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Editorial

In situ Hybridization: Applications in the Realm of Neuroscience

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Editorial

In situ hybridization

In situ hybridization is a technique widely used in neuroanatomy for mapping anatomical distribution and characterization of neurochemicals in brains of murine and human models. However, the use of this technique is not just confined to the realm of neurosciences and is widely employed in other fields of biological sciences like Cell biology and Genetics.

The technique was originally developed by Gall and Purdue and independently by John et al., in 1969, with in the span of five decades, the technique has developed by leaps and bounds and since then many different methods have been developed.

As the name implies, the technique uses radioactive or non-radioactive nucleic acid probes, which hybridize mRNA or DNA species of a certain biochemical entity such as neurotransmitter or neuropeptide, in the tissue of interest followed by measurement of signals.

Originally, radioactive isotopes of elements Phosphorous and Sulphur were the only available labels for the preparation of the probes, consequently, the technique implementation was largely hampered by safety issues, short life span due to radioactive decaying of probes and long duration exposure required (1-2 months) for development of measurable signals in the auto radiographs for analysis. Despite these impediments, the high sensitivity of the radioactively labelled probes for detection of low copy numbers of nucleic acids, sustained its continuous but limited use. However, the usefulness of the technique gave an impetus for the development of flurochromes and haptens (Digoxygenin and Biotin) in the last two decades, which, revolutionaries this technique, increasing its application in the field of biological science by many folds. Consequently, there has been an exponential rise in publications with this technique since, 1999 [1].

Fluorescence *In situ* Hybridization (FISH) involves the enzymatic incorporation of flourophore-modified bases to form fluorescence probes, which can be further conjugated with hapten and Biotin. Now a combination of Florescin, Biotin and Digoxygenin labels can be used for detection of as many as 2 to 12 probes in a single experiment with good sensitivity and resolution. FISH capitalizes on the basic principle of *In situ* hybridization but has the advantage of high sensitivity detection, low cost and facile preparation of probes and shorter durations required for result analysis. The added features of automated data collection and analysis have played a major role in making *In situ* hybridization a foremost biological assay.

In situ hybridization has an edge over other genomic expression techniques like RT-PCR, unlike them, it does not only measures expression levels of the genes but gives a wealth of information regarding its spatial location and pattern of expression. These gene patterns can be correlated with particular cellular phenotypes, which are important in studies of disease progression.

In situ hybridization has played an instrumental role in unearthing neuroanatomical distributions and characterization of a number of physiologically important neuropeptides like NPY, Orexin, CRF and the recently discovered NPS [2-5]. These neuropeptides have been found to be downstream factors in the progression of many psychiatric conditions [6]. Detailed anatomical tracing of these neuropeptides not only endow us with morphology of neurons, but cross talk with other neurotransmitters is also revealed. The detailed neuroanatomical mapping of Neuropeptide S system is an interesting example of it.

Neuropeptide S is a small, 20 amino acid peptide. In 2004, NPS was identified to be the ligand for the previously discovered GPCR 154, since then this receptor is termed as NPSR Interestingly, NPS can said to be the last neuropeptide discovered up to date through receptor deorphanization technique. With the help of *In situ* hybridization based studies, detailed neuroanatomical mapping of Neuropeptide S system in rat brain [5] and later in mouse brain [7], revealed that the NPS expression is confined to only two nuclei in mouse brain i.e. the periocular region ventromedial to the Locus coeruleus and the Kolliker Fuse nucleus and unlike rats is absent in the principal sensory 5 nucleus of the trigeminal tract in the brain stem region. In comparison to rather limited expression of the ligand, radioactive labeled probes revealed the widely distributed expression of the receptor in highly relevant brain region for sleep, anxiety and energy balance regulation. Later, functional studies confirmed the role of NPS/NPSR system in the regulation of these and many other physiological functions [6]. Intrigued by the pronounced expression of NPSR in brain regions associated with reward and motivation, we investigated the link between the endogenous NPS system, morphine intoxication and withdrawal in the rats [8]. *In situ* hybridization analysis revealed statistically significant increase in NPSR levels in the ventral tegmental area, of the morphine dependent rats decapitated 12 hours after the last administration and checking of the physical withdrawal signs VTA is an important brain region forming part of the circuitry implicated in morphine addiction. Moreover, in rats subjected to protracted withdrawal of 7 days, increase in transcript levels was observed in the Basolateral Amygdala Region (BLA). Additionally, at 7 days reduction in NPSR gene expression was found

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in the Bed Nucleus Stria Terminalis (BNST). These areas are part of a common neurocircuitry that plays a critical role in the regulation of stress response and anxiety. Hence, this shows that in the context of NPS being pro arousal in nature, regulation in expression of NPSR mRNA in these areas is an attempt to compensate for the increase in anxiety in response to protracted morphine withdrawal.

Hence, investigations intrigued by the abundant expression of the neuropeptides in functionally relevant brain regions have unearthed important physiological functions of these neuropeptides.

Despite being a 50-year-old technique, *In situ* hybridization is still a technique of choice for brain mapping. Automacity and availability of nonisotopically labelled probes like flourescin and Haptens have further augmented its applications in clinical and diagnostic setups. The present era has seen the advent of many cutting edge technology and techniques like Optogenetics and Neuroimaging; nevertheless, *In situ* hybridization will continue to be an important biological assay in neuroscience due to its relatively simple principle and ability to give huge amount of data in a single experiment.

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