Table 2. Coupling of "non-communicating" primary neighbours (NCPN) to secondary neighbours

	NCPN coupled to injected secondary neighbour		NCPN not coupled to injected secondary neighbour	total No. of injections
control	17 (54.8%)		14	31
chi (th.)		1 : 1		P = 0.8992
EG (18 h)	10 (33.3%)		20	30
chi. (th.)		1 : 2		P = 1.0000

Procedure: a secondary neighbour directly adjacent to a tested "non-communicating" primary neighbour was injected and the coupling of the tested non-communicating primary neighbour to the injected secondary neighbour was evaluated. Those injected secondary neighbours that were not coupled to any other secondary neighbour were not included.
chi (th.) – theoretical values of χ^2

In order to evaluate whether the original cell population contained clones with different communication capabilities, we tested this value in six clones that had been derived independently, and the results were compared to those of the standard cell line. Moreover, we also tested the response of these clones to the treatment with EG. The results are shown in Fig. 2. Although the average values were rather variable, only one clone (XV) gave a significantly different communication capability (t-test, $P < 0.05$) as compared to the original cell line. The response of the clones to exposure to EG was more variable and three out of six clones were found to be significantly different from the original population (III, VI, XVI).

The population distribution of cells with different probabilities of communication to their primary neighbours in the original population and its clones in both standard conditions and after 18-h treatment with EG are depicted in Fig. 3 (non-communicating injected cells were omitted). The untreated standard population as well as clone VI showed a distinctly normal distribution of the probability of coupling between an injected cell and its primary neighbours with a median from 41 to 60%. The next two clones (II, IV) had similar medians and their population distribution appeared to be near normal, while the medians of the rest of the clones (III, XIV, XV) were different from the median of the original population. The extreme population distribution was found for clone XV, with a median between 81 and 100%. Our results indicate that the original population contains some clones with different communication capability (e.g. XV) and the distribution of cells with different probabilities to couple to their primary neighbours may also vary among the clones.

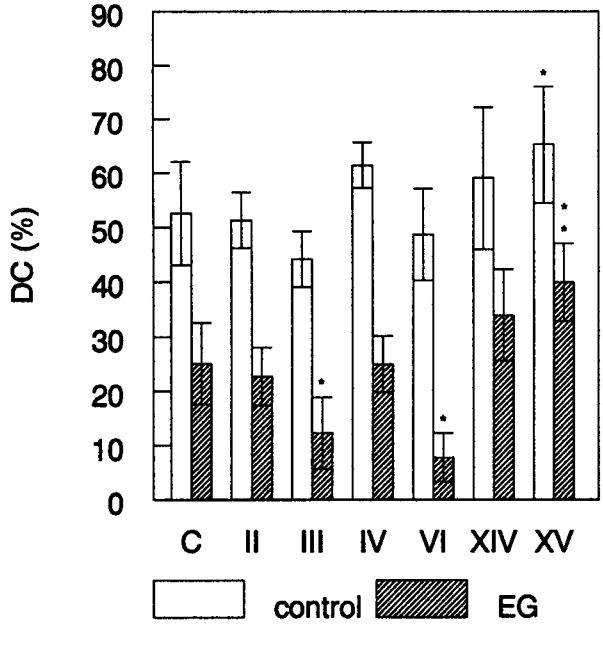

Fig. 2. Communication capability (DC%) of the original cell line (C) and its clones (II, III, IV, VI, XIV, XV) under standard conditions (control) or after 18-h exposure to EG
* = $P < 0.05$ (t-test) vs. value of the original population
*
* = $P < 0.01$ (t-test) vs. value of the original population

In Table 3 we compare the frequency of non-communicating injected cells in clones and in the standard population. While in the original population only 1% of the cells did not communicate to any of their primary neighbours, the corresponding values in all clones tested were found higher with a maximum of 10.7%. These findings again support the conclusion that the standard cell population contains some clones with the gap junctional communication capabilities different from those of the cell line as a whole. A similar situation was found when the frequency of non-communicating cells was evaluated after 18-h exposure to EG, where the maximum even reached the value of 70.7% (VI). Consequently, EG increases the frequency of non-communicating cells, which results in a decrease in the communication capability of the population.

The immunocytochemical detection of connexins present in the cell population used has shown that all cells contained connexin (cx43) present on the cell membranes adjacent to all primary neighbours, although the cells communicated to only a half of them (Fig. 4).

In two of the clones (XIV, XV) the variability of the communication capability in time was assessed. Clone

Table 3. Frequency of non-communicating injected cells (DCO) in the original population (V79-4) and its clones (II–XV) under standard conditions (control) or after 18-h exposure to EG.

cells	control		EG	
	DCO/ No. of injections	%	DCO/ No. of injections	%
V79-4	1/100	1.0	14/60	23.3
II	4/75	5.3	18/75	24.0
III	6/75	8.0	40/75*	53.3
IV	1/75	1.3	24/75	32.0
VI	8/75*	10.7	53/75**	70.7
XIV	21/225*	9.3	61/240	25.4
XV	7/375	1.9	42/300	14.0

* = $P < 0.05$ (χ^2) vs. value of the original population
** = $P < 0.01$ (χ^2) vs. value of the original population

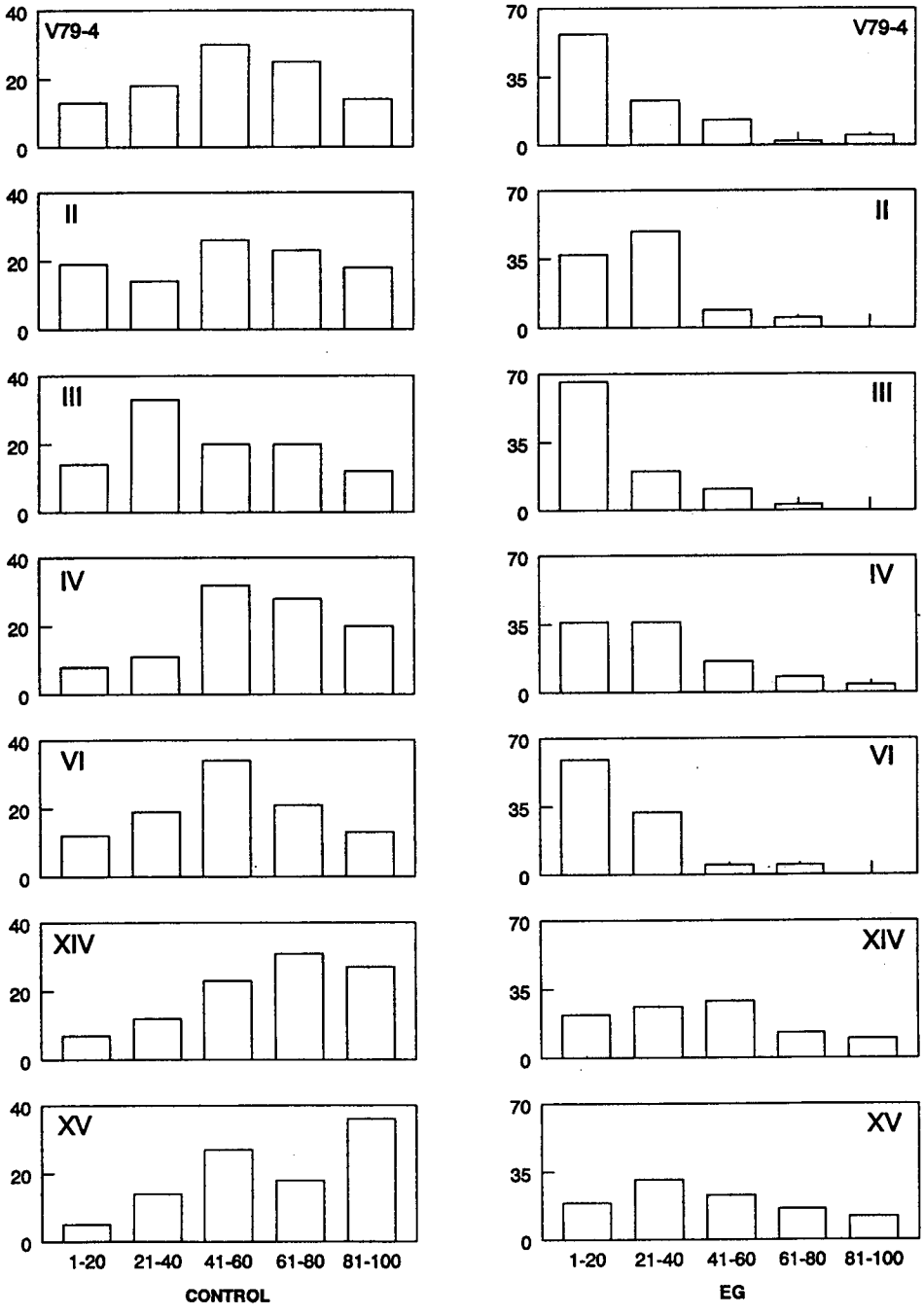

Fig. 3. Population distributions of cells with different communication capability for the original cell line (V79-4) and its clones (II–XV) under standard conditions (control) or after 18-h exposure to EG (non-communicating cells not included)
X-axis - percentage of primary neighbours coupled to an injected cell (%)
Y-axis - percentage of injected cells (%)