dict the precise shape of the envelope, so we cannot rule out other factors contributing to the envelope shape.

We have solved numerically the one-dimensional Schrödinger equation for surfaces A, B, and C as a function of the dissociation energy to determine the energy-dependent phase differences, $(\varphi_A - \varphi_B)$ and $(\varphi_B - \varphi_C)$, in analogy to the simple example shown in Fig. 2. The photofragment orientation, $Im[\mathbf{a}_1^\dagger]$, is linearly proportional to $\sin(\varphi_A - \varphi_B)$ and $\sin(\varphi_B - \varphi_C)$. Comparison of the photodissociation-wavelength dependence of these functions (Fig. 4, B and C) to experiment shows that the interference pattern is caused predominantly from dissociation via surfaces A and B. Overall, we conclude that at a photolysis wavelength of 490 nm, ICl dissociates predominantly via the B state; at 560 nm, ICl dissociates predominantly via the A state; and at intermediate wavelengths, the dissociation proceeds coherently through both states. The oscillations of $\sin(\varphi_A - \varphi_B)$ shown in Fig. 4B match very well the experimental oscillations in Fig. 4A, in contrast to the calculations shown in Fig. 4C. We conclude that the C state does not significantly participate in the photodissociation. The match at short wavelengths between Figs. 4A and 4B indicates that the calculated surfaces A and B are quite accurate; however, at longer wavelengths the calculated oscillations of $\sin(\varphi_A - \varphi_B)$ are too rapid. We suggest that this discrepancy may indicate that the shapes of the surfaces A and B need to be slightly improved.

To our knowledge, this study represents the first observation that the photolysis of a molecule with linearly polarized light causes the production of oriented photofragments. Previously, oriented photofragments have been observed using photolysis with circularly polarized light (*17–22*), but such orientation does not necessarily arise from matter-wave interference. This technique is not limited to diatomic molecules (*23*) and can be used to study excited states and dissociative processes with unprecedented sensitivity. Conventional spectroscopy is sensitive to the shape and nature of bound electronic states. In contrast, the interference pattern of the photofragment orientation is sensitive to the shape and nature of dissociating states. This technique can be used to investigate the unimolecular decomposition of chemical systems involving multiple electronic states by coherently preparing more than one electronic state of different symmetry. As such, the technique may become an important probe of wave-packet dynamics on multiple dissociative surfaces.

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6. In the celebrated Young's two-slit experiment, the interference of light passing through two slits causes a bright and dark fringe pattern to appear on a screen, the intensity of which varies as $\cos\Delta\phi$ (where $\Delta\phi$ is the phase difference between the two paths). If instead, the light used is initially linearly polarized, and crossed polarizers are placed in front of the two slits (at 45° to the linear polarization so that both slits transmit equally), then the light on the screen no longer shows an intensity fringe pattern, but shows instead a polarization fringe pattern. This polarization pattern can be separated into components with linearly polarized variations (proportional to $\cos\Delta\phi$) and circularly polarized variations (proportional to $\sin\Delta\phi$). The analog of this special two-slit experiment in molecular photolysis is that in which the interference of matter waves from potential surfaces of different symmetry causes angular momentum polarization of the photofragments [for discussions and examples of $\cos\Delta\phi$ variations in the alignment of photofragments, see E. Flemming, O. Wilhelmi, H. Schmoranzer, M. Glass-Maujean, *J. Chem. Phys.* **103**, 4090 (1995) and (*4, 5, 25*)]. The experiment described in the present report is an example of the $\sin\Delta\phi$ variations in photofragment helicity.
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11. In such crystals, the wavelength and speed of light that is linearly polarized parallel to the optical axis of the crystal differs from that which is linearly polarized perpendicular to the optical axis. Light that is polarized at some angle (such as 45°) to the optical axis has components that are parallel and perpendicular to the optical axis. These components, which were in phase upon entering the crystal, change phase as the light propagates through the crystal. A phase difference of a quarter wave upon exiting the crystal produces pure circularly polarized light.
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16. The combined system of uniformly distributed ICl molecules and a beam of linearly polarized light has no net helicity; therefore, no net helicity can appear after photolysis. In the axial recoil approximation, no angular momentum appears in the translational motion of the photofragments, and the helicity of the Cl atoms must be exactly counterbalanced by an opposite helicity of the I atoms. Therefore, unless the I atoms from $I^{35}Cl$ and $I^{37}Cl$ can be distinguished, the orientation of the I atoms will reflect the 3:1 population-weighted sum of the $^{35}Cl$ and $^{37}Cl$ orientations and reflect similar information.
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23. For the photolysis of polyatomic systems, photofragment helicity should be detectable under the following conditions: one photofragment is detected in a state-specific manner, and its velocity distribution is detected with an energy resolution greater than the internal energy spacings of the undetected fragment. For poorer energy resolution, some reduction in the photofragment helicity is expected but may not be severe, depending on how rapidly the photofragment helicity varies with the internal state of the undetected photofragment.
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# Reversal of Phencyclidine Effects by a Group II Metabotropic Glutamate Receptor Agonist in Rats


**Bita Moghaddam* and Barbara W. Adams**



**Glutamatergic abnormalities have been associated with several psychiatric disorders, including schizophrenia and addiction. Group II metabotropic glutamate receptors were targeted to normalize glutamatergic disruptions associated with an animal model of schizophrenia, the phencyclidine model. An agonist of this group of receptors, at a dose that was without effects on spontaneous activity and corticolimbic dopamine neurotransmission, attenuated the disruptive effects of phencyclidine on working memory, stereotypy, locomotion, and cortical glutamate efflux. This behavioral reversal occurred in spite of sustained dopamine hyperactivity. Thus, targeting this group of receptors may present a nondopaminergic therapeutic strategy for treatment of psychiatric disorders.**


Several lines of evidence suggest that glutamatergic mechanisms contribute to the pathophysiology of schizophrenia (*1–3*). For example, phencyclidine (PCP) and other antagonists of *N*-methyl-D-aspartate (NMDA) receptors have psychotomimetic

properties in healthy individuals (*4*) and exacerbate preexisting symptoms of schizophrenia (*5*). However, drugs that target ionotropic glutamate receptors are not considered therapeutically useful because of the ubiquitous involvement of these receptors in mediating fast synaptic transmission throughout the central nervous system. Metabotropic glutamate receptors (mGluRs) on the other hand may provide important pharmacotherapeutic targets for psychiatric disorders associated with increased or decreased glutamatergic neurotransmission. These receptors modulate synaptic neurotransmission, and the heterogeneous localization of at least eight subtypes of mGluRs (mGluR1 to mGluR8) with distinct functional properties suggests that glutamatergic neurotransmission may be modulated in an anatomically and functionally distinct manner (*6*). The subtypes of mGluRs are currently classified within three groups (I to III) on the basis of sequence homology and pharmacology. The group II family of mGluRs, which consists of mGluR2 and mGluR3, is primarily distributed in forebrain regions (*6*, *7*). Stimulation of this group of mGluRs mediates presynaptic depression and decreases evoked release of glutamate, suggesting that these receptors regulate activated glutamate release by presynaptic mechanisms (*8*–*10*).

PCP and other psychotomimetic NMDA antagonists increase glutamate efflux (*11*, *12*) and may produce their cognitive and locomotor effects, at least in part, by potentiating glutamatergic neurotransmission at non-NMDA receptors (*2*, *11*). In rodents (*11*, *13*) and humans (*14*), antagonists of non-NMDA receptors or pretreatments that attenuate glutamate release reduce mnemonic and other behavioral effects of NMDA receptor antagonists. Hence, reduction of presynaptic glutamatergic activity by targeting group II mGluRs may present an approach for reversing those behavioral effects of PCP associated with increased glutamatergic activity.

Animals were treated with the systemically active and highly selective agonist of group II mGluR (+)-2-aminobicyclo-[3.1.0]-hexane-2,6,-dicarboxylate monohydrate (LY354740) (*15*) or vehicle before they received PCP (*16*). The highest dose of LY354740 used [10 mg of LY354740 per kilogram of body weight intraperitoneal (ip) injection] has been shown to not affect basal glutamate efflux but to normalize depolarization-induced activation of glutamate release (*9*). This dose also does not affect neuromuscular coordination, spontaneous locomotor activity, or learning and memory as assessed by passive avoidance responding (*15*).

The increase in glutamate efflux in the prefrontal cortex produced by PCP was abolished in animals that were pretreated with the mGluR agonist LY354740 (Fig. 1). In contrast, activation of dopamine release by PCP in the prefrontal cortex or the nucleus accumbens was not reduced by this pretreatment (Fig. 2).

Stimulation of group II mGluRs nearly abolished PCP-induced locomotor activity and significantly reduced the total stereotypy score, primarily because of a decrease in head rolling, a hallmark of behavioral activation by NMDA receptor antagonists (Fig. 3). This behavioral reversal at a dose of LY354740 that is without an effect on spontaneous locomotion (*15*) is distinct from similar effects reported with monoamine receptor antagonists, including the antipsychotic drugs haloperidol and clozapine, in that this latter class of drugs reduces locomotor activation by PCP at doses that, by themselves, produce marked locomotor inhibition (*17*).

The effect of LY354740 was also assessed on PCP-induced impairment of discrete-trial delayed alternation task. This is a rodent working memory paradigm that involves continuous changing of strategy and thus is not prone to overtraining as in ordinary T-maze delayed alternation tasks (*12*). Pretreatment with LY354740 reduced the deficits induced by 5 mg of PCP per kilogram of body weight and abolished the deficits induced by a PCP dose of 1 mg/kg (Fig. 4).

The reduction in PCP-induced glutamate efflux by the mGluR agonist suggests that this drug ameliorates the behavioral effects of PCP by attenuating presynaptic glutamatergic activity, a mechanism consistent with reports that non-NMDA receptor antagonists and glutamate release inhibitors reduce the behavioral effects of PCP or ketamine (*11*, *13*, *14*). However, because in vivo selectivity of

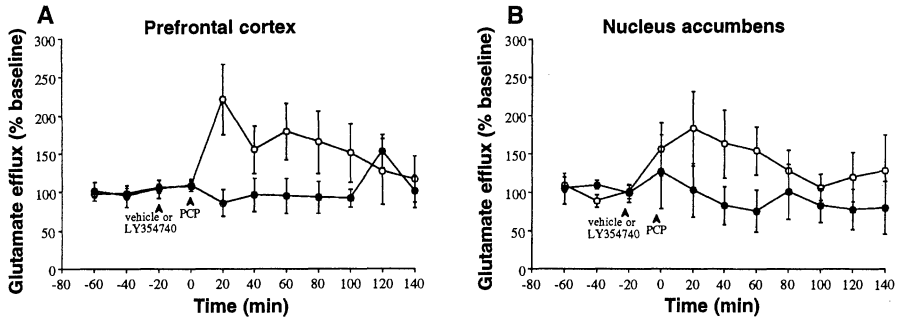
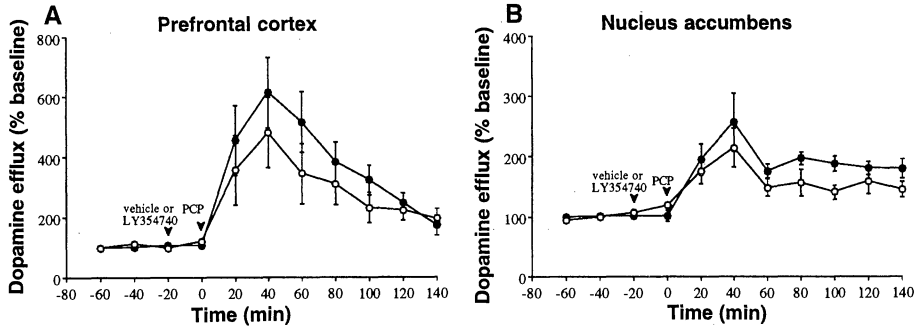



**Fig. 1.** Effect of pretreatment with the group II mGluR agonist LY354740 (10 mg/kg ip injection) (●; $n = 6$) or vehicle (○; $n = 7$) on stimulation of glutamate efflux by PCP (5 mg/kg ip injection) in the (**A**) prefrontal cortex and (**B**) nucleus accumbens of freely moving rats. PCP injection after vehicle (water) produced a significantly greater effect on extracellular glutamate concentrations in the prefrontal cortex ($P < 0.05, f = 2.2$) than in animals pretreated with LY354740. Similar results were observed in the nucleus accumbens; however, because of a greater degree of variability, the difference in the two groups did not reach statistical significance.



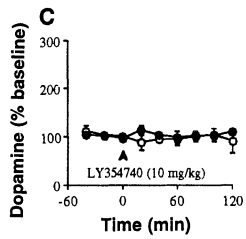




**Fig. 2.** Effect of pretreatment with the group II mGluR agonist LY354740 (10 mg/kg ip injection) (●) or vehicle (○) on stimulation of dopamine efflux by PCP (5 mg/kg ip injection) in the (**A**) prefrontal cortex or (**B**) nucleus accumbens of freely moving rats. Animals pretreated with LY354740 exhibited a significant increase in dopamine release in the nucleus accumbens ($P < 0.001, f = 6.4$) and prefrontal cortex ($P < 0.001, f = 7.2$). ●, $n = 6$ (A) and 8 (B); ○, $n = 7$ (A) and 8 (B). (**C**) The lack of effect of LY354740 (10 mg/kg) by itself on dopamine release in both regions is shown. Prefrontal cortex, ●; nucleus accumbens, ○.

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LY354740 and the source of the activated extracellular glutamate by PCP is presently unknown (*11*, *12*), other mechanisms involving glial cells or postsynaptic interactions between mGluRs and NMDA receptors cannot be ruled out.

Although the psychotomimetic and cognitive effects of PCP are often attributed to the effects of this drug on dopamine neurotransmission (*3*), attenuation of the behavioral effects of PCP by the mGluR agonist was not accompanied by a reduction in dopamine release. This observation is consistent with findings indicating that dopaminergic neurotransmission is dissociated from locomotor and other behavioral effects of NMDA receptor antagonists (*12*, *18*) and questions a direct role for dopamine in the psychotomimetic effects of PCP.

Although extrapolation of data from rodent models to complex human syndromes such as acute PCP intoxication or schizophrenia may be problematic, the behavioral measures in the present study are generally considered relevant to the clinical phenomenology. Locomotor activity in the rodent may be a useful indicator of the propensity of a drug to elicit or exacerbate psychosis in humans (*3*, *19*), and cognitive tasks with a working memory component may model the frontal lobe dysfunctionality and working memory deficits associated with schizophrenia (*19*, *20*). Furthermore, stereotyped tendencies have been reported in schizophrenics, and this behavior may be of importance to schizophrenic symptomatology, not merely in the context of movement, but also relating to organization of thought and perseveration (*21*). The present observation that LY354740 reduced the head-rolling stereotypy in the rodents may be of particular importance because repetitive head rolling is commonly reported in individuals suffering from PCP psychosis (*22*). Thus, our finding that stimulation of group II mGluRs reduces all the above behavioral effects of PCP suggests that targeting these receptors may ameliorate symptoms of acute PCP psychosis in humans and, assuming that PCP psychosis is a valid model of schizophrenia, may be effective in treating related symptoms of schizophrenia. Of note, the putative peptide neurotransmitter *N*-acetyl-aspartyl-glutamate, whose levels have been reported to be abnormal in schizophrenic brains (*23*), is an endogenous agonist for group II mGluRs (*24*).

The important functional characteristic of this group of mGluR agonists, which has relevance for their clinical utility, is that, unlike dopamine antagonists or drugs that target the ionotropic glutamate receptors, a significant amelioration of the cognitive and motoric effects of PCP was observed at a dose that was without a measurable effect on animals' spontaneous activity or on dopaminergic neurotransmission. This observation is consistent with an autoregulatory function for these receptors whereby they inhibit glutamate release under conditions of high agonist availability. Thus, targeting mGluR receptors may offer exquisite functional selectivity by specifically modulating those presynaptic sites where glutamatergic neurotransmission is abnormally regulated.

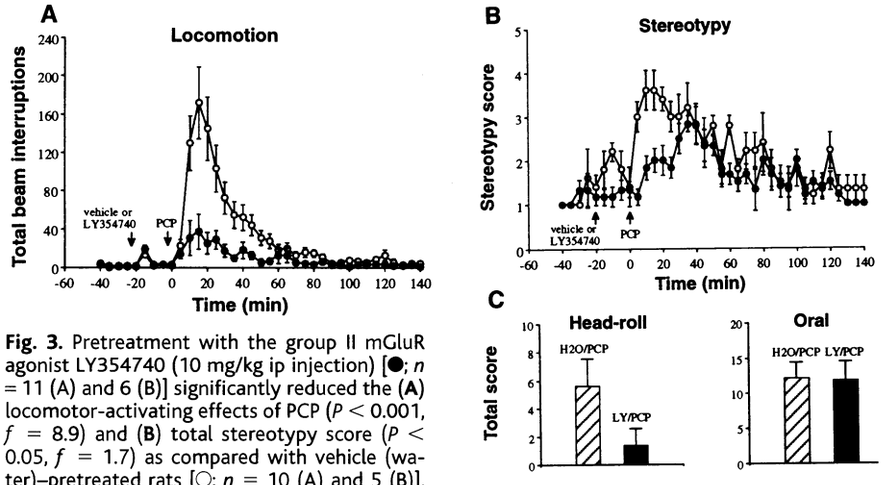


**Fig. 3.** Pretreatment with the group II mGluR agonist LY354740 (10 mg/kg ip injection) [●; $n$ = 11 (A) and 6 (B)] significantly reduced the (**A**) locomotor-activating effects of PCP ($P < 0.001$, $f = 8.9$) and (**B**) total stereotypy score ($P < 0.05$, $f = 1.7$) as compared with vehicle (water)–pretreated rats [○; $n$ = 10 (A) and 5 (B)]. (**C**) The effect of this pretreatment on oral stereotypy and head rolling scores totaled for the 2-hour period after PCP injection is shown. A lower dose of LY354740 (1 mg/kg ip injection) was without a significant effect on the locomotor activations produced by PCP (*25*).

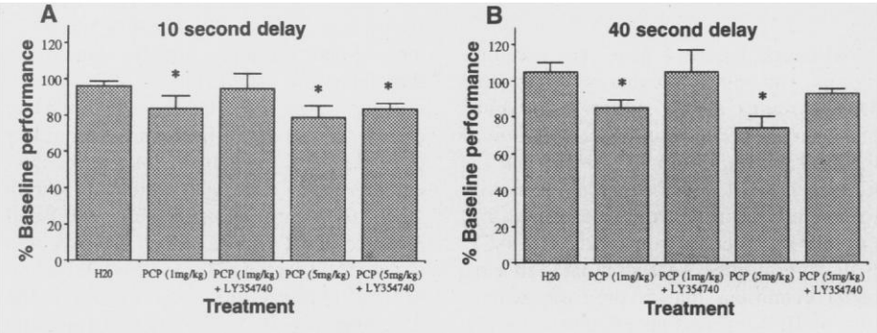


**Fig. 4.** Effect of vehicle (water), PCP (1 or 5 mg/kg ip injection), and PCP + LY354740 (10 mg/kg ip injection) on discrete-trial delayed alternation performance in rats. Animals were trained until their performance of percentage of correct choice (total of 10 choice runs) at (**A**) 10- and (**B**) 40-s delays was stabilized for at least five consecutive days (**C**). A "baseline performance" at each delay was defined for individual animals as the average of correct choices on three consecutive days before drug injection. Water ($H_2O$) or LY354740 (10 mg/kg) was injected immediately before PCP and 50 to 60 min before testing. Treatment with both doses of PCP impaired the performance at 10- (○) and 40-s (▲) delays. Pretreatment with LY354740 significantly reversed the impairment of a PCP dose of 1 mg/kg at both delays and a PCP dose of 5 mg/kg at the 40-s delay (*, $P < 0.05$, as compared with water-treated group; $n$ = 5 to 9).


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16. Animals were treated with vehicle or LY354740 before they were injected with a moderate dose of PCP (1 and 5 mg/kg ip injection). Measurements were made of (i) in vivo extracellular glutamate concentrations in the prefrontal cortex and nucleus accumbens, (ii) in vivo extracellular dopamine concentrations in the prefrontal cortex and nucleus accumbens, (iii) locomotor activity, (iv) stereotypy, and (v) performance in a T-maze discrete-trial delayed alternation task, a rodent working memory paradigm. Animal use procedures were in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, Bethesda, MD, 1996) and were approved by the Yale University Animal Care and Use Committee. Microdialysis and behavioral measures were performed in awake, freely moving rats (male Sprague-Dawley, 275 to 325 g) as described in (*12*). Microdialysis and locomotor data were analyzed by repeated measures of analysis of variance (ANOVA). The delayed alternation data were compared by multifactorial ANOVA followed by Tukey post hoc analysis. Significance was set at $P < 0.05$.
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# Independent and Epigenetic Regulation of the Interleukin-4 Alleles in CD4$^+$ T Cells

**Mark Bix and Richard M. Locksley***

How an individual effector T cell acquires a particular cytokine expression pattern from many possible patterns remains unclear. CD4$^+$ T cells from $F_1$ mice, which allowed assignment of the parental origin of interleukin-4 (IL-4) transcripts, were divided into clones that expressed IL-4 biallelically or monoallelically from either allele. The allelic pattern was transmitted as a stable epigenetic trait. Regulation of cytokine expression by a mechanism that treats each allele independently suggests a probabilistic process by which a diverse repertoire of combinatorially assorted cytokine gene expression patterns could be generated among the clonally related daughters of a single precursor cell.

Descriptions of T helper ($T_H$) cell types that express cytokine patterns distinct from the classic $T_H1$ and $T_H2$ subsets are not readily explained by current models of T cell differentiation from naïve to cytokine-expressing effector cells (*1*). The unusual cytokine patterns appear as if generated by combinatorial assortment of probabilistically expressed genes (*2*, *3*). We hypothesized that a probabilistically regulated gene would have two chances to be expressed in diploid cells and that, if the two alleles were regulated independently, a mixture of cells that used either one or both alleles should exist within a population expressing the gene.

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Although the IL-4 gene was nonpolymorphic among a number of traditional *Mus musculus* inbred strains, a polymorphism in exon 1 allowed discrimination of the IL-4 cDNA of inbred strains from the CAST/Ei strain, by differential sensitivity to the restriction enzyme Bsg I (Fig. 1A) (*4*). CD4$^+$ T cells from (129 × CAST/Ei)$F_1$ hybrid mice were stimulated in vitro under conditions that favored the generation of IL-4–expressing effector cells (*5*). Even under such conditions, the frequency of IL-4–expressing cells is less than 5% (*3*). We used a limiting-dilution approach to screen for monoallelic IL-4 gene expression (*6*), a strategy used to demonstrate monoallelic expression among olfactory receptor genes (*7*).

Under these conditions of limiting template, the semi-nested polymerase chain reaction (PCR) approach was, on average, capable of detecting IL-4 transcripts 30% of the time from a repeatedly screened cDNA aliquot. Consequently, the PCR assay was done multiple times on each of the nine samples that revealed the presence of a single allele (*8*). As demonstrated by differential sensitivity to Bsg I digestion, three samples screened repeatedly revealed only the CAST/Ei allele, whereas six revealed only the BALB/c allele [Fig. 1B and (*9*)]. These data were consistent with monoallelic expression and prompted further studies with cloned cells.

Analysis of cloned cells allows the direct investigation of gene expression in individual cells and thus avoids the statistical imprecisions of the limiting-dilution approach. We examined a panel of 30 CD4$^+$ T cell clones generated from (BALB/c × CAST/Ei)$F_1$ hybrid mice by stimulation with allogeneic H-2$^b$ cells in the presence of recombinant IL-4 and IL-12 monoclonal antibody (mAb), conditions that favor the establishment of IL-4–producing clones (*10*). Of these alloreactive clones, 25 expressed IL-4, and 12 could be expanded and maintained long-term. Seventeen hours after activation of resting clones with immobilized mAbs to the T cell receptor (TCR) and CD28 (*11*), RNA was isolated and screened for the parental origin of the IL-4 transcripts. Twelve (48%) of the 25 clones revealed monoallelic expression (eight BALB/c, four CAST/Ei) and 13 revealed biallelic expression [Fig. 2A and (*9*)]. The twofold bias in favor of monoallelic expression of the BALB/c rather than the CAST/Ei allele was not statistically significant. These data confirmed the suggestion from the limiting-template analysis that monoallelic IL-4 expression does occur. Because some clones expressing IL-4 from either one or both alleles were obtained from a single animal [experiment A (*10*)], the data suggested a process distinct