from Charles River (C57) did not show any significant behavioral differences in reversal training, reinforcer devaluation, or odor detection. Both WT and C57 mice were used as controls.

Table S2. Comparison of sucrose and polycose licks for low- and high-effort conditions during testing for reward hedonics by effort

	Sucrose (licks ± SEM)	Polycose (licks ± SEM)	Statistics
High-effort condition			
Control	390 ± 36 ($n = 6$)	304 ± 37($n = 6$)	$t(10) = 1.68$, $P = 0.12$
DN-DISC1	226 ± 32 ($n = 4$)	152 ± 46($n = 3$)	$t(5) = 1.39$, $P = 0.22$
Low-effort condition			
Control	272 ± 51 ($n = 5$)	192 ± 32 ($n = 5$)	$t(8) = 1.31$, $P = 0.23$
DN-DISC1	198 ± 66 ($n = 4$)	195 ± 28.57 ($n = 4$)	$t(6) = 0.84$, $P = 0.46$

No differences in preference (no. of licks ± SEM) for the two rewards were revealed by either control [$F(1,20) = 3.39$, $P = 0.08$] or DN-DISC1 [$F(1,13) = 2.36$, $P = 0.14$] mice. In addition, there was no significant Group × Reward interaction in either the high- or low-effort condition ($F < 1$; $P > 0.76$).