FAST BLUE AND DIAMIDINO YELLOW AS RETROGRADE TRACERS IN PERIPHERAL NERVES: EFFICACY OF COMBINED NERVE INJECTION AND CAPSULE APPLICATION TO TRANSECTED NERVES IN THE ADULT RAT

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ABSTRACT

Capsule application of Diamidino Yellow (DY) to the cut end of the sciatic nerve immediately followed by capsule application of Fast Blue (FB) resulted in about 95% double-labelled dorsal root ganglion neurones (DRGn) and motoneurones (Mn). Nerve injection of DY followed either immediately or two months later by capsule application of FB resulted in about 90% double-labelled DRGn and Mn, indicating that DY and FB label similar populations of DRGn and Mn, and that insignificant DY fading occurred during this period. Inversing the order of application, however, i.e. nerve injection of FB followed immediately by capsule application of DY, resulted in double labelling in only about 10% of the DRGn and Mn. These percentages increased to 70% of the DRGn and 60% of the Mn when the FB injection was followed one or two months after by the DY application, indicating that DY uptake is blocked by recent administration of FB. The results indicate that DY and FB might be useful for sequential labelling before and after nerve injury as a tool to investigate the accuracy of sensory and motor regeneration.

INTRODUCTION

Regrowth of injured peripheral nerve axons to appropriate nerve branches after nerve injury is likely to be important for the restoration of adequate motor control and stimulus localisation. The extent to which such regrowth is correct has been indirectly studied by several anatomical methods. In one of these methods (Aldskogius and Thomander, 1986; Aldskogius et al., 1987), retrograde tracers are applied to regenerating axons in a branch of an injured peripheral nerve. As the motor cell bodies of origin of the axons belonging to the different branches are normally topographically organised, a disrupted organisation after regeneration reflects inappropriate regrowth of the regenerating axons. In another method of investigation that involves several steps, a retrograde tracer is first applied to an uninjured branch of a peripheral nerve. After a few days of survival to allow retrograde transport of the tracer to the cell body, the parent nerve of the labelled branch is injured such that the injured axons may grow either into the previously injured nerve branch or into other branches. Then, a second retrograde tracer with different labelling characteristics is applied to either the previously labelled nerve branch (Madison et al., 1996), or to another previously unlabelled branch (Shenaq et al., 1989; Molander and Aldskogius, 1992). The proportion of cell bodies with axons that have regrown into the original nerve branch or into a new branch can then be determined by counting the number of cell bodies labelled by both tracers or either tracer alone.

Much of the reliability of these methods depends on whether or not the tracers label the same fibre populations with similar efficacies and, if used in combination, whether or not they interact with each other. Similar labelling efficacies have been claimed for the combination of fluorescent dyes coupled to dextran amines (Fritzsch and Sonntag, 1991), for Fluorogold and DiI (Harsh et al., 1991), and for fluorogold and HRP (Brushart, 1993). These

tracer combinations have the disadvantage, however, that they can not be observed by the same microscopic illumination conditions. Furthermore, dextran amines and DiI are not taken up by undamaged axons and therefore require application to a transected nerve rather than nerve injection. This may be a disadvantage in experiments where the method of successive labelling is used, as described above, as it requires an extra injury.

With the aim to study the rate of appropriate regrowth of injured axons to nerve branches after injury to the parent nerve, we were interested in finding a pair of fluorescent tracers which: (i) are likely to have similar labelling efficacies, (ii) may be administered by either nerve injection or in a capsule applied to the proximal end of a transected nerve**,** (iii) can be observed at the same time with the same filter combination by showing different colours and by accumulation in separate compartments of the cell body. The combination of True Blue (TB) or Fast Blue (FB) which show a blue stain primarily in the cytoplasm, and Nuclear Yellow (NY) or Diamidino yellow (DY) which show a yellow stain primarily in the nucleus, both in ultraviolet (UV) light (Kuypers et al., 1980), is such a candidate. The combination of FB and DY has previously been used for tracing in the central nervous system (Bentivoglio et al., 1980; Keizer et al., 1983), for simultaneous tracing of the connections of multiple peripheral nerves (Crockett et al., 1987; Gordon and Richmond, 1990), for quantitative studies of the sympathetic reinnervation of the eye (Hendry et al., 1986), and for studies of the relative amount of appropriate motor reinnervation of the anterior tibial muscle (Rende et al., 1991). For DY and TB used together, it was reported that DY seems to be less efficient than TB for retrograde labelling of sciatic neurones (Haase and Payne, 1990), but so far there is no such information available with regard to the combination of DY and FB in the same nerve.

The aim of the present study has been to test the usefulness of the combination of FB and DY for retrograde tracing from the same peripheral nerve as a tool to investigate the accuracy of sensory and motor regeneration. More specifically, we have tested the hypothesis

that they are taken up and transported by the same neuronal populations after both nerve injection and capsule application to transected nerves, and also the possibility that application of one tracer affects the uptake and transport of the other.

MATERIALS AND METHODS

Nineteen Sprague Dawley rats weighing 270-350 were used in the present study. All animals were obtained from Harlan Interfauna Iberica S.A., maintained in the Animal Care Service, Faculty of Medicine, University of Barcelona, and treated in accordance with the ethical guidelines of the University. Anaesthesia was initiated with ether and then continued with chloral hydrate (300mg/kg) during surgical procedures and perfusion.

Unilateral injections showed that no contralateral DRG labelling of the injected tracer occurred (unpublished). This enabled the use of bilateral experiments, thereby reducing the number of animals used.

Tracer application

Capsule application of DY to transected nerve followed by capsule application of FB

Three rats (bilateral or unilateral experiments, n=5) were used to examine the possible interaction between FB and DY after successive capsule application to transected nerves. The sciatic nerve was exposed at the level of the thigh and transected by means of pairs of sharp scissors. A swab was placed below the nerve to absorb any spread of tracers. The proximal cut end of the nerve was then inserted into a plastic capsule containing 1.5 µl of 5% of DY (EMS-Polyloy), kept in this position for 30 minutes and then removed. After that, the nerve was directly (without wash or new nerve transection) inserted into a new capsule containing 1.5 µl of 5% FB (Sigma), kept in this position for 30 minutes and removed. Finally, the proximal cut end was cleaned, the skin was sutured and the rat was allowed to recover from the anaesthesia. In

this study, we did not do capsule application of FB followed by capsule application of DY (see discussion).

Nerve injection of tracer immediately followed by capsule application of a second tracer to the same nerve after nerve transection

Another serie of experiments were designed to examine if tracer administration by nerve injection labels the same neuronal population as administration by capsule application to a transected nerve. In the first subgroup (four rats, n=5) the tibial nerve was dissected at the level of the knee joint and a swab was placed below the nerve. The tip of a 25 gauge needle attached to a 10 µl Hamilton syringe was introduced about 4mm distal to the origin of the gastrocnemius branches, pushed 1-3 mm in proximal direction followed by injection of 1 µl of 5% DY (EMS-Polyloy). After this, the tibial nerve was transected just distal to the point of needle introduction and the proximal cut end was placed inside a capsule containing 1.5µl of 5% FB for 30 minutes. After that, the capsule was removed, the distal cut end cleaned and the skin sutured. In the other subgroup (three rats, n=5) tracers were inverted. Thus, 1 µl of FB was injected and 1.5 µl of DY was placed in the capsule.

Nerve injection of tracer followed by delayed capsule application of a second tracer to the same nerve after nerve transection

The final series of experiments were designed to examine the possibility of tracer interactions after nerve injection followed by delayed capsule application. In the first subgroup (5 rats, n=9), 1 µl of 5% DY was injected in the tibial nerve about 4 mm distal to the origin of the gastrocnemius nerves, and the rats allowed to recover. After two months, the previously injected tibial nerve was reexposed, transected immediately proximal to the DY injection and a

capsule containing 5% FB was applied at the cut nerve end. In the second subgroup (5 rats, n=10), the tracers were inverted so that injection of 1 μl of FB was followed one (n=4) or two months (n=6) later by nerve transection and application of a capsule containing DY to the transected nerve end.

Fixation, sectioning, microscopic examination and statistical analysis

Five days after the last tracer application the rats were anaesthetised as described above and a thoracotomy was performed. An intracardial injection of 1000 UI of heparin/kg body weight was followed by perfusion through the ascending aorta with 100 ml saline and then with 1000 ml 4% paraformaldehyde and 10% sucrose in phosphate buffer (PB, pH=7.40) for twenty minutes. The lumbar dorsal root ganglia (DRGs) L3-6 and corresponding spinal cord segments were removed, postfixed for three hours in the same fixative and then immersed in 15% sucrose in PB at 4ºC overnight. The L4-6 DRGs were cut on a cryostat in 10μm thick longitudinal sections, and the spinal cord and L3 DRGs were cut in 30 μm thick serial longitudinal sections. Sections were all thaw-mounted on chrom-alum gelatinised (5%) slides and coverslipped using an antifading solution containing 1% paraphenylendiamine and 10% phosphate buffered saline in glycerol.

The sections were examined in an Olympus Vanox fluorescent microscope equipped with violet light filters (DM 455 dichroic mirror and BP 405 exciter filter which gives 405 nm excitation, and 455 nm emission wavelengths). All neuronal profiles with an identifiable nucleus were counted in every tenth DRG section and in every fourth spinal cord section. The counting was performed the same day as the sectioning took place. The counting was not carried out in a blinded fashion.

At short survival times after administration, DY positive cells showed an intense yellow fluorescence in the nucleus and less staining in the cytoplasm. At two months,

however, many of the DY positive cells also showed intense granular labelling in the cytoplasm. FB labelled cells showed an intense blue fluorescence in the cytoplasm and usually also in the nucleus. Because of the intensity of the FB nuclear labelling, it was sometimes difficult to identify DY nuclear labelling in FB/DY double-labelled neurones. In those cases, identification was facilitated by exposing the sections with violet light filters for up to half a minute until the nuclear FB had faded and the DY could be observed more clearly. The DY nuclear labelling was found to be less intense but more resistant to fading than FB. The cytoplasmatic FB labeling was still visible after this procedure. The total number of labelled profiles counted in each case using every tenth section of DRGs L4-6 as well as means and standard deviations from total cell counts and percentages are presented in the table. No corrections were made for the possibility of counting split cells twice in different sections.

Paired Wilcoxon W tests were used to compare mean double labelling percentages between DRG and spinal cord in the same rats, while Mann-Whitney U tests were used to compare percentages between different experiments. Total cell counts after different exposure periods were compared by means of Student's t-test after verifying the normality of the variable by the Kolmogorov-Smirton test. Some animals (R124, R125, R126, R141) showed very faint FB cytoplasmatic labelling in most DRG cells. The difference in labelling intensity between these cells and those labelled from axonal transport was very striking, and was not considered to cause any problem regarding identification. Thus, these cases were also included.

No such FB labelling was visualised in any case in spinal cord. None of the cases showed DY labelling of this kind in the DRGs or spinal cord.

RESULTS

Capsule application of DY to transected nerve followed by capsule application of FB

Capsule application of DY followed by a second capsule with FB to the proximal end of the transected sciatic nerve resulted in double labelling (FB/DY) in 96.6% of the dorsal root ganglion cells and 96.7% of the motoneurones of the spinal cord (Table I, Exp 1; Fig 1 A and B). No significant difference (p=0.6858) in the percentages of double labelling was found between motoneurones and DRG neurones. In the DRG, 1,9% of the neurones were only FB positive and 1.5% showed only DY. Similar results were found in the spinal cord; 0.7% of all labelled cells were only FB positive and 2.4% of all positive cells were single DY labelled.

Nerve injection of tracer immediately followed by capsule application of a second tracer to the same nerve after nerve transection

Injection of DY in the tibial nerve immediately followed by nerve transection and capsule application of FB resulted in double labelling (FB/DY) of 97.3% of all DY labelled DRG neurones, and of 93.7% of all DY labelled motoneurones (Table I, Exp 2). No significant difference (p=0.0679) in the percentages of double labelling was found between motoneurones and DRG neurones.

The inverse procedure, i.e. injection of FB immediately followed by capsule application of DY on the transected nerve, resulted in double labelling (FB/DY) of 10.6% of all FB labelled DRG neurones, and of 13.3% of all FB labelled motoneurones (Table 1, Exp 3). No significant difference (p=0.6858) in the percentages of double labelling was found between motoneurones and DRG neurones.

The differences in the rate of double labelling resulting from injection of DY followed by capsule application of FB and vice versa (table I, Exp 2 and 3) were significant, both for DRG neurones (p=0.0079) and motoneurones (p=0.0079).

Nerve injection of tracer followed by delayed capsule application of a second tracer to the same nerve after nerve transection

Injection of DY in the tibial nerve followed two months later by nerve transection and application of a capsule with FB resulted in double labelling (FB/DY) of 92.3% of all DY labelled DRG neurones and 86.9% of all DY labelled motoneurones (Table I, Exp. 4). The difference in rate of double labelling between DRG neurones and motoneurones in this experimental situation was found to be statistically significant (p=0.0109). In all these cases, many nuclei appeared to be slightly less stained with DY than the short survivors. Furthermore, intensely stained granules which appeared to have a slightly different colour than the nuclei, were found in the periphery of the nucleus and/or evenly distributed in the cytoplasm in some DY labelled cells. Such granulae were never observed in cells without DY nuclear labelling. In these cases, other double-labelled neurones showed a diffuse mixture of FB and DY in the both the cytoplasm and nuclei (Fig.1C). No significant difference in the total number of DY positive DRG neurones (p=0.991) or motoneurones (p=0.130) were found when comparing counts obtained five days and 2 months, respectively, after injection of DY.

The inverse procedure, i.e. injection of FB followed one or two month later by capsule application of DY on the transected nerve, resulted in double labelling (DY/FB) of 73.2% of all FB labelled DRG neurones, and of 59,5% of all FB labelled motoneurones (Table I, Exp 5). The cell counts obtained from the rats that survived one and two months, respectively, from nerve injection to capsule application are presented together here, as we did not see any differences between these groups, neither in the rate of double-labelled neurones (DRGs, p=0.6698; motorneurone pool, p=0.8312), nor in the total number of FB labelled neurones (DRGs, p=0.3938; motoneurone pool, p=0.6698). The results after delayed application of

DY are significantly different from the corresponding results obtained after FB injection immediately followed by capsule application of DY, for both DRG neurones (p=0.0013) and for motoneurones (p=0.0027). No significant difference in the total number of FB positive DRG neurones (p=0.425) or motoneurones (p=0.714) were found when comparing counts obtained after five days, and after one or two months, respectively, following injection of FB.

DISCUSSION

The main results of the present study show that the number of neurones retrogradly labelled by FB and DY after successive administration to a peripheral nerve depends on both the way of administration and the order in which the tracers are administered.

Capsule application of DY followed by capsule application of FB, each for 30 minutes to the proximal end of the cut sciatic nerve, resulted in a high rate of neuronal colocalisation of tracers in the DRG (96.5%) and spinal cord ventral horn (96.7%). A small number of neurones were single labelled with either DY or FB. This suggests similar tracer efficacies for these tracers when used with this technique, i.e. uptake by injured axons of DY does not seem to obstruct subsequent uptake of FB. The failure for reaching a 100% of double labelling is most likely due to technical limitations such as incomplete nerve contact with the dyes inside the capsules or to reduction in the ability of some axons to take up tracers as a result of axonal damage during transection and introduction of the nerve into the capsule.

For technical reasons, it is reasonable to assume that dye administered by nerve injection reaches fewer fibres than administration by capsule applied to a transected nerve (Taylor et al., 1983). We tested the validity of this assumption in a second series of experiments where injection of one dye was followed by either immediate or delayed nerve transection and application of a capsule with a second dye to the proximal nerve end. Injection of DY into the tibial nerve immediately followed by nerve transection and application of a capsule with FB

resulted in DY labelling in more than 90% of all FB labelled neurones. This finding indicates that most neurones previously labelled by DY readily take up and transport also FB. Furthermore, less than 10% of all FB labelled neurones were FB single labelled, showing that most of the fibres labelled by capsule application of FB had taken up and transported the previously injected DY. This indicates that injection of DY is nearly as effective as capsule application of DY after nerve transection for labelling as many fibres as possible in the nerve, given the amounts and concentrations used in this study. This finding is of interest in experimental situations where a reduction of the effects of nerve trauma is desired.

Unexpectedly, when the procedure was inverted so that the injection of FB was followed by immediate transection and capsule application of DY, the resulting rates of double labelling were considerably lower. Only 10.6% of the FB positive DRG neurones and 13.3% of the motoneurones contained also DY. This suggests that the injected FB blocked the uptake and/or transport of the subsequently administered DY, perhaps by a toxic effect or by a competition at the transport level, favouring FB. This finding is possibly related to an earlier report that injection of a mixture of DY and TB into the sciatic nerve resulted more neurones labelled with TB than with DY (Haase and Payne, 1990). In this study, we did not test whether a similar block would be obtained by capsule application of FB followed by capsule application of DY, mainly because we did not expect this approach to be useful in our future regeneration experiments after realising the blocking effect of FB in the injection experiments as described above. We did, however, test the hypothesis that such negative effects of FB on subsequent uptake of DY would be decreased if the DY were administered at a delayed time point when less FB would be expected to be present at the administration site. In support of this hypothesis, we found that the rate of DY labelled FB neurones rose to 73.2% in the DRG and 59.5% in the spinal cord ventral horn after delayed administration of DY, but was still considerably lower than after injection of DY followed by capsule application of FB. The increase was unrelated to the number of FB

labelled cells which was found to be unaltered compared to the short survivors. This is in agreement with previous reports showing that FB remains in the cell bodies for long periods of time (Shenaq et al., 1989; Garret et al., 1991; Molander and Aldskogius, 1992; Novikova et al., 1997). It should be noted, however, that the blocking of DY by FB may depend on the source of DY. We have tried to use also samples of DY originally obtained from Dr Illing (kindly supplied by Dr. Gonzalo, Centro Médico, Soria, Spain) with higher yield of double labelling together with FB. Unfortunatly, DY from this source is no longer available.

We also tried to inject DY followed two months later by capsule application of FB. In these cases, the mean percentage of FB labelled DY neurones was 92.3 % in the DRG and 86.9% in the ventral horn of the spinal cord. These percentages are similar to those observed after immediate capsule application. Furthermore, the finding that the mean number of DY labelled neurones was similar after five days and two months indicates that the tracer remained in the labelled neurones and did not spread significantly to surrounding neurones. This finding is in accordance with Keizer et al. (1983) who demonstrated labelling in neurones of the central nervous system for at least three weeks after injection of DY, which was the longest survival time used by these investigators. However, our results are not in accordance with a previous report in the axolotl (Wigston and Kennedy, 1987), showing a considerable increase in the number of DY labelled motoneurones two-three months following DY administration to a peripheral nerve, compared to nine-eleven days. These authors suggested that the increase they observed may be explained by transfer between axons within nerves *en route* to the spinal cord or a direct transfer of DY to adjacent neurones and glia within the spinal cord.

Our interpretation that the number of DY labelled neurones in our material did not increase is also supported by the finding of a high rate of double labelling, indicating that only neurones in the same originally labelled peripheral nerve were included.

The cellular distribution of DY at short survival times after adminstration was mainly

restricted to the nuclei. This is in accordance with previous reports (cf. Keizer et al. 1983). However, at two months after DY administration the intensity of the nuclear staining appeared fainter. In addition, yellow granulae appeared also in the cytoplasm. This does not seems to have been reported previously. The yellow granular labelling was seen only in neurones with yellow labelling in the nucleus, indicating that the phenomenon was restricted to neurones previously labelled with DY. The granulae did not show the typical lipofuscin color, but it is not possible from our results to exclude that some of the granular labelling in these cells is lipofuscin rather than cytoplasmatic accumulations of DY. Even if the granulae reflect a general degradation process in these neurones, the high rate of double labelling found indicates that the transport capacity is preserved. Furthermore, as the number of DY labelled neurones was similar to that found five days of exposition to the dye, it is conceivable that cell death did not occur or was insignificant at this time point. Thus, we find it reasonable to assume that the results of the countings in this study would have been very similar regardless of whether the granuale correspond to DY or accumulation of lipofuscin in which case it would have been restricted to neurones previously exposed to DY. Further experiments are needed to assess whether exposure to DY changes the regenerative capacity of these neurones.

An attractive approach to study regeneration accuracy after peripheral nerve injury is to retrogradly label cell bodies of afferents and efferents in a particular nerve branch before and after injury to its parent nerve trunk using two different tracers. This approach has been tried in several previous studies (Madison et al., 1996; Brushart, 1993; Fritzsch and Sonntag, 1991). Theoretically, a high rate of double-labelled and few single labelled cell bodies would indicate that the injured axons have regenerated across the injury into their former nerve branch. The results of this study where we tested the usefulness of this method for uninjured axons show important aspects of the methodological difficulties and advantages involved in the use of FB and DY for such studies. Capsule application of DY immediately followed by capsule

application of FB resulted in a high rate of double-labelled neurones, indicating that both tracers label the same types of primary afferents and motoneurones. Both FB and DY were found to remain in the neuronal cell bodies in the DRG and spinal cord ventral horn for months after nerve injection, suggesting that either one might be used as a first tracer to label original normal connections before experimental nerve injury. Furthermore, a high rate of double labelling results from injection of DY followed two months later by capsule application of FB to the same nerve whereas the inverse approach, injection of FB followed two months later by capsule application of DY resulted in fewer double-labelled neurones, indicating interaction between these tracers. Thus, in experiments designed to investigate regeneration accuracy using DY and FB, administration of DY before administration of FB is to be preferred in order to reduce the risk of such tracer interaction.

Further potential problems associated with the use of sequential injections of fluorescent dyes for studies of regeneration accuracy after nerve injury are possible toxic effects of the long-lasting tracer in regenerating neurones, different ability of regenerating as compared to uninjured neurones to take up the second tracer, and the possibility that injected dyes remain available for uptake at the injection site when regenerating fibres reach the previously injected nerve branch. These problems are now under investigation.

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TABLES

Table 1. Numbers of FB, DY, FB/DY and percentages of FB/DY double-labelled profiles of the L4-6 DRGs and spinal cord segments in the different experimental situations (Exp.). Note that the numbers given resulted from counting in every tenth DRG section and every fourth spinal cord section and do not represent estimations of the true total numbers of neurones. In Exp. 2,3,4, and 5 the percentages of double-labelled profiles is related to the total number of cells containing the tracer injected. Exp.1: capsule application of DY followed by capsule application of FB; Exp. 2: DY injection in the tibial nerve immediately followed by capsule application of FB just distal to the injection site; Exp. 3: FB injection in the tibial nerve immediately followed by capsule application of DY just distal to the injection site; Exp. 4: DY injection in the tibial nerve followed two months later by capsule application of FB just proximal to the injection site; Exp 5: FB injection in the tibial nerve followed one to two months later by capsule application of DY just proximal to the injection site.

DRG					SPINAL CORD			
Exp 1.	**%FB/DY**	FB	DY	FB/DY	**%FB/DY**	FB	DY	FB/DY
Mean	**96.6%**	25.0	24.8	1425.4	**96.7%**	4.0	15.6	475.4
± S.D	**1.8%**	18.8	31.3	217.3	**2.5%**	2.6	19.0	126.9
Exp 2.	**FB/DY / FB/DY+DY**	FB	DY	FB/DY	**FB/DY / FB/DY+DY**	FB	DY	FB/DY
Mean	**97.3%**	94.6	22.8	882.2	**93.7%**	22.4	10.0	179.0
± S.D	**1.8%**	82.3	15.2	169.8	**4.0%**	18.8	6.6	68.6
Exp 3.	**FB/DY / FB/DY+FB**	FB	DY	FB/DY	**FB/DY / FB/DY+FB**	FB	DY	FB/DY
Mean	**10.6%**	903.4	5.8	95.2	**13.3%**	203.6	1.0	33.6
± S.D	**12.3%**	275.5	4.1	105.8	**11.2%**	65.5	1.1	27.1
Exp 4.	**FB/DY / FB/DY+DY**	FB	DY	FB/DY	**FB/DY / FB/DY+DY**	FB	DY	FB/DY
Mean	**92.3%**	57.67	67.56	836.00	**86.9%**	19.4	40.1	211.2
± S.D	**1.5%**	31.10	18.15	233.54	**5.2%**	27.3	30.1	62.4
Exp 5.	**FB/DY / FB/DY+FB**	FB	DY	FB/DY	**FB/DY / FB/DY+FB**	FB	DY	FB/DY
Mean	**73.2%**	227.1	68.4	679.4	**59.5%**	95.2	29.1	152.0
± S.D	**18.6%**	138.5	117.5	257.3	**23.7%**	19.5	22.2	72.9

FIGURE LEGENDS

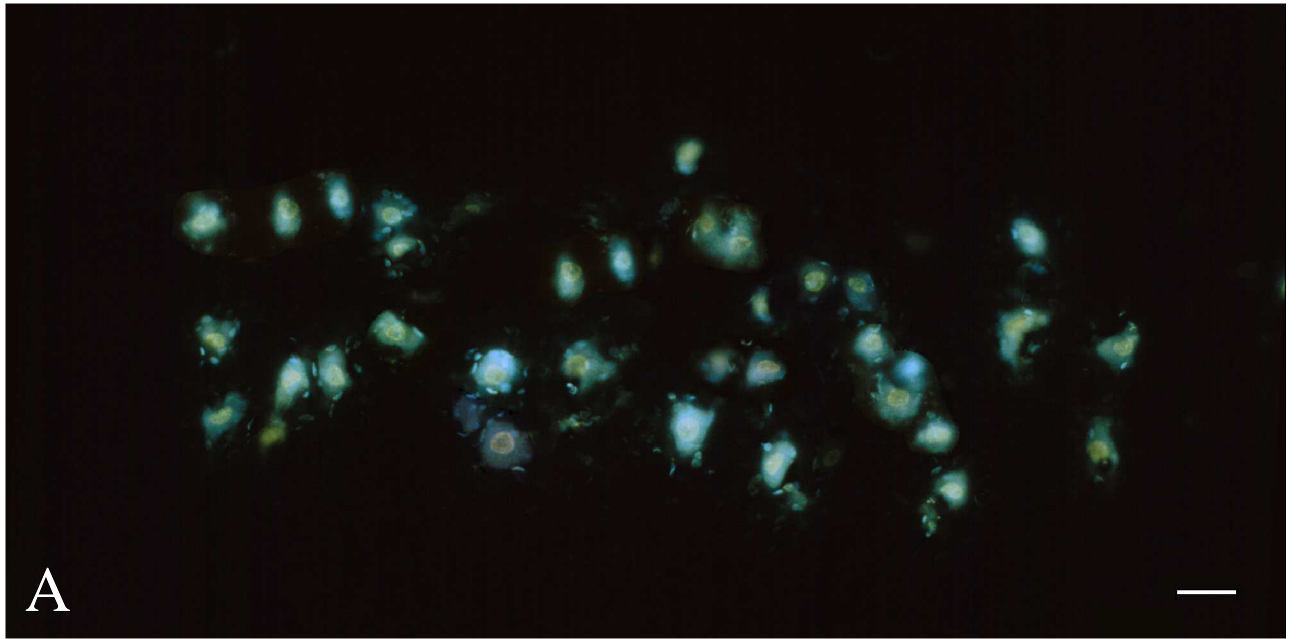
Fig. 1.

A. Photomicrograph of part of longitudinal section of the lumbar spinal cord showing retrogradly labelled motoneurones after sequential capsule application of DY and FB to the cut end to the sciatic nerve. Note that the DY nuclear labelling is surrounded by FB labelled cytoplasm.

B. Photomicrograph of part of longitudinal section of a lumbar DRG showing retrogradly labelled cell bodies of primary afferent neurones after sequential application of DY and FB to the cut end of the sciatic nerve. Most neurones are double-labelled (FB/DY).

C. Photomicrograph of part of longitudinal section of a lumbar DRG showing retrogradly labelled cell bodies of primary afferent neurones after nerve injection of DY followed two months later by capsule application of FB. Many neurones show yellow cytoplasmatic granules, which often appear more intense than the nuclei (large arrows; compare with figs A and B). The apparent difference in color between these granulae and the more faintly labelled nuclei is a result of differences in intensity. These granules may also appear evenly distributed in the cytoplasm (arrowheads). Some double-labelled cells showed a mixture of FB and DY labelling in both cytoplasm and nuclei (thin arrows).

Scale bar: 50 µm in all sections.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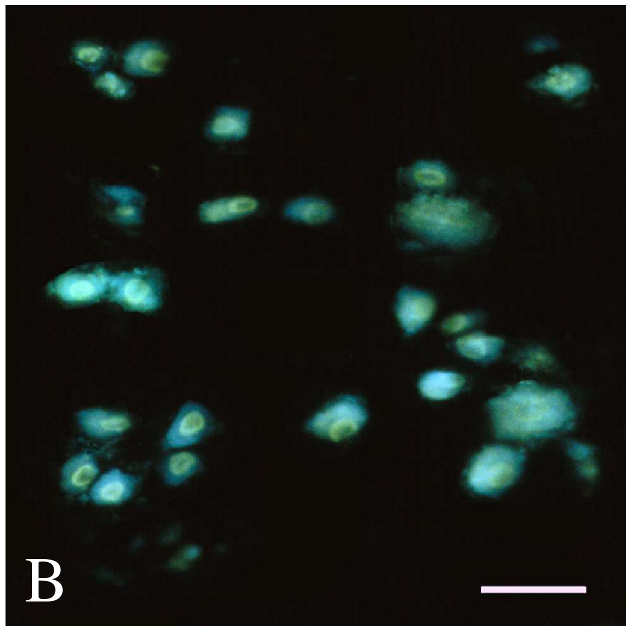
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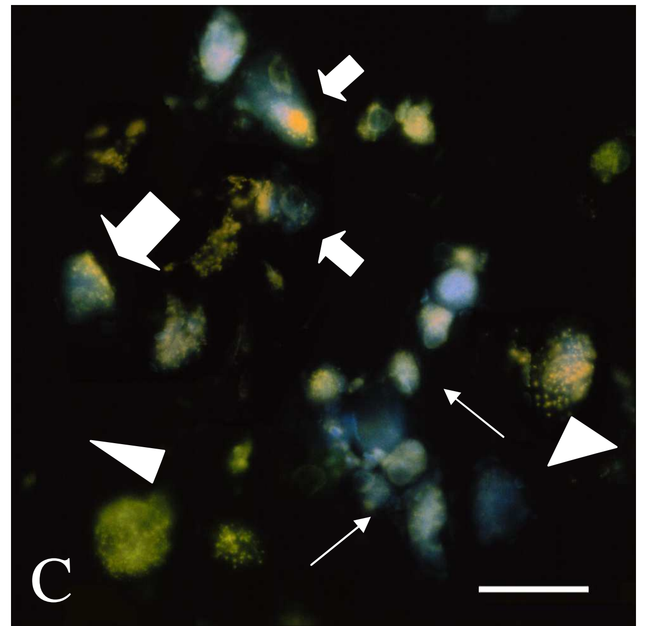
C