Trisomy of the G protein-coupled K^+ channel gene, *Kcnj6*, affects reward mechanisms, cognitive functions, and synaptic plasticity in mice

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Edited* by Lily Yeh Jan, University of California, San Francisco, CA, and approved January 3, 2012 (received for review June 7, 2011)

G protein-activated inwardly rectifying K+ channels (GIRK) generate slow inhibitory postsynaptic potentials in the brain via $G_{i/o}$ protein-coupled receptors. GIRK2, a GIRK subunit, is widely abundant in the brain and has been implicated in various functions and pathologies, such as learning and memory, reward, motor coordination, and Down syndrome. Down syndrome, the most prevalent cause of mental retardation, results from the presence of an extra maternal chromosome 21 (trisomy 21), which comprises the *Kcnj6* gene (GIRK2). The present study examined the behaviors and cellular physiology properties in mice harboring a single trisomy of the *Kcnj6* gene. *Kcnj6* triploid mice exhibit deficits in hippocampal-dependent learning and memory, altered responses to rewards, hampered depotentiation, a form of excitatory synaptic plasticity, and have accentuated long-term synaptic depression. Collectively the findings suggest that triplication of *Kcnj6* gene may play an active role in some of the abnormal neurological phenotypes found in Down syndrome.

electrophysiological recordings | neuronal culture | fear conditioning | sucrose preference | open-field

Down syndrome (DS) is caused by a trisomy of chromosome 21 (HSA21) with the frequency of 1 for every 733 births. The major neurological DS phenotypes include mental retardation, muscle hypotonia, and appearance of Alzheimer disease neuropathology after the age 35 (1, 2). In addition, DS individuals also exhibit hyperactivity in youth (3) and abnormal pain perception (4). It is accepted that the observed abnormalities result from increased gene dosage of the 420 genes located HSA21 (5–8), and that the neurological manifestations may be due to changes in synaptic structure and function (9). Because many HSA21 gene products are known or predicted to have functional role in critical neurological processes, much effort was devoted in recent years in assigning critical genes or regions for DS phenotypes, by mainly using mouse models (10). Identifying the smallest region of overlap in individuals who are trisomic for only part of HSA21 (segmental trisomy 21), and share the same subset of DS features, has been used as a basis for defining DS critical region (11). The DS critical region in human genes list includes the *Kcnj6* gene, which encode for the G protein-coupled inwardly rectifying K^+ channel type 2 (GIRK2), yet currently, it is not clear what are the physiological and cognitive effects associated with this gene trisomy.

In the brain, GIRK channels generate slow inhibitory postsynaptic potentials (12) and play an important role in the control of resting membrane potential and regulation of cellular excitability (13). In the CNS, GIRK channels are activated by various *Pertussis* toxin-sensitive $G_{i/o}$ protein-coupled receptors, such as $GABA_B$, A_1 adenosine receptor and D_2, D_3, and D_4 dopaminergic receptors (14, 15), through the release of the Gβγ subunits of the G protein (16, 17). Neuronal GIRK channels consist mainly of GIRK1 and GIRK2 heteromers, and are widely expressed in the CNS, mainly at postsynaptic sites (18). In some brain regions, like the hippocampus, heteromers of GIRK1 and GIRK3 are also expressed, and in others, like the substantia nigra, homomeric channels consist of GIRK2 are present (18). Studies of null mice for the various GIRK-channel subunits implicated these channels in brain mechanisms that relates to hyperalgesia, seizure susceptibility, cognitive functions, reward mechanisms, and anxiety (18). Most of the information collected so far has been obtained using animal models that lack one or two of the GIRK channel subunits; however, very little is known about the pathological consequences associated with an increase in gene number. More specifically, little is known about the consequences of GIRK2 gene trisomy and its relation to DS.

A mouse line with trisomy for *Kcnj6* alone (285E6/67) was established (19) that displays long-term spatial-memory deficits (20) with intact long-term potentiation (LTP) in hippocampal slices (21). The present study sought to investigate the effects of *Kcnj6* trisomy in mice using behavioral and cellular physiology assays. It was evident that *Kcnj6* triploid mice exhibit deficits in hippocampal-dependent recall of contextual fear conditioning, modified reward mechanisms, hampered postsynaptic depotentiation (DP), and accentuated long-term depression (LTD). We propose that increased dose of GIRK2 channels may have significant consequences that can lead to some of the neurological abnormalities found in DS patients.

Results

GIRK2 Trisomy Affects Reward Mechanisms. Because others (e.g., refs. 22 and 23) have associated various GIRK channel subunits with altered reward mechanism, the present study assessed the effects of an extra copy of *Kcnj6* on sucrose preference, a well-established index of reward mechanism activity (24). First, we weighted the animals and found no difference between the triploid and diploid weight (Fig. 1*A*). Initial assessment compared triploid and diploid *Kcnj6* mice water and sucrose consumption separately (i.e., when either water or sucrose was the only available liquid to consume). Triploid mice drank significantly less ($P < 0.05$) water (Fig. 1*B*) than diploid mice, yet no difference was evident between triploid and diploid mice sucrose consumption (Fig. 1*C*). Interestingly, when *Kcnj6* diploid and triploid mice were allowed to freely consume either water or sucrose, the triploid mice drank significantly ($P < 0.01$) more sucrose than diploid mice, yet drank comparable amounts of water (Fig. 1*D*). Overall, it appears that triploid *Kcnj6* mice display altered responses to the availability of sucrose that may be related to altered function of reward mechanisms.

Author contributions: A. Cooper, G.G., M.M.T., A. Chen, and E.R. designed research; A. Cooper, G.G., and L.G.-D. performed research; M.M.T. contributed new reagents/analytic tools; A. Cooper, G.G., and M.M.T. analyzed data; and A. Cooper and E.R. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1109099109/-/DCSupplemental.

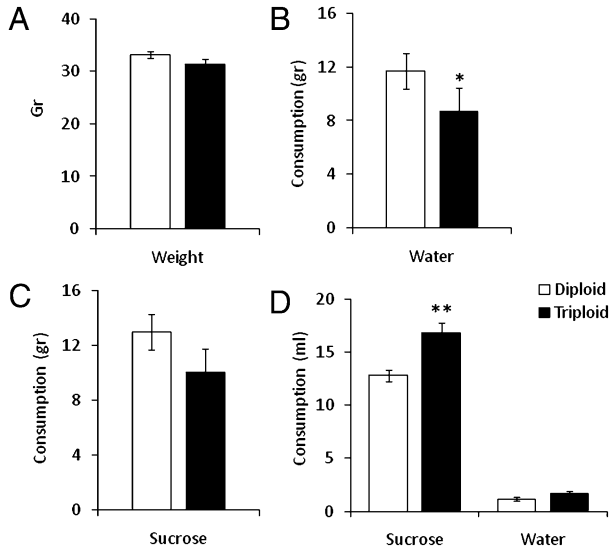

Fig. 1. Increased sucrose consumption on the sucrose preference test. (*A*) Body weight of adult mouse in grams ($n = 12$ for each group). (*B*) Amount of water consumed over 3 d in grams when water was available solely ($n = 8$ for each group). (*C*) Amount of sucrose consumed over 3 d in grams when sucrose was available solely (diploid: $n = 7$, triploid: $n = 8$). (*D*) Amount of sucrose and water consumed over 3 d in milliliter on a "choice" test ($n = 12$ for each group). $^{*}P < 0.05$, $^{**}P < 0.005$.

Normal Motility, Anxiety-Like Behavior, and Circadian Cycle Activity in Triploid Mice. To rule out altered freezing responses because of altered motor functions, and altered hippocampal function because of increased anxiety, the effects of an extra copy of *Kcnj6* on motility and anxiety-like behavior were assessed. Anxiety-like behaviors were assessed using three well-established behavioral assays: open-field (OF), elevated plus-maze (EPM), and dark-light transfer (DLT). In all these tests, mice exploratory behavior in a novel setting is monitored; the more a mouse explores this new environment, the longer duration it spends in the "risky" sections of the apparatus (OF center, EPM open arms, and DLT light section) or the more quickly and frequent it visits these sections, the less it is considered anxious (25). No differences were observed between triploid compared with diploid *Kcnj6* mice in relative time in the OF center (Fig. S1*A*) or time spent in the EPM different arms (Fig. S1*B*). In the DLT the triploid and diploid *Kcnj6* mice did not differ in distance traveled and time spent in the light section (Fig. S1 *C* and *D*), yet compared with diploid *Kcnj6* mice, triploid mice visited the light section significantly fewer times ($P < 0.05$) (Fig. S1*D*). Collectively, the anxiety indices over all assessments (OF, EPM, and DLT) suggest that an extra *Kcnj6* copy is insufficient to alter anxiety-like behaviors in a substantial manner. Further assessments monitored general home-cage locomotion activity throughout the circadian cycle, and found no difference between the diploid and the triploid mice (Fig. S1*E*).

GIRK2 Trisomy Affects Hippocampal-Dependent Recall. Because the triplication of the HSA21 fragment in mice was shown to affect cognitive functions (26), we evaluated the contribution of a single gene, *Kcnj6*, trisomy on learning and memory processes, using the auditory fear-conditioning paradigm (27) assessing contextual, hippocampal-dependent, and cue, amygdala, and hippocampal-dependent, recall. The cue-recall assessments (cue-test) indicated no differences in freezing duration at 2 h, 26 h, or 1 wk posttraining (Fig. 2*A*), suggesting unaffected amygdalar functions in these triploid mice. In contrast, *Kcnj6* triploid mice exhibited significant ($P < 0.05$) less freezing than *Kcnj6* diploid mice in the context test, specifically at 24 h following the conditioning (Fig. 2), suggesting altered hippocampal-dependent learning and memory processes. No differences were evident 1 wk following the conditioning.

To conclude so far, in comparison with *Kcnj6* diploid mice, the triploid mice exhibited a specific deficit in the hippocampal-dependent contextual-fear recall task, which was coupled by an altered response to sucrose rewards; yet *Kcnj6* triploids and diploids did not differ substantially in anxiety-like behaviors, motility, circadian cyclicity, and body mass.

Normal Resting Potential and Increased Basal- and Adenosine- but Not Baclofen-Induced GIRK Current in Triploid Hippocampal Neurons. In light of the observed altered behaviors, we were interested in the cellular properties of triploid versus diploid neurons in the hippocampus. Therefore, electrophysiological recordings were performed on primary hippocampal neurons obtained from diploid and triploid mice. We found no difference in resting-membrane potential between the diploid and the triploid neurons with -47.8 ± 2.1 mV ($n = 18$) and -47.0 ± 2.2 mV ($n = 20$), respectively (Fig. 3*A*). Under conditions where the extracellular solution contained 60 mM KCl, barium-sensitive inward currents (-70 mV holding potential), measure of basal GIRK currents, the triploid neurons displayed significantly larger ($P < 0.05$) inward currents compared with the diploid neurons with 8.26 ± 1.87 pA/pF ($n = 8$) and 15.33 ± 0.85 pA/pF ($n = 9$), respectively (Fig. 3 *B* and *C*). The effect of GIRK2 trisomy on receptor activated GIRK currents (at -70 mV, by either 100 μM adenosine or 50 μM baclofen), was diverse. Although adenosine-induced GIRK current was elevated in triploid neurons (6.67 ± 0.91 pA/pF, $n = 8$) compared with diploid (3.88 ± 0.53 pA/pF; $n = 9$), baclofen-induced current remained the same with 7.32 ± 1.41 pA/pF ($n = 11$) and 10.10 ± 1.93 pA/pF ($n = 10$) for the triploid and the diploid neurons, respectively (Fig. 3*C*).

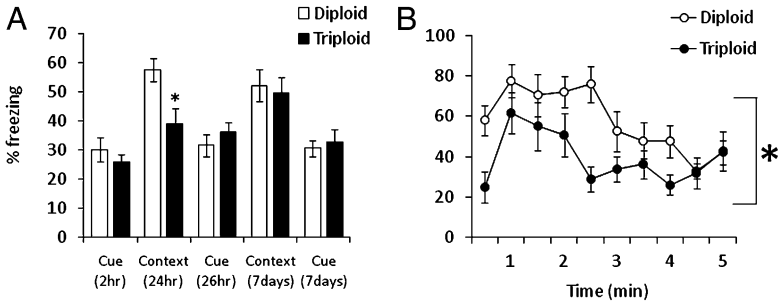

Fig. 2. Abnormal memory of the context a day after training on the fear-conditioning paradigm. (*A*) Percent of freezing in a 5-min session of fear conditioning, on cue test [2 h (diploid: $n = 8$, triploid: $n = 6$), 26 h ($n = 8$ for each group) and 7 d posttraining ($n = 7$ for each group)] and context test [24 h (diploid: $n = 8$, triploid: $n = 6$) and 7 d posttraining)]. (*B*) Percent of freezing on the context test a day after training in bins of 30 s. $^{*}P < 0.05$.

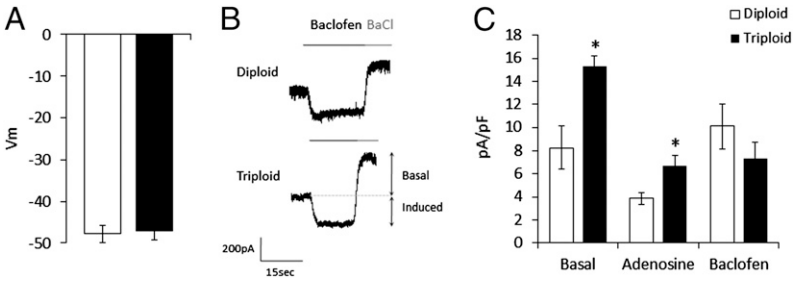

Fig. 3. Normal resting potential and increased basal and adenosine but not baclofen-induced GIRK current in triploid neurons. (*A*) Neuronal resting potential (Vm) (diploid: $n = 18$, triploid: $n = 20$). (*B*) Sample traces of inward GIRK currents recorded at −70 mV with high concentration of K^+ at the external solution. GIRK current was induced by Baclofen (50 μM), $GABA_B$ agonist, and blocked by $BaCl_2$ (3 mM). (*C*) Average of basal current (diploid: $n = 8$, triploid: $n = 9$) and current induced by adenosine (diploid: $n = 8$, triploid: $n = 9$) and baclofen (diploid: $n = 10$, triploid: $n = 11$). $^*P < 0.05$.

Blunted DP of Basal-GIRK Current in Triploid Hippocampal Neurons. GIRK2 was also found as key player in DP, a form of synaptic plasticity that regulates the induction of LTP (28). In this process, the activation of NMDA receptor (NMDAR) elicits an increase in GIRK currents by the elevation of GIRK2 channel number on the plasma membrane (29, 30). The molecular and cellular mechanisms of DP were described in detail but its physiological relevance still remains unknown. We were interested to find whether *Kcnj6* trisomy affects this process. We mimicked DP by comparing GIRK currents in hippocampal neurons that were exposed to 0.2 mM (2R)-amino-5-phosphonovaleric acid (APV), an NMDAR antagonist, for 24–48 h and 15 min following the removal of APV (30). Basal GIRK currents were significantly increased in diploid and triploid neurons after the 15-min wash of APV with 0.71 ± 0.45 pA to 33.33 ± 9.12 pA for the diploid ($P < 0.05$) and 13.05 ± 4.75 pA to 33.67 ± 9.58 pA for the triploid ($P < 0.05$) (Fig. 4*A*). Interestingly, the basal currents of the triploid neuron before the removal of APV were significantly higher that of the diploid neurons ($P < 0.05$), but was not different 15 min after APV removal. On triploid neurons the basal current did not increase significantly as a result of APV wash, probably because of the initial higher current density. These results suggest hampered DP in triploid neurons, a probable cause for some of the behavior abnormalities described above. We next tested whether the receptor activated GIRK currents. Adenosine-induced current was larger in triploid neurons than diploid ($P < 0.05$) (Fig. 4 *B* and *C*). The removal of APV did not cause increase in adenosine-induced currents in cells from both genotypes. In contrast, the baclofen-induced current was similar in neurons before and 15 min after APV removal, for both the diploid and the triploid mice (Fig. 4 *B* and *D*).

GIRK2 Triploid Mice Have Hampered Synaptic DP and Are More Prone to LTD. The effect of GIRK trisomy on synaptic plasticity has also been examined by electrophysiological recordings from hippocampal slices. Field excitatory postsynaptic potentials (EPSPs) were recorded in stratum radiatum of the CA1 region of hippocampus to measure DP. First, we induced LTP (tetanized stimulation at 100 Hz for 1 s), which was normal in the triploid slices, with 1.83 ± 0.06% and 1.93 ± 0.12% for the diploid and triploid mice, respectively (Fig. 5 *A* and *B*), as was shown before (21). Then, DP was induced by low-frequency stimulation (LFS), 2 Hz for 10 min. The EPSP decline, as a measure of DP, was obstructed in the triploid slices (Fig. 5 *A* and *B*). The DP was unexpectedly transient on the diploid slices, perhaps because of genetic background difference from a previous report (30); nevertheless, in the triploid mice induction of DP was greatly attenuated, with 1.13 ± 0.09% ($n = 7$) and 1.39 ± 0.07% ($n = 7$) of the EPSP level right after LFS for the diploid and triploid mice, respectively. Similar results, of normal LTP and abnormal transient DP, were also obtained when the LFS was delivered 15 min after the tetanized stimulation (Fig. S2) ($n = 4$ for each group). In addition, when LFS was applied to a non-LTP–induced pathway in the same slice, we detected a small reduction in EPSP for the diploid slices and stabilization back to baseline levels within 7 min, as expected (Fig. 5*C*). Interestingly, the same stimulation initiated LTD in the triploid slice that was stable for the duration of the experiment ($P < 0.05$; F value, 3.46) (Fig. 5*D*). Overall, these results indicate of abnormalities in synaptic plasticity in the *Kcnj6* triploid mice.

Elevation in GIRK1 and GIRK2 Expression but Not GIRK3 in Triploid Hippocampus. Given the above results, we were interested in testing whether the GIRK channel levels were affected by the *Kcnj6* trisomy. The levels of protein expression in triploid and diploid hippocampus were measured for the three subunits of GIRK that are expressed in this hippocampus (GIRK1 to 3), using Western blot analysis. For quantitative analysis, we calculated the amount of GIRK relative to a control membrane

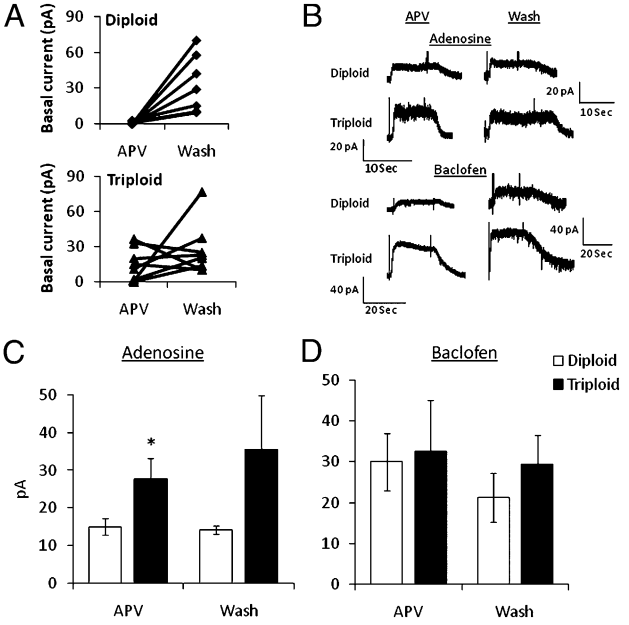

Fig. 4. Blunted DP of basal-GIRK current in triploid neurons. (*A*) Basal GIRK current with APV and after 15-min wash. Three traces of Tertiapin-sensitive current were averaged for each cell on each condition (diploid: $n = 7$, triploid:$n = 9$). (*B*) Sample traces of outward GIRK currents recorded at −50 mV from the same neuron before and 15 min after removal of APV. The GIRK current was induced by adenosine (100 μM) and baclofen (50 μM). Averaged adenosine (diploid: $n = 10$, triploid: $n = 9$) (*C*) and baclofen ($n = 5$ for each group) (*D*) -induced GIRK current with APV and after 15-min wash. $^*P < 0.05$.

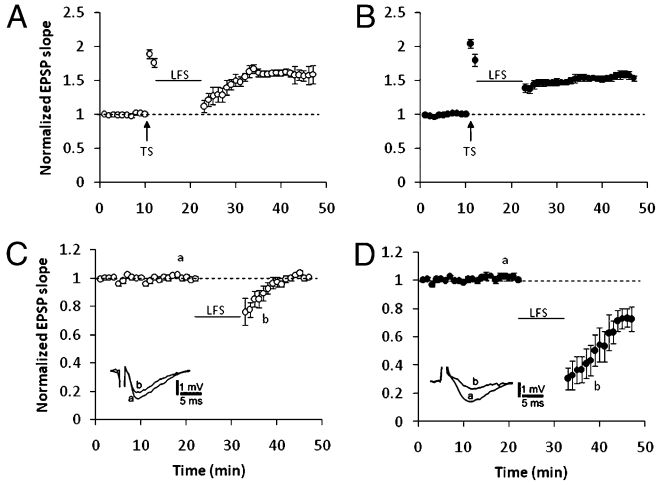

Fig. 5. Hampered DP and accentuated LTD. EPSP slope (normalized to baseline) of DP, induced in diploid (*A*) and triploid (*B*) brain slices by tetanus stimulation (TS) followed by LFS after 2 min (n = 7 for each group) and representative sample traces. EPSP slope (normalized to baseline) of diploid (*C*) and triploid (*D*) brain slices that were stimulated only by LFS (n = 7 for each group) and representative sample traces.

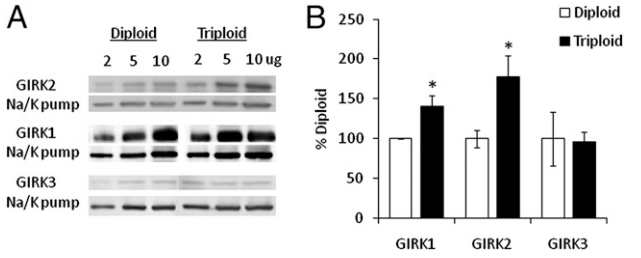

Fig. 6. Elevation in GIRK1 and GIRK2 protein expression but not GIRK3 in triploid Hippocampi. (*A*) Western blot of hippocampus total protein for GIRK1-3 and the Na/K ATPase for reference. (*B*) Quantification of GIRK1 to -3 protein expression levels normalized to Na/k ATPase, as percentage of control (diploid). *$P < 0.05$ (n = 3 mice per group).

protein, Na/K ATPase pump. The expression levels of GIRK1 and GIRK2 were higher in triploid hippocampi relative to diploid ($P < 0.05$) (Fig. 6). No difference was found in the expression level of the GIRK3 subunit.

Discussion

The *Kcnj6* gene encodes for the GIRK2 potassium channels that serves as one of the end points of translating chemical transmission to electrical signaling at the postsynaptic site, and is the main mechanism for generating slow inhibitory postsynaptic potentials (12). One can raise the possibility that an increase in GIRK2 currents may affect synaptic function early on in development, and in the mature animal, to reshape the electrical balance in the brain. Previously, Ts65Dn, TS1Cje, and Ts1Rhr mouse lines have been extensively used to characterize neurological abnormalities associated with the triplications of many genes in the HSA21 region (31–39). In cases where GIRK currents have been studied, the data indicated an increase in current density, as expected from a triplication of the *Kcnj6* gene in these animal models. In addition, LTP was decreased and LTD was increased. It is of note that the above noted extensive research used mouse models with multiple gene triplication, thus raising the need to investigate the role of a single *Kcnj6* gene triplication to better delineate the specific effects of an access of this gene in the neurological manifestation associated with animal models for DS. This study sought to characterize the contribution of the *Kcnj6* gene trisomy to neurological manifestation seen in DS phenotype. The findings indicated that the presence of an extra copy of *Kcnj6* gene affects both hippocampal-dependent memory, reward mechanisms, and at the cellular level, synaptic DP and LTD. These behavioral and cellular effects have been associated with some of the neurological malfunctions in DS patients (26).

Effect of GIRK2 Trisomy on the Mouse Behavior. Comparing the behavioral phenotype of the GIRK2 triploid mouse model with that of DS mouse models, Ts65Dn (10), indicated several differences and similarities. Although the Ts65Dn mouse model tends to be overactive (10), GIRK2 triploid mice show normal exploration and motility. Furthermore, the circadian cycle activity of GIRK2 triploid mice was also normal, but Ts65Dn mice displayed increased activity levels during the dark phase of a 12/12-h light/dark cycle (35). The *Kcnj6* triploid model also differed from the Ts65Dn model in the effect on body weight; although Ts65Dn mice weighed less than euploid (40), no differences were observed in body weight of GIRK2 triploid and diploid mice. However, in accordance with the Ts65Dn model (41), GIRK2 triploid mice exhibited normal anxiety-like behaviors.

In a similar manner to Ts65Dn mice (42), GIRK2 triploid mice expressed a specific deficit in contextual recall—but not cued recall—following fear conditioning, suggestive of hippocampal dysfunction. Several hippocampal-dependent learning paradigms, including different forms of spatial learning (43) and contextual fear conditioning (42), indicated cognitive deficits among Ts65Dn mice (see ref. 10 for review). It is of note that this deficit was transient (i.e., evident 1 d following the conditioning but not 1 wk later). Because others (e.g., refs. 44–46) have suggested that the hippocampus is necessary for retaining context-dependent memories until they are consolidated in neocortical structures, these findings suggest altered hippocampal functions that affected the transient phase of hippocampal-dependent contextual memory retention. Alternatively—yet not excluding—according to Dudai and colleagues' (47) theory of "trace dominance," the transient deficit may be due to a weak "memory trace" at 24 h posttraining that was amplified, rather than undergoing extinction, during the test the day after the conditioning . Nevertheless, Chabert et al. (20) did report impaired long-term memory in the Morris water maze task, suggesting hippocampal-dependent memory dysfunction that corresponds with the current findings. The current results are discordant with previous reports (20), indicating this line of mice as displaying normal contextual fear conditioning, yet methodological differences in the conditioning paradigm, apparatus, and freezing quantification method between the present study and the previous report may account for this discrepancy. Furthermore, these methodological considerations may also account for the discrepancies in cued fear conditioning; Ts65Dn exhibited deficits in cued fear conditioning (31), but GIRK2 triploid mice freezing in response to the cue did not differ from those of GIRK2 diploid mice, suggesting intact amygdala-dependent learning and memory.

GIRK2 has been implicated frequently with the reward-system function, especially in assays of drugs addiction; for example GIRK2 and GIRK3 knockout mice exhibit decreased self-administration of cocaine (22, 48, 49). Indeed GIRK2 trisomy affected sucrose consumption in the current study. GIRK2 triploid mice drank significantly less water when it was the only available liquid, tended to drink less sucrose when it was the only available liquid, yet drank significantly more sucrose when they could choose between the two. Taken together, these findings suggest irregularity in their reward circuits, which coincide with the substantial presence of GIRK2 in dopaminregic neurons (50).

Effect of GIRK2 Trisomy on Cellular Activity and Properties. Coinciding with altered hippocampal-dependent learning and

memory, we observed a significant increase in the expression levels of GIRK2 in the triploid hippocampus. Previously it was found that GIRK1 expression levels were also increased in a similar manner in Ts65Dn mice, suggesting that an increase in GIRK1/2 currents densities may affect the balance between inhibitory and excitatory transmission, to cause potentially adverse consequence in cognitive function (51, 52). Our data regarding GIRK3 expression levels indicated no difference. Therefore, it is suggested that learning and memory of contextual fear conditioning and different forms of activity-dependent synaptic plasticity in the hippocampus may involve GIRK channels composed of GIRK1 and GIRK2 but not GIRK3 currents.

Having established at this stage of the study that GIRK2 trisomy alters hippocampal-dependent learning and also increases GIRK1 and GIRK2 expression levels, we were interested in the potential cellular mechanisms underlying these effects. To this end, we measured the activity and properties of triploid neurons compared with diploid in primary hippocampal culture using the whole-cell variation of the patch-clamp technique. We found normal resting potential of the triploid neurons but the basal (nonreceptor-induced) and adenosine-induced GIRK current were significantly higher. However, baclofen-induced GIRK current were not changed. It seems that adenosine-evoked currents depend on GIRK channel quantity in a special manner, because it was also reported that only adenosine-evoked currents increase as a result of GIRK channel elevation in DP, even though DP can be triggered chemically by other GIRK-coupled receptors, such as 5-HT activators (53).

Because the behavioral abnormalities observed in the GIRK triploid mice could not be related to impaired LTP (21), we assessed the effects of GIRK2 trisomy on a different type of synaptic plasticity mechanism (i.e., DP, a form of excitatory synaptic plasticity). The elevation in basal GIRK current upon NMDAR activation, which was shown before to be the mechanism of DP (29, 30), is hampered in triploid neurons. Evidence for hampered DP was detected in brain slices as well, because no reduction in EPSP was observed in the triploid mice after LFS. NMDAR activation did not alter GIRK-induced currents by A1R, in contrast to our expectation, because of previous reports of adenosine involvement in DP (28, 30). $GABA_B$-evoked current did not increase as a result of NMDAR activation, in accordance with previous results that demonstrated internalization of $GABA_B$ receptor upon NMDAR activation (54). Interestingly, LFS induced LTD in the triploid slices but not on the diploid ones, probably because of elevation in GIRK channels, which caused elevation in potassium efflux. One might expect that elevation in potassium channels would lead to more depression in the DP, as was evident in the LTD induction; however, in GIRK trisomy, hampered DP was probably because of lack of NMDAR stimulation to elevate the number of GIRK channels on the membrane, as they were already at their maximum. Irregularities in two types of synaptic plasticity, DP and LTD, however, can explain, in part, the changes in the behavior of these animals.

To summarize, the trisomy of GIRK2 causes deficits in hippocampal-dependent learning and memory of contextual fear conditioning and altered motivation to consume sucrose. These effects were coupled by an increase in the expression levels of GIRK1 and GIRK2 in the hippocampus. Furthermore, trisomy of GIRK2 resulted in alterations of several cellular properties, such as elevation in basal- and adenosine-induced GIRK current, hampered DP, and accentuated LTD. This study provides additional critical physiological effects of the overdose of the *Kcnj6* gene in a devastating genetic disease. The partial display of DS phenotypes strengthens the idea that it is a complex syndrome mediated by a contribution of many genes (55). A better understanding of the contribution of triplication of specific genes located in DS critical region to DS neurological abnormalities would lead to better mouse models and bring us closer to find therapy.

Materials and Methods

Animals. The strain YAC 285E6 (Tg67) was generated on an FVB inbred background by refs. 19 and 21 and had integrated one copy of the human *Kcnj6* gene. The strain was purchased from the European Mouse Mutant Archive (http://www.emmanet.org/index.php) as cryopreserved embryos. The embryos were implanted in surrogate FVB mothers. The FVB background that was used to microinject the transgenic fragments carries a recessive mutation (rd) inducing retinal degeneration. To avoid any impact of *rd* affecting visual cues, we used F1 male offspring born from C57BL/6J females and transgenic FVB males (21). Mice were maintained in a pathogen-free temperature-controlled room (22 ± 1 °C) with food and water available ad libitum on a 12-h light-dark cycle. All experimental protocols were approved by the Institutional Animal Care and Use Committee of The Weizmann Institute of Science.

Behavioral Assessments. ***Fear conditioning.*** This paradigm was described previously (56); briefly, percent-time spent freezing, indexing fear was assessed in mice throughout the habituation session, the conditioning session, the contextual memory test, and the cue memory test. Apparatus: a computer-controlled fear-conditioning system (TSE Systems) monitors the procedure while measuring the mice freezing behavior. Procedures: day 1, habituation; day 2, conditioning. During this 5-min training session, mice were exposed to two pairings of a coterminating tone and shock.

Context test. Context-dependent memory was assessed by reexposure to the conditioning box for 5 min without any stimuli. The context test was performed 1 d and 1 wk after conditioning.

Cued test. Presentation of the tone alone was twice (2 and 3 min from the beginning of the session) in a novel context. The cue test was performed 2 h postconditioning and 2 h after each context test (meaning 2 h, 1 d and 1 wk after conditioning).

Statistical Analysis. All data are reported as mean ± SEM. Sample size (*n*) refers to the number of animals in a group, neurons analyzed in electrophysiological recordings from cultured hippocampal neurons, slices analyzed in electrophysiology recordings from hippocampal slices, or the number of animals used to analyze levels of protein expression. Between-groups effects were assessed by independent Student *t* tests or one-way ANOVA; within group effects were assessed by paired-sample Student *t* tests.

ACKNOWLEDGMENTS. We thank Ruth Meller and Elisha Shalgi for technical help and the E.R. laboratory for helpful comments. The work was supported in part by the Josef Cohn Center for Biomembrane Research, Israeli Science Foundation Grant 207/09, The David and Fela Shapell Family Center for Genetic Disorders Research, The Nella and Leon Benoziyo Center for Neurological Diseases, and the Foundation Jerome le Jeune.

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