Prion Protein Modulates Monoaminergic Systems and Depressive-like Behavior in Mice*

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Background: The prion protein (PrPSc) functions as a scaffold for cell surface signaling systems, and plays a role in neurodegenerative diseases that include clinical depression among their symptoms.

Results: PrPSc-null mice showed depressive-like behavior concomitant with functional changes in monoaminergic systems.

Conclusion: PrPSc regulates functions of monoaminergic synapses.

Significance: PrPSc may be involved in major depression and related neuropsychiatric disorders.

We sought to examine interactions of the prion protein (PrPSc) with monoaminergic systems due to: the role of PrPSc in both Prion and Alzheimer diseases, which include clinical depression among their symptoms, the implication of monoamines in depression, and the hypothesis that PrPSc serves as a scaffold for signaling systems. To that effect we compared both behavior and monoaminergic markers in wild type (WT) and PrPSc-null (PrPSc−/−) mice. PrPSc−/− mice performed poorly when compared with WT in forced swimming, tail suspension, and novelty suppressed feeding tests, typical of depressive-like behavior, but not in the control open field nor rotarod motor tests; cyclic AMP responses to stimulation of D1 receptors by dopamine was selectively impaired in PrPSc−/− mice, and responses to serotonin, but not to norepinephrine, also differed between genotypes. Contents of dopamine, tyrosine hydroxylase, and the 5-HT5A serotonin receptor were increased in the cerebral cortex of PrPSc−/− mice, as compared with WT mice. Microscopic colocalization, as well as binding in overlay assays were found of PrPSc with both the 5HT5A and D1, but not D4 receptors. The data are consistent with the scaffolding of monoaminergic signaling modules by PrPSc, and may help understand the pathogenesis of clinical depression and neurodegenerative disorders.

The term prion, an abbreviation of proteinaceous infectious particle, underscores the prevailing concept that an infectious, self-propagating, protein-only particle is the cause of transmissible spongiform encephalopathies, a group of neurodegenerative disorders that include scrapie, Creutzfeldt-Jakob disease, fatal familial insomnia, bovine spongiform encephalopathy, and the chronic wasting disease of cervids (1, 2). The disease-related aggregates of abnormally folded protein, often referred to as prion scrapie (PrPSc), arise by structural conversion of the endogenous cellular protein known as the prion protein (PrPSc), encoded by the human gene PRNP (Prnp in mouse) (3, 4). Although the abnormal conformers have historically received the most attention, there is growing interest in the physiological roles of the native, endogenous PrPSc, due to its pleiotropic functions and more recently to the links with Alzheimer disease and cancer (see Refs. 5–7 for reviews).

The prion protein is mainly, albeit not exclusively, expressed at the surface of nerve and immune cells and, similar to other glycosylphosphatidylinositol-anchored proteins, PrPSc associates with lipid rafts amid continuous trafficking around distinct plasma membrane domains and intracellular vesicles (8, 9). Experimental studies show that PrPSc participates in events such as cell proliferation, differentiation, and survival through various signaling pathways (5, 6).

An increasing body of evidence supports the hypothesis that PrPSc functions as a cell surface scaffold protein, that is, it serves as a hub for the assembly of diverse signaling modules involved in a variety of physiological functions (5, 10). Indeed, the scaffold hypothesis is consistent with the often controversial functional properties assigned to the prion protein, which extend far beyond the nervous system, including immune responses (11, 12), cancer biology (6, 13), heart oxidative stress (14), glucose homeostasis (15), and stem cell regulation (16, 17). Such widespread influence probably depends on the ability of PrPSc to bind several partners, varied combinations of which populate the cell surface of distinct cell types (5, 10).

Functional consequences of the direct binding of PrPSc to several neurotransmitter receptors were reported (18–21), and neurotransmission may be further affected by PrPSc through co-regulation of the expression of neurotransmitter receptor subunits (22). In particular, antibody-mediated engagement of PrPSc reduced signaling through adenylyl cyclase, phospholipases C and A2, from at least three subtypes of the G protein-coupled serotonin (5HT) receptor and modulated the crosstalk among these pathways in a cell line that expresses 5HT receptors as well as PrPSc (23, 24).

Monoaminergic systems are disturbed as diseases progress in animal models of transmissible spongiform encephalopathies.

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‡ The abbreviations used are: PrPSc, prion protein; 5HT, serotonin; AD, Alzheimer disease; SERT, serotonin transporter; TPH, tryptophan hydroxylase; TH, tyrosine hydroxylase; FST, forced swim test.
Changes were reported in PrPSc-infected brains, of the levels of serotonin, dopamine, and norepinephrine (25–27), as well as in the activity of monoaminergic receptors (28) and related enzymes, such as monoaminooxidase B (29) and adenylyl cyclase (30). Moreover, serotonergic dysfunction was observed in human clinical cases of fatal familial insomnia (31), and dopaminergic depletion was reported in patients with Creutzfeldt-Jakob disease, associated with rapid cognitive decline and movement disorders (32, 33). Almost 40% of patients of sporadic Creutzfeldt-Jakob disease showed symptoms of major depression, a disorder linked to monoaminergic neurotransmission (34, 35). Notably, PrPc-null mice reportedly showed signs of depressive-like behavior, which were corrected by imipramine, a tricyclic antidepressant that acts primarily as a monoamine reuptake inhibitor (36).

On the other hand, the prion protein has also been identified as a ligand of oligomers of the β-amyloid peptide (37), which are closely tied with signal corruption in the brains of patients of Alzheimer disease (AD) (38, 39). Clinical depression has been associated with AD, both as a symptom and as a risk factor (40, 41), and despite current controversy as to the role of biogenic amines in AD-associated depression (42), both neuropathological and in vivo imaging studies demonstrated links between monoaminergic systems and AD (43–45).

A phage display screen conducted in our laboratory suggested that PrPc may bind both the 5HT5A serotonergic receptor and the serotonin transporter SERT.3 In light of the apparent connections among monoaminergic neurotransmission, cell surface PrPc, and behavioral output, we hypothesized that monoaminergic signaling might be affected in the absence of PrPc. The present study both extends behavioral analysis to Prnp knock-out mice of a well controlled genetic background, as well as discloses novel neurochemical changes that may underlie their depressive-like behavior.

**Experimental Procedures**

**Animals**

Experiments were done using male, 8–12 weeks old, C57BL/10 and PrPc-knock-out mice (Npu Prnp<sup>−/−</sup>; 107), backcrossed to C57BL/10 mice for at least 10 generations. The founders of our colony were kindly provided by Drs. Bruce Chesebro and Richard Race (Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT). Heterozygous mice were mated and homozygous F1 descendants were crossed to generate PrPc-null (Npu Prnp<sup>−/−</sup>) embryos and their respective wild-type controls (Npu Prnp<sup>+/+</sup>). Animals were housed in groups of five per cage, under a 12-h light/12-h dark cycle, with controlled room temperature and humidity. Food and water were provided ad libitum. All procedures followed institutional guidelines and were approved by the Animal Care and Use Committee of the Federal University of Rio de Janeiro.

**Behavior**

**Forced Swim Test (FST)**—The FST were conducted as described by Porsolt (46), with minor modifications (47). Mice were individually forced to swim for 6 min in an open cylinder (65 cm height, 30 cm diameter) filled with water (24 ± 1 °C) to a height of 45 cm. The testing sessions were preceded, 1 day earlier, by an habituation session in which each mouse was placed in the swimming cylinder for 15 min, as a means to reduce stress and/or anxiety on the next day. Water was changed between subjects, and after testing animals were dried under a warm light for 20 min. Immobility time was recorded along the 6 min of testing, by a trained researcher blind to group assignment. Mice were considered immobile when they ceased struggling and remained floating motionless in the water, making only movements necessary to keep their head above water.

**Tail Suspension Test**—Mice were suspended individually to 60 cm above the floor by adhesive tape placed ~1 cm from the top of the tail. Immobility time induced by tail suspension was recorded during 6 min according to the method described by Steru et al. (48) with minor modifications (49). Immobility was defined as the absence of any limb or body movements, except for those caused by breathing.

**Novelty Suppress Feeding Test**—Mice were deprived of food for 24 h before testing. At the time of testing, one food pellet was placed on a white filter paper located in the middle of a test arena (30 × 30 × 45 cm). One at a time, mice were placed in one of the corners of the arena and allowed to explore for a maximum of 10 min. Their latency to visit and begin chewing the food pellet was recorded. Immediately after the test, animals were transferred to their home cages and allowed to feed freely.

**Locomotor Activity Assay (Open-field Test)**—Mice were placed in the corner of a 30 × 30 × 45-cm box divided in 9 smaller squares. The number of squares crossed with all paws (crossings) and the raising the forepaws (rearings) were scored in 5-min sessions. The box was thoroughly cleaned with a solution of 30% alcohol and dried between subjects.

**Accelerating Rotarod Test**—Motor performance was tested using an accelerating Rotarod (Insight, Brazil). All mice were pre-trained in the apparatus to reach a stable performance and to minimize novelty stress. Training consisted of 3 sessions on 3 consecutive days, each session including 3 separate runs. In each training run, animals were placed on the rods at an initial speed of 6 rpm for 30 s. The speed was then increased gradually to 37 rpm over 5 min. If a mouse fell from the apparatus during a training session, it was immediately placed back on, until the end of the run. Between runs, mice rested for at least 2 min, to reduce stress and fatigue. On the day of testing, 5 consecutive trials were made with 15-min inter-trial intervals, and in each trial the speed was slowly increased from 6 to 37 rpm within 5 min. Results were expressed as latency of animals to fall from the apparatus.

**Assay of Accumulation of cAMP**

Mice were euthanized in a CO2 chamber and immediately decapitated. Brains were removed, and the cerebral cortex or striatum dissected out and cut into 400-μm slices using a tissue chopper. Slices were selected and distributed equally in 12-well plates filled with aCSF (126 mM NaCl, 12 mM MgCl2, 2.5 mM KCl, 21.4 mM NaHCO3, 11.1 mM glucose, 1.2 mM NaH2PO4, and 400 μM ascorbic acid), saturated with a 95% O2, 5% CO2 mixture, just before the experiment. A phosphodiesterase
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inhibitor (3-isobutyl-1-methylxanthine, 50 μM) was added for 15 min and then each well received a 40-min stimulus with forskolin, serotonin, dopamine, norepinephrine, SKF-38393, SCH-23390, SB-699551, or raclopride. Generation of cAMP was completely stopped by the addition of 10% trichloroacetic acid, and then assayed radiometrically, as previously described (50).

Western Blots

Mice cerebral cortex was dissected out in ice-cold PBS, and homogenized in a solution containing 2% SDS, 20 mM EDTA, and 62.5 Tris-HCl, pH 7.5. Samples were centrifuged at 10,000 × g for 10 min at 4 °C. Supernatants were collected and protein concentrations were determined using the BCA method. For each sample, 30 μg of protein were used for SDS-PAGE, transferred to PVDF membranes, and probed with: anti-PrPC (1:1000), anti-TPH (1:1000), anti-tryptophan hydroxylase (TPH) (1:500), anti-D1 (1:400), anti-SERT (1:500), anti-5HT5A (1:400), and anti-TH (1:1000), anti-Phospho-TH Ser40 (Cell Signaling, number 2791, 1:1000), anti-5HT1A (Santa Cruz, sc-1459, 1:100). Anti-α-tubulin (Sigma, T6074, 1:50,000) and anti-GAPDH (Millipore, MAB374, 1:3000) antibodies were used as loading controls.

Extraction and Quantification of Monoamines

Brain monoamines and their immediate metabolites were measured by HPLC coupled with electrochemical detection (HPLC-ED), following a protocol adapted from Arita and co-workers (51). Briefly, animals were decapitated, brains were removed, and the cerebral cortex or striatum dissected out in ice-cold PBS. Perchloric acid was added to each sample to a final concentration of 0.1 M. Samples were sonicated (2 pulses of 5 s, 50 Hz) and then centrifuged (10,000 × g) for 10 min to remove precipitated proteins. Supernatants were used for HPLC. For normalization, pellets were resuspended and protein concentrations were quantified with a BCA kit (Thermo Fisher Scientific, Waltham, MA). Fast isocratic separation was achieved with the following mobile phase: 20 mM sodium phosphate dibasic; 20 mM citric acid, 10% methanol, 0.12 mM Na2EDTA, and 566 mg/liter of heptanesulfonic acid, pH 2.64.

Immunohistochemistry

Animals used for immunohistochemistry were anesthetized and transcardially perfused with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed and placed in phosphate buffer containing increasing concentrations of sucrose (10, 15, and 30%), for cryoprotection. Frozen coronal sections (40 μm) were cut using a cryostat (Leica Microsystems, Wetzlar, Germany) and stored in TBS-AF (Tris-buffered saline, 0.05% sodium azide, 30% glycerol, and 15% sucrose) at 4 °C. For free-floating immunohistochemistry, sections were washed in PBS and blocked for 2 h with 0.25% Triton, 3% BSA, and 3% normal donkey serum. The following primary antibodies were then incubated overnight at 4 °C: anti-PrPC (Cayman, SAF83, 1:200), anti-D1 (Merck, US324390, 1:400), anti-SERT (Sigma, SAB4200039, 1:500), anti-5HT5A (Sigma, SAB2101110, 1:400), anti-TH (Millipore, MAB318, 1:400), and anti-TPH (Millipore, AB1541, 1:500). After washing with PBS, sections were incubated for 2 h at room temperature with Alexa Fluor secondary antibodies (Life Technologies), briefly stained with DAPI (1 μg/ml), and mounted on glass slides with n-propyl gallate.

Overlay Assay

Recombinant mouse PrP<sup>C</sup> was separated by SDS-PAGE in various lanes (5 μg/lane), and transferred to a nitrocellulose membrane. Protein migration was verified with Ponceau S staining. The membrane was incubated for 16 h at 4 °C in a solution containing 100 μg of a total protein extract from the cerebral cortex of wild type mice, in 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM CaCl<sub>2</sub>, 1% BSA, and 0.5% Nonidet P-40. Then, individual lanes were cut, and each was subject to Western blotting with the appropriate antibody. In addition to D1, 5HT5A, and SERT, we also probed overlay membranes with an antibody to dopamine receptor D4 (Santa Cruz, SC-1439, 1:1000), as a control. Target proteins are detected only if binding to the previously identified PrP<sup>C</sup> band.

Statistical Analysis

Except where stated otherwise, data are presented as mean ± S.E. We used Student’s t test, or analysis of variance followed by Bonferroni’s post hoc test, to compare means among treatments. Differences between treated and control groups were considered significant when p < 0.05.

Results

We first tested for depressive-like traits in PrP<sup>C</sup>-null mice as compared with control littermates. In the “forced swim test,” PrP<sup>C</sup>-null mice were immobile for significantly longer periods than wild-type controls (Fig. 1A). Similar results were obtained with the “tail suspension test” (Fig. 1B). Such behavior, giving up struggling and remaining immobile in adverse situations, is commonly interpreted as a measure of “hopelessness,” and is susceptible to antidepressant treatments (52), thus likely indicative of a depressive-like state. A more complex procedure, known as “novelty suppressed feeding test,” also showed a difference between groups. Food-deprived PrP<sup>C</sup>-null mice had a higher latency to seek out a food pellet, when compared with wild-type animals (Fig. 1C). This increased latency may be interpreted as unwillingness to eat despite being hungry, a depressive-like behavior analogous to anhedonia (53).

To test whether motor deficits might explain the behavioral effects, we compared the performances of both wild-type and PrP<sup>C</sup>-knock-out mice in the “open field test” and in the more demanding “accelerating rotarod test.” No significant differences were found between genotypes either in the number of crossings/rearings measured in the open field, or in the latency to fall from the rotarod apparatus, thus indicating similar motor capacities in both groups (Fig. 1, D and E).

Changes in synaptic functions are likely to explain the behavioral differences between the mouse genotypes. Therefore, we assessed parameters of cortical monoaminergic transmission, through measurements of neurotransmitter-induced cAMP accumulation. As expected, serotonin, dopamine, and norepinephrine elicited accumulation of cAMP when administered to
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For the dose-dependent loss of wild-type response is unknown, but may be related to nonspecific activation of other G-protein-coupled receptors. Moreover, such high doses of SKF-38393 were shown to be potentially toxic and cause internalization of D1 receptors (54).

The D1-like receptor antagonist SCH-23390 fully prevented dopamine-mediated accumulation of cAMP in wild-type slices, but it had no effect on PrP<sup>C</sup>-knock-out tissue (Fig. 3B). To test whether an overactivation of D2-like receptors, rather than the apparent lack of D1-receptor response, is at play in the absence of PrP<sup>C</sup>, we examined the effect of the D2-like receptor antagonist raclopride. At 2 μM, raclopride had no effect on the net accumulation of cAMP induced by 100 μM dopamine in cortical slices of either genotype (Fig. 3C). We also tested the effects of a 5HT5A receptor antagonist. The relatively higher cAMP production stimulated by 1 nM serotonin in PrP<sup>C</sup>-null tissue when compared with wild-type (Figs. 2A and 3D) was partially prevented in the presence of SB-699551-A, upon which no significant difference was found between genotypes (Fig. 3D).

Western blots of monoamine-related proteins showed similar levels of SERT, TPH, 5-HT1A, and D1 in both genotypes. 5HT5A levels, however, were found increased by ~30% in knock-out mice, and a similar increase was detected in the levels of TH (Fig. 4, B–D and F), albeit the proportion of TH phosphorylation in the Ser-40 residue was unchanged between groups (Fig. 4, A and E). In addition, we compared the total levels of 5HT, dopamine, noradrenaline, and their metabolites 5-hydroxyindoleacetic acid and 3,4-dihydroxyphenylacetic acid, in extracts from the cerebral cortex of wild-type and PrP<sup>C</sup>-null mice. A significant increase was found in total dopamine levels of PrP<sup>C</sup>-null tissue, whereas the other neurotransmitters and metabolites were similar in both genotypes (Fig. 5A). Again, there were no differences between the levels of either dopamine or 3,4-dihydroxyphenylacetic acid in the striatum of mice of both genotypes (Fig. 5B).

Finally, we examined the pattern of immunostaining of proteins related to serotonergic and dopaminergic synapses, and compared those with the prion protein. In both genotypes, the general patterns of immunostaining were similar for tryptophan hydroxylase (TPH), the 5HT transporter (SERT), 5HT5A receptor, tyrosine hydroxylase (TH), as well as for the most abundant dopamine receptor in the cortex, D1R. Simultaneous PrP<sup>C</sup> immunostaining was strongly positive in the cerebral cortex of wild-type mice, and it often co-localized with either D1R or 5HT5A (Fig. 6). These results were consistent with an overlay assay that resulted in binding of PrP<sup>C</sup> to D1R, but not to D4R, and to 5HT5A, as well as binding to SERT (Fig. 7).

Discussion

In the present study, we used 8–12-week-old wild-type and PrP<sup>C</sup>-null mice both to further explore a previous report of depressive-like traits in PrP<sup>C</sup>-deficient animals (36), and to examine monoaminergic signaling in the cerebral cortex. Among our results, the higher immobility in PrP<sup>C</sup>-null mice subject to the FST and tail suspension test, as well as their increased latency in novelty suppress feeding test, support the hypothesis that the lack of prion protein leads to depressive-like
behavior in mice. Differing, however, from the study by Gadotti and co-workers (36), we did not find significant differences in motor function between genotypes.

Consistent with our results, no difference had been detected by other authors in the same motor tests when PrP-null mice were compared with their wild-type littermates of a distinct genetic background, although an increase in locomotion and exploratory behavior was reported in mice that overexpress PrP (56). An earlier study had, however, reported increased numbers of crossings in an open field by PrP-null mice when compared with wild-type, but the data presented were not compelling (57). It should be noted that in both the latter studies, wild-type mice were descendants of the mating of C57/BL6 and 129/sv mice, whereas the PrP-null mice were from an independent colony derived from a Prnp knock-out colony derived several years before (58). In turn, our colony derived from heterozygous mice has been inbred for several generations into the C57/BL10 background, thus leading to a higher degree of congenicity (59).

Our results are especially consistent with reports that motor deficits detectable by either rotarod or treadmill testing in PrP-null mice are age-dependent. Thus, differing results in such tests were absent between wild-type and PrP-null genotypes at 8–12 weeks of age, but apparent in older animals (60, 61). Other groups also reported age-dependent motor deficits restricted to aged PrP-null mice (62, 63).

FIGURE 2. Monoamine-induced accumulation of cAMP in the cerebral cortex is genotype-related. A, serotonin induced an increase in cAMP only at 1 nM, at which point, Prnp-null cortical tissue showed a significantly larger response, compared with wild-type; B, responses to dopamine were significantly larger in wild-type at 100 μM or higher when compared with the unresponsive Prnp-null tissue. C, noradrenaline responses were similar in both genotypes. D and E, both concentration dependence (D) and kinetics (E) of adenylyl cyclase activation induced by forskolin in the cerebral cortex were similar in both genotypes, as were the responses to stimulation of striatum slices with dopamine (DA), SKF-38393 (SKF), or forskolin (FSK) (F). n = 3–6. *, p < 0.05; **, p < 0.01.
On the other hand, in a study of wild-type and PrPC-null mice inbred into the FVB genetic background, no difference between 8–12-week-old mice of either genotype were found in a forced swimming test (64), which differs from both our results (Fig. 1A), as well as of others (36). Notably, however, the FST in the Massimino study (64) was run for 3 min only, which differs greatly from the usual procedures, where the first 2 of a standard 6-min test are sometimes even discarded due to the hyperactivity of mice when introduced in the water pool (65). In addition, differing from our experiment, no pretraining/habituation was reported (64), whereas such pretraining helps prevent novelty stress and anxiety associated with the swimming test. Mice

FIGURE 3. Roles of D1-like and 5HT5A receptors in the differing cAMP responses of the cerebrocortical tissue from distinct genotypes. A, a D1-like receptor agonist elicited cAMP accumulation in wild-type, but not in Prnp-null tissue. B, a D1-like receptor antagonist completely blocked cAMP accumulation induced by 100 μM dopamine in wild-type tissue, with no effect in Prnp-null. C, a D2-like receptor antagonist had no effect on cAMP responses. D, a 5HT5A receptor antagonist partially prevented the larger increase in cAMP of Prnp-null tissue treated with 1 nM serotonin. n = 3–6, *, p < 0.05; **, p < 0.01.

FIGURE 4. Differing contents of monoamine-related proteins in wild-type and PrPC-null mice. Western blots of protein extracts from the cerebral cortex show no significant differences between genotypes for TPH, SERT, 5HT1A, or D1 (B–D and F). Notice, however, that both 5HT5A and TH contents were significantly increased in Prnp-null mice (A and E). Relative level of phosphorylation of TH at the Ser-40 residue was similar in both groups (E). GAPDH was used as control in C, because the α-tubulin band overlapped with 5HT1A. n = 3–7; *, p < 0.05.
of the FVB strain, along with other Swiss-derived strains, are hyperactive, and show a rather low level of immobility even in standard FST (66, 67). Both differing genetic backgrounds and experimental protocols may, therefore, explain the distinct results found in our FST experiments, as compared with those of Massimino and co-workers (64). Overall, our data are consistent with the hypothesis that 8–12-week-old PrP<sup>C</sup>-null mice show a depressive-like behavior without motor defects, when compared with age-matched wild-type mice.

Notwithstanding that the monoamine hypothesis may be an oversimplification unlikely to fully explain the complexity of human depression (68–71), unbalance of monoaminergic systems is considered a key factor of the disease, and a prominent neurochemical feature of animal models (72, 73). Here we found several distinctions in monoaminergic function between wild-type and PrP<sup>C</sup>-null mice.

Two among six marker proteins of monoaminergic systems, the levels of which were estimated by Western blots, were found at distinct levels between PrP<sup>C</sup>-null and wild-type mice. The contents of both the 5HT5A serotonin receptor, and the limiting enzyme for dopamine synthesis, TH, were ~30% higher in PrP<sup>C</sup>-null mice when compared with wild-type. Notably, the relative amounts of the main phosphorylated form of TH (pTHSer-40) were similar in both genotypes. Consistent with these findings, we also found a significant increase of total dopamine levels in PrP<sup>C</sup>-null cerebral cortex. Although phosphorylation of TH is the primary regulator of the production of dopamine in synapses (74), it is likely that the increased level of total TH, rather than a shift in phosphorylation, explains the chronically increased dopamine content.

Cyclic AMP is a common second messenger of monoaminergic signaling associated with depression (see Ref. 75 for review). Interestingly, when compared with the wild-type response, cerebrocortical tissue lacking PrP<sup>C</sup> responded with a significantly larger increase in cAMP to treatment with a low concentration of serotonin. In contrast, a drastically reduced cAMP response was observed in PrP<sup>C</sup>-null tissue stimulated with moderate concentrations of dopamine. The increases in cAMP induced either by norepinephrine or the potent adenylyl cyclase activator forskolin did not differ between knock-out and wild-type tissue, indicating that effects on monoaminergic transmission were neither generalized for metabotropic receptors, nor due to changes in the functional capacity of adenylyl cyclase. Differing from cerebrocortical tissue, slices of striatum from either PrP<sup>C</sup>-null or wild-type mice responded with similarly increased levels of cAMP, and had undistinguishable dopamine contents. These data are consistent with the lack of change in motor functions found in our behavioral experiments.

The D1-like receptors are the most abundant dopamine receptor in the cerebral cortex, and our experiments with selective agonists and antagonists showed that a lack of D1-like

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**FIGURE 5.** Neurotransmitter and metabolite contents are selectively altered in cerebrocortical tissue. A, levels of dopamine were increased in Prnp-null cerebrocortical tissue when compared with wild-type, whereas neurotransmitter serotonin (5-HT) and noradrenaline (NA), as well as metabolites 5-hydroxyindoleacetic acid (5-HIAA, from serotonin) and 3,4-dihydroxyphenylacetic acid (DOPAC, from dopamine) were similar in both genotypes. B, total dopamine and DOPAC contents in the striatum were, however, similar in both genotypes. n = 6–12, *p < 0.05.

**FIGURE 6.** Distribution and colocalization of immunostaining to prion and monoamine-related proteins in the cerebral cortex. Labeling is shown for TPH, monoamine transporter (SERT), 5HT5A serotonin receptor, TH, or D1 dopamine receptor (red), and the prion protein (green) in confocal micrographs of coronal sections taken from the parietal cortex of wild-type (left) and Prnp-null (right) mice. Notice colocalization of PrP<sup>C</sup> with 5HT5A (C), and with D1 (E).

**FIGURE 7.** D1R, 5HT5A, and SERT bind to PrP<sup>C</sup>. Overlay assays of cerebrocortical protein extracts against recombinant PrP<sup>C</sup> were positive for phage display hits 5HT5A and SERT, and for D1 dopamine receptor, but not for D4 dopamine receptor.
activity is mainly responsible for the unresponsiveness found in PrP<sup>C</sup>-null cortical slices. A plausible hypothesis is that the increased content of dopamine, due to the higher content of tyrosine hydroxylase in the brains of PrP<sup>C</sup>-null mice, may lead to sustained desensitization of D1 receptors (76). Alternatively, or perhaps in addition to desensitization, the presence of PrP<sup>C</sup> may be required for full responsiveness of D1-like receptors to dopamine, either due to direct regulation or to interfering with heterodimerization with D2-like receptors (77). The latter hypothesis is particularly interesting in view of the evidence that attributes the role of the dopaminergic system in clinical depression basically to the D2-like class of receptors (78).

In turn, there are numerous subtypes of serotonin receptor and their responses are difficult to isolate or reduce to a simple modulation of cAMP levels (79). Indeed, we found that the 5HT5A antagonist SB-699551-A appeared to diminish, albeit slightly, the serotonin-induced accumulation of cAMP in PrP<sup>C</sup>-null tissue, which fails to correlate with the reported negative coupling of this receptor type to adenyl cyclase described upon transfection into cell lines (80–84). The 5HT5A receptor is relatively rare, apparently more abundant in astrocytes than in neurons (80, 85), and only recently a link began to be established between mood and cognition, and the presence of this receptor in cortical neurons (86).

The behavioral consequences of serotonin signaling through 5HT5A, relative to other receptor types, are in fact unclear. For example, SB-699551-A had no effect in the forced swim test, but induced sedation in rats (87), and, whereas increased cAMP has been linked to positive effects on memory consolidation (88), an impairment of this trait was reported by other investigators, in mice treated with the same 5HT5A antagonist (89). Furthermore, 5HT5A has been identified as an inhibitory autoreceptor that regulates the release of serotonin, if only in the absence of concurrent signaling through 5HT1A receptors (90). Therefore, the specific contribution of 5HT5A to serotonin-induced accumulation of cAMP in the cerebral cortex is difficult to assert. In this respect, it is particularly relevant to our results that the prion protein was also shown to modulate the activity of at least 3 other serotonin receptors, namely 5HT2B, 5HT1B/D, and 5HT2A, in an inducible serotoninergic cell line (23, 24).

Although the changes reported here in monoaminergic control of intracellular cAMP in the cerebral cortex are insufficient to fully explain the depressive-like behavior of PrP<sup>C</sup>-null mice, they clearly demonstrate the involvement of monoaminergic systems in the behavioral effects of PrP<sup>C</sup>. The overlay assay showed that both the 5HT5A serotonin receptor and the SERT transporter, previously detected as potential ligands in a phage display screen, are indeed able to bind the prion protein, and confocal microscopy showed colocalization of PrP<sup>C</sup> with 5HT5A, albeit not with SERT. In addition, evidence for binding of PrP<sup>C</sup> to the D1 dopamine receptor was found in the overlay assay, and the two proteins colocalized in confocal micrographs. Both the 5HT5A and D1 receptors, therefore, add to a growing list of potential functional partners of PrP<sup>C</sup> consistent with the scaffold hypothesis, which already includes several neurotransmitter receptors, as well as other extracellular molecules (19, 20, 91–95). These findings further strengthen the hypothesis that PrP<sup>C</sup> provides a cell surface scaffold for multiple signaling modules (5, 10).

On the other hand, the changed contents of monoaminergic marker proteins are intriguing. Evidence has been reported of co-regulation of expression of the Prnp gene with both glutamate and γ-aminobutyric acid receptors (22), but protein content was not included in that study. Notably, whereas the content of both 5HT5A and TH were increased in PrP<sup>C</sup>-null tissue when compared with wild-type, no difference was detected for 5HT1A, D1 receptor, the SERT transporter, nor the TPH enzyme, all of which pertain to the same neurochemical pathways. Thus, the effect of PrP<sup>C</sup> upon proteostasis is selective, similar to other results of our laboratory that showed an effect of PrP<sup>C</sup> upon cellular content of a purinergic receptor but not of a metabotropic glutamate receptor, both of which bind to the prion protein.4 These data suggest that PrP<sup>C</sup> may interact and affect the functional properties of signaling systems not only at the cell surface, but also at the level of proteostasis.

Synaptic dysfunction and damage are prominent features of prion pathology, as seen both in post-mortem tissue of human patients and in animal models of the disease (96, 97). Several reports emerged in the last decade associating PrP<sup>C</sup> to synaptic function, structure, and plasticity (98) as well as memory and behavioral processes (99), often through the cAMP/PKA pathway. Notably, in elderly human subjects, variability in PRNP, the human gene encoding PrP<sup>C</sup>, was associated with a decline in cognitive performance (100, 101).

Human prion diseases, in particular the variant Creutzfeldt-Jakob, may present initial clinical symptoms involving psychiatric and behavioral disorders such as depression, anxiety, insomnia, and hallucinations (102). Much has been speculated that such deficits are related with the monoaminergic system (103, 104). It is possible that, as PrP<sup>C</sup> begins to propagate, early loss of function of PrP<sup>C</sup> at the neuronal surface may interfere with monoaminergic signaling by modulating G-protein-coupled receptor responses. In addition, the evidence that implicates PrP<sup>C</sup> as a binding partner of Aβ peptide oligomers (37) extends the potential relevance of our data to neuropsychiatric manifestations of Alzheimer disease (105). Indeed, the behavioral deficits that indicate a depressive-like behavior of PrP<sup>C</sup>-null animals are reminiscent of those described in mice subject to injections of Aβ peptide oligomers (106). Further to the importance to specific neurodegenerative diseases, modulation of monoaminergic neurotransmission by PrP<sup>C</sup> may also be a potential target in major depression and related disorders.

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4 M. V. Carneiro, T. A. Americo, M. F. Santiago, M. Z. Guimarães, and R. Linden, unpublished data.
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