Modulation of cognition and neuronal plasticity in gain- and loss-of-function mouse models of the schizophrenia risk gene Tcf4

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Abstract

The transcription factor TCF4 was confirmed in several large genome-wide association studies as one of the most significant schizophrenia (SZ) susceptibility genes. Transgenic mice moderately overexpressing Tcf4 in forebrain (Tcf4tg) display deficits in fear memory and sensorimotor gating. As second hit, we exposed Tcf4tg animals to isolation rearing (IR), chronic social defeat (SD), enriched environment (EE), or handling control (HC) conditions and examined mice with heterozygous deletion of the exon 4 (Tcf4Ex4δ+/−) to unravel gene-dosage effects. We applied multivariate statistics for behavioral profiling and demonstrate that IR and SD cause strong cognitive deficits of Tcf4tg mice, whereas EE masked the genetic vulnerability. We observed enhanced long-term depression in Tcf4tg mice and enhanced long-term potentiation in Tcf4Ex4δ+/− mice indicating specific gene-dosage effects. Tcf4tg mice showed higher density of immature spines during development as assessed by STED nanoscopy and proteomic analyses of synaptosomes revealed concurrently increased levels of proteins involved in synaptic function and metabolic pathways. We conclude that environmental stress and Tcf4 misexpression precipitate cognitive deficits in 2-hit mouse models of relevance for schizophrenia.

Introduction

TCF4 encodes an ubiquitously expressed class I basic helix-loop-helix (bHLH) transcription factor which has been implicated in several neurodevelopmental disorders, mental retardation, intellectual disability, and schizophrenia (reviewed in ref. 1). The TCF4 gene has a complex structure harboring multiple 5′ initial exons that generate longer and shorter TCF4 protein variants2–5. TCF4 haploinsufficiency leads to Pitt–Hopkins syndrome (PTHS) characterized by mental and developmental retardation, episodic hyperventilation, and distinct facial features4–6.

In most cases, PTHS causing chromosomal deletions or frame shift mutations map to the 3′ end of the gene and cause TCF4 protein variants that lack the bHLH domain and/or are incapable of DNA binding7. A mouse mutant haploinsufficient for Tcf4 3′ exons 16 and 17 (which encode the bHLH domain) has been considered a model for PTHS8. More 5′ located deletions in TCF4 have been identified in individuals with intellectual disability who lack other features of PTHS5,9.

Large genome-wide association studies (GWAS) consistently identified several non-coding single nucleotide polymorphisms (SNPs) in the 5′ located introns of the TCF4 gene contributing to an increased risk for SZ11–14 and more recently also to major depressive disorder (MDD)15,16. TCF4 transcript levels have been shown to be moderately increased in blood cells of SZ patients and in neurons derived from induced pluripotent stem cells.
ings of hippocampal plasticity, as well as structural and negative symptoms\textsuperscript{18,29,30} although sample sizes must be implicated in cognitive performance and potentially also malformations as well\textsuperscript{28}. Thus, the view emerges that loss-of-deep behavioral pro

to IR to study gene-dosage effects. We complemented long
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liorate symptoms\textsuperscript{31,34} considered robust environmental risk factors, while intact animal models, chronic social isolation and defeat are increased to allow de
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totranscriptionally by neuronal activity\textsuperscript{24}. Recent shRNA-mediated knock-down analyses have provided evidence that TCF4 modulates neuronal development and function by repressing neurexins and the ion channels SCN10A and KCNQ1, respectively\textsuperscript{25,26}. In addition, high levels of overexpression of the long TCF4 variant TCF4B by in-utero electroporation has been shown to disturb cortical laminar development in an activity-dependent fashion\textsuperscript{27} and haploinsufficient mice display cortical malformations as well\textsuperscript{28}. Thus, the view emerges that loss-of TCF4 is implicated in embryonic neurodevelopment as well as postnatal neuroplasticity. Studies in humans have shown that risk alleles associated with TCF4 may be implicated in cognitive performance and potentially also negative symptoms\textsuperscript{18,29,30} although sample sizes must be increased to allow definitive conclusions. According to the gene–environment interaction (GxE) model for psychiatric diseases\textsuperscript{31,32}, it is likely that diverse environmental factors cooperate with TCF4 risk alleles and/or associated mechanisms. Thus far, only smoking has been identified as environmental risk factor modulating auditory sensory gating together with TCF4 risk alleles\textsuperscript{53}. In patients and animal models, chronic social isolation and defeat are considered robust environmental risk factors, while intact social structures and support have been shown to ameliorate symptoms\textsuperscript{31,34–36}. Moreover, an enriched environment positively influences rodent behavior and protects from psychopathologies\textsuperscript{36,37} whereas social isolation and social defeat (a model of chronic psychosocial stress) induce in mice a set of somatic, behavioral and molecular changes considered to be relevant endophenotypes of SZ\textsuperscript{35,38–40}.

To unravel GxE interactions, we performed comprehensive behavioral phenotyping of Tcf4tg and wildtype mice subjected to environmental stress by post-weaning isolation rearing (IR) or social defeat (SD) in contrast to group housing in an enriched environment (EE) or handling control (HC). We also analyzed heterozygous Tcf4 loss-of-function mice (Tcf4Ex4\textsuperscript{\delta/\textsuperscript{\delta}+}) in which heterozygous deletion of exon 4 reduces the expression of long Tcf4 transcript variants. These mice were subjected to IR to study gene-dosage effects. We complemented deep behavioral profiling by electrophysiological recordings of hippocampal plasticity, as well as structural and proteomic analyses characterizing gene-dosage dependent modulation of cognition and plasticity by TCF4.

**Materials and methods**

**Behavioral profiling**

The overall approach of the data calibration and dimensional reduction strategy and housing conditions were described previously\textsuperscript{35}. Analyses were done in R software version 2.15.2 using the R-package nlme and R-functions gls and anova. Graphs were generated using R-package plotrix, exported as.eps files and edited in Adobe Illustrator CS5. Behavioral tests were in general following published procedures\textsuperscript{22,35}. The investigator was blinded at the time of experimental procedures and the genotype decoding was performed at the end of the corresponding set of experiments. Further details and description of all mouse strains used are given in the Supplementary Information.

**Electrophysiology**

Microisland autaptic culture preparation, acute brain slice preparations, and electrophysiology were performed according to published procedures\textsuperscript{41}. LTP and LTD measurements were performed with hippocampal slices obtained from 3 to 4 week old Tcf4tg and Tcf4Ex4\textsuperscript{\delta/\textsuperscript{\delta}+} mice according to published procedures (see Supplementary Information for details).

**STED nanoscopy and proteomics**

The stimulated emission depletion (STED) nanoscopy experiment was performed essentially as described\textsuperscript{42}. Synaptosomes were isolated according to refs.\textsuperscript{43,44}. All Proteome analysis of cytosolic fractions (S1) and synaptosomes (S4) and the western blots were performed according to established protocols\textsuperscript{12,45} (see Supplemental Information for details).

**Results**

**Experimental design and Tcf4Ex4\textsuperscript{\delta/\textsuperscript{\delta}+} mouse model**

In this study, we combined analyses of TCF4 gain- and loss-of-function mouse models: Tcf4tg mice, which mildly overexpress Tcf4 in postnatal forebrain; and Tcf4Ex4\textsuperscript{\delta/\textsuperscript{\delta}+} mice, with heterozygous deletion of exon 4. To study the behavioral consequences of psychosocial stress in these mice, we analyzed three cohorts of wildtype (wt) and Tcf4 mouse models exposed to different environmental conditions starting from the perinatal period (Fig. 1a–d). In cohort 1, wt and Tcf4tg mice were exposed to enriched environment (EE) and isolation rearing (IR); in cohort 2, wt and Tcf4tg mice were subjected to social defeat (SD) by a 3-weeks daily exposure to an aggressive resident mouse, and to daily handling as a control (HC). In cohort 3, wt and Tcf4Ex4\textsuperscript{\delta/+} were subjected to IR. Subsequently, all animals were subjected to deep behavioral phenotyping in adulthood (Fig. 1b).
By homology, exon 4 of the mouse *Tcf4* gene corresponds to exon 5 in the human *TCF4* gene which is located in the middle of the 5′ variable region (exons 1–9), which has been demonstrated to generate multiple mRNA isoforms by differential splicing and 5′ initial exons (Fig. 1c). The 3′ constant region encompasses exons 10–21 which are common to all known TCF4 splice variants (Fig. 1c). Deletions within the 5′ variable region have been shown to be associated with intellectual disability, whereas most mutations associated with Pitt–Hopkins–Syndrome (PHS) are found in the 3′ constant region that encodes the bHLH dimerization and DNA binding domain. Several SNPs thought to confer an increased risk of SZ are found in the 5′ variable region flanking human exon 5 and 6 (corresponding to murine exons 4 and 5) (Fig. 1c, d).
and Supplementary Fig. 1a). Therefore, we hypothesized that the Tcf4Ex4Δ+/- mouse line might be of relevance in the context of cognitive disability and SZ. Molecular analysis showed that truncated exon 4 of Tcf4 was detectable only in Tcf4Ex4Δ+/- and not in control mice (Supplementary Fig. 1b). To measure the mRNA level of different Tcf4 exons in Tcf4Ex4Δ+/- mice, we performed qRT-PCR on transcripts from prefrontal cortex (PFC, Fig. 1e) and hippocampus (Hi, Fig. 1f). Expression levels of exons 1–2 in Tcf4Ex4Δ+/- mice were comparable to wt in both brain regions whereas exons 5–6 containing transcripts, located directly downstream of the deleted exon 4, were clearly reduced in both PFC and Hi (p < 0.0001 and p = 0.0277, t-test, respectively). The expression levels of consecutive exons were higher with increasing distance from exon 4, i.e. exons 7–9 containing transcripts showed less pronounced decrease in FCx (p = 0.0174, t-test) and similar tendency in Hi (Fig. 1e, f). This shows that only the long Tcf4 transcript variants are reduced in Tcf4Ex4Δ+/- mice. In wt mice, the exons most abundant in PFC are exons 7–9, and in hippocampus exons 5–6 (Supplementary Fig. 1c, d). Tcf4Ex4Δ+/- hypomorphic mice are viable, breed well and show neither increased mortality nor major developmental impairments. We detect no abnormal facial or body features of Tcf4Ex4Δ+/- mice that might reflect PHS-like phenotype (Supplementary Fig. 1e–i).

**Tcf4 modulates cognition in a gene-dosage and environment-dependent manner**

We profiled Tcf4 gain and loss-of-function mutants in the context of GXE interactions with a comprehensive test battery assessing various behaviors’ and condensed these to behavioral “domains” and “superdomains” adapted from selected research domain criteria (RDoc) for psychiatric disease models. We focused on cognitive (spatial and flexibility learning, fear memory, working memory), affective (negative and positive valence) and activity-related domains according to a multivariate statistical strategy based on stepwise data calibration and reduction combined with multiple-testing corrections (see Table 1 and Supplementary Table 1 for details) as described previously. The effects discussed below always refer to multiple-testing adjusted multivariate analyses unless stated otherwise.

A global test revealed highly significant effects of Tcf4 expression and environmental stress factors IR and SD, with p < 0.001 for all symptom classes (Supplementary Table 1). Most behavioral subdomains were significantly affected by environment, although marked differences were observed for cohort 1 (IR vs EE) and cohort 2 (HC vs SD) (see details under “Environmental Effects” column in Supplementary Table 1 and for selected tests Supplementary Fig. 2). We examined genetic main effects of Tcf4tg in EE, IR, SD, HC, and genetic main effects of Tcf4Ex4Δ+/- in IR, in comparison to respective wt controls. To comprehensively analyze these effects towards clinical symptom groups, we collapsed all individual behavioral tests (Supplementary Table 1) into behavioral domains that are depicted in Table 1 (for brevity, genetic main effects in HC (see Supplementary Table 1) are not displayed in Table 1 as they did not reach significance at the symptom class level). In EE, we obtained a significant difference between Tcf4tg and wt mice in the negative symptom class (p = 0.001), which was exclusively driven by the curiosity tests (rearing in the open field and hole board test, indicating reduced curiosity of Tcf4tg mice) (Supplementary Table 1). Note, that the effects for the individual tests failed to pass the significance threshold after Bonferroni correction whereas the multivariate and sum score analyses reached significance (Table 1 and Supplementary Table 1).

In IR (Tcf4tg and Tcf4Ex4Δ+/-) and SD (Tcf4tg), we did not obtain significant genetic main effects of Tcf4 alterations at the level of affective and activity domains (Table 1). Nonetheless, given the recent association of TCF4 with depression, we further inspected the subdomain level and identified that Tcf4tg mice exhibited under HC and SD significantly reduced fighting times in the tail suspension test monitoring depressive-like behavior (p < 0.001, Supplementary Table 1, Supplementary Fig. 3). The effect size of these observations, however, was not sufficient to obtain a significant result in the subdomain “Motivation” (Table 1, Supplementary Table 1).

In contrast to the affective and activity domains, the genotype significantly influenced cognitive symptoms. Tcf4tg mice displayed more severe cognitive impairments under IR (Tcf4tg: p = 0.005; Tcf4Ex4Δ+/-: p < 0.001) and SD (Tcf4tg: p < 0.001) than respective wt controls (Table 1, Supplementary Table 1). Housing mice in EE prevented cognitive deficits in Tcf4tg mice (p = 0.663) while daily handling (HC) partially ameliorated them (p = 0.068) (Table 1, Supplementary Table 1). In contrast to EE, HC was insufficient to prevent deficits in spatial learning (which became most pronounced under SD, Dataset Supplementary Fig. 1), possibly because of the missing social and sensory stimulation. We further inspected the genotype effects on the cognitive subdomains by visualizing the complete spectrum of cognition-related tests in radar charts (Supplementary Fig. 4).

The most pronounced environment-dependent cognitive deficits of the Tcf4 mouse models manifested in spatial learning, as assessed in Morris Water Maze, in the initial learning (Tcf4tg in SD, p = 0.001, and Tcf4Ex4Δ+/- in IR, p < 0.001) and flexibility learning (Tcf4tg in IR, p = 0.005; Tcf4tg in SD, p < 0.001; and Tcf4Ex4Δ+/- in IR, p < 0.001)(Fig. 2, Supplementary Fig. 4, Table 1). The
Table 1  Genetic main effects in Tcf4tg and Tcf4Ex46^{+/−} mice in different environments.

| Superdomain       | Domain                  | Measure | P\_global | EE environment tg/wt | IR environment tg/wt | SD environment tg/wt | IR environment Tcf4Ex46/wt \* | P  |
|-------------------|-------------------------|---------|-----------|-----------------------|----------------------|----------------------|-------------------------------|----|
|                   |                         | Effect  | Statistic | P                     | Effect t             | Statistic | P                     | Effect t             | Statistic | P |
|                   |                         | tg      | t60 = −0.438 | 0.663 | −1.811 | t66 = −2.890 | 0.005 | −1.997 | t66 = −4.372 | <0.001 | −1.632 | t66 = −3.723 | <0.001 |
|                   |                         | Statistic |          | P          | Statistic |          | P          | Statistic |          | P          |
| COGNITIVE DOMAIN  |                         |         | 0.127     | 0.015     | 0.093     | 0.621     | 0.465     | 0.413     | 0.47   | 0.104     | 0.104 |
|                   | Spatial Learning        |         | t40 = 0.040 | 0.969 | −4.103 | t50 = −2.397 | 0.019 | 3.899 | t56 = −6.578 | <0.001 | −4.112 | t56 = −6.670 | <0.001 |
|                   | Initial learning        |         | t40 = −0.128 | 0.899 | 2.598 | t54 = −1.469 | 0.148 | 3.035 | t56 = −3.378 | 0.001 | −4.834 | t56 = −4.815 | <0.001 |
|                   | Flexibility learning    |         | t40 = −0.849 | 0.400 | 7.877 | t54 = −2.919 | 0.005 | 4.698 | t56 = −6.020 | <0.001 | −6.862 | t56 = −4.632 | <0.001 |
|                   | Recall                  |         | 0.127     | 0.015     | 0.093     | 0.621     | 0.465     | 0.413     | 0.47   | 0.104     | 0.104 |
|                   | Fear memory             |         | t54 = −0.939 | 0.352 | −0.807 | t58 = −2.056 | 0.044 | 0.068 | t58 = 0.179 | 0.858 | 0.806 | t58 = 1.433 | 0.158 |
|                   | Context memory          |         | t52 = −0.830 | 0.410 | 0.066 | t56 = −0.144 | 0.886 | 0.318 | t56 = −1.129 | 0.271 | 0.763 | t56 = 1.512 | 0.137 |
|                   | Cue memory              |         | t54 = −0.729 | 0.469 | 1.548 | t58 = −3.437 | 0.001 | 0.453 | t58 = 0.801 | 0.431 | 0.95 | t58 = 1.585 | 0.119 |
|                   | Social fear memory      |         | ns        | −         | −         | −         | −         | −         | 1.553 | 0.2099 | 0.040 |
|                   | Working memory          |         | 0.127     | 0.015     | 0.093     | 0.621     | 0.465     | 0.413     | 0.47   | 0.104     | 0.104 |
|                   | Working memory          |         | t40 = 0.040 | 0.969 | −4.103 | t50 = −2.397 | 0.019 | 3.899 | t56 = −6.578 | <0.001 | −4.112 | t56 = −6.670 | <0.001 |
| AFFECTIVE DOMAIN  | Pain sensitivity        |         | t10 = −0.359 | 0.001 | −0.389 | t11 = −0.682 | 0.497 | −0.159 | t11 = 0.573 | 0.598 | −0.018 | t11 = 0.028 | 0.978 |
|                   | Anxiety                 |         | t40 = −0.935 | 0.358 | 0.773 | t40 = −0.348 | 0.730 | −         | −         | −         | −         | 0.120 | t40 = 0.098 | 0.923 |
|                   | Curiosity               |         | t40 = −1.251 | 0.216 | −0.115 | t40 = −0.490 | 0.626 | 0.371 | t40 = 0.641 | 0.523 | −0.082 | t40 = 1.271 | 0.207 |
|                   | Motivation              |         | t40 = −2.742 | 0.008 | 1.560 | t40 = 1.238 | 0.221 | 0.486 | t40 = 0.482 | 0.411 | −         | t40 = 0.251 | 0.621 |
| ACTIVITY DOMAIN   | Hyperactivity           |         | t40 = −0.824 | 0.413 | −0.224 | t50 = −0.266 | 0.791 | 0.163 | t50 = 0.560 | 0.577 | 0.490 | t50 = 1.070 | 0.289 |
|                   | Ambulation              |         | t40 = −0.850 | 0.376 | −0.768 | t50 = −0.391 | 0.360 | 0.776 | t50 = 1.205 | 0.231 | 0.447 | t50 = 1.832 | 0.407 |
|                   | Speed                   |         | t40 = −1.553 | 0.127 | −0.087 | t50 = −0.071 | 0.944 | −0.413 | t50 = −1.180 | 0.243 | 0.380 | t50 = 0.944 | 0.349 |

Significant differences are marked in bold and with stars in the case of P\_global. Numbers of degrees of freedom are marked for each statistic in subscript. Statistics depicted are from the multivariate analyses (see Supplementary Table 1 for all details).
Fig. 2 Tcf4 gene-dosage modulates cognition in environment-dependent manner. Tcf4tg mice (cohort 1, a, b, cohort 2, d, e) and Tcf4Ex4δ−/+ mice (cohort 3, g, h) were submitted to spatial learning in the water maze task. Cumulated results from water maze and other behavioral profiling (Table 1) are depicted as radar charts for Tcf4tg (c, f) and Tcf4Ex4δ−/+ (i) mice. a In the initial learning task in Morris Water Maze, Tcf4tg and wt mice in isolation rearing (IR) learned slower than mice in enriched environment (EE) (p < 0.001, environmental effect), as measured by the latency to reach a hidden platform. The effect was independent of genotype (p = 0.210, G×E interaction test). b During reversal learning, Tcf4tg mice subjected to IR needed more time to reach the platform than IR subjected wt littermates (p = 0.005), indicating an impairment of cognitive flexibility. c Behavioral profiles show impaired spatial learning upon IR (blue) and no cognitive deficits upon EE (green) in Tcf4tg mice compared to wt mice from the corresponding environment (black). Green and blue stars indicate significant differences (see Table 1 and Dataset Supplementary Fig. 1 for details). d Among genotype and housing conditions, all passing multiple-testing correction). However, at the superdomain level, displayed here, only nominal significance was reached (spatial learning p = 0.019, fear memory p = 0.044, blue stars in brackets). e In initial learning, socially defeated (SD) Tcf4tg mice displayed longer platform latencies from the handling control (HC) group or wt animals (p ≤ 0.001). Tcf4tg HC mice showed slightly delayed platform latencies than IR animals (p = 0.007, not reaching significance after Bonferroni correction). f In reversal learning (i.e. flexibility learning), Tcf4tg mice needed significantly more time to reach the platform than wt animals in both SD (p < 0.001) and HC groups (p = 0.002). g Behavioral profiles of Tcf4tg mice from SD and HC. Spatial learning in Tcf4tg mice is significantly impaired upon SD (red) and mildly in HC (gray) compared to wt mice in the corresponding conditions (black) as indicated by stars of corresponding colors (multiple-testing adjusted significance, see Table 1 and Dataset Supplementary Fig. 1 for details). Pain sensitivity was not assessed in this cohort (as indicated by white circle). g, h Tcf4Ex4δ−/+ mice housed in IR displayed higher platform latencies than controls in initial learning (p < 0.001)(g) and flexibility learning (p < 0.001)(h). i Behavioral profiles of Tcf4Ex4δ−/+ mice (blue) show that spatial learning and working memory are impaired compared to wt mice (black), as indicated by blue stars (multiple-testing adjusted significance, see Table 1 and Supplementary Table 1 for details). a, b, d, e, g, h Data represent mean ± SEM. n = 12–16 mice per genotype and housing conditions. See Table 1 and Dataset Supplementary Fig. 1 for detailed statistics.
transmission and/or neuronal plasticity may be compromised upon gain and loss of TCF4 function. To study glutamatergic neurotransmission at the single-cell level, we used the well-established autaptic culture paradigm of primary hippocampal neurons isolated from wt, Tcf4tg and Tcf4Ex4−/− mice. With this system, we studied several pre- and postsynaptic properties including short-term plasticity but none of these measurements revealed a genotype-dependent alteration (Supplementary Fig. 5).

To address the functional consequences of altered Tcf4 expression levels for synaptic transmission at the network level, we next studied synaptic transmission and plasticity at Schaffer collateral-CA1 pyramidal (SC-CA1) synapses in acute hippocampal slices from 4- to 5-week-old Tcf4tg and Tcf4Ex4δ−/− mice and their wildtype littermates (Fig. 3). First, we measured input–output curves at the Schaffer collateral synapses by extracellular field recordings to reveal the impact of TCF4 on the basal synaptic transmission. There was no difference detected between slices obtained from Tcf4tg and Tcf4Ex4δ−/− mice and corresponding wt controls (Fig. 3a, b), showing that different expression levels of TCF4 have no effect on basal synaptic transmission. Next, to understand the function of TCF4 on long-term synaptic plasticity, we measured hippocampal long-term potentiation (LTP) induced by high-frequency stimulation (100 Hz, 1 s) and long-term depression (LTD) induced by low-frequency stimulation (1 Hz, 15 min) at SC-CA1 synapses (Fig. 3c–f). LTP was unaltered in slices from Tcf4tg mice (mean at 35–40 min in Tcf4tg 150 ± 5% and in wt 151 ± 3%; p = 0.186) (Fig. 3c) but was significantly elevated in slices obtained from Tcf4Ex4δ−/− mice (mean at 35–40 min in Tcf4Ex4δ−/− 151 ± 3% versus wt 141 ± 3%; p = 0.001) (Fig. 3d). Moreover, Tcf4tg slices displayed a profound increase in LTD level (mean at 35 to 40 min in Tcf4tg 73 ± 2% versus wt 83 ± 2%; p = 0.001) (Fig. 3e), whereas in the Tcf4Ex4δ−/− slices LTD was unchanged (mean at 35–40 min in Tcf4Ex4δ−/− 79 ± 1% versus wt 79 ± 2%; p = 0.143) (Fig. 3f).

Thus, slight dysregulation of Tcf4 expression levels does not alter basic neurotransmission but shapes long-term plasticity in hippocampal neuronal networks. Moreover, changes in Tcf4 gene-dosage can mediate a differential impact on LTD and LTP.

**Synapse-related alterations in Tcf4tg mice**

Based on the altered synaptic plasticity and the evident cognitive deficits in adult Tcf4tg mice which were particularly prominent in the reversal “flexibility” learning paradigm (Fig. 2b, c), we aimed at identifying cellular and molecular correlates in synaptic structures. For these analyses, we focused on frontal cortical structures (anterior cingulate cortex, ACC; and medial-prefrontal/orbitofrontal cortex, OFC) implicated in associative and flexibility learning and known for its importance in schizophrenia. First, we applied super-resolution STED microscopy to quantitatively assess spine morphologies and densities on dendrites from Tcf4tg and wt control mice where sparse numbers of neurons were genetically labeled with EYFP. Spine morphology was analyzed in 4- and 12-week-old Tcf4tg and wt control mice in EE and 12-week-old Tcf4tg and wt mice subjected to SD (Fig. 4a). Tcf4tg mice showed increased total spine density at 4 weeks of age (p = 0.031), but no such difference was observed in 12-week-old animals (Fig. 4b). SD stress during puberty caused a significant decrease in spine density in 12-week-old mice (p = 0.003) independent of the genotype (Fig. 4b). We further analyzed the relative distributions and densities of five subtypes of spines with increasing morphological complexity: filopodium/stripe, stubby/stump, mushroom/racket, cup/sickle, and branched spines, as previously reported (Supplementary Fig. 6a). The relative distribution of different spine types was similar across the genotypes and experimental groups (Supplementary Fig. 6b, d, f). In accordance with the total spine analysis (Fig. 4b), spine densities of all subtypes showed a subtle yet overall significant increase in 4 weeks old Tcf4tg mice (p = 0.0055, two-way ANOVA) with the most prominent difference detected at immature “stubby/stump” like spines (p < 0.01, post hoc) (Supplementary Fig. 6c). Again, no significant differences between the genotypes were observed at 12-week-old animals independent of stress conditions (Supplementary Fig. 6e, g).

We performed electron microscopy on tissue sections from 4-week-old Tcf4tg and wt controls to assess abundance and morphologies of synapses in the ACC and OFC at the ultrastructural level (Supplementary Fig. 7). The total number of asymmetric (i.e. mature glutamatergic) synapses was not altered between the genotypes and regions (Fig. 4c). Moreover, a close inspection of several ultrastructural characteristics of synapses (shape of the postsynaptic density and active zone, number and distribution of synaptic vesicles) did not reveal any quantitative differences (Supplementary Fig. 7d–l), as was the number of mitochondria and myelinated axons unchanged (Supplementary Fig. 7m–q).

Finally, we applied a quantitative isoform-coded mass spectrometric proteomic analysis of cytoplasmic fractions and synaptosomes (enriched for pre- and postsynaptic components associated with the postsynaptic density) isolated from frontal cortex tissue of 4 weeks old Tcf4tg and wt littermates. After stringent filtering (fold-change >1.5<1.5, corr. p-val.<0.05, ≥2 peptides), we identified 38 up- and 10 down-regulated proteins in the synaptosomal fraction of Tcf4tg mice (Supplementary Table 2). In the cytoplasmic fraction, we detected 13 up- and 24 down-regulated proteins in Tcf4tg (Supplementary Table 2). As
expected, the overlap was low with only 4 proteins detected in both subcellular fractions (Supplementary Table 2). We performed a gene ontology pathway enrichment analysis and visualized the results as a highly connected network of pathway-node associations (Fig. 4d). This analysis identified synapse-function and interconnected metabolism associated pathways as the two major subclusters (Fig. 4d).

Taken together, our results imply that moderately dysregulated levels of TCF4 expression—50% increase in Tcf4tg mice\(^{22}\) and 50% decrease of the “long isoforms” in Tcf4Ex4δ\(^{+/-}\) mice—have a profound impact on higher...
order cognition likely caused by disturbed synaptic plasticity rather than severe structural alterations in the corresponding neuronal networks.

**Discussion**

In this study, we investigated gene–environment interactions in two mouse models with slightly altered Tcf4 transcript levels. This may mimic the changes in human TCF4 expression levels linked to risk variants in intronic TCF4 regions associated with SZ, MDD, and intellectual disability. By applying a “domain-oriented” deep behavioral profiling, we detected behavioral deficits in these mice, which were induced by the combination of the genetic and psychosocial risk factors. We saw that isolation rearing (IR) was more detrimental for Tcf4-overexpressing (Tcf4tg) than for wt mice, while enriched environment (EE) masked this genetic vulnerability. Similar effects were obtained when we compared Tcf4tg and wt mice subjected to social defeat (SD), a chronic psychosocial stress paradigm, with mice handled once daily but otherwise kept in isolation (HC). In both comparisons, most behavioral domains were affected except activity, which was assessed by “ambulation” (i.e. speed and distance running) in several behavioral tests. These were used as the only surrogate parameter of a hyperactive state often observed during a psychosis-associated phase in patients, possibly reflecting an aspect of positive symptoms51. This clearly indicates a limitation of rodent models because prototypic positive symptoms cannot be investigated. Nonetheless, we believe that our findings of strong
GxE interaction provide evidence that the “2-hit” Tcf4/SD and Tcf4/IR approaches represent valid mouse models for the cognitive subdomain of relevance for SZ and possibly also MDD. Tcf4tg mice used for behavior were on F1 hybrid background while Tcf4Ex4+$^{+/−}$ were kept on a pure C57b6 background. Thus, we cannot formally rule that this may have an impact on the direct comparison of the different lines, we consider this as rather unlikely since we have previously shown that Tcf4tg mice backcrossed for several generations on the C57b6 background performed highly similar in behavioral testing compared to F1 hybrids. The “pairing” of risk gene alterations with environmental stress conditions has been suggested as promising strategy to improve validity of psychiatric mouse models. Given the strong associations of TCF4 and psychosocial stress with schizophrenia and MDD, it seems likely that relevant mechanisms are affected in our 2-hit Tcf4 gain- and loss-of-function mouse models.

TCF4 has recently been identified by the PsychEncode consortium as a key “hub” gene in human brain development, and was found to be expressed at increased levels in the Hi and PFC of SZ patients from independent postmortem sample collections. On the other hand, both increased and reduced TCF4 expression in blood has been detected in independent studies and subject groups suffering from psychosis and MDD, it seems likely that relevant mechanisms are affected in our 2-hit Tcf4 gain- and loss-of-function mouse models.

Calcium signals from ligand and voltage-gated channels play a central role in coupling synaptic activity to the downstream signaling cascades, and several members of both groups (NMDA- and AMPA-receptors and L-type Ca-channels, in particular) have been strongly associated with psychotic disorders. The relationship between the strength of synaptic activity and the equivalent calcium signal on one hand and the sign (potentiation versus depression) and degree on the other is described by the Bienenstock-Cooper-Munro (BCM) model. This model is in agreement with the findings that sliding calcium levels activate different calcium/calmodulin-depend-ent protein kinases and/or other calcium-modulated synaptic proteins, which partially determine the differential induction of LTP or LTD. Several such candidates have been identified in our proteome analysis. Based on our findings, follow up experiments are needed to substi-tuate the hypothesis that Tcf4 gene dosage determines postsynaptic Ca$^{2+}$ levels and to identify the responsible target genes of TCF4. We conclude that the presented 2-hit mouse models represent valid tools for pre-clinical treatment trials, e.g. with cognitive enhancers that target cognitive processing units of relevance for affective and non-affective psychoses.

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Author contributions
D.M.B., M.M.B., and A.C. performed behavioral analyses. D.M.B. performed electron-microscopic analyses. N.K. performed bioinformatic analyses of omics data. P.D., H.S., and K.W. performed S.T.E.D. imaging and analysis. M.M.B., D.M., and C.W.T. performed synaptosome isolations and proteomic measurements. P.F., A.S., and S.P. contributed conceptually. J.S.R. performed electrophysiological measurements. D.M. performed multiparametric statistical analysis. P.F., A.S., and S.P. contributed conceptually.

Conflict of Interest
The authors declare that they have no conflict of interest.

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