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EdU, a new thymidine analogue for labelling proliferating cells in the nervous system

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Labelling and identifying proliferating cells is central to understanding neurogenesis and neural lineages *in vivo* and *in vitro* .The standard method of labelling proliferating cells uses the thymidine analogue, bromodeoxyuridine (BrdU), which incorporates into the DNA during S-phase of the cell cycle. A disadvantage of this method is that the immunochemical processing requires pre-treatment of the cells and tissue with heat or acid to reveal the antigen. This pre-treatment reduces reliability of the method and degrades the specimen, reducing the ability for multiple immuno-fluorescence labelling at high resolution. We report here the utility of a novel thymidine analogue, ethynyl deoxyuridine (EdU), detected with a fluorescent azide via the "click" chemistry reaction (the Huisgen 1,3-dipolar cycloaddition reaction of an organic azide to a terminal acetylene). The detection of EdU requires no heat or acid treatment and the incorporated EdU is covalently conjugated to a fluorescent probe, using a copper-catalysed chemical reaction. The reaction is quick and compatible with fluorescence immunochemistry and other fluorescent probes. We show here that EdU efficiently labels proliferating cells of embryos and adult animals. It effectively labels cells during neurogenesis and the progeny may be identified at least 30 days later, thus allowing the tracking and quantification of proliferating cells in multiple neurogenic regions including the olfactory neuroepithelium[1]. We demonstrate its utility, superseding BrdU as a cell proliferation marker, as it markedly improves the detection of proliferating cells and allows concurrent high resolution fluorescence immunochemistry.

[1] Chehrehasa et al 2008, J Neuroscience Methods, *In Press*.