

# Greenhouse Gases in Intensive Agriculture: Contributions of Individual Gases to the Radiative Forcing of the Atmosphere

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Agriculture plays a major role in the global fluxes of the greenhouse gases carbon dioxide, nitrous oxide, and methane. From 1991 to 1999, we measured gas fluxes and other sources of global warming potential (GWP) in cropped and nearby unmanaged ecosystems. Net GWP (grams of carbon dioxide equivalents per square meter per year) ranged from 110 in our conventional tillage systems to -211 in early successional communities. None of the annual cropping systems provided net mitigation, although soil carbon accumulation in no-till systems came closest to mitigating all other sources of GWP. In all but one ecosystem, nitrous oxide production was the single greatest source of GWP. In the late successional system, GWP was neutral because of significant methane oxidation. These results suggest additional opportunities for lessening the GWP of agronomic systems.

Radiative forcing of Earth's atmosphere is increasing at unprecedented rates, largely because of increases in the greenhouse gases CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O (1). Agriculture plays a major role in the global fluxes of each of these gases and has been promoted as a partial means for slowing further increases in radiative forcing through the potential for soil C sequestration in cropping systems under reduced tillage (2, 3) and organic (4) management regimes. Crop management also affects N<sub>2</sub>O and CH<sub>4</sub> fluxes (5), and changes to these fluxes can also affect the impact of agriculture on radiative forcing (6), as can the use of CO<sub>2</sub>-producing crop subsidies such as fertilizer (7), lime, and fuel. A complete understanding of agriculture's impact on radiative forcing requires field-level analysis of all contributors to the net global warming potential (GWP) of these systems.

During 1991-99, we measured N<sub>2</sub>O production, CH<sub>4</sub> oxidation, and soil C sequestration in a replicated series of cropped and unmanaged ecosystems in the Midwest United States (8). We studied four corn-wheat-soybean rotations managed (i) with conventional chemical inputs and tillage, (ii) with conventional inputs and no tillage, (iii) with reduced chemical inputs, and (iv) organically with no chemical inputs (9). The latter two treatments included a winter legume cover

crop following the corn and wheat portions of the rotations to provide nitrogen and mechanical cultivation to control weeds.

We also examined two perennial crops—alfalfa (*Medicago sativa*) and poplar (*Populus* sp.) trees—and four types of unmanaged vegetation, including a set of early successional ecosystems abandoned from agriculture in 1989, two types of mid-successional systems, and a set of late successional forested sites. One of the two midsuccessional types was abandoned from agriculture around 1950, and another was established in 1959 by converting a small never-tilled woodlot to an annually mowed grassland (10). Productivity and soil properties of all sites are consistent with their management histories (Table 1).

With the exception of our conventionally managed system, all of our cropping systems accumulated soil C over the decade since their establishment. The no-till system accumulated 30 g C m<sup>-2</sup> year<sup>-1</sup>, which is an average value for no-till rotations in the Midwest United States (11), and the organic-based systems accumulated 8 to 11 g C m<sup>-2</sup> year<sup>-1</sup> (Table 1). Reduced tillage is the likely cause of C sequestration in the no-till system (12); in the low-input systems, both of which were plowed and cultivated as part of their normal management regime, sequestered C is likely the result of a winter cover crop that in two of three rotation years added an additional 1 to 2 megagrams ha<sup>-1</sup> of unharvested plant biomass to soil.

The poplar and alfalfa systems added 32 to 44 g C m<sup>-2</sup> year<sup>-1</sup> to the soil C pool, similar to that added by the no-till system, and 60 g C m<sup>-2</sup> year<sup>-1</sup> was sequestered in the early successional community abandoned from agricul-

ture in 1989 (Table 1). We detected minor soil C changes in the midsuccessional old field (about 0.9 g C m<sup>-2</sup> year<sup>-1</sup>) and no changes in the never-tilled successional or late successional forest communities.

We made N<sub>2</sub>O and CH<sub>4</sub> flux measurements using a static chamber approach (13, 14) at weekly to monthly intervals during portions of the year when soils were not frozen. N<sub>2</sub>O fluxes were, on average, three times higher from the annual crop and alfalfa sites than from any of the unmanaged successional sites (3.5 ± 0.21 compared with 1.1 ± 0.05 g ha<sup>-1</sup> day<sup>-1</sup> N<sub>2</sub>O-N; Fig. 1) (15). N<sub>2</sub>O fluxes from the organic system (3.49 ± 0.44 g N ha<sup>-1</sup> day<sup>-1</sup>) were not significantly different from either the low-input (3.73 ± 0.61) or the two conventionally fertilized systems (3.35 ± 0.30 g N ha<sup>-1</sup> day<sup>-1</sup>). Nor did the conventionally fertilized systems differ: Fluxes of N<sub>2</sub>O from the no-till systems were similar or slightly higher than those under conventional tillage management (3.22 ± 0.45 compared with 3.48 ± 0.39 g N ha<sup>-1</sup> day<sup>-1</sup>). Spring fluxes in the fertilizer-based systems peaked after fertilization, which occurred for corn both at planting (25% of total fertilizer input) and 3 to 4 weeks later (the remaining 75%). In the low-input and organic systems, there was a less pronounced but longer lived flux associated with the decomposition of the winter-legume cover crop, similar in pattern to seasonal differences in soil nitrogen availability among these systems (16).

Rates of CH<sub>4</sub> oxidation did not differ among any of the cropped sites (-1.80 ± 0.06 g CH<sub>4</sub>-C ha<sup>-1</sup> day<sup>-1</sup> on average), but were substantially different from fluxes in the forest sites (-9.17 ± 0.25 g CH<sub>4</sub>-C ha<sup>-1</sup> day<sup>-1</sup> on average; Fig. 1). CH<sub>4</sub> oxidation recovered slowly after the onset of succession. Early successional sites had rates as low as the cropped sites, and rates of oxidation in midsuccessional sites were only 60% of those in forest sites 50 years after abandonment.

That N<sub>2</sub>O fluxes were similar among all annual cropping systems suggests that it is neither fertilizer per se nor tillage that leads to accelerated N<sub>2</sub>O fluxes from cropping systems, but rather high soil nitrogen availability. This notion is corroborated by results from the alfalfa and poplar systems. Although alfalfa receives no N fertilizer nor is tilled—high productivity is maintained by internal N<sub>2</sub> fixation rather than fertilizer inputs—N<sub>2</sub>O fluxes were equivalent to those from the annual crops (Fig. 1). N<sub>2</sub>O fluxes from the poplar sites, on the other hand, were the lowest of any system measured, despite the fact that average productivity was among the highest (Table 1). In this case, low fluxes were likely the result of very conservative soil nitrogen cycling—on average, soil nitrate and min-

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eralizable N were 3 to 10 times lower in the poplar sites than in any of the high-N<sub>2</sub>O sites (Table 1).

The difference in N<sub>2</sub>O production between cropped and successional systems provides an estimate of background fluxes in agriculture now missing from current global flux estimates. The current Intergovernmental Panel on Climate Change (IPCC) methodology for assessing direct N<sub>2</sub>O emissions from agricultural fields (17) multiplies total N inputs (from synthetic fertilizer, manure, legumes, and crop residue) by an N<sub>2</sub>O emission factor calculated as the difference between N<sub>2</sub>O flux from fertilized versus unfertilized experimental plots plus a background flux equivalent to that of the unfertilized plot. The difference between the estimated background flux and the actual preagricultural flux is missing (18). In our site, the N<sub>2</sub>O flux difference between the unfertilized early successional site and the late successional forest (15) would add 40% to estimates of background N<sub>2</sub>O emissions, or about 20% to estimates of annual crop emissions based on IPCC emission factors alone (19). The magnitude of this increase further underscores the importance of contemporary agriculture, as suggested in recent revisions of the global N<sub>2</sub>O budget (18). A 20% increase in the total flux attributed to cultivated soils in the most recent IPCC assessment (1) adds 0.7 Tg N year<sup>-1</sup> to the global N<sub>2</sub>O flux.

We used current IPCC factors (20) to estimate the GWP for each of these systems based on contributions of individual gases. GWP provides a measure of the cumulative radiative forcing of various greenhouse gases relative to some reference gas, usually CO<sub>2</sub>, over a specific time horizon, here 20 years (21). We calculated net CO<sub>2</sub> flux on the basis of changes in soil organic matter and the CO<sub>2</sub> cost of agronomic inputs—N fertilizer, lime,

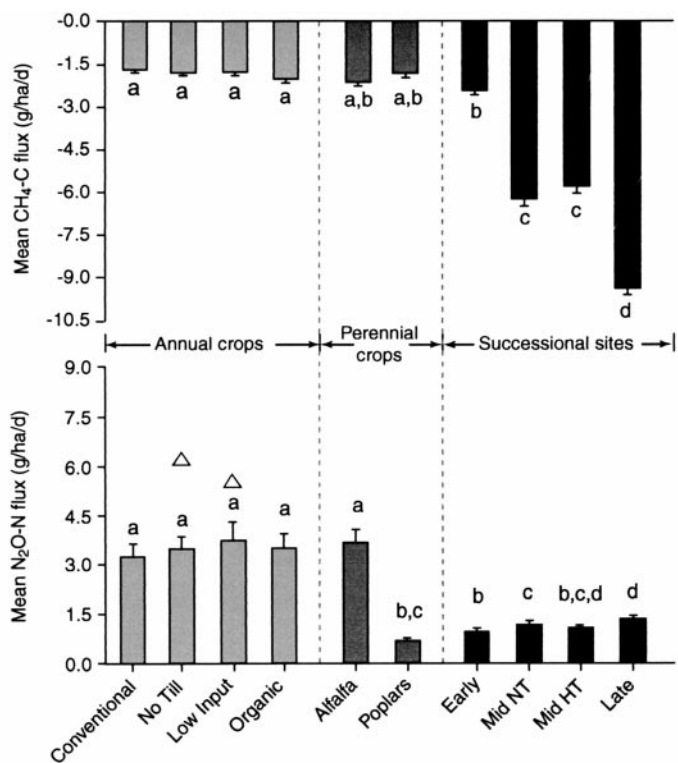
and fuel. Changes in soil organic matter reflect the difference between net C uptake by plants and losses of carbon from crop harvest and from the microbial oxidation of crop residues and soil organic matter (22).

The conventional tillage system exhibited a net GWP of 114 g CO<sub>2</sub> equivalents m<sup>-2</sup> year<sup>-1</sup> (Table 2). About half of this potential was contributed by N<sub>2</sub>O production (52 g CO<sub>2</sub> equivalents m<sup>-2</sup> year<sup>-1</sup>), with an equivalent amount (50 g CO<sub>2</sub> equivalents m<sup>-2</sup> year<sup>-1</sup>) contributed by the combined effects

of fertilizer and lime. The CO<sub>2</sub> cost of fuel use was also significant but less than that of either lime or fertilizer. No soil C accumulated in this system, nor did CH<sub>4</sub> oxidation significantly offset any GWP sources.

The net GWP of the no-till system (14 g CO<sub>2</sub> equivalents m<sup>-2</sup> year<sup>-1</sup>) was substantially lower than that of the conventional tillage system, mostly because of increased C storage in no-till soils. Slightly lower fuel costs were offset by somewhat higher lime inputs and N<sub>2</sub>O fluxes. Intermediate to

**Fig. 1.** CH<sub>4</sub> oxidation (top) and N<sub>2</sub>O production (bottom) in annual and perennial cropping systems and unmanaged systems. Annual crops were managed as conventional cropping systems, as no-till systems, as low-chemical input systems, or as organic systems (no fertilizer or manure). Midsuccessional systems were either never tilled (NT) or historically tilled (HT) before establishment. All systems were replicated three to four times on the same or similar soil series; fluxes were measured over the 1991–99 period. There are no significant differences ( $P > 0.05$ ) among bars that share the same letter on the basis of analysis of variance. Triangles indicate average fluxes when including the single day of anomalously high fluxes in the no-till and low-input systems in 1999 and 1991, respectively (15).



**Table 1.** Patterns of aboveground net primary production (ANPP), soil nitrogen availability, and soil organic carbon (30) among study sites (10). Values are means ( $\pm$ SE) of annual ecosystem averages ( $n = 8$  years), except that organic C values are 1999 means.

Ecosystem management	ANPP (MT ha <sup>-1</sup> year <sup>-1</sup> )	NO <sub>3</sub> -N <sup>†</sup> (μg g <sup>-1</sup> )	N mineralization potential <sup>‡</sup> (μg g <sup>-1</sup> day <sup>-1</sup> )	Organic C <sup>‡</sup> (%)	Organic C <sup>‡</sup> (kg m <sup>-2</sup> )	ΔC (g m <sup>-2</sup> year <sup>-1</sup> )
Annual crops (Corn-soybean-wheat rotation)						
Conventional tillage	9.24 (1.41)	6.54 (0.53)	0.13 (0.05)	1.00 (0.07)	0.94 (0.05)	0.0
No till	9.19 (1.48)	4.74 (0.32)	0.17 (0.03)	1.24 (0.05)	1.24 (0.06)	30.0
Low input with legume cover	8.84 (1.39)	4.34 (0.21)	0.23 (0.02)	1.08 (0.03)	1.05 (0.01)	11.0
Organic with legume cover	7.79 (1.11)	3.83 (0.20)	0.21 (0.02)	1.09 (0.05)	1.02 (0.04)	8.0
Perennial crops						
Alfalfa	8.18 (1.67)	2.53 (0.17)	0.26 (0.02)	1.30 (0.05)	1.38 (0.08)	44.0
Poplar	10.17 (4.00)	0.30 (0.02)	0.04 (0.01)	1.40 (0.14)	1.26 (0.11)	32.0
Successional communities						
Early successional	4.24 (0.37)	0.63 (0.04)	0.08 (0.01)	1.63 (0.06)	1.54 (0.05)	60.0
Midsuccessional (HT)*	2.60 (0.27)	0.37 (0.05)	0.16 (0.04)	1.61 (0.19)	1.37 (0.14)	0.9
Midsuccessional (NT)*	4.93 (0.22)	0.47 (0.03)	0.03 (0.02)	3.63 (0.28)	2.84 (0.22)	0.0
Late successional forest	5.26 (0.11)	1.84 (0.11)	0.28 (0.03)	2.93 (0.47)	2.29 (0.21)	0.0

\*HT, historically tilled; NT, never tilled.

<sup>†</sup>0- to 25-cm depth.

<sup>‡</sup>0- to 7.5-cm depth.

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the conventional and no-till systems were the low-input and organic-based systems. These latter systems also sequestered C, but soil C gains were not sufficient to offset the substantial GWP of N<sub>2</sub>O production, even accounting for the lower CO<sub>2</sub> cost of N fertilizer and lime inputs in these systems. Thus, the low-input and organic-based systems had a total GWP of 41 to 63 g CO<sub>2</sub> equivalents m<sup>-2</sup> year<sup>-1</sup>, which is about half the GWP of the conventional tillage system.

In contrast to the annual cropping systems, all of the perennial crops and successional communities had a net negative or neutral GWP (Table 2). The 10-year-old early successional community had the highest mitigation potential (-211 g CO<sub>2</sub> equivalents m<sup>-2</sup> year<sup>-1</sup>) owing to a high rate of soil C storage, no CO<sub>2</sub>-producing agronomic activities, and very low rates of N<sub>2</sub>O production. However, these rates of soil C storage did not appear to persist beyond a few decades—in our 50-year-old midsuccessional sites, soil C storage rates were about 15% of rates in the early successional sites, yielding a net mitigation potential of only -31 g CO<sub>2</sub> equivalents m<sup>-2</sup> year<sup>-1</sup>. Neither the midsuccessional never-tilled sites nor the late successional forests stored detectable amounts of soil C; in both cases, the GWP of N<sub>2</sub>O production was largely offset by higher rates of CH<sub>4</sub> oxidation, yielding net GWPs close to zero.

The mitigation potential of perennial crops differed mainly as a result of agronomic inputs and N<sub>2</sub>O fluxes. Alfalfa stored substantial amounts of soil C (161 g CO<sub>2</sub> equivalents m<sup>-2</sup> year<sup>-1</sup>), but almost all of these gains were offset by CO<sub>2</sub> released from lime and by N<sub>2</sub>O production, leaving a net GWP

of -20 g CO<sub>2</sub> equivalents m<sup>-2</sup> year<sup>-1</sup>. Poplars stored less soil C but in contrast to alfalfa received no lime and had very low N<sub>2</sub>O fluxes, yielding a mitigation potential more than five times that of alfalfa and about half that of our early successional sites.

If all U.S. cropland (186 × 10<sup>6</sup> ha) (23) had a net GWP similar to that of our conventional tillage system, the annual CO<sub>2</sub> cost would total 0.06 Pg C equivalents. Over the most recent 6-year period for which data are available, U.S. fossil fuel emissions of 1.4 Pg C year<sup>-1</sup> grew at a rate of 0.02 Pg year<sup>-1</sup> (24). Agriculture thus plays a minor role in the GWP economy of the U.S., yet net mitigation of agricultural fluxes could offset the current annual increase in fossil fuel CO<sub>2</sub> emissions.

Whole-system GWP analysis reveals a number of management options for mitigation. In our annual crop systems, the high net GWP of conventional tillage was largely neutralized by soil C storage during no-till management. C was also sequestered by the use of cover crops: Despite intensive cultivation, soil C storage in our low input and organic systems provided about one-third the mitigation benefit of no till.

But soil C storage is only half of the story: In our no-till system, other GWP sources more than offset mitigation gains from soil C, suggesting other mitigation potentials. By substituting biological N<sub>2</sub> fixation for synthetic fertilizer use, for example, our organic system saved an amount of CO<sub>2</sub> equivalent to about 25% of that mitigated by no-till soil C capture. A lower need for lime in these systems doubled this effect. In cropping systems where N fertilizer use is less conservative [e.g., (25)], mitigation gains could be even greater. Efforts to minimize N<sub>2</sub>O release and

maximize CH<sub>4</sub> oxidation could also substantially lower the net GWP of annual cropping systems.

Many of these alternative mitigation strategies are related to tightening the nitrogen cycle of cropped ecosystems, a nontrivial challenge in light of the importance of N to crop yields. N fertilizer is not now used to sequester soil C, so reducing fertilizer use to provide greenhouse gas mitigation will require careful management of cover crops, residues, and the microbial and physical processes that regulate soil N availability (26) if high yields are to be maintained. The use of N fertilizer to sequester soil C is unlikely to result in net mitigation.

Maximum mitigation is provided by removing land from production. The strong mitigation potential of our early successional system will persist into midsuccession as carbon is also allowed to accumulate in unharvested wood (27). This option may be practical for marginal lands such as those in the U.S. Conservation Reserve Program; for productive cropland that must meet the food needs of a burgeoning global population, mitigation of agricultural impacts will better depend on policies that address multiple sources of GWP in cropping systems.

### References and Notes

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8. Sites were located at the W. K. Kellogg Biological Station (KBS) Long-Term Ecological Research (LTER) Site (<http://lter.kbs.msu.edu>). KBS is in southwest Michigan in the northern portion of the U.S. corn belt (85°24'W, 42°24'N; 920 mm year<sup>-1</sup> precipitation). Soils are well-drained Typic Hapludalfs; conventional crop yields are equivalent to average yields for the U.S. Department of Agriculture North Central region.
9. Treatments receiving conventional chemical inputs received pre- and postemergence herbicide, nitrogen, phosphorus, and potassium fertilizer, lime, and insecticides as called for by regional best management practices and integrated pest management (IPM) protocols. Nitrogen was applied to corn (on average, 123 kg N ha<sup>-1</sup>) and to wheat (56 kg N ha<sup>-1</sup>) but not to soybeans. See note (7) in supplemental materials (28).
10. The cropping systems and early successional community (seven ecosystems) were replicated as six 1-ha plots in a randomized block design established in the same contiguous area farmed as a single unit for decades previously. The midsuccessional never-tilled (NT) sites ( $n = 4$  plots) were located within a single 2-ha area 300 m from the cropping systems on the same soil series. The midsuccessional historically

**Table 2.** Relative GWPs for different management systems based on soil carbon sequestration, agronomic inputs, and trace gas fluxes. Units are CO<sub>2</sub> equivalents (g m<sup>-2</sup> year<sup>-1</sup>) based on IPCC conversion factors (20–22). Negative values indicate a global warming mitigation potential. All systems are replicated as described in Table 1 and (10).

Ecosystem management	CO <sub>2</sub>				N <sub>2</sub> O	CH <sub>4</sub>	Net GWP
	Soil C	N fertilizer	Lime	Fuel			
<b>Annual crops (corn-soybean-wheat rotation)</b>							
Conventional tillage	0	27	23	16	52	-4	114
No till	-110	27	34	12	56	-5	14
Low input with legume cover	-40	9	19	20	60	-5	63
Organic with legume cover	-29	0	0	19	56	-5	41
<b>Perennial crops</b>							
Alfalfa	-161	0	80	8	59	-6	-20
Poplar	-117	5	0	2	10	-5	-105
<b>Successional communities</b>							
Early successional	-220	0	0	0	15	-6	-211
Midsuccessional (HT)*	-32	0	0	0	16	-15	-31
Midsuccessional (NT)*	0	0	0	0	18	-17	1
Late successional forest	0	0	0	0	21	-25	-4

\*HT, historically tilled; NT, never tilled.



tilled (HT) and the forest systems were each replicated as three separate sites within 2 km of the other systems on the same or similar soil series. See note 2 in supplemental materials (28) for additional site histories.

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14. Gas sampling protocols appear in note 6 in supplemental materials (28).
15. For mean values and statistical details, see Web tables (28).
16. Biweekly inorganic N analyses show high levels of N in all cropping systems in late spring. More inorganic N occurs in the conventional systems than in the organic-based systems, but the increase persists several weeks longer in the organic-based systems.
17. IPCC, OECD, IEA, *Revised 1996 IPCC Guidelines for National Greenhouse Gas Inventories* (Organization for Economic Cooperative Development, Paris, 1997).
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19. The average daily N<sub>2</sub>O flux in our conventional cropping system (3.22 g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup>) extrapolates to 1.17 kg N<sub>2</sub>O-N ha<sup>-1</sup> year<sup>-1</sup>. Of this, 0.37 to 0.53 kg N<sub>2</sub>O-N is apparent background flux (based on fluxes in our successional systems), or 31 to 45% of the 1.17 kg N total. Over a 3-year corn-soybean-wheat rotation in this system, IPCC methodology (17, 18) calculates our N inputs to average 139 kg ha<sup>-1</sup> year<sup>-1</sup>, for a calculated emission factor of 0.0055 to 0.0067 kg N<sub>2</sub>O-N per kg N input, within the lower portion of the IPCC range of 0.0025 to 0.0225.
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21. We calculated GWP using IPCC factors of 1 for CO<sub>2</sub>, 280 for N<sub>2</sub>O, and 56 for CH<sub>4</sub> (20). Calculation details for all GWP sources appear in Web tables (28).
22. Soil carbon storage in surface horizons provides a conservative estimate of in situ CO<sub>2</sub> sequestration for cropped systems. Ignored are potential changes in dissolved C leached to groundwater, changes in soil C deeper in the profile due to root decomposition, and erosion transfers to reservoir sediments. Also unaccounted is C captured by woody biomass in our poplar and successional systems because we expect that this C will be oxidized when the wood is eventually harvested.
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## Signal Transduction Through Prion Protein

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The cellular prion protein PrP<sup>c</sup> is a glycosylphosphatidylinositol-anchored cell-surface protein whose biological function is unclear. We used the murine 1C11 neuronal differentiation model to search for PrP<sup>c</sup>-dependent signal transduction through antibody-mediated cross-linking. A caveolin-1-dependent coupling of PrP<sup>c</sup> to the tyrosine kinase Fyn was observed. Clathrin might also contribute to this coupling. The ability of the 1C11 cell line to trigger PrP<sup>c</sup>-dependent Fyn activation was restricted to its fully differentiated serotonergic or noradrenergic progenies. Moreover, the signaling activity of PrP<sup>c</sup> occurred mainly at neurites. Thus, PrP<sup>c</sup> may be a signal transduction protein.

Although much progress has been made over the past few years regarding the involvement of the scrapie prion protein (PrP<sup>Sc</sup>) in transmissible spongiform encephalopathies (TSEs) (1), the biological function of the cellular, nonpathogenic isoform of PrP, PrP<sup>c</sup>, still remains enigmatic. PrP<sup>c</sup> is an ubiquitous glycoprotein expressed strongly in neurons (2). PrP-deficient mice are viable and develop normally, but they display minor defects that differ according to the null strain (3). In contrast, mice expressing an NH<sub>2</sub>-terminally truncated version of PrP<sup>c</sup> in a null background show neuronal degeneration soon after birth, suggesting that PrP<sup>c</sup> may play an important role in the maintenance and/or regulation of neuronal functions (4). Recent data have focused on the copper-binding ability of PrP<sup>c</sup> (5), and an involvement of PrP<sup>c</sup> in the regulation of the presynaptic copper concentration and of synaptic transmission has been proposed (6). The attachment of PrP<sup>c</sup> to the plasma membrane through a glycosylphosphatidylinositol (GPI) anchor may also be consistent with a role in cell-surface signaling or cell adhesion. Indeed, the 37-kD laminin receptor binds PrP<sup>c</sup> (7).

Because PrP<sup>c</sup> may act as a cell-surface receptor, we investigated whether signal transduction pathways coupled to PrP<sup>c</sup> after antibody-mediated cross-linking (8). Our experimental strategy relies on the neuronal differentiation model 1C11 (9). The 1C11

clone is a committed neuroectodermal progenitor with an epithelial morphology that lacks neuron-associated functions (9). Upon induction, 1C11 cells develop a neural-like morphology, acquire neuronal markers, and convert into either 1C11<sup>\*/5-HT</sup> (serotonergic) cells or 1C11<sup>\*\*/NE</sup> (noradrenergic) cells. The choice between the two differentiation pathways depends on the set of inducers used (9). Within 4 days, 1C11<sup>\*/5-HT</sup> cells implement a complete serotonergic phenotype, including 5-HT synthesis, storage, transport, catabolism, as well as three functional serotonergic receptors of the 5-HT<sub>1B/D</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2A</sub> subtypes (9). The noradrenergic phenotype of 1C11<sup>\*\*/NE</sup> cells is complete within 12 days, with a progressive onset of catecholamine synthesis, storage, transport, catabolism, and an α<sub>1D</sub>-adrenoceptor (9). The differentiation events involve almost 100% of the cells and follow a well-characterized time course.

PrP<sup>c</sup> is constitutively expressed in the 1C11 progenitor and throughout differentiation (10). Thus, the 1C11 cell line offers the opportunity to study PrP<sup>c</sup> in relation to the onset of neuronal functions and within an integrated neuronal context. The effects of antibody-mediated ligation of PrP<sup>c</sup> at the cell surface of the 1C11 progenitor or its fully differentiated 1C11<sup>\*/5-HT</sup> and 1C11<sup>\*\*/NE</sup> progenies were followed. PrP<sup>c</sup> cross-linking did not induce any specific phosphoinositide (PI) hydrolysis or nitric oxide production, nor did it promote the activation of p21ras or phospholipase A2 in the 1C11 cell line within 30 min of cross-linking (11). In contrast, ligation of PrP<sup>c</sup> with specific antibodies induced a marked decrease in the phosphorylation level of the tyrosine kinase Fyn in

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