

# Nonlinear grassland responses to past and future atmospheric CO<sub>2</sub>

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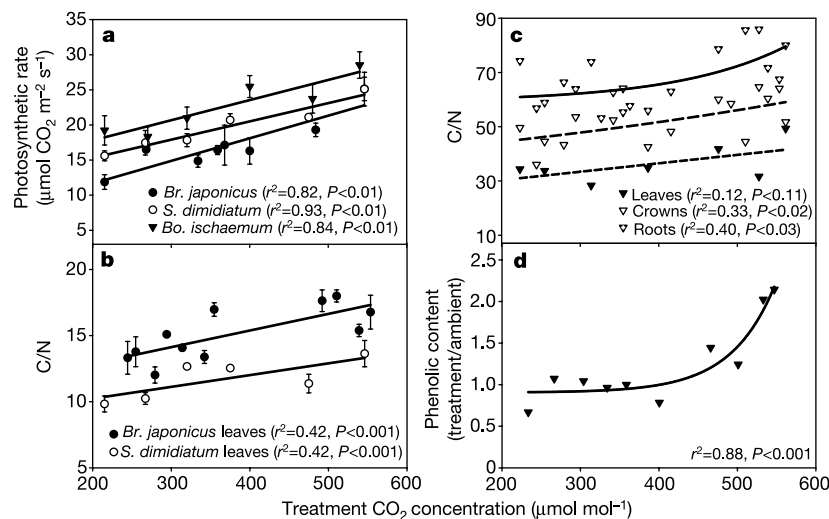
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Carbon sequestration in soil organic matter may moderate increases in atmospheric CO<sub>2</sub> concentrations (C<sub>a</sub>) as C<sub>a</sub> increases to more than 500 μmol mol<sup>-1</sup> this century from interglacial levels of less than 200 μmol mol<sup>-1</sup> (refs 1–6). However, such carbon storage depends on feedbacks between plant responses to C<sub>a</sub> and nutrient availability<sup>7,8</sup>. Here we present evidence that soil carbon storage and nitrogen cycling in a grassland ecosystem are much more responsive to increases in past C<sub>a</sub> than to those forecast for the coming century. Along a continuous gradient of 200 to 550 μmol mol<sup>-1</sup> (refs 9, 10), increased C<sub>a</sub> promoted higher photosynthetic rates and altered plant tissue chemistry. Soil carbon was lost at subambient C<sub>a</sub>, but was unchanged at elevated C<sub>a</sub> where losses of old soil carbon offset increases in new carbon. Along the experimental gradient in C<sub>a</sub> there was a nonlinear, threefold decrease in nitrogen availability. The differences in sensitivity of carbon storage to historical and future C<sub>a</sub> and increased nutrient limitation suggest that the passive sequestration of carbon in soils may have been important historically, but the ability of soils to continue as sinks is limited.

The concentration of CO<sub>2</sub> in the atmosphere has increased dramatically since the Last Glacial Maximum, most recently owing to fossil fuel burning and land conversion to agriculture.

This increase in C<sub>a</sub> has focused attention on the role of terrestrial ecosystems in sequestering anthropogenic CO<sub>2</sub> (refs 2, 5, 7, 11, 12). The long-term consequences of rising C<sub>a</sub> on C sequestration are highly dependent on feedbacks between plant responses to C<sub>a</sub> and nutrient dynamics<sup>7,8,13</sup>. Plant growth is often enhanced with increases in C<sub>a</sub> (refs 6, 14), sometimes leading to changes in plant tissue chemistry and organic inputs to soils<sup>15,16</sup>. These and other feedbacks controlled by microbial processes may either increase<sup>13,17</sup> or decrease<sup>7,8,18</sup> nutrient availability, and mediate the long-term ability of ecosystems to sequester C<sup>7,8,19</sup>. For C sequestration to be important at decadal and century timescales, nutrient availability must not hinder higher plant production and new organic C must be stabilized in soil pools with relatively long turnover times. The partitioning of C among soil organic matter (SOM) pools with different turnover rates is thus a crucial determinant of C sequestration in many systems and is tightly coupled with plant tissue chemistry and nutrient dynamics<sup>13,16,18</sup>.

A field experiment<sup>9</sup> in an intact C<sub>3</sub>/C<sub>4</sub> grassland in central Texas provided a continuous gradient of C<sub>a</sub> from 200 to 550 μmol mol<sup>-1</sup> permitting the measurement of critical threshold and nonlinear responses to past, present and future atmospheric CO<sub>2</sub>. Plant and ecosystem properties, including water-use efficiency, photosynthesis, respiration rates and primary productivity, often change with rising C<sub>a</sub>, but it is not likely that all such responses were or will be linear<sup>3,20,21</sup>. Physiological thresholds<sup>20</sup>, transient or acclimatory responses<sup>22</sup>, and the strong coupling of plant and soil responses<sup>18</sup> are examples of mechanisms that may drive nonlinear processes in nature<sup>23</sup>. Nonlinear and threshold responses are the focus of several new international programmes<sup>23</sup> and may explain some of the apparent contradictory results observed in recent CO<sub>2</sub> studies<sup>8,13</sup>. Furthermore, research on how intact ecosystems respond to both past and future C<sub>a</sub> provides a context that can demonstrate the sensitivity of C dynamics to changes that have already occurred as well as those forecast for the coming century. Extrapolation from experiments that impose step changes in C<sub>a</sub> is complicated by the possibility that plants may evolve as C<sub>a</sub> changes more slowly in nature. There is some evidence, however, that perennial plants have not evolved quickly enough to be closely adapted to current C<sub>a</sub> (ref. 24).



**Figure 1** Effects of CO<sub>2</sub> treatment on various species. **a**, Maximum CO<sub>2</sub> assimilation for three species (*Bothriochloa ischaemum*, *Solanum dimidiatum*, *Bromus japonicus*) in 1999, showing a significant positive relationship between maximum CO<sub>2</sub> assimilation and treatment CO<sub>2</sub> in all species (P < 0.01). **b**, C/N ratio for leaves from the two C<sub>3</sub> species show a positive, linear increase for both species with increasing treatment CO<sub>2</sub>. **c**, C/N ratio for *Bo. ischaemum* roots, crowns and leaves. Roots showed an exponential increase

in C/N ratio with increasing CO<sub>2</sub> (P < 0.03); crowns showed a positive, linear increase (P < 0.05). **d**, Relative change in phenolic concentrations in *Bo. ischaemum* roots (expressed relative to ambient values). There was a strong, exponential increase in root phenolic content (P < 0.001), with an apparent threshold at C<sub>a</sub> slightly above ambient levels.

**Table 1** Calculated carbon production and storage

	Year	Elevated (550–350 $\mu\text{mol mol}^{-1}$ )	Subambient (365–200 $\mu\text{mol mol}^{-1}$ )	$R^2$ ( $P$ value)	Superambient/ subambient	Pretreatment superambient/subambient
Aboveground net primary production ( $\text{g m}^{-2} \text{yr}^{-1}$ )	1996–2000	1,047.5 (64.9)	683.9 (52.2)	0.35 (0.006)	1.52	0.83
Belowground net primary production 0–30 cm ( $\text{g m}^{-2} \text{yr}^{-1}$ )	1998–1999	294 (24.6)	185 (22.8)	0.16 (0.09)	1.59	1.03
Soil $\text{CO}_2$ flux ( $\mu\text{g m}^{-2} \text{sec}^{-1}$ )	1996–2000	4.02 (0.13)	2.85 (0.17)	0.46 (0.001)	1.41	1.13
Root biomass 0–30 cm ( $\text{g m}^{-2}$ )	1998	173.0 (39.4)	102.0 (16.2)	0.25 (0.02)	1.69	0.84
Soil organic carbon 0–15 cm ( $\text{g m}^{-2}$ )	1996–2000	4,442 (175)	3,656 (120)	0.32 (0.05)	1.22	1.05

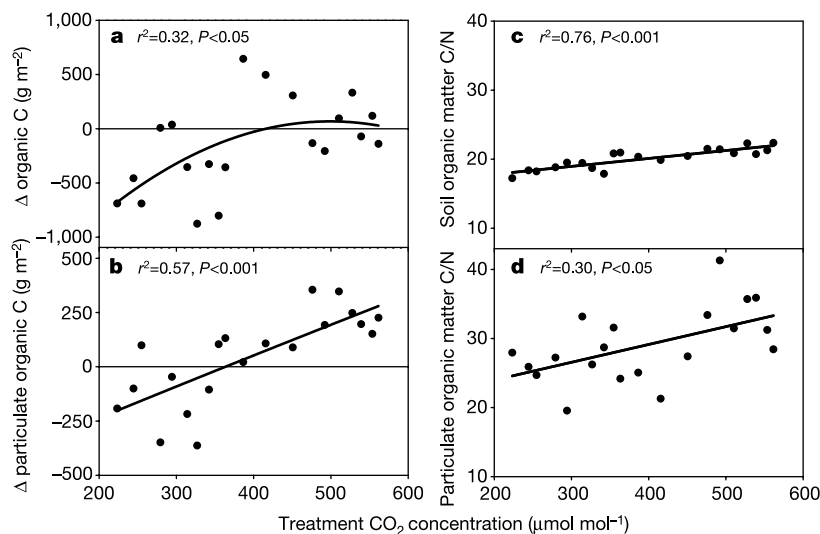
$R^2$  and  $P$  values are from best-fit regressions of variables on  $C_a$  over subambient through elevated concentrations. s.e.m., the standard error of the mean, shown in parentheses after the mean value. For all data other than root biomass, s.e.m. is determined as the standard error for annual means; for root biomass it is the standard error between section means ( $n = 10$ ).

Along the experimental gradient, plants responded to higher  $C_a$  by increasing photosynthesis and net primary production (Fig. 1a, Table 1). As treatment  $\text{CO}_2$  increased, maximum  $\text{CO}_2$  assimilation rates increased linearly for both  $C_3$  and  $C_4$  plants<sup>10</sup> (Fig. 1a;  $P < 0.01$ ). Associated with this increase in  $\text{CO}_2$  assimilation was a 50% increase in above- and belowground net primary production at elevated  $\text{CO}_2$  compared to subambient  $\text{CO}_2$  (Table 1). Tissue chemistry was altered as well, with an increase in tissue C/N with higher  $C_a$  and an exponential increase in phenolic concentration (Fig. 1b–d).  $C_a$  and species type were highly significant predictors of C/N, with C/N positively correlated with  $C_a$  (analysis of covariance (ANCOVA):  $P < 0.001$  for  $C_a$ ;  $P < 0.001$  for species). The concentration of phenolic compounds in roots of one of the dominant species in the system, the  $C_4$  grass *Bothriochloa ischaemum*, showed a strong threshold effect, with little variation in plants grown at subambient  $C_a$ , but an exponential increase above ambient  $\text{CO}_2$  (Fig. 1d,  $P < 0.001$ ).

Soil C storage and belowground metabolism were greatly altered. Despite a linear increase in photosynthesis along the gradient, soil C storage was much more sensitive to subambient than to elevated  $C_a$  (Fig. 2a). At subambient  $C_a$ , bulk soil C stocks decreased by 11%, or  $450 \text{ g m}^{-2}$ , between 1996 and 2000 (Table 2). However, there was no concomitant increase in soil C storage at elevated  $C_a$  (Fig. 2a), with soil C increasing by a modest  $3.3\%$  ( $144 \text{ g m}^{-2}$ ) over the same time period (Table 2). The relationship between treatment  $\text{CO}_2$  and the change in bulk soil organic C over three years follows an asymptotic function (Fig. 2a,  $P < 0.05$ ), suggesting that the ability of soils to act as sinks for anthropogenic  $\text{CO}_2$  will slow or reach saturation.

Accompanying altered soil C storage was an important change in soil organic matter chemistry. Total organic matter C/N was linearly associated with treatment  $C_a$  (Fig. 2c,  $P < 0.01$ ), in a pattern similar to that observed for plant tissue chemistry. There was also a divergence in patterns of soil respiration at super- versus subambient  $C_a$ . Soil  $\text{CO}_2$  flux at peak plant growth was 40% higher at elevated than at subambient  $C_a$ , suggesting that much of the increase in C fixed with rising  $C_a$  is lost to microbial or root respiration<sup>5</sup> (Table 1).

The changes observed in particulate organic matter (POM) demonstrate a shift in the balance between new and old SOM. POM is a relatively labile class of SOM, with a residence time of between 10 and 50 years<sup>11,25,26</sup>. The 14% loss in POM carbon at subambient  $C_a$  parallels the loss in total organic C (Table 2). However, in contrast to total organic C, POM C increased linearly with treatment  $\text{CO}_2$ , even at elevated  $C_a$  (Fig. 2b). These findings indicate that at elevated  $C_a$ , increases in POM C were largely offset by losses in the older, mineral-associated organic matter<sup>27</sup> (Table 2). Even within the POM class, there were increases at elevated  $C_a$  in the two most labile fractions (free and macroaggregate POM), while there was a decrease in the most recalcitrant fraction (microaggregate POM)<sup>26</sup> (Table 2). This represents a change in ecosystem C partitioning to faster cycling organic matter<sup>11,16,26</sup>, which may explain why higher C assimilation and production did not lead to increased C sequestration. Our result is similar to those of other studies that reported that at low nutrient availability and elevated  $\text{CO}_2$ , carbon was lost from the mineral-bound fraction of SOM<sup>25</sup>. Similarly, an annual grassland exposed to a doubling of  $C_a$  had



**Figure 2** Effect of  $\text{CO}_2$  treatment on soil carbon storage. **a**, Change in organic C stocks (0–15 cm) between 1997 and 2000. Values are the difference between section means in 1997 and 2000 determined using four subsamples per 5-m section per year. There was a quadratic relationship between the change in soil C stocks and treatment  $\text{CO}_2$  ( $P < 0.05$ ). The linear fit for these data was not significant. **b**, Significant, linear change in particulate

organic matter (POM) carbon between 1997 and 2000 ( $P < 0.001$ ). **c**, There was a significant, linear increase in bulk soil organic matter (SOM) C/N with treatment  $\text{CO}_2$  in December 2000 ( $P < 0.001$ ). **d**, POM C/N for samples collected in December 2000 ( $P < 0.05$ ). Values are the means of four subsamples per section.

**Table 2 Pools and changes in soil organic carbon and particulate organic carbon**

	Treatment leg	December 2000 0–15 cm (g m <sup>-2</sup> )	Change (g m <sup>-2</sup> ) 1997–2000	Relative change (%)
Total soil organic matter C	Superambient	4,442 (175)	144 (92)	3.3
	Subambient	3,656 (120)	-450 (100)	-11
Particulate organic matter C—Free	Superambient	186.1 (23.7)	86.5 (22.0)	70
	Subambient	158.2 (16.8)	-26.9 (6.3)	-16
Macroaggregate	Superambient	723.4 (38.7)	193.3 (33.9)	36
	Subambient	626.6 (39.8)	-104.0 (56.8)	-14
Microaggregates	Superambient	87.5 (6.7)	-21.6 (22.6)	-24
	Subambient	65.9 (6.4)	-5.3 (5.5)	-9
Total particulate organic matter C	Superambient	975.4 (33.9)	258.2 (70.1)	35
	Subambient	857.3 (56.8)	-132.3 (88.2)	-14
Mineral-associated organic matter C	Superambient	3,719 (195)	-123 (96)	-3.3
	Subambient	3,030 (118)	-346 (121)	-9.8

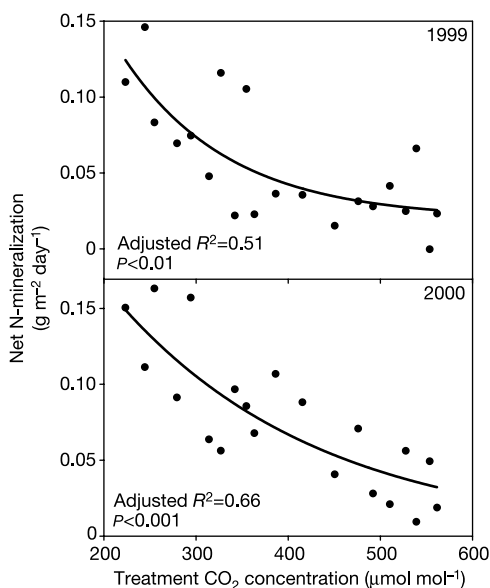
s.e.m, the standard error of the section means ( $n = 10$ ), shown in parentheses after the mean value.

higher ecosystem C uptake and belowground allocation but little extra C storage<sup>5</sup>. Much of the increased C was partitioned to rapidly cycling pools that make a negligible contribution to long-term storage because of their small size and relatively high turnover rates.

The feedback between plant responses to  $C_a$  and nutrient dynamics is vital in determining C sequestration in ecosystems<sup>7,8,18</sup>. Nitrogen mineralization rates decreased dramatically and non-linearly with increasing  $CO_2$  ( $P < 0.01$ ), with the largest changes occurring at subambient concentrations (Fig. 3). Net N mineralization was three times higher at 200–240  $\mu\text{mol mol}^{-1}$   $CO_2$  than at 530–550  $\mu\text{mol mol}^{-1}$ . Because of the changes in the chemical composition of detritus and increased C supply, microbes at high  $CO_2$  may need to mineralize older, mineral-associated SOM to meet their nutritional requirements. As a result, there was a decrease in plant-available N as a consequence of microbial immobilization and a loss in C stored in mineral-associated fractions of organic matter. Some workers have concluded that suppressed N availability under elevated  $CO_2$  may increase C storage by suppressing decomposition rates<sup>8,18</sup>, but we found that there were only modest gains in soil C storage at the lowest N availability. In contrast to other grassland

$CO_2$  studies<sup>6,14,21</sup>, our results are apparently a consequence of altered plant litter chemistry rather than an indirect effect of altered soil water status, as increases in plant water-use efficiency along the gradient<sup>10</sup> were offset by higher plant biomass (data not shown). Increases in  $C_a$  resulted in higher nitrogen-use efficiency by plants<sup>10</sup>, but a threefold decrease in nitrogen availability will probably have a detrimental effect on long-term plant productivity and, ultimately, on ecosystem carbon storage.

Higher net primary productivity<sup>5,7</sup>, altered plant tissue chemistry<sup>27</sup>, modifications of SOM composition and stocks<sup>5,11,25</sup>, and changes in nutrient availability<sup>13,18</sup> with increases in  $C_a$  suggest that both forests and grasslands are sensitive to rising  $CO_2$ . The capacity of future ecosystems to act as sinks for anthropogenic  $CO_2$  will be determined by feedbacks among ecosystem processes<sup>7,18</sup> and will be sensitive to the location of specific thresholds that influence the magnitude of the change in ecosystem dynamics<sup>23</sup>. In this grassland, soil C stocks and net N mineralization are much more sensitive to subambient than elevated  $C_a$ , indicating that we are currently at an important threshold. Soils may have played a role in passively sequestering C since the last interglacial period, but their ability to continue to act as a C sink may be limited by nutrient availability. To assess the impacts of rising  $CO_2$  on carbon sequestration patterns and nutrient dynamics requires knowledge of potential threshold responses and the legacy of historical and prehistorical changes. □



**Figure 3** Net N mineralization during the 1999 and 2000 growing seasons. There was a significant, negative exponential relationship between net N mineralization and treatment  $CO_2$  during midsummer in both years ( $P < 0.001$ ). During spring and autumn there were no significant differences in N mineralization rates for the subambient and elevated chambers.

**Methods**

**Experimental system**

Two parallel, elongated chambers (1 m tall × 1 m wide × 60 m long) were constructed on a grassland dominated by the  $C_4$  perennial grass *Bothriochloa ischaemum* (L.) Keng and Ambient air plus the  $C_3$  perennial forbs *Solanum dimidiatum* Raf. and *Ratibida columnaris* (Sims) D. Don. pure  $CO_2$  was injected into the eastern chamber to initiate the elevated gradient (550–350  $\mu\text{mol mol}^{-1}$ ), while ambient air was injected into the western chamber, initiating the subambient  $CO_2$  gradient (365–200  $\mu\text{mol mol}^{-1}$ ). Gradients have been maintained during the growing season since May 1997 by altering flow rate through the chambers. At night, air flow in the chambers is reversed, maintaining a  $C_a$  gradient at 150  $\mu\text{mol mol}^{-1}$  above daytime concentrations. The chambers are divided into 5-m sections, and air is cooled and dehumidified in each section to maintain air temperature and vapour pressure deficit near ambient conditions. Our results span pre-treatment data (1996–1997) and the three complete growing seasons during which the grassland was exposed to a  $C_a$  gradient (1998–2000).

**Soil analyses**

Soil respiration was evaluated monthly using a LI-COR 6200. Total inorganic and organic soil carbon was determined using a two-temperature combustion procedure designed specifically for calcareous Blackland Prairie soils<sup>28</sup>. Four soil cores were collected from each of the 20 sections in stratified, random positions. Total C and N were measured using a CE Instruments NC 2100 elemental analyser (ThermoQuest Italia). We measured POM in two aggregate size classes (macroaggregates (>250  $\mu\text{m}$ ); microaggregates (250–53  $\mu\text{m}$ )) using the method described in ref. 26 to determine POM C. Mineral-associated C was determined by difference between total C and POM C. We determined POM C using four soil samples from each section ( $n = 80$ ) that were collected in September 1997 and December 2000. We used a month-long, *in situ* open-core incubation method described in ref. 29 to measure net nitrogen mineralization.

## Statistical considerations

The experimental system is constructed to resolve the shape of ecosystem responses to a gradient in CO<sub>2</sub>. The experimental design uses a regression approach to test for significant CO<sub>2</sub> effects based on changes in slope along the gradient. We used regression to test for a significant relationship between C<sub>a</sub> and the response variable using the regression wizard in SigmaPlot 5.0 for Windows (SPSS Inc.) We tested linear, logarithmic, power and hyperbolic functions to fit the data, and selected the model with the highest adjusted R<sup>2</sup> after examining the residual plots for normality and homoscedasticity. When models were nearly the same in their explanatory value (R<sup>2</sup> values within 0.05), we report results for the linear model. Because we had only a single experimental system oriented in one direction across the landscape, it is possible that the measured responses may have been influenced by some unquantified factor covarying with CO<sub>2</sub> treatment. However, extensive pretreatment data, including such ecosystem characteristics as soil C stocks, net primary productivity and soil respiration, revealed no such trends before fumigation (Table 1 and additional data not shown). Furthermore, the system design ensured that key environmental variables (photosynthetically active radiation, T, relative humidity, and so on) remained similar across the gradient<sup>9</sup>. The absence of strong threshold responses at the transition between the two chambers provides further evidence that neither landscape position nor position within the chamber significantly influenced observations. To control for any pre-existing variation in soil organic matter, we evaluate the change in soil C stocks between 1997 and 2000 rather than absolute levels (Table 2).

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## Competing interests statement

The authors declare that they have no competing financial interests.

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# Dopamine-mediated modulation of odour-evoked amygdala potentials during pavlovian conditioning

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Pavlovian conditioning results when an innocuous stimulus, such as an odour, is paired with a behaviourally relevant stimulus, such as a foot-shock, so that eventually the former stimulus alone will elicit the behavioural response of the latter. The lateral nucleus of the amygdala (LAT) is necessary for the emotional memory formation in this paradigm<sup>1–4</sup>. Enhanced neuronal firing in LAT to conditioned stimuli emerge in parallel with the behavioural changes<sup>5–11</sup> and are dependent on local dopamine<sup>12–15</sup>. To study the changes in neuronal excitability and synaptic drive that contribute to the pavlovian conditioning process, here we used *in vivo* intracellular recordings to examine LAT neurons during pavlovian conditioning in rats. We found that repeated pairings of an odour with a foot-shock resulted in enhanced post-synaptic potential (PSP) responses to the odour and increased neuronal excitability. However, a non-paired odour displayed PSP decrement. The dopamine antagonist haloperidol blocked the PSP enhancement and associated increased neuronal excitability, without reversing previous conditioning. These results demonstrate that conditioning and habituation processes produce opposite effects on LAT neurons and that dopamine is important in these events, consistent with its role in emotional memory formation.

In male rats (Sprague–Dawley, 250–350 g) anaesthetized with 8% chloral hydrate, odour-evoked depolarizing responses can be observed in neurons of the LAT, as well as responses evoked by a foot-shock (Fig. 1). Repeated presentation of an odour resulted in a gradual attenuation of the odour-evoked PSPs (Fig. 2;  $n = 4$ ,  $P < 0.01$ ,  $F = 10.9$ , degrees of freedom (d.f.) = 6, repeated measures analysis of variance (ANOVA)), as well as causing a significant suppression of membrane fluctuations during the odour presentation to below the level of spontaneous activity (baseline odour-evoked PSP  $3,287.5 \pm 459.2$  mV ms; after six presentations  $-1,302.3 \pm 175.7$  mV ms). In contrast, in a separate group, pairing of an odour with a train of foot-shocks in a pavlovian