Schizophrenia-related cognitive dysfunction in the Cyclin-D2 knockout mouse model of ventral hippocampal hyperactivity

Running title: Modelling cognitive impairments in the prodromal state

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ABSTRACT

Elevated metabolism and neural activity at the output stage of the anterior hippocampus has been described as a physiological endophenotype of schizophrenia, and its development maps onto the transition from the prodromal to the psychotic state of this disease. Interventions that halt the spreading glutamatergic over-activity in this region and thereby the development of overt schizophrenia may be promising therapeutic strategies. However, animal models with high construct validity to support such pre-clinical development are lacking. We here describe a large-scale phenotyping of the Cyclin-D2 knockout (CD2-KO) mouse model which shows a hippocampal Parvalbumin-interneuron dysfunction and a pattern of hippocampal over-activity similar to that seen in prodromal patients. We found, that CD2-KO mice displayed novelty-induced hyperlocomotion (a correlate in the positive symptom domain), that was largely resistant against D1- and D2-dopamine receptor antagonism, but fully responsive to the mGlu2/3R-agonist LY379268. In the negative symptom domain, CD2-KO mice showed no impairment in social interaction, nest building, or incentive motivation, but transient anhedonia in the sucrose-preference task. Also, unconditioned anxiety, perseveration, and motor impulsivity were normal. However, in the cognitive domain, CD2-knockouts showed reduced executive function in assays of rule-shift and rule-reversal learning, but also an impairment in working memory, that was resistant against LY379268. In contrast, sustained attention and forms of spatial and object-related memory that are mediated by short-term habituation of stimulus-specific attention were intact. Our results suggest, that CD2-KO mice are a valuable model in translational research targeted at the cognitive symptom domain in causal relation to hippocampal over-activity in the prodrome-to-psychosis transition.

INTRODUCTION

The specific re-creation of endophenotypes of psychiatric diseases in animal models has been proposed as a promising strategy to meet the current challenge of identifying rodent models with predictive validity for drug discovery [1]. In schizophrenia, hyperactivity of the output stages of the anterior hippocampus represents a physiological endophenotype that is unique in predicting disease progression from the prodromal state to overt schizophrenia, characterized by psychosis [2,3]. More specifically, baseline metabolic hyperactivity in the anterior CA1 region is elevated in prodromal patients that will develop positive symptoms in the near future, compared to individuals that do not. The development of hyperactivity in the anterior subiculum, the output stage of the hippocampus that is directly excited by CA1, in turn, coincides with the onset of psychosis and is accompanied by atrophy of the anterior hippocampus, which might manifest the disease state irreversibly [2]. Therefore, it has been suggested, that interventions that ameliorate anterior hippocampal hyperactivity in the prodromal state and thereby halt further disease progression could be a promising therapeutic strategy [4].

To support this research, rodent models that display hyperactivity of the ventral hippocampus (vHC, which is homologous to the human anterior hippocampus) are necessary. In rats, two approaches have been taken, namely direct electrical or chemical stimulation of the vHC [5–10] as well as developmental models such as pre-natal Methylazoxymethanol acetate (MAM) injections [11–13], which lead to vHC hyperactivity later (but also refrontal aberrations). The focus of investigations in these models was the positive symptom domain. It is well established, that their vHC hyperactivity entails a hyper-dopaminergic state of the mesolimbic (ventral tegmental area to Ncl. accumbens) projection and thereby deficits of novelty- and amphetamine-induced hyperlocomotion, latent inhibition and pre-pulse inhibition.

In mice however, only few studies have been conducted, and those focused largely on the contribution of a hypofunction of inhibitory interneurons to vHC hyperactivity. It had been suggested previously, that a homeostatic downregulation of the GABAergic output from Parvalbumin-positive (PV) interneurons of the CA1-region of the hippocampus leads to an effective disinhibition of the pyramidal neurons of CA1, which in turn over-activate the excitatory projection neurons of the ventral subiculum, that project to multiple target areas in the brain that are relevant for affective and cognitive functions [14]. In one case such failure of inhibition in the vHC has been re-created directly by chemogenetic silencing of either all interneurons or only Parvalbumin-positive ones [15]. Regarding rodent correlates of the positive symptom domain, the broad disinhibition lead to the induction of hyper-locomotion, which was sensitive to haloperidol and also to selective D1-receptor antagonism by SCH23390, while the PV-neuron silencing only decreased pre-pulse inhibition. Both interventions impaired spontaneous alternation (a potential measure of short-term memory), but not social interaction [15].

A different set of studies has focused on the Cyclin-D2 knockout mouse model (*Ccnd2*$^{-/-}$, or CD2-KO)[16] that displays a reduced number of PV-cells in the hippocampus and some areas of the neocortex, but not the medial prefrontal cortex [17,18]. The reported reduction of PV-cells varies between 20-40 % in hippocampus and it is particularly prominent in the CA1 region. In sensory and motor cortex, the reported average decrease of PV-cells varied between an insignificant 10 % and 30 %, and is particularly prominent in superficial layers. While the deletion of *Ccnd2* affects cell development in the subventricular zone of the medial ganglionic eminence where PV-cells originate from, Somatostatin-positive interneurons that derive from the same region, are not decreased in their numbers in the adult brain [18]. Physiologically, these animals recapitulate the schizophrenia-related endophenotype of hyperactivity of the vHC, particularly in the dentate gyrus, CA2/CA3 and CA1 – but not the ventral subiculum; a pattern that is more akin to the prodromal state (see above) in which patients already present with cognitive symptoms [17]. However, cognitive deficits related to

schizophrenia have not been systematically examined in this mouse line yet (see Table 1 for a summary on previous behavioural assessments of *Ccnd2*$^{-/-}$ mice) or, in fact, in other rodent models of ventral hippocampal hyperactivity. However, consistent with rat models of vHC hyperactivity, *Ccnd2*$^{-/-}$ mice have been shown to display rodent correlates of the positive symptom domain such as novelty- and amphetamine-induced locomotor hyperactivity [17,19].

Importantly, the same mouse line also shows a lack of neurogenesis in the adult dentate gyrus (and the olfactory bulb) and has therefore been studied in the context of depression and potentially neurogenesis-related dysfunction in spatial learning. Surprisingly, however, this mouse line did not show a depression-related phenotype at baseline as assessed by the forced-swim test, the tail suspension test, and sucrose preference (in fact, it showed reduced immobility in tests of behavioural despair) [19,20]. *Ccnd2*$^{-/-}$ mice did, however, show some aversion towards novel material, including nesting material, which might underlie an observed nest building deficit [17,19]. In line with results from other mouse models with *selectively* impaired neurogenesis, *Ccnd2*$^{-/-}$ mice did not show deficits in basic spatial learning in the water-maze, Barnes maze, or the plus-maze. However, they do show less precision and efficiency in their search strategies and a deficit in spatial reversal learning hinting towards impaired cognitive flexibility [19,21–23].

The dual cellular pathology of impaired adult neurogenesis and reduced hippocampal PV-cell numbers, strengthens the translational value of the CD2 knockout model for schizophrenia, since both phenotypes are linked to the disease: while the role of PV cell dysfunction is well established [25,26], reduced adult neurogenesis was only recently discovered in post-mortem brains from patients with schizophrenia – but, surprisingly, not with major depression [27]. The reduction of neurogenesis by inflammation could be a mechanistic link between schizophrenia and its genetic [28] and environmental immunological risk factors [29]. Furthermore, PV-cell hypofunction could be contributing to the neurogenesis deficit, since the innervation of new born cells by PV interneurons is critical for their survival

and development [30]. In reverse, new born granule cells activate strong dentate gyrus-feedback and CA3-feedforward inhibition [31,32], implying that reduced neurogenesis might lead to hippocampal hyperactivity independently from PV-cells.

Importantly, however, a deficit in contextual fear-conditioning seen in one study in the CD2-KO mouse, was fully rescued by implantation of GABAergic precursor cells specifically into the ventral hippocampus [17]. This implies that – even though impaired contextual fear-conditioning would also be expected to occur due to impaired neurogenesis [33] - restoration of inhibition (not neurogenesis) was sufficient to ameliorate its behavioural consequences. Similarly, the enhanced sensitivity to amphetamine in *Ccnd2*$^{-/-}$ mice and hyperactivity of dopamine neurons in the VTA, were rescued by transplantation of interneurons into their vHC.

This suggests that the *Ccnd2*$^{-/-}$ mouse model is well-suited to study the role of ventral hippocampal hyperactivity in the causation of schizophrenia-related deficits and to preclinically develop drug candidates that target this pathology. To that end, we have subjected *Ccnd2*$^{-/-}$ mice to a large battery of schizophrenia-related deficits in the positive, negative and cognitive symptom domain.

METHODS

Animals

Male and female *Cyclin-D2* knockout (*Ccnd2*$^{-/-}$, CD2-KO) mice [16] and wild-type littermate controls were generated by crossing heterozygous *Ccnd2*$^{-/+}$ mice. Altogether, 18 male and 12 female knockouts as well as 19 male and 9 female wildtype controls were used. Animals were group-housed in Type II-Long individually ventilated cages (Greenline, Tecniplast, G), enriched with sawdust, Sizzle nest and cardboard houses (Datesand, UK) and subjected to a 13 h light / 11 h dark cycle. All experiments conformed to the German Animal Rights Law

(Tierschutzgesetz) 2013, the EU Directive 2010/63/EU, and the Federal Ethical Review Committee (Regierungsprädisium Tübingen) of Baden-Württemberg.

Behavioural tests

Detailed protocols for all behavioural tests are given in Supplementary Methods and the majority of tests (novel-object recognition, Y-maze novelty preference, T-maze working memory, novelty-induced locomotion, 5-choice-serial-reaction-time-task, spatial reference learning, elevated plus-maze, sucrose preference and nest building) were conducted as previously described [34].

In brief, in the *novel-object recognition test*, mice were exposed to two identical objects A1 and A2 in the sample phase (10 min) and – after a delay of 2 min – to an identical copy of the familiar object, A3 and a novel object B1 in the test phase (5 min). Direct physical interaction between the snout or whiskers with the object were scored manually by an observer blind to genotype, and the extent of novel-object recognition was determined according the ratio of time spent with B1 to time spent with B1 and A3 combined. *Spatial novelty preference in the Y-maze* was conducted analogously: animals explored the start arm and a pseudo-randomly selected goal arm in the sample phase (5 min), while they had access to all arms (including the previously unvisited novel arm) in the test phase (2 min) that followed after a 1 min-delay period during which the sawdust in the maze was mixed to remove olfactory landmarks. In the *T-maze test of working memory*, 10 trials were conducted per day in food-deprived (85-90 % of free-feeding weight) animals, while each trial consisted of a sample run with a forced choice into one of the goal arms and a choice run in which the previously unvisited arm was baited and its visit was counted as a correct choice. Animals were trained up over 5 days in a blocked paradigm with a delay between sample and choice run of 5 s and an inter-trial interval (ITI) of 3-10 min. Subsequently, the protocol was altered every second day starting with a massed design with a delay of 1 s and an ITI of 25 s, followed by protocols in a blocked design with delays of 5, 20 and 60 s. The assessment of

spatial reference learning and rule-shift and rule-reversal were conducted in a plus-maze where the start arm (North or South) was chosen pseudo-randomly and one of the goal arms (East or West) was baited according to the respective rule and its visit counted as a correct choice. 10 trials were given per day with a delay of 5-10 min between them. Animals were first trained up on an egocentric rule (*turn left* or *turn right*). After achieving the performance criterion of 85 % correct trials in 20 consecutive trials, animals were switched to an allo-centric rule (*go West* or *go East*) to assess spatial rule-shift learning. After reaching the same 85 % performance criterion the allo-centric rule was reversed (e.g. from *go West* to *go East*, or vice versa) to assess reversal learning. *Unconditioned anxiety* was measured on the elevated plus-maze, where animals could explore two open and two closed arms elevated 75 cm above ground. *Novelty-induced locomotion* was assessed by exposing animals to a new Typ III cage (Tecniplast, G) with clean sawdust for 2 h. *Reciprocal social interaction* was assessed in similar cages but with darker walls (Tecniplast, G), into which a younger adult stimulus mouse of the same sex and strain had been introduced. Social interactions (sniffing, taxing with snout or whiskers, but not aggression or sexual behaviour) were scored manually and blind to genotype over a period of 16 min in 2 min intervals. Arenas used in the mentioned tasks had mostly walls of transparent Perspex, except for the elevated plus-maze and the novel object recognition test where non-transparent grey plastic walls were used. Light levels were always set to 100 lux at the brightest spot in the arena. Movement was video-tracked using ANY-maze (San Diego Instruments, CA, USA) in the Y-maze, the elevated plus-maze and during novelty-induced locomotion.

Sucrose preference was assessed over night in animals that were single-housed on the first day of the testing sequence, on which they were presented with two bottles of drinking water. Over the 4 subsequent nights one of the two bottles contained either 1 % w/v (2 nights) or 10 % w/v (2 nights) sucrose in drinking water, while only the water bottle remained in the cages during the day and for the sixth night. During this last night, animals received a

Nestlet™ (Datesand, UK) of 2.3-2.6 g and nests were scored according to an established *nest building* rating system next morning [35].

For the *5-choice-serial reaction time task* (5-CSRTT) mice were trained up in operant chambers (Med Associates Inc., VT, USA) over 5 stages with increasing attentional difficulty until mice reached a consistent performance (≥80 % accuracy, ≤50 % omissions, ≥30 trials) on the stage 5 baseline protocol where the stimulus duration was 4 s and the inter-trial interval (waiting) time was 5 s [34]. Subsequently, various challenge protocols (attention challenges: reduction of the stimulus duration to 1 s or distracting by random switching of house light, or both; impulsivity challenge: extension of the waiting time to 9 s) were applied over 2 consecutive days, followed by 1-3 d of baseline training before the next challenge. Afterwards, mice were trained on a simplified version of this task where only hole 2 or 4 was illuminated in each trial until obtaining at an accuracy of ≥80 % in two consecutive trials. Subsequently, the rule was shifted from a cued to a spatial version, whereby only hole 2 (or, in other mice, hole 4) was rewarded irrespective if hole 2 or 4 was illuminated in a given trial. To assess *rule-shift learning* in this operant paradigm, mice were trained until they had reached a criterion of 80 % accuracy on trials where the correct hole was unlit on 3 consecutive days. Condensed milk diluted with drinking water was used as reward on rewarded maze tasks, strawberry milk was used in operant box paradigms.

Behavioural pharmacology

To assess the effects of the D1-antagonist SCH23390, the D2-antagonist raclopride and the mGlu2/3R-agonist LY379268 on novelty-induced locomotion in CD2-KO mice, each drug was injected i.p. at an injection volume of 10 ml/kg prior to a 1 h exposure in a novel open-field, identical to those used for the prior assessment of novelty-induced locomotion (see above). S(-)Raclopride-(+)tartrate salt (Sigma, G) was diluted in 0.3 % TWEEN80/saline and injected 60 min before the start of the run. SCH23390 hydrochloride and LY379268 disodium salt (Tocris, G) were diluted in saline and injected 30 min or 45 min, respectively, before the

run. For dose-response assessment of these drugs, separate adult male C57BL/6 cohorts were used in a between-subject design (4-8 mice per sub-group); either raclopride, SCH23390 or LY379268 were injected 30 min, 30 min, or 45 min, respectively, before a 30 min exposure to the novel openfield, followed by injection of 0.2 mg/kg MK-801 maleate (in saline). For the T-maze test, LY379268 was injected 50-60 min before testing commenced. Additionally, amphetamine-induced locomotion was tested by injection of D-amphetamine hemisulfate (Sigma, diluted in Saline), 30 min after an acclimation period in the novel open field, immediately followed by another hour of post-injection exposure. Latin-square designs with a fixed decreasing order of doses, counterbalanced for starting dose and genotype, were used for all within-subject studies.

RESULTS

Sustained novelty-induced hyperlocomotion

Novelty-induced hyperlocomotion was assessed in 22 males (9 knockouts, 13 controls) and 20 females (12 knockouts, 8 wildtype controls). A repeated-measures ANOVA over 24 5-minute intervals revealed a significant effect of genotype ($P < 0.0005$) and a trend for gender ($P = 0.051$), but no interaction between the two factors ($P = 0.791$), indicating that KO mice are hyperlocomotive (Figure 1a, b; see Supplementary Table S1 for statistical details on this and all subsequent results). Additionally, the number of rotations, an indicator of stereotypies, was significantly elevated in knockouts of both genders ($P_{genotype} = 0.001$; $P_{gender} = 0.237$, $P_{genotype*gender} = 0.932$; univariate ANOVA, Figure 1c).

Amphetamine-induced hyperlocomotion

In a similar locomotor activity paradigm, injection of 2 mg/kg amphetamine after an acclimation period of 30 min resulted in a significant subsequent increase of locomotion in both genotypes (P (dose) < 0.0005, P (genotype) = 0.015, P (genotype * dose) = 0.957),

univariate ANOVA; between-subject design; Figure 1d-e). Univariate ANOVA within each dose revealed that the higher locomotion of CD2-KOs relative to controls seen qualitatively at each dose, only reached significance after vehicle injection (P = 0.007) but not after amphetamine injection (P > 0.1). Likewise, when expressing locomotor activity post-injection relative to locomotor activity before injection there was no difference between genotypes (P = 0.759), while the effect of dose remained (P < 0.005). This suggests, that CD2-KO mice are not more sensitive to amphetamine than controls; if anything, amphetamine aligns wildtype with knockout levels of locomotor activity (Figure 1f).

Incomplete reduction of novelty-induced locomotion by the D2/3 antagonist raclopride

Signalling through D2-type receptors in the nucleus accumbens mediates spontaneous locomotor activity in rodents [36] and antagonism of these receptors is also the suggested mechanism of the antipsychotic action of neuroleptics. We therefore tested, if the novelty-induced hyperlocomotion in CD2-KO mice is indeed responsive to the D2-receptor antagonist raclopride as supposed for a rodent correlate of psychosis. We first confirmed in a dose-response study in a separate group of male C57BL/6J mice, that the doses 0.33, 1 and 3 mg/kg raclopride were effective in reducing novelty and MK-801-induced hyperlocomotion, while 0.11 mg/kg was statistically ineffective (Figure 1g). Using the two lowest effective doses 0.33 or 1 mg/kg, we firstly performed a between-subject study in CD2-KO mice and controls. While raclopride at both doses significantly reduced locomotion (distance moved) in both genotypes, surprisingly, knockout mice remained significantly hyperlocomotive at both doses compared to wildtype controls (P < 0.0005, univariate ANOVA; P < 0.05 for ANOVAs at each dose, Figure 1h). That means, although 0.33 and 1.0 mg/kg could reduce locomotion in knockouts to the level of vehicle-injected controls, these doses could not reduce locomotion to control levels *within* each dose. Also, a dose of 0.33 mg/kg could significantly reduce the number of rotations in wildtype mice compared to vehicle (P = 0.004, Tukey post-hoc test), but not in knockout mice (P = 0.646), suggesting that the drug is even less effective in the latter (Supplementary Table S1).

To investigate this further, a subset of mice were continued in a within-subject regime with vehicle and the 4 doses 0.11, 0.33, 1 and 3 mg/kg. Again, at all doses, knockout mice remained hyperlocomotive compared to wildtype controls ($P < 0.0005$, repeated-measures ANOVA; $P < 0.05$ for univariate ANOVAs), and across all 5 doses a significant dose*genotype interaction was apparent ($P = 0.033$, Figure 1i), indicative of differential efficacy of raclopride. Expressing the amount of locomotion as percentage of the locomotion each mouse displayed in its vehicle-trial revealed that the higher doses, 1 and 3 mg/kg, reduced novelty-induced locomotion significantly *less* in knockout mice compared to wildtype controls ($P < 0.05$, ANOVA, Figure 1j), supporting the view that the drug is less effective in CD2 knockouts. This suggests, that while D2-receptors may mediate baseline locomotion which is reduced in both genotypes, there is an additional, D2-independent mechanism of increasing locomotor drive in CD2 knockouts. This is reminiscent of the hyperlocomotion produced by electrical (20 Hz) stimulation of the ventral hippocampus in rats, which is mediated by D1- rather than D2-receptors [5].

Incomplete reduction of novelty-induced locomotion by the D1 antagonist SCH23390

Therefore, we repeated this experiment with the D1-antagonist SCH23390. Assessing the efficacy of 0.011, 0.033, 0.1 and 0.3 mg/kg against novelty- and MK-801-induced locomotion in a separate cohort of C57BL6/J males revealed that the two intermediate doses 0.033 and 0.1 mg/kg had the strongest, yet statistically moderate, effects (between-subject design; Figure 1k). Those two doses were tested against novelty-induced locomotion in CD2-KO mice and controls (within-subject design). While the drug significantly reduced locomotion dose-dependently in both genotypes, KO mice again remained hyperlocomotive relative to wildtype controls receiving the same dose ($P_{genotype} < 0.0005$, $P_{dose} < 0.0005$, $P_{dose*genotype} = 0.016$, repeated-measures ANOVA; Figure 1l-m). Expressing locomotor activity under each dose relative to distance moved after injection of vehicle revealed that the drug was less effective in reducing hyperlocomotion in CD2-KO mice compared to wildtypes ($P_{genotype} = 0.02$, repeated-measures ANOVA; Figure 1n).

Normalization of novelty-induced locomotion by the mGlu2/3 agonist LY379268

mGlu2/3 agonists have been proposed to be able to scale down glutamatergic transmission and one compound from this family, LY379268, has been shown to normalize ventral hippocampal hyperactivity evoked by chronic ketamine application in mice [2]. In a separate cohort of male C57BL6/J mice, we found that a dose of 1 mg/kg LY379268 can reduce novelty-induced but not MK-801-induced hyperlocomotion, while 3 mg/kg strongly reduces novelty-induced locomotion but only moderately normalizes MK-801 induced locomotion (Figure 1o). Testing both doses in CD2-KO mice (within-subject design), we found that 3 mg/kg can completely normalize novelty-induced locomotion in CD2-KO mice relative to wildtype controls receiving the same dose ($P_{genotype}$ = 0.941), while 1 mg/kg achieved a partial reduction ($P_{genotype}$ = 0.029 at 1 mg/kg and $P_{genotype}$ < 0.0005 at vehicle, ANOVA, Figure 1p-q). Indeed, LY379268 appeared to reduce locomotion stronger in knockouts than in wildtypes ($P_{genotype*dose}$ = 0.01) leading to significantly less locomotion relative to the vehicle trial at 3 mg/kg compared to wildtype controls ($P_{genotype}$ = 0.043, ANOVA; Figure 1r). We confirmed the finding of a significant interaction between dose (vehicle vs 3 mg/kg) and genotype in a between-subject study in an extended cohort ($P_{genotype*dose}$ = 0.015, n: 15 KO, 20 WT; not shown).

CD2-KO mice are impaired in various forms of cognitive flexibility

Impairments of executive function are a hallmark of schizophrenia and reduced capacity to learn a rule-shift in a spatial version of the attentional set-shifting task conducted in the plus-maze has been demonstrated in the *Ccnd2*$^{-/-}$ previously [22]. Firstly, we sought to confirm this finding by replicating the testing of a rule-shift from ego-centric to allo-centric associative learning. KO mice (10 males) acquired a spatial long-term memory according to an egocentric rule similarly well as wildtypes (14 males). The number of trials needed to reach the criterion of 85 % correct of the last consecutive 20 trials did not differ significantly

between genotypes (P = 0.232, t-test), and the learning curves were similar ($P_{genotype}$ = 0.367, repeated-measures ANOVA over the second half of the 12 training blocks conducted by all animals; Figure 2a-c). This indicates, that CD2-KO mice can process spatial information and acquire a spatial memory largely normally.

Once animals had reached criterion, the associative rule was shifted to an allo-centric one. In this task, KO mice needed significantly more trials to reach criterion again (P = 0.04, t-test) and average performance was lower compared to wildtypes (P = 0.048, repeated-measures ANOVA over the second half of the 14 training blocks conducted by all animals; Figure 2d-f).

Thirdly, once mice had reached criterion, the capacity for rule reversal learning was assessed: the allo-centric spatial location that was associated with a reward was switched. Similarly to the rule-shift, KO mice required more trials than controls to reach criterion again (P = 0.021, t-test) and their performance was inferior (P = 0.01, repeated-measures ANOVA over the second half of the 16 training blocks conducted by all mice).

Given that rule-shift learning is usually attributed to prefrontal rather than hippocampal dysfunction, the finding that KO mice were impaired in the rule-shift paradigm was surprising. Therefore, we sought confirmation of this impairment by conducting a newly developed operant-box paradigm that bears more resemblance to the Wisconsin card-sorting test indicative of prefrontal dysfunction in humans than the rodent attentional set-shifting task by implementing a rule-shift *within* the visual domain (Figure 2j): mice that had been trained up in the 5-choice-serial reaction time task (see below) were subsequently trained further to poke into illuminated holes 2 or 4, as indicated, of the 5-choice wall. Once mice had reached a criterion of an accuracy of 80 % on two consecutive days in this task, the rule was shifted from a cue-indicated to a spatial association: while the random illumination of hole 2 or 4 was continued with equal probability, only poking into one specific hole (2 or 4), which was kept constant for each mouse for the remainder of the training, was

rewarded. While all mice achieved an accuracy close to 100% in the 50% of trials that the rewarded hole was also illuminated, they required continued training in order to chose the rewarded hole in trials where it was not illuminated. KO mice required significantly more trials to reach the criterion of 80 % accuracy in the choice of the unlit rewarded hole on three consecutive days (P = 0.006, univariate ANOVA) and showed lower accuracy during training (P = 0.006, repeated measures ANOVA over the second half of the 16 training blocks conducted by all mice, Figure 2k-l; 1 wildtype and 2 CD2-KO mice were excluded as they did not reach criterion within 23 sessions).

CD2-KO mice are unimpaired in object-related and spatial short-term habituation

Dysfunction of sustained attention and stimulus-specific attention (attentional control) have both been related to schizophrenia [37,38], and could explain deficits in other cognitive tests conducted in this study. We first assessed a form of spatial short-term memory that is largely mediated by short-term habituation of attention using the spatial novelty-preference test in the Y-maze. In this test, habituation is measured as a preference for spending time in a novel as opposed to a familiar choice arm (or 'space'). In the sample phase of the task, KO mice showed significant hyperlocomotion (P = 0.018, univariate ANOVA) but spent a similar amount of time in the to-be-familiar arm (P = 0.425) as wildtype mice suggesting equal levels of exploration (Figure 3a-c). In the test phase, mice of both genotypes explored the novel arm significantly more than the familiar arm, in terms of both entries and time (P < 0.0005, repeated-measures ANOVA, Figure 3d-e), and there was no genotype-related difference in the preference ratios for the novel arm (P > 0.2, univariate ANOVA; Figure 3f). Additionally, preference ratios were significantly above chance level in both genotypes (P < 0.005, one-sample t-test).

Novel-object recognition represents a non-spatial, object-related version of the novelty-preference test of habituation-driven short-term memory (Figure 3g). CD2-KO mice showed

strongly increased exploration of the objects in both, the sample phase as well as the test phase compared to controls ($P < 0.01$; ANOVA; Figure h-j). However, mice of both genotypes showed significantly higher exploration of the novel compared to the familiar object in the test phase ($P < 0.0005$, repeated-measures ANOVA), and did not differ in their preference for the novel object ($P > 0.4$, ANOVA; Figure 3k). In summary, this indicates, that CD2-KO mice are not impaired in short-term habituation of attention.

CD2-KO mice are not impaired in sustained attention, motivation, perseveration or impulse control

To assess sustained attention, CD2-knockouts (8 females, 7 males) and wildtype controls (5 females, 11 males) were trained on the 5-choice serial reaction time task (5-CSRTT) through 4 stages of increasing difficulty, before being challenged with different protocols (see Methods). In the baseline protocol, which was conducted with a stimulus duration (SD) of 4 s and an inter-trial-interval (ITI) of 5 s, knockout mice appeared significantly better in the primary measure of attention, % accuracy ($P = 0.038$; ANOVA), although in each of the gender groups alone there was no significant effect of genotype ($P > 0.05$, *t*-test; Figure 3l). In the secondary measures of attention, % omissions, % correct and number correct responses, female knockout mice performed worse than female wildtype mice ($P < 0.05$, *t*-test), although, qualitatively, the opposite was observable in the male cohort, yielding significant genotype * gender interactions in all three measures ($P < 0.02$, ANOVA; Figure 3m-o). The basic measure of motivation, reward latency, did not differ between genotypes ($P > 0.3$, ANOVA), but females, irrespective of genotype, had a longer reward latency than males ($P_{gender} < 0.05$, $P_{gender*genotype} = 0.307$, ANOVA; Figure 3p). Additionally, perseveration, indicated by unrewarded repetition of nose-pokes into previously rewarded holes, was not significantly higher in CD2-KO mice compared to controls ($P = 0.303$, ANOVA; Figure 3q).

After baseline training, 4 challenge protocols were conducted, out of which three were targeted at sustained attention (shortening of the stimulus duration, distraction during the ITI,

and both), and one at impulse control (increasing the ITI to 9 s). Within 60 sessions, 2 wildtype males and 7 females (KO and WT) did not reach the criterion in the baseline stage to proceed to challenge protocols, which were therefore only conducted in males.

Each challenge protocol was conducted over two consecutive days and was preceded and succeeded by sessions with the baseline protocol. For analysis of each parameter, the values from the two challenge days were averaged and a repeated measures ANOVA was calculated using the performance of the preceding baseline session and the average of the two challenge days.

Regarding impulse control, all challenges increased the primary measure of motor impulsivity, % premature responses ($P < 0.0005$ for all challenges except the exclusive distraction ($P = 0.015$), repeated-measures ANOVA). Similarly, all challenges decreased attentional accuracy ($P < 0.0005$ for all attention challenges, $P = 0.001$ for the impulsivity challenge). A trend of slightly better attention in CD2-KO mice at baseline (see above) was seen in the baseline sessions of most protocols (Figure 3r-u). However, in knockout mice attentional accuracy was affected significantly stronger by the first (and therefore unexpected) attentional challenge ($P_{challenge*genotype} < 0.05$ for %accuracy and %correct), and a similar trend ($P_{challenge*genotype} = 0.066$ for %accuracy) was seen with the first distraction challenge (Figure 3r, t). However, that effect was absent at the later combined SD-reduction/distraction challenge (Figure 3u), suggesting it does not reflect a genuine attentional deficit.

With respect to %premature, there was no significant effect of genotype nor a genotype * challenge interaction ($P > 0.05$) detectable on any of the protocols suggesting that knockout mice are not more impulsive than controls (Figure 3r-u).

In summary, CD2-knockout mice appear largely normal on all psychiatrically relevant parameters measured by the 5-CSRTT. While knockouts are unaltered in measures of motivation, perseverance and impulsivity, they show slightly improved sustained attention at

baseline, which is then, however, somewhat stronger affected by unexpected challenges. Also, this task shows again, that knockout mice are unimpaired in acquiring an associative memory during operant learning.

CD2-KO mice show an impairment in working memory that is resistant against LY379268

In order to assess spatial working memory, mice were trained on a rewarded alternation (delayed-non-match-to-place) task on the T-maze over 5 days in a blocked paradigm with a delay (intra-trial interval) of 5 s and an inter-trial interval of at least 3 min. While female knockouts did not differ from controls in this initial training paradigm, male knockouts performed worse across those training sessions (females: $P_{genotype}$ = 0.497; males: $P_{genotype}$ = 0.005; both genders: $P_{genotype}$ = 0.013, P_{gender} = 0.002, $P_{\text{genotype*gender}}$ = 0.123, P_{day} = 0.001, repeated-measures ANOVA; Figure 4a-c). Subsequently, mice were challenged by different alterations of the paradigm each performed in two consecutive sessions: firstly, the delay was maximally reduced ("1 s") and the paradigm changed to massed trials with an inter-trial interval of 25 s. Then blocked paradigms with increasing delay (5 s, 20 s, 60s) were conducted. Across paradigms and genders, knockout mice were significantly impaired (P < 0.0005), and females were significantly better (P < 0.0005; repeated-measures ANOVA). When assessing individual challenges by univariate ANOVA across genders, all protocols except the 60s-delay revealed significantly reduced performance in knockouts (P < 0.05; Figure 4d). (The 60 s-protocol strongly reduced performance in wildtype mice, especially in males.) When analysing the genders individually, effects of genotype dropped below significance level (P < 0.05, t-test) in the 20 s protocol in females, as well as in the 5 s (P = 0.059) and the 60 s-protocol in males (Figure 4e).

We next examined, if LY379268 was equally effective against the working memory deficit in CD2-KO mice as it was against their hyperlocomotion. However, across doses their lower

performance remained apparent ($P_{genotype}$ = 0.007, P > 0.05 for effects of dose, gender and all interactions, repeated-measures ANOVA, within-subject design, Figure 4f).

CD2-KO mice show normal unconditioned anxiety

During the testing of novelty-induced locomotion in an open field (see above), we found no effect of genotype on the average distance to the cage walls (P > 0.1 for all factors, ANOVA, Figure 5a), suggesting similar levels of unconditioned anxiety. Likewise, on the elevated plus-maze, animals of both genotypes spent significantly more time in the closed compared to the open arms (P_{arm} < 0.0005, repeated-measures ANOVA, Figure 5b). Accordingly, the preference ratio for the open arm (time spent in open arms divided by time spent in all arms) was not significantly different between genotypes (P = 0.488), but was significantly lower in females compared to males (P = 0.003, univariate ANOVA, Figure 5c). Preference ratios calculated from the number of entries did not differ between any groups (P > 0.1, ANOVA, Figure 5d). These results confirm, that anxiety is not altered in CD2 KO mice.

Sucrose preference

Sucrose preference was assessed over 5 consecutive nights during which single-housed mice were presented with two drinking bottles. One of the two bottles (always placed in the same position) contained either 1 % (night 2 and 3) or 10% (night 4 and 5) sucrose in drinking water instead of normal drinking water (night 1).

Analysing the preference ratio for sucrose (consumed volume of liquid from sucrose bottle relative to total consumed liquid) over the 5 nights, we found a strong effect of day (P < 0.0005) but also a day*genotype interaction (P = 0.007) without an effect of genotype (P = 0.647, repeated-measures ANOVA), suggesting that both genotypes develop a strong preference for sucrose but with a different trajectory (Figure 5e-g). This divergence, resulted

largely from the first encounter with sucrose (night 2), where the consumed liquid volume as affected significantly by an interaction between genotype and bottle (water vs sucrose, P = 0.001), an absolute difference between consumed volume of sucrose water (P = 0.014) and a significantly reduced sucrose preference in knockouts (P = 0.012, ANOVAs) were apparent. This phenotype corresponds to a mild anhedonia at the beginning of sucrose-preference testing in CD2-KO mice, as described previously [19].

Nest building

We measured nest building as a compound indicator of hippocampus-dependent social and motivational functioning [35]. Nests built by single-housed mice from pressed Nestlets (2.3-2.5 g) were scored according to a published rating scale and the amount of untorn material (> 0.1 g) was weighed, in addition. Neither the nest score nor the amount of untorn material were significantly different between genotypes (P > 0.2 for within-gender comparisons of both parameters, Mann-Whitney-U-test, P > 0.05 for ANOVA across genders in both parameters; Figure 5h-j).

Reciprocal social interaction

To assess reciprocal social interaction, test mice were introduced into open field cages and left to interact for 16 min, during which the number of interactions and total time of interacting were scored offline blind to genotype in 2 min intervals. Surprisingly, the total interaction time and the number of interactions were significantly higher in CD2-KO mice compared to wildtypes ($P_{genotype}$ < 0.05, ANOVA; Figure 5k-l). Males showed more interaction than females (P_{sex} < 0.0005 for both parameters), and the increased interaction in CD2-KO mice reached significance only in the male subgroup in both parameters (P_{males} < 0.02, $P_{females}$ > 0.1, *t*-tests). This difference was mainly driven by the early stages of the test, in which CD2-KO mice showed a sustained high level of interaction time ($P_{interval*genotype}$ = 0.003, repeated-measures ANOVA; Figure 5n).

DISCUSSION

We here did a comprehensive behavioural assessment of schizophrenia-related deficits across the three symptom domains of schizophrenia in the cyclin-D2-KO mouse model of ventral hippocampal hyperactivity. It was shown previously, that this model gains its construct validity from (1) a hypofunction of Parvalbumin-positive interneurons, (2) reduced hippocampal volume, and (3) a lack of adult neurogenesis, three cellular pathologies observed in schizophrenia [2,17–19,23,27,39]. But it also displays a mesolimbic hyperdopaminergic state, behavioural hyperactivity, and baseline hyperactivity of hippocampal subfields CA1-3, which could all be corrected by increasing inhibition in the ventral hippocampus through transplantation of GABAergic precursor cells [17]. This previous work suggests that CD2-KO-mice could provide a unique tool to understand the potential causal relationship between the endophenotype of hippocampal hyperactivity, analogously observed in patients with schizophrenia at baseline [2,3,40–43] and during sensory processing [44,45], and the various symptoms of the disease. Hippocampal hyperactivity correlates with positive, but also with cognitive symptoms, while conflicting results exist regarding the negative symptom domain [3,43]. While a causal relationship between ventral hippocampal hyperactivity and rodent correlates of positive symptoms has been well established in rats, a causation of cognitive deficits by this endophenotype has not been systematically demonstrated which is why tests of cognition were the main focus of this study.

With respect to the cognitive symptom domain, our present data demonstrates impairments in cognitive flexibility, affecting both rule-shift and rule-reversal learning (extending earlier findings, see Table 1), and uncovers a previously unknown impairment in spatial working memory in CD2-KO mice. The latter aligns with a negative correlation between hippocampal hyperactivity and working memory seen in schizophrenia patients [43]. In contrast to patient data, however, we could not find an indication that CD2-KO mice are impaired in sustained

attention or short-term habituation of stimulus-specific attention. Notably, the latter has been suggested to underlie aberrant salience [38], and thereby psychosis [46], which implies that these mice do probably not model the psychotic state of schizophrenia. This is in line with the profile of their ventral hippocampal hyperactivity, which spares the ventral subiculum [17], akin to the prodromal (as opposed to psychotic) state in patients [2]. It also aligns with their normal response to amphetamine.

Nevertheless, CD2-KO mice did display robust novelty-induced hyperlocomotion, which is usually considered a rodent correlate of the positive symptom domain (note however, that minor positive symptoms can also be seen in prodromal patients, and that the extent of hyperlocomotion in this mouse model appears considerably smaller than in a mouse model with impaired short-term habituation, the *Gria1*-knockout mouse [38,47,48]). Interestingly, the elevation of locomotion relative to controls was maintained even after treatment with D1- or D2-receptor antagonists, in line with the unresponsiveness of baseline hippocampal hyperactivity to antipsychotic medication in patients [3]. In contrast, novelty-induced hyperlocomotion was completely normalized by the mGlu2/3-agonist LY379268, supporting the notion, that hippocampal glutamatergic activity can be scaled down by this pharmacological intervention [2]. Interestingly, mice also showed increased exploration of unfamiliar objects and mice in other tests.

We furthermore show, that this mouse line does not display deficits in the negative domain, including social interaction, nest building, anhedonia, or incentive motivation, nor altered motor impulsivity, anxiety or perseveration. The deficit profile of CD2-KO mice therefore resembles schizophrenia, more than any other psychiatric disorder, although the particular presence of cognitive symptoms rather than underpinnings of positive symptoms suggests that this models rather the prodromal state.

Based on the presented phenotyping, this mouse model appears very well suited to develop therapeutical interventions targeted at an overactive hippocampus, resulting cognitive

deficits, and, more generally, at halting the transition from the prodromal to the psychotic state [4].

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Conflict of Interest

The authors declare that they have no conflict of interest relating to this study.

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FIGURE LEGENDS

Figure 1. Pharmacology of novelty-induced hyperlocomotion in CD2-KO mice. (**a, b**) Locomotion in a novel environment in 5 min intervals (a) and total over 2 h (**b**). (**c**) Number of rotations in the same experiment. (**d, e**) Locomotion after injection of vehicle (0), 1 or 2 mg/kg amphetamine in 5 min intervals (**d**) and in total over 2 h (**e**). (**f**) Same data as in (e) but expressed as speed (m/min) relative to the speed in the 30 min preceding the amphetamine injection. (**g, k, o**) Dose-response measurements on the efficacy of raclopride (**g**), SCH23390 (**k**) and LY379268 (**o**) in reducing novelty-induced (left) and MK-801-induced (right, dark-blue indicates injections of 0.2 mg/kg MK-801, light-blue is vehicle) hyperlocomotion using the stated doses (x-axes, in mg/kg). (**h**) Between-subject assessment of the efficacy of raclopride at the stated doses on novelty-induced locomotor activity in CD2-mice in 5 min intervals (total distance moved and statistics not shown). (**i, m, q**) Within-subject assessment of raclopride (**i**), SCH23390 (**m**) and LY379268 (**q**) at the stated doses on novelty-induced locomotion, plotting total distance moved in 1 h. (**j, n, r**) Within-subject assessment of raclopride (**j**), SCH23390 (**n**) and LY379268 (**r**) at the stated doses on novelty-induced locomotion, plotting distance moved in 1 h (as %) relative to the vehicle (0) value in each animal. (**l, p**) Same data as in (m, q), within-subject assessment of SCH23390 (**l**) and LY379268 (**p**) at the stated doses on novelty-induced locomotion, plotting distance moved in 5 min intervals. Wildtype controls, grey; CD2-KO, black; *f*, females, *m*, males; numbers in brackets denote *n* for each group. All doses stated in mg/kg. * $P < 0.05$, ** $P < 0.01$, *** $P \leq 0.001$. For trends, *P* values are stated as numbers. Error bars, S.E.M..

Figure 2. Impairments of cognitive flexibility in CD2-KO mice. (**a, d, g**) Sequential learning paradigms in the plus-maze starting with the acquisition of an egocentric appetitive spatial reference memory (**a**), followed by a rule-shift towards an allocentric associative memory (**d**) and a rule-reversal within the allocentric rule (**g**); specific rules (arrows) are

shown as examples only. Goal arms are denoted West (W) and East (E), start arms North (N) and South (N). (**b, e, h**) Number of correct choices in blocks of 5 trials for the number of blocks that were done by all mice in the cohort in the reference learning (**b**), the rule-shift learning (**e**) and the rule-reversal learning (**h**). (**c, f, i**) Blocks of 5 trials needed to reach the criterion of 85 % in the last 4 blocks (20 trials) correct choices in the reference learning (**c**), the rule-shift learning (**f**) and the rule-reversal learning (**i**). (**j**) Operant paradigm of rule-shift learning from a hole indicated by the cue-light to a hole defined by its spatial position (while cue lights are still presented but bare no prediction). (**k**) Accuracy for the correct choice in trials where the incorrect hole is illuminated in each session. (**l**) Number of sessions needed to reach the criterion of ≥80 % accuracy in three consecutive sessions. Wildtype controls, grey; CD2-KO, black. * $P < 0.05$. Error bars, S.E.M..

Figure 3. Intact sustained attention and short-term habituation in CD2-KO mice. (**a**) Paradigm of the spatial-novelty preference test. (**b**) The total distance moved and (**c**) time spent in the to-be-familiar choice arm in the sample phase. (**d**) Time spent in the indicated choice arms and (**e**) number of entries into them during the test phase. (**f**) Preference for the novel arm according to either time or entry measures. (**g**) Paradigm of the novel-object recognition task, different colours indicate different types of objects. (**h**) Amount of time the animals investigated both objects in the sample phase. (**i, j**) Interaction with the novel and the familiar object in the test phase in terms of time (**i**) and number of contacts (**j**). (**k**) Preference for the novel object according to either time or entry measures. (**l-q**) Performance in measures of attention (accuracy (**l**), percent omissions (**m**), percent (**n**) and number of correct responses (**o**)), incentive motivation (latency to reward receptacle (**p**) and perseveration (percent perseverative responses into the correct hole, (**q**)) in the baseline protocol (4 s stimulus duration, SD; 5 s inter-trial-interval, ITI; average of the last 4 sessions before the challenges started). (**r-u**) Measures of attention (accuracy, left; %correct, middle) and impulsivity (percent premature responses relative to all initiated trials, right) various

challenge protocols (right value in all subpanels, average of 2 consecutive sessions) relative to the baseline protocol immediately before the challenge (left value in all subpanels). Attention was challenged by a reduction of the SD to 1 s (**r**), a random distraction by switching of the house light for 0.5 s during the ITI (**t**) and a combination of SD-reduction to 1 s and random switching of the house light for 1 s (**u**). Impulsivity was challenged by increasing the waiting time (ITI) until the holes were illuminated to 9 s (**s**). Wildtype controls, grey; CD2-KO, black; *f*, females, *m*, males; numbers in brackets denote *n* for each group. $^{\#}$ $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P \leq 0.001$. In within-subject data (line graphs), symbols on horizontal lines indicate an effect of the within-subject factor (not drawn in (r-u) for clarity), symbols on vertical lines indicate effects of genotype, and symbols on the connecting lines indicate an interaction between both factors. The yellow lines indicate chance-level performance.Error bars, S.E.M..

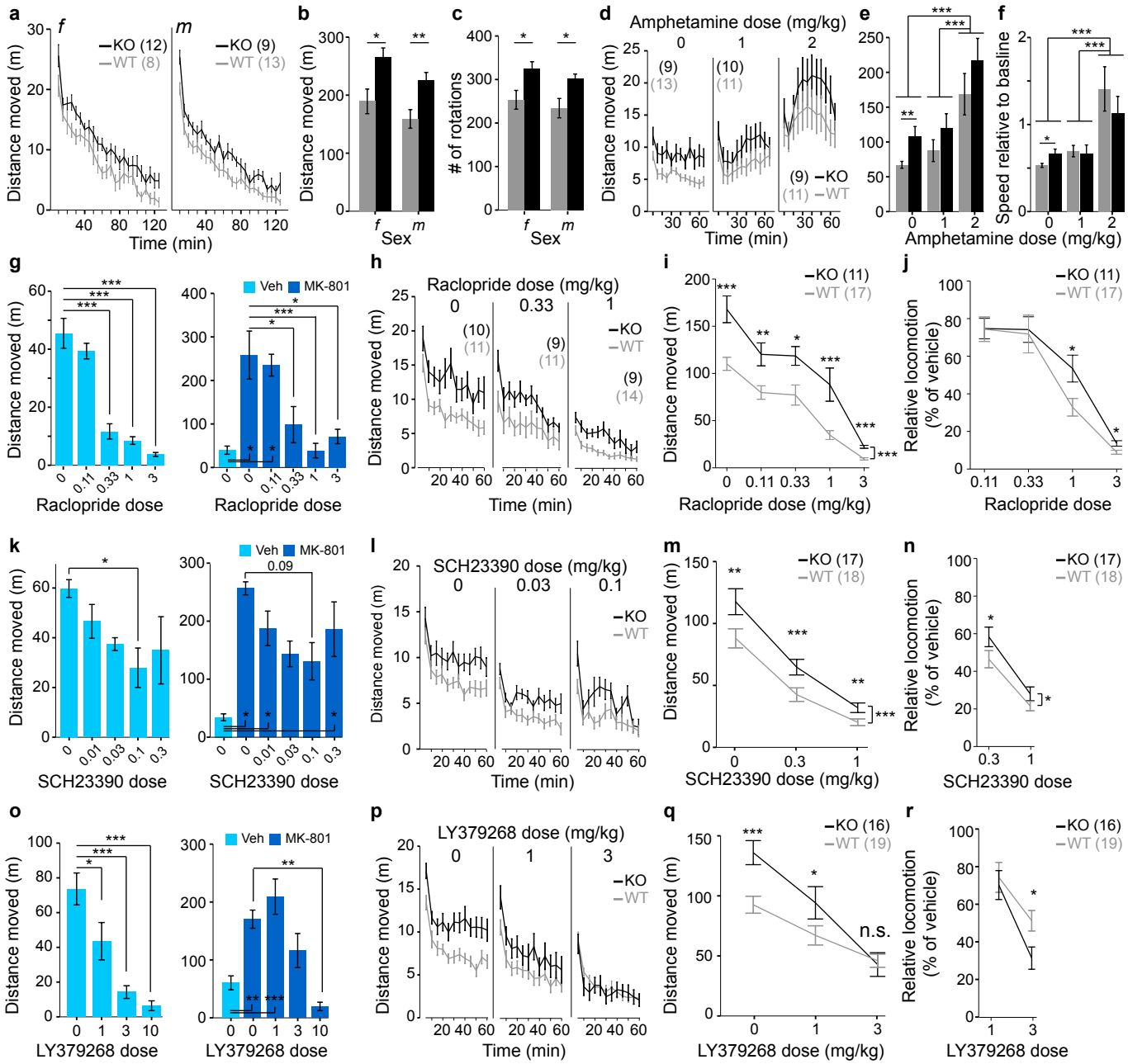
Figure 4. Impaired spatial working memory in CD2-KO mice. (**a**) Paradigm of the spatial working memory test. (**b, c**) Performance (correct choices out of 10 trials, in %) over the 5 initial training days shows a significant effect of genotype across the total cohort (**b**), which is driven by the male subgroup (**c**, right), rather than the females (**c**, left). (**d, e**) Performance under 4 different challenge protocols conducted in the same order as plotted with 2 consecutive days per protocol (averaged for the display) shown for the whole cohort (**d**) and the genders separately (**e**); protocols differed by their design (massed vs blocked) and the delay between sample and choice trial as indicated on the x-axes. (f) After a re-training (identical to the previous one (b), data not shown) either vehicle (0), 1 or 3 mg/kg LY379268 was injected prior to testing sessions in the massed design (1 s delay), with interleaved re-training sessions (blocked design, 5 s delay, not shown). Wildtype controls, grey; CD2-KO, black; *f*, females, *m*, males; numbers in brackets denote *n* for each group. * $P < 0.05$, ** $P < 0.01$. In within-subject data (line graphs), symbols above data points indicate an effect of

group in the indicated condition, symbols on vertical lines indicate effects of genotype across conditions. The yellow lines indicate chance-level performance. Error bars, S.E.M..

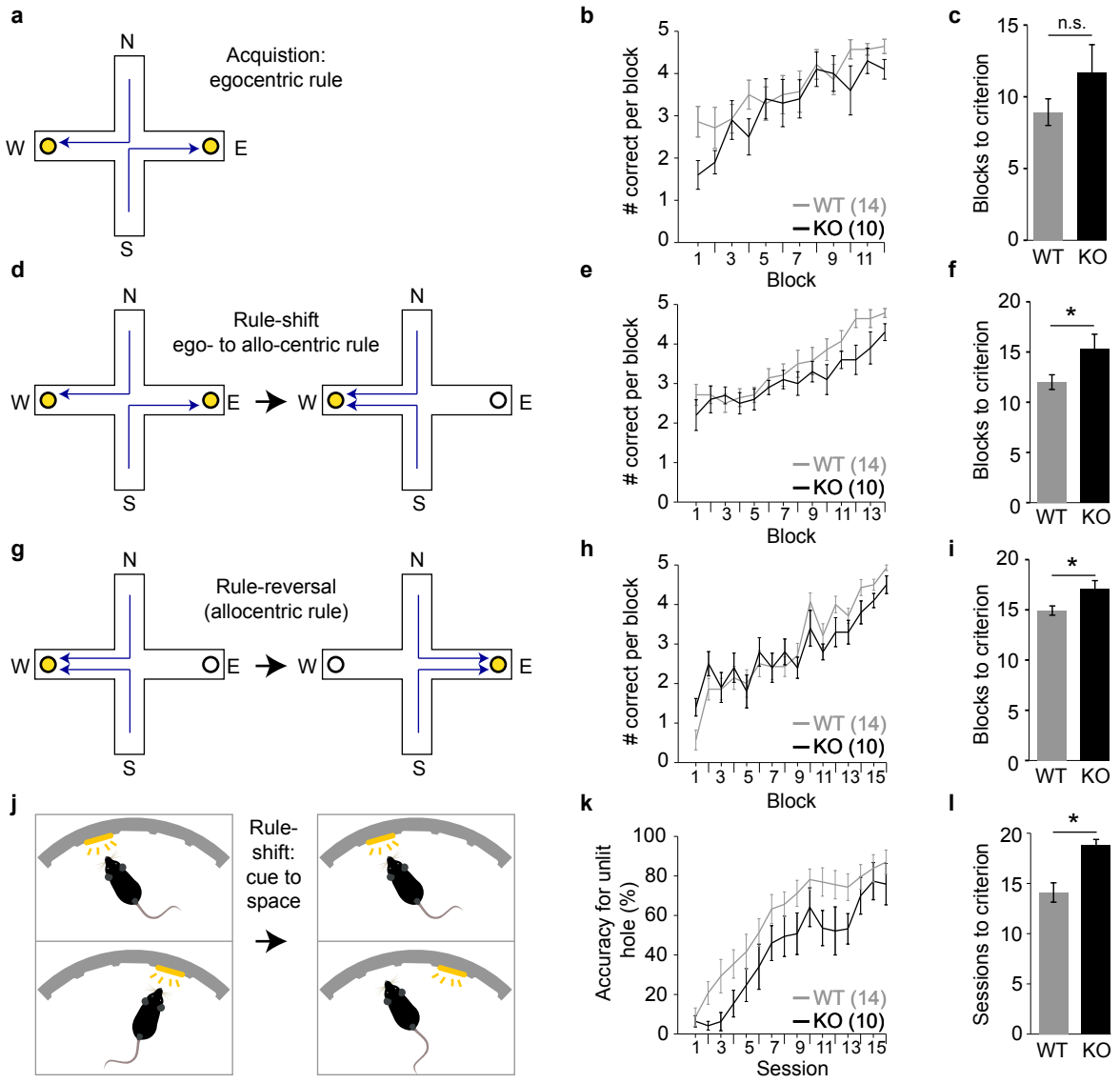
Figure 5. Intact affective functioning of CD2-KO mice. (**a**) Paradigm and data of measuring unconditioned anxiety according to the average distance from the wall of an open field during 2 h of exploration. (**b-d**) Paradigm and data of measuring unconditioned anxiety in the elevated plus-maze. (**b**) Time spent in the open and closed arms as indicated, and (**c**) the preference ratio calculated from those times (time spent in open arms/time spent in all arms). (**d**) Preference ratio calculated as in (c) but from the number of entries into open and closed arms. (**e-g**) Paradigm and data from the sucrose preference test of anhedonia. (**f**) Absolute consumption volumes from the bottles with indicated sucrose concentration in drinking water over 5 nights, and (**g**) preference ratios (consumed volume of sucrose-water/total consumed liquid) over the 5 nights, with the yellow line indicating lack of preference. Note that both bottles contained water in the first night. (**h-j**) Paradigm and data for nest-building assessment according to (**i**) the qualitative nest score (1-5 rating scale with 5 indicating the best nests [35]), and (**j**) left-over untorn material relative to weight of the original nestlet. (**k-n**) Paradigm and data for assessment of reciprocal social interaction with an unknown younger adult mouse of the same sex and strain. (**l**) Total time of social interactions and (**m**) total number of interaction over 16 min. (**n**) Interaction time in 2 min bins. Wildtype controls, grey; CD2-KO, black; *f*, females, *m*, males; numbers in brackets denote *n* for each group. * $P < 0.05$, ** $P < 0.01$, *** $P \leq 0.001$. For trends, *P* values are stated as numbers. Error bars, S.E.M..

Psychological Function	Behavioural test	Phenotype
Spatial associative learning	Water maze	Normal [23] but less precise/efficient searching[21]
	Plus-maze	Normal [22]
	Barnes maze	Normal, but more errors during training [24]
	Place preference (IntelliCage)	Normal [23]
Spatial reversal learning	Water maze	Impaired (delayed acquisition/higher perseverance) [21]
Spatial reversal learning	Plus-maze	Normal [22]
Spatial rule-shift learning	Plus-maze (ego→allocentric)	Impaired (delayed acquisition, but not due to perseverative errors) [22]
Aversive associative learning	Cue FC	Normal [19,23]; enhanced freezing in novel context [19], or reduced freezing in novel context after repeated cue presentation [17]
	Context FC	Normal [19,23] or reduced [17] freezing
	Trace FC	Improved [23]
Operant learning	Nose-poking for reward	Normal [23]
Procedural learning	(various)	Normal [23]
Anhedonia	Sucrose preference	Normal, but delayed preference [19]
Behavioural despair	Forced swim test, Tail suspension test	Mildly anti-depressive (reduced immobility) [20]
Nesting	Nest building	Reduced [19], potentially due to avoidance of novel nesting material [17]
Sensitivity to psychostimulant	Amphetamine-induced locomotion	Enhanced [17]
Stereotypy	Stereotypic movements	Decreased [19]
Processing of novelty	Novelty-induced locomotion	Enhanced [19] or normal [23]
	Novelty-induced exploration	Enhanced [19]
	Exploration of novel material, incl. bedding	Avoidance of novel material [17]
	Digging and marble burying	Reduced [19]
Unconditioned anxiety	Elevated Plus-maze	Normal [23]
	Open field	Normal [23]
Olfaction	(Various)	Normal [17] or reduced [23]
Visions, tactile sense	(Various)	Normal [17]
Posture, gait, muscle tone	(Various)	Normal [17]

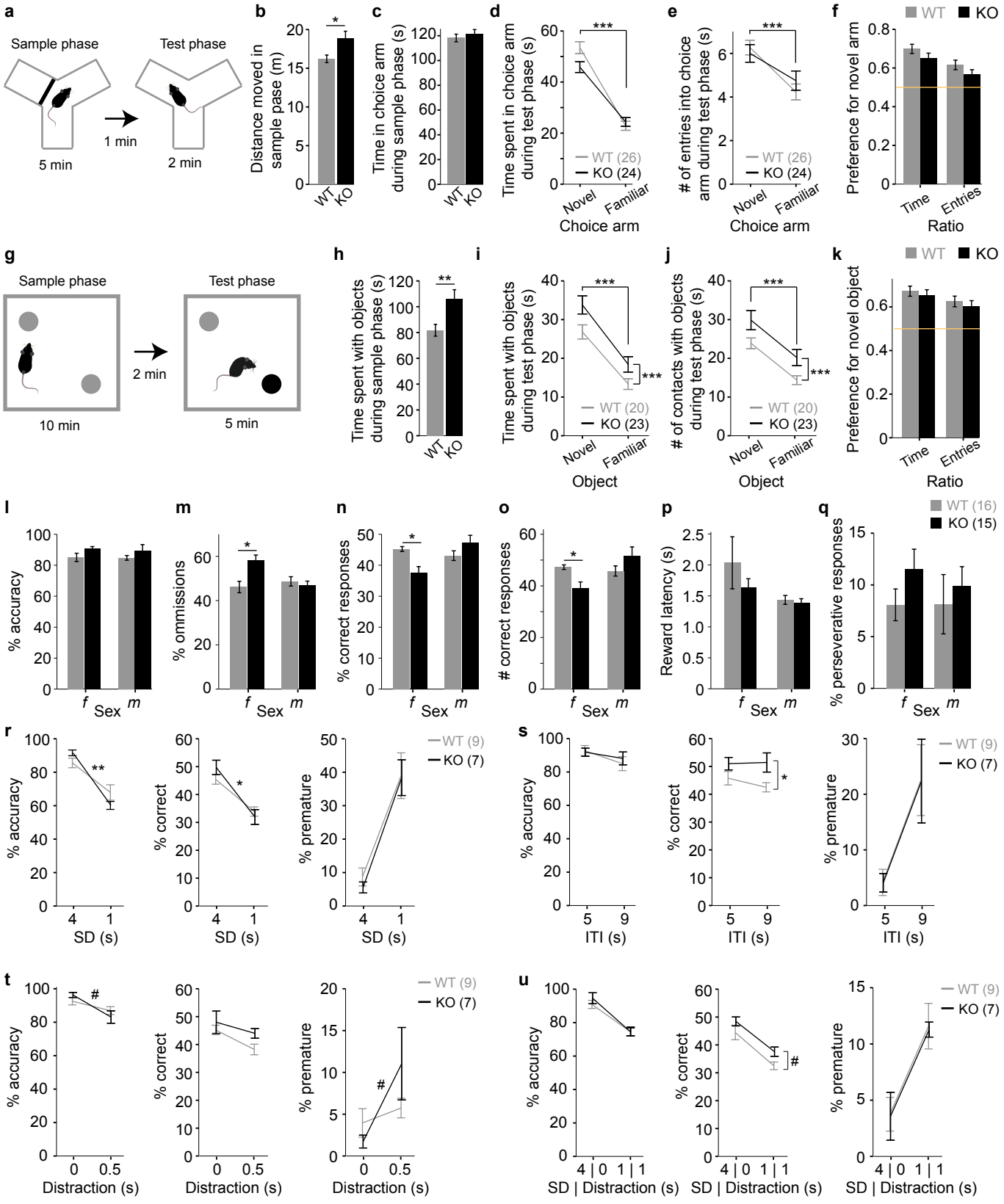
Table 1: Overview over previous behavioural studies in CD2-KO mice. FC, fear-conditioning. Numbers refer to citations in the reference list.



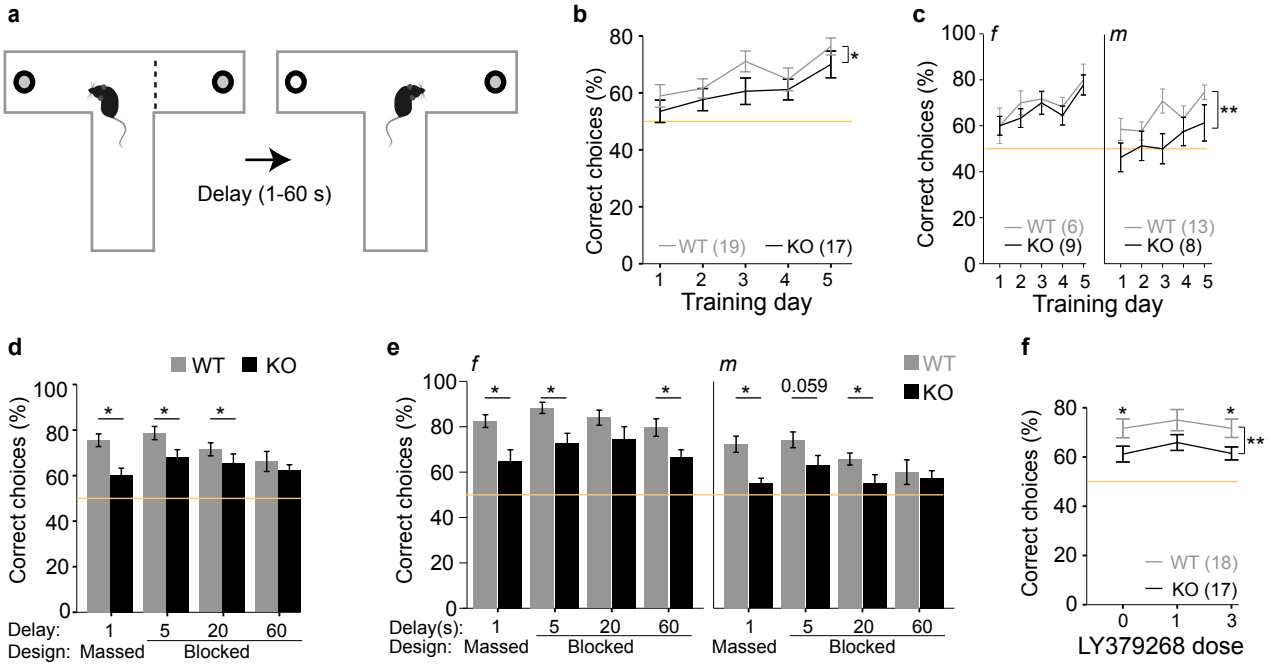
Grimm et al., Figure 1



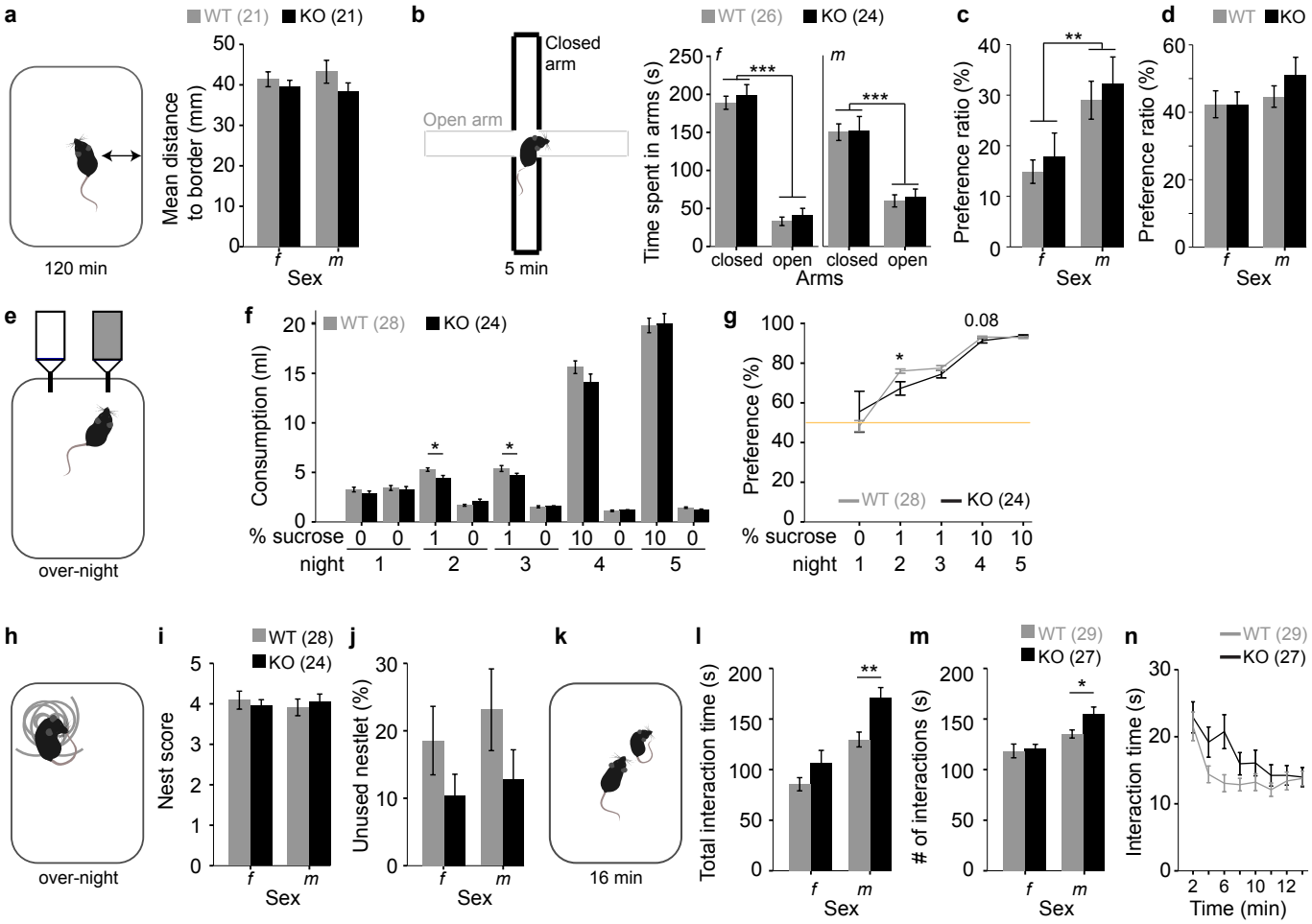
Grimm et al., Figure 2



Grimm et al., Figure 3



Grimm et al., Figure 4



Grimm et al., Figure 5