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Research Papers

Simultaneous visualization of Nuclear yellow and iron–dextran complex for demonstration of branched neurons by retrograde axonal transport

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Retrogradely transported iron–dextran complex and Nuclear yellow could be demonstrated simultaneously on the same sections of rat CNS. Using combined bright-field and ultraviolet illuminations, double labeled neurons were observed. They exhibited a fluorescent nucleus and cytoplasmic Prussian blue granules. Thus, the combination of these two tracers appeared to be a convenient method for the anatomical demonstration of collaterals.

Introduction

Recent neuroanatomical methods based upon retrograde axonal transport of exogenous substances have allowed the demonstration of axon collaterals. A number of combinations of tracers has been proposed for such a purpose (Stewart et al., 1977; Van der Kooy et al., 1978; Cesaro et al., 1979; Hayes and Rustioni, 1979, 1980; Berkley et al., 1980; Brodal et al., 1980; De Holmos and Heimer, 1980; Illing, 1980; Steindler and Deniau, 1980; Bentivoglio et al., 1980, 1981; Jeffery et al., 1981; Kuypers et al., 1979, 1980; Wong and Kelly, 1981; Yezierski and Bowker, 1981; Katan et al., 1982; Nguyen-Legros et al., 1982). Among the available fluorescent tracers recently developed (Kuypers et al., 1980), Nuclear yellow (NY) appears to be of special interest because of its predominant localization within neuronal nuclei after axonal transport. Actually, the majority of retrograde tracing experiments result in cytoplasmic labeling. It can be supposed that a competition exists between different tracers involving the same mechanisms of uptake, transport and storage, rendering the multiple labeling somewhat hazardous. Moreover, it can be difficult to observe simultaneously several tracers filling the perikarya of neurons. The use of a

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substance with different affinity and localization, such as Nuclear yellow, may avoid this saturation phenomenon and greatly facilitate the detection of doubly or multiply labeled neurons.

The present experiments were designed in order to demonstrate the possible use of Nuclear yellow together with iron–dextran complex, a tracer used previously for double labeling after retrograde axonal transport (Cesaro et al., 1979; Nguyen-Legros et al., 1982).

Materials and Methods

Eight male Sprague–Dawley rats (280–300 g) were used and two types of experiments were performed: (1) to test the technical possibility of demonstrating the two tracers within the same neuron in a single tissue section, 4 rats received a striatal injection (coordinates A 8.5, L 2.5, H + 6, according to the atlas of Albe-Fessard et al., 1966) of 0.3 μl of concentrated iron–dextran complex (ID, Dexionex, Pharmacia France), followed by 3 injections of 0.2 μl of 1% Nuclear yellow (NY, Hoechst S 769121) into the same area, 4 days later; and (2) to ascertain the validity of the method for the demonstration of branched neurons, 4 rats received multiple injections of 0.03–0.1 μl of ID into the anterior cortex, followed by 3 injections of 0.2 μl of NY into the stiatum 4 days later.

All injections were made through a glass micropipette as described elsewhere (Cesaro et al., 1979). Since NY does not dissolve easily in saline but forms a suspension, small multiple injections were preferred to avoid plugging of the cannula.

The rats were anaesthetized with Ketamine (100 mg/kg) 16–18 h after NY injections, and perfused through the left ventricle successively with 500 ml of saline-phosphate buffer (0.1 M, pH 7.4), 600 ml of 4% buffered paraformaldehyde and 500 ml of 10% sucrose in the same buffer. The brains were dissected out and frozen frontal sections (40 μm) were cut immediately. It was crucial to process the tissues rapidly in order to minimize in vitro migration of NY from neuronal nuclei into cytoplasm and surrounding glial cell nuclei (Bentivoglio et al., 1981; Wong and Kelly, 1981). The sections were collected in 0.1 M phosphate buffer, mounted on gelatin-coated slides and air-dried. Alternate sections were processed by Perl's reaction for the histochemical demonstration of ferric iron. After air-drying, the sections were either mounted with Entellan or observed with immersion objectives without coverslipping.

Results

In the two types of experiments, labelled neurons were observed in intralaminar thalamic nuclei as well as in substantia nigra. Almost all neurons were doubly labeled after double striatal injection, whereas, as previously described (Cesaro et al., 1979), only scattered neurons were doubly labeled in the centralis lateralis nucleus

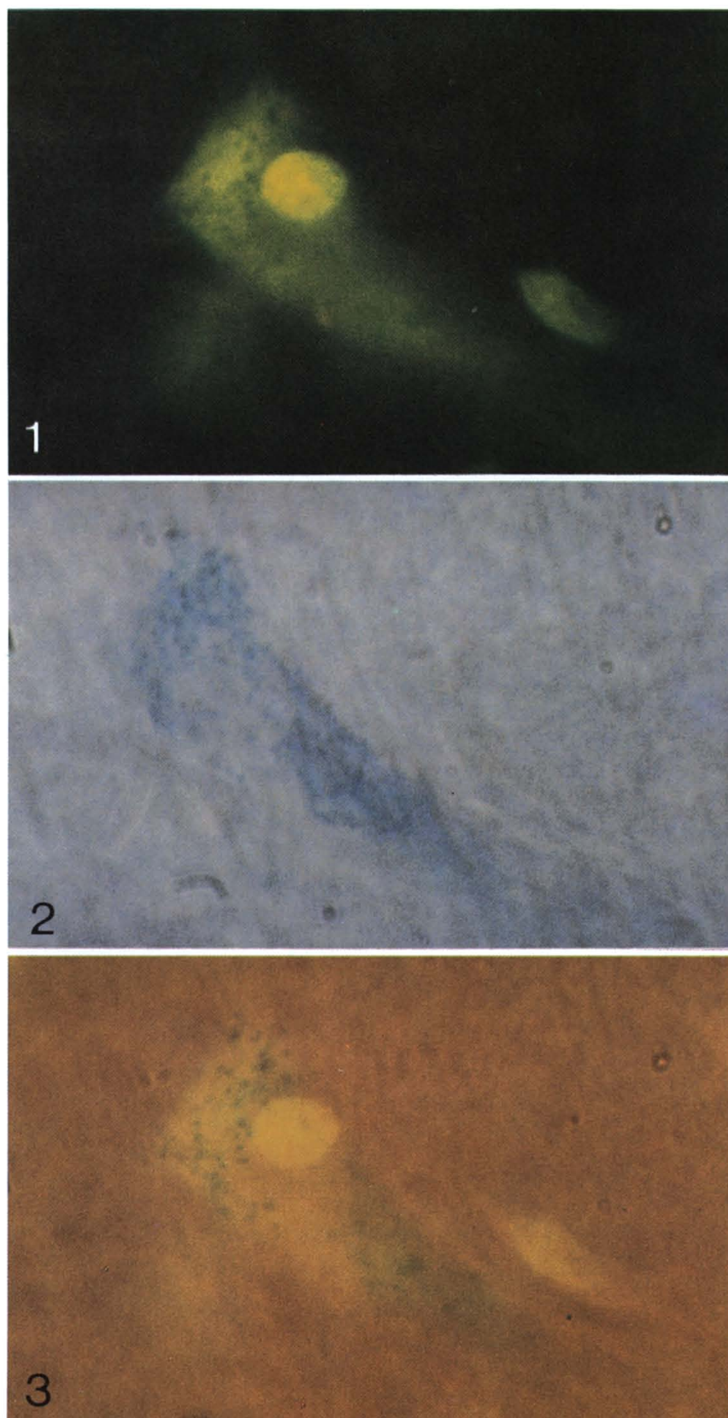

Fig. 1–3. The same doubly labeled neuron is observed. Fig. 1. Doubly labeled neuron seen with ultraviolet illumination. Fig. 2. doubly labeled neuron seen with bright-field illumination. Fig. 3. Doubly labeled neuron seen with combined ultraviolet and bright-field illuminations. This last combination allows discrimination of fluorescent yellow nucleus and cytoplasmic Prussian blue granules. ($\times 2000$.)

and the centrum medianum parafascicularis complex, and none in the substantia nigra after cortical and striatal injections. The percentage of doubly labeled neurons vs neurons labeled with NY or ID only, was quite comparable to that obtained with ID and horseradish peroxidase (HRP) in the same system. Identical extent of thalamic neuronal labeling was observed after NY as compared with HRP after striatal injection (Cesaro et al., 1982).

Ultraviolet excitation light allowed to observe the bright fluorescence of NY within neuronal nuclei, and sometimes to a less degree within the cytoplasm, while Prussian blue granules appeared as dark cytoplasmic holes (Fig. 1). Bright-field illumination revealed the Prussian blue cytoplasmic granules (Fig. 2). The combination of ultraviolet epifluorescence and dim bright-field illumination allowed the simultaneous observation and discrimination of the two tracers (Fig. 3).

The sections could be processed for Perl's reaction without imparing the bright nuclear fluorescence of NY. However, a weak cytoplasmic fluorescence was more frequently observed in Perl's-treated sections as compared with untreated ones. This may be due to a faint migration of NY induced in vitro by the acidic Perl's reactive. In spite of this minor drawback, the use of NY in combination with ID proved to be convenient for the demonstration of branched neurons by retrograde axonal transport. Since it has been reported that the use of NY was compatible with HRP tracing (Wong and Kelly, 1981; Katan et al., 1982), it seems likely that NY could be used concurrently with both HRP and ID for the demonstration of several collaterals.

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