of BME cells to invade collagen gels and form capillary-like structures[8], at concentrations comparable to those that inhibited *in vitro* proliferation of endothelial cells (Fig. 2*a*–*e*); the IC_{50} of *in vitro* angiogenesis was ~0.35 μM (Fig. 2*e*).

The inhibitory effects of 2-methoxyoestradiol were not restricted to endothelial cells. The proliferation of low-density cultures of various normal (bovine granulosa, NIH3T3 mouse embryonic fibroblast and HFK2 human skin fibroblast) and tumour (SH-EP human neuroblastoma, A204 human rhabdomyosarcoma and Y-79 human retinoblastoma) cells were inhibited, with IC_{50} concentrations of 0.35–2.2 μM (Fig. 1 and data not shown). Again, quiescent confluent monolayers of HFK2 cells were virtually unaffected at doses up to 100 μM (Fig. 1). These results indicate that actively proliferating cells are the target of 2-methoxyoestradiol, and that the compound probably interferes with a proliferation-associated cellular event.

The *in vitro* effects of 2-methoxyoestradiol on angiogenesis and proliferation of malignant cells prompted us to investigate its *in vivo* properties. When administered orally in mice, 2-methoxyoestradiol inhibited the growth of tumours arising from subcutaneously injected Meth A sarcoma and B16 melanoma cells (Fig. 3*a*, *b*). This tumour-suppressing effect seemed to be due to inhibition of tumour-induced angiogenesis rather than direct inhibition of the proliferation of tumour cells. This was assessed by quantifying the vasculature of tumours using microspheres injected in the left ventricle shortly before the mice were killed and the tumours excised. After the tumours had been weighed, the tumour tissue was dissolved and the microparticles were counted. There was a significant reduction in the number of microspheres per g tumour tissue in the treated compared with the control tumours (Fig. 3*c*); the result was confirmed by visualization of the capillaries with Indian ink (Fig. 3*d*, *e*). Apart from their marginally lower weight (~15%), the treated mice showed no apparent signs of toxicity and were all alive after 12 days of daily treatment. We have not observed hair loss, intestinal disturbance or infection, all toxic side-effects associated with conventional chemotherapy. The lack of toxicity is consistent with the *in vitro* results showing no effect on quiescent, non-dividing cells (Fig. 1). The marked difference in the tumour weight of treated and control animals (75.7%) cannot be explained by the 15% weight loss of the mice.

Unlike the angiostatic steroids of corticoid structure[5], heparin or sulphated cyclodextrins are not required for the antiangiogenic activity of 2-methoxyoestradiol, indicating a different mechanism of action. The results in Table 1 and the negligible affinity of 2-methoxyoestradiol for the oestrogen receptor[9] also exclude the possibility that the effects of this steroid on endothelial cells are mediated through interactions with the oestrogen receptor. We have observed, by immunofluorescence, disruption of microtubules, but not actin microfilaments, vimentin intermediate filaments or vinculin-containing adhesion plaques in endothelial cells treated with 2-methoxyoestradiol (data not shown); this suggests that abnormal microtubule assembly might be responsible for its effects on proliferating cells[10,15]. We have also observed that 2-methoxyoestradiol reduces the bFGF-induced increase in urokinase-type plasminogen activator activity in BME cells, without affecting levels of activity of tissue-type plasminogen activator or plasminogen activator inhibitor-1; this effect is partially mimicked by colchicine and other microtubule-disrupting agents (M.S.P *et al.* manuscript in preparation). Because increased endothelial cell proteolysis is required for extracellular matrix invasion during angiogenesis, the selective reduction in urokinase-type plasminogen activator activity may be responsible in part for the antiangiogenic effect of 2-methoxyoestradiol.

Antiangiogenic therapy of human diseases is a promising new concept that has already been applied in the treatment of refractory haemangiomas with α-interferon[11,12] and nerve-sheath tumours with AGM-1470 (ref. 13). The *in vitro* and *in vivo* inhibitory effects of 2-methoxyoestradiol on angiogenesis suggest that this compound is a novel antiangiogenic therapeutic agent which could be used in the treatment of solid tumours and other angiogenic diseases such as paediatric haemangiomas, rheumatoid arthritis, psoriasis and diabetic retinopathy. □

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Facial shape and judgements of female attractiveness

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THE finding that photographic[1–4] and digital[5] composites (blends) of faces are considered to be attractive has led to the claim that attractiveness is averageness[5]. This would encourage stabilizing selection, favouring phenotypes with an average facial structure[5]. The 'averageness hypothesis' would account for the low distinctiveness of attractive faces[6] but is difficult to reconcile with the finding that some facial measurements correlate with attractiveness[7,8]. An average face shape is attractive but may not be optimally attractive[9]. Human preferences may exert directional selection pressures, as with the phenomena of optimal outbreeding and sexual selection for extreme characteristics[10–14]. Using composite faces, we show here that, contrary to the averageness hypothesis, the mean shape of a set of attractive faces is preferred to the mean shape of the sample from which the faces were selected. In addition, attractive composites can be made more attractive by exaggerating the shape differences from the sample mean. Japanese and caucasian observers showed the same direction of preferences for the same facial composites, suggesting that aesthetic judgements of face shape are similar across different cultural backgrounds. Our finding that highly attractive facial configurations are not average shows that preferences could exert a directional selection pressure on the evolution of human face shape.

The averageness hypothesis was tested directly by comparing the attractiveness of composite faces that had different shapes but identical skin textures. A composite image made from many individuals of a group should maintain any consistent visual attributes possessed by that group. If attractiveness is an index

of averageness, a composite with a shape derived from highly attractive faces should not differ from a composite with the average face shape. Alternatively, if attractive faces are systematically different from average, a composite with a shape derived from highly attractive faces should be preferred. A further prediction of the averageness hypothesis is that, if any face shape is exaggerated away from average, it should become less attractive.

These predictions were first examined with a collection of 60 caucasian female face images. The images, presented in random order, were rated for attractiveness on a 7-point Likert scale (1 very unattractive, 7 very attractive) by caucasian subjects (26 female, age 18–45 yr, and 10 male, age 19–24 yr). Ratings were correlated across subject gender (Spearman's $r_{(58)}=0.75$, $P<0.00005$) and were consistent across subjects (mean inter-rater correlation $r_{(58)}=0.41$, $P<0.005$; estimated by correlating each subject's ratings with those of one other randomly chosen subject of the same gender and averaging standard deviate (z) score transformed correlation coefficients).

The 'average' face shape was defined as the mean shape of the entire sample (see Fig. 1 for methods). The 'high' face shape was derived from the 15 faces with highest attractiveness ratings. The high shape was then caricatured by exaggerating the shape differences from the average by 50% to create a 'high + 50%' shape. Composite images with average, high and high + 50%

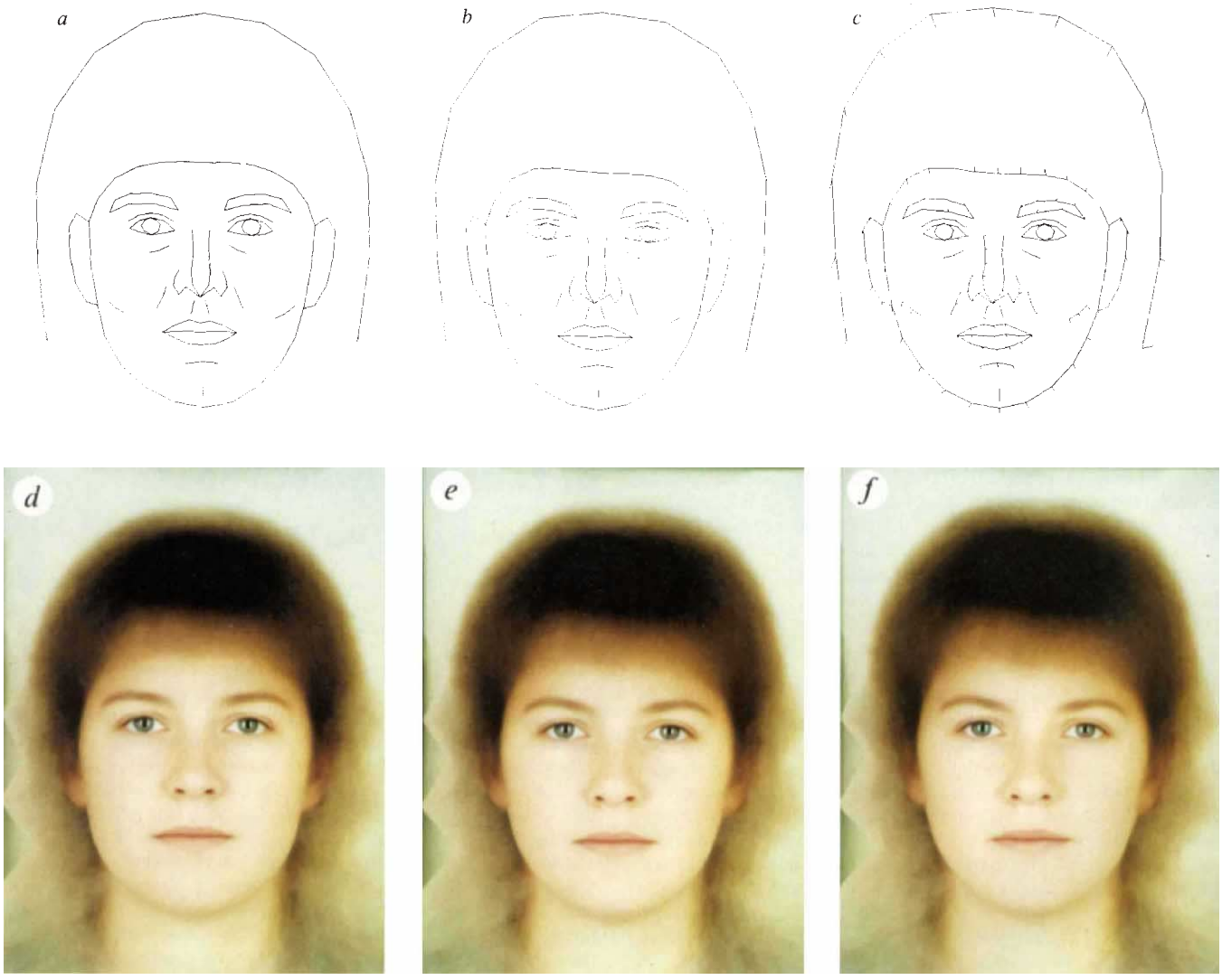

FIG. 1 *a–c*, Shape of average, attractive and caricature of attractive caucasian female faces. Images of 60 UK caucasian female faces (age 20–30 yr; posing with neutral expression) were frame-grabbed in 24-bit colour (531 horizontal by 704 vertical pixel resolution). The shape of facial features of the 60 faces was defined by manually marking 224 predefined feature points (for example, tip of the nose) for each digital face image[18]. The average position of each feature point was calculated for the entire sample of 60 faces and the appropriate points were joined to produce a line-drawn representation of the 'average' face shape (*a*). The mean shape of attractive faces ('high' shape; *b*) was defined by averaging the positions of the feature points for the 15 faces with highest attractiveness ratings. To caricature[18] any distinctive attributes of the high shape, the differences between the 224 feature positions in the high and average shapes were increased by 50% to define the 'high + 50%' shape (*c*). The differences in feature positions between average and high + 50% shapes are indicated in *c* as superimposed vector differences calculated after scaling, rotating and translating these two shapes so that the pupils were aligned. *d–f*, Composite images. Each of the 60 original images was distorted[19] (that is, 'warped' or 'morphed') into the 'average' shape. To achieve this, the feature positions defining the shape of each face were used to divide original images into triangular tessellations. Images were distorted by taking each individual triangle and remapping the enclosed pixels to fit the shape of the corresponding triangle in the tessellation structure for the average shape. The 60 images with the same remapped shape were then blended together[20] to form the average composite (*d*) by averaging the colours and intensities of corresponding pixels in constituent images. High and high + 50% composite images (*e* and *f*) were similarly obtained by remapping the 60 original images into the high and high + 50 shapes before blending.

shapes, but identical skin textures, were generated from the 60 original faces (see Fig. 1*d–f* for methods).

A new set of 36 UK caucasian subjects (age 22–46 yr; 18 female, 18 male) was given pairs of composites and asked to choose the most attractive in each pair. Male subjects preferred the high to the average composite (16/18 subjects; binomial test, $P<0.001$) and preferred the high + 50% to the high composite (15/18; $P<0.005$). Female subjects expressed the same preferences (15/18, $P<0.005$; 14/18, $P<0.02$, respectively). These results show that attractiveness is not averageness: first, the high composite was preferred over the average; second, when the high composite was caricatured to increase the difference from average, the attractiveness increased.

To examine the generality of the effects across faces, we extended the study to a set of Japanese female faces rated for attractiveness by Japanese subjects. Average, high and high + 50% composites were created using similar methods (Fig. 2). A new group of 26 Japanese subjects (undergraduates in Japan, aged 19–29 yr; 9 female, 17 male) preferred the high to the average composite (21/26 subjects, $P<0.01$) and preferred the high + 50% to the high composite (19/26, $P<0.02$). Thus, exaggeration of the differences between the high and average shapes again enhanced attractiveness.

To examine the cross-cultural consistency of the manipulations, the composite Japanese faces were shown to 36 caucasian subjects (undergraduates in the UK, aged 19–26 yr; 25 female, 11 male). These subjects also preferred the high to the average composite (25/36, $P<0.02$) and preferred the high + 50% to the high composite (25/36, $P<0.02$). Thus, caucasian and Japanese subjects showed the same pattern of preferences with the same face stimuli. This is consistent with previous findings of greater similarities than differences in cross-cultural judgements of facial attractiveness[15,16].

Figure 1*c* shows the differences in physical dimensions between average and attractive (high + 50%) caucasian female face shapes. The more attractive shape had higher cheek bones, a thinner jaw and larger eyes relative to the size of the face than the average shape. The attractive shape also had shorter distances between the mouth and chin and between nose and mouth. Average and attractive (high + 50%) Japanese face shapes showed similar differences in the eyes, mouth and chin (Fig. 2*c*). The Japanese face shapes also showed more pronounced differences in eyebrow shape/position and an additional change in height on the nasal side of the cheek.

We found similar preferences for a non-average shape with male faces. High and average composites were made with methods described in Fig. 1 from a set of 59 UK caucasian male faces (age 20–30 yr) initially rated for attractiveness by UK caucasian subjects (age 18–30 yr; 15 male, 15 female; ratings correlated across gender of rater, $r_{(57)}=0.92$, $P<0.0001$, and across individual rater, estimated mean correlation $r_{(57)}=0.45$, $P<0.005$). A new set of caucasian subjects (age 18–30 yr; 18 male, 18 female) preferred the high to the average composite (14/18 male subjects, $P<0.02$; 13/18 female subjects, $P<0.05$). The attractiveness of the high composite was not enhanced by caricaturing.

Thus, our results show that highly attractive faces are systematically different in shape from average. The similarity of attractive facial characteristics across two cultures is consistent with the claim that such characteristics are functionally significant. Viewpoints vary on the functional significance of attractiveness in humans[7,8,17] and preferences in general[5,10–14]. Attractive facial features may signal sexual maturity and fertility, emotional expressiveness or a 'cuteness' generalized from parental protectiveness towards young[7,8]. If human facial attractiveness affects fitness in an evolutionary sense, then the preference for non-

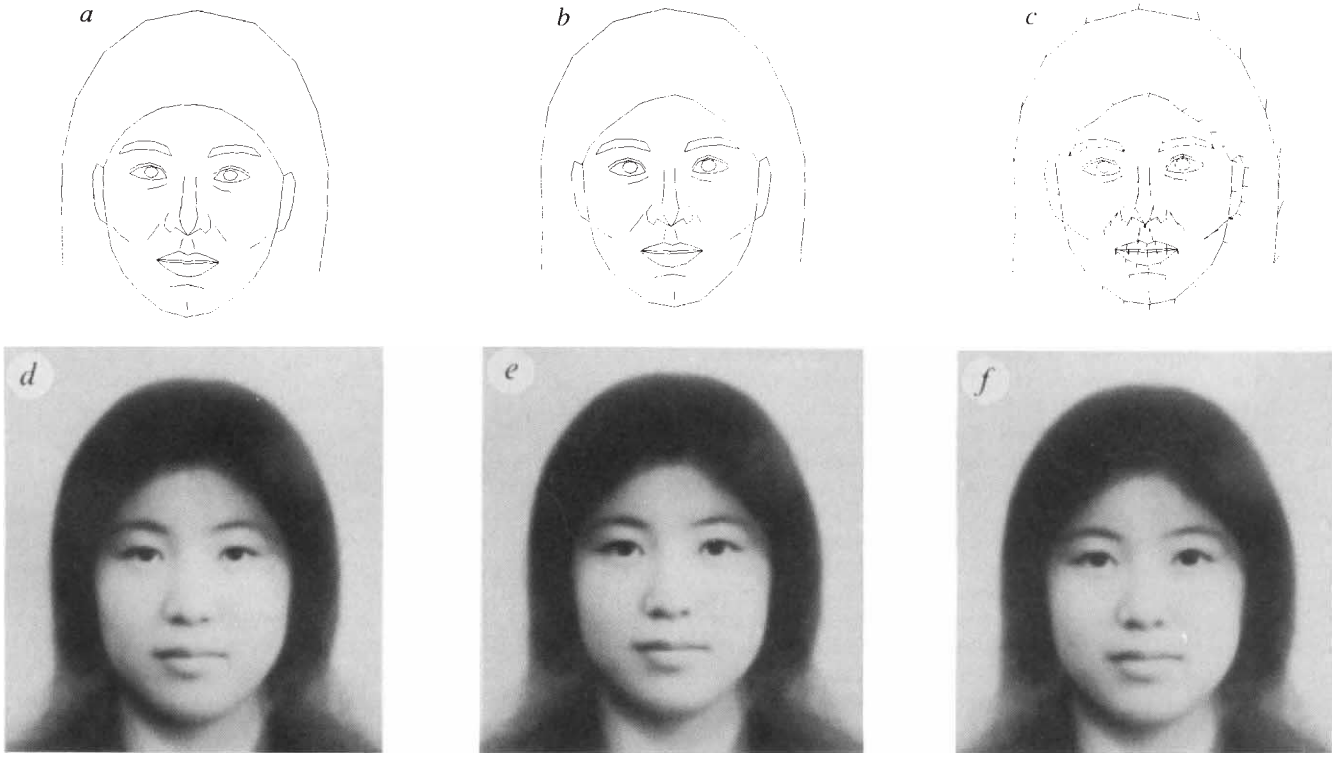

FIG. 2 Shapes and composite images of Japanese faces. The faces of 342 Japanese high-school girls (age 18–19 yr; posing with neutral expression) were photographed and rated for attractiveness on a 7-point Likert scale by 12 Japanese subjects (age 23–28 yr; 6 female, 6 male). The photographs were frame-grabbed in 8-bit grey-scale format. Using methods described in Fig. 1, line drawings were derived for the average shape (*a*), the high shape (*b*) from the 16 faces with highest attractiveness ratings and the caricatured high + 50% shape (*c*). Changes in feature positions from average to high + 50% shape are included in *c*. Composite face images with average (*d*), high (*e*) and high + 50% (*f*) shapes were obtained using the same methods as for Fig. 1.

average face shapes would exert a directional selection pressure away from the population mean. Selection pressures relating to other facial functions (for example, ingestion, respiration) may well work in different directions, limiting any evolutionary effects of preferences on face shapes. □

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Simultaneous LTP of non-NMDA- and LTD of NMDA-receptor-mediated responses in the nucleus accumbens

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THE nucleus accumbens (NA), a ventral extension of the striatum, plays a role in several complex behaviour patterns[1] and also is a major site of action of drugs of abuse such as cocaine[1–3]. Intrinsic NA cells are predominantly quiescent[4,5] and their activity depends on excitatory input from cortical and subcortical limbic afferents[6,7]. Here we examine the mechanisms of synaptic plasticity at the synapse between prelimbic cortical afferents and cells in the core region of the NA[8–10]. Manipulations that induce a Ca^{2+}-dependent long-term potentiation (LTP) of non-NMDA (*N*-methyl-D-aspartate)-receptor-mediated responses also produce a simultaneous long-term depression (LTD) of NMDA-receptor-mediated responses. These results indicate that in a single cell the same change in postsynaptic Ca^{2+} concentration can have opposite effects on non-NMDA- and NMDA-receptor-mediated synaptic responses. This may be particularly important in the NA, where NMDA receptors are critical for mediating the behavioural actions of drugs of abuse[11–13].

Prelimbic cortical afferents make direct monosynaptic connections with cells in the NA core region and elicit an excitatory postsynaptic potential (e.p.s.p.) mediated mainly by non-NMDA receptors (non-NMDARs)[9,10,14]. Tetanic stimulation of this input (100 Hz, 1 s) reliably induced LTP of both extracellular field e.p.s.ps (62 ± 20%; mean ± s.e.; $n = 14$; Fig. 1*a*) and e.p.s.ps recorded using whole-cell recording techniques (68 ± 9%; $n = 17$; Fig 1*b*). LTP induction was blocked reversibly by application of the NMDA receptor (NMDAR) antagonist D(−)-2-amino-5-phosphonovalerate[15] (D-APV, 25 μM) (field e.p.s.p., Fig. 1*a*; $n = 4$; whole-cell e.p.s.p., $n = 6$; results not shown). LTP could also be induced by pairing moderate postsynaptic depolarization with low-frequency afferent stimulation (2 Hz for 2 min) (60 ± 6%; $n = 7$; Fig. 1*c*). To determine whether LTP in NA cells requires a rise in postsynaptic calcium ion concentration ($[Ca^{2+}]_i$), electrodes were filled with the calcium chelator 1,2-bis(2-aminophenoxy)ethane-*N*, *N*, *N*′, *N*′-tetraacetic acid (BAPTA, 10 mM). Tetanic stimulation did not elicit LTP in cells that had been loaded with BAPTA (Fig. 1*d*), whereas the increase in the simultaneously recorded field e.p.s.ps confirmed that the tetanic stimulation did induce LTP in surrounding cells ($n = 4$). Thus, like several forms of LTP found in hippocampus[16] and in cortex[17], LTP in the NA requires an NMDAR-dependent increase in $[Ca^{2+}]_i$.

Because NMDA receptors contribute to synaptic transmission at these synapses[4,5,8], we examined whether tetanic stimulation also induced LTP of NMDAR-mediated synaptic responses. Surprisingly, tetanic stimulation caused a long-term depression (LTD) of the NMDAR-mediated e.p.s.p. (−41 ± 6%; $n = 4$; Fig. 2*a*, *b*) which was recorded in the presence of the selective non-NMDAR antagonist 6-cyano-7-nitroquinoxaline-2,3,-dione (CNQX; 10 μM) and 0.1 mM extracellular Mg^{2+}. To test whether this change from LTP to LTD was due to the removal of Mg^{2+} from the perfusing medium, we isolated the NMDAR-mediated e.p.s.p. in normal medium (1.2 mM Mg^{2+}) by depolarizing the cell in the presence of CNQX. Again, tetanic stimulation induced LTD of the NMDAR-mediated e.p.s.p. (−37 ± 10%; $n = 6$; Fig. 2*c*, *d*). Following its induction, LTD routinely lasted for the duration of the recording (50–70 min).

The induction of LTD versus LTP may depend on the postsynaptic $[Ca^{2+}]_i$, with lower $[Ca^{2+}]_i$ causing LTD and higher $[Ca^{2+}]_i$ being required for LTP[18,19]. Because non-NMDARs were blocked in the previous experiments, tetanic stimulation may not have caused as large an increase in postsynaptic $[Ca^{2+}]_i$ as that elicited in the absence of CNQX. To test this possibility, we examined the effects of pairing low-frequency afferent stimulation with direct postsynaptic depolarization and found that this protocol also induced LTD of the NMDAR-mediated e.p.s.p. (−48 ± 12%; $n = 5$) (Fig. 3*a*, *b*). LTD was blocked by loading cells with BAPTA (10 mM; $n = 7$; Fig. 3*c*) and also required NMDAR activation, as application of the tetanus in the presence of D-APV (25 μM) resulted in the complete recovery of the NMDAR-mediated response following D-APV washout ($n = 3$; results not shown).

Although the same induction protocols produced opposite effects on the two components of the e.p.s.p., an important question is whether LTP and LTD can be generated simultaneously in a single cell. To monitor non-NMDAR- and NMDAR-mediated synaptic components simultaneously, we took advantage of their different voltage dependence and kinetics[20,21]. Voltage-clamping cells at −80 mV permitted measurement of non-NMDAR-mediated fast inward excitatory postsynaptic currents (e.p.s.cs), and holding the membrane potential at +20 to +30 mV allowed measurement (45 ms after stimulation) of predominantly NMDAR-mediated outward e.p.s.cs (Fig. 4*A*). After baseline e.p.s.cs were obtained at −80 mV, the cell was

FIG. 1 The induction of LTP of the non-NMDAR-mediated e.p.s.p. *a*, ► Bath application of D-APV (25 μM) blocks the induction of LTP in field e.p.s.ps recorded in the core region of the nucleus accumbens. After 10–15 min wash-out of D-APV, LTP could be induced ($n = 4$). In this and other figures, 'up' arrows represent times at which tetanic stimulation was applied. Insets show superimposed field e.p.s.ps taken at the indicated times. The first negative peak in the field e.p.s.p reflects direct

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