DOWNLOAD PDF 27 NEUROANATOMICAL TRACING OF NEURONAL PROJECTIONS WITH FLUORO-GOLD

Chapter 1 : Neurological function following intra-neural injection of fluorescent neuronal tracers in rats

The study of neuronal connectivity requires the ability to trace axons from the neuronal cell body to its axon terminal (anterograde tracing) and from the terminal back to the soma (retrograde tracing). Such neuroanatomical tracing is frequently used to identify neurons on the basis of their pre- or.

Received Oct 29; Accepted May To view a copy of this license, visit http: However, such clearing works by removing lipids and, as an unintended consequence, also removes lipophilic dyes. To remedy this wash-out, the molecular structure of the dye can be altered to adhere to both membranes and proteins so the dye remains in the tissue after lipidâ€"clearing. Nevertheless, the capacity of such modified dyes to remain in tissue has not yet been tested. Here, we test dyes with molecular modifications that make them aldehyde-fixable to proteins. We use the challenging adult, myelin-rich spinal cord tissue, which requires prolonged lipidâ€"clearing, of rats and mice. Tracing the position of an extracellular electrode after electrophysiological experiments in the nervous system is important to verify both the brain region and the distance between electrode and nearby neurons, which are often identified via either genetically expressed reporters or immunohistochemistry. The tracing is commonly accomplished using a non-toxic fluorescent and lipophilic dye, as the widely used carbocyanine dye DiI, which is painted on the electrode shank prior to insertion. The electrode then leaves remnants of the dye that can be easily be detected by fluorescent microscopy in histological preparations 1 , 2 , 3. An additional widespread application of lipophilic fluorescent dyes is for tracing neuronal connections and projections in the nervous system 4 , 5 especially since these dyes, e. DiI, are well-suited for immunohistochemistry 6 and have minimal photoâ€"bleaching 7. DiI can be transported retrogradely 8 and has been used for post-mortem axonal labelling in animals 9 and humans 10 , Nevertheless, this approach has an unfortunate limitation due to the need to do serial sectioning of the tissue, which is imprecise and labour intensive. The tracing can require several sections if the cutting angle is not parallel to the electrode track or neuronal trace and includes the risk of distorting the samples. These clearing techniques work essentially by washing away the lipids with detergents and solvents. This leaves molecules with amine ends, i. These lipidâ€"clearing processes introduce a caveat when combined with the axonal tracing or electrode marking: The lipophilic dyes are also washed out since they adhere to the lipids 19 , 20 , CLARITY and similar clearing techniques are thus currently incompatible with the employment of lipophilic dyes in tracing and marking. Clearing alternatives such as ClearT, 17 , 22 do not wash out the lipids and therefore leaves the lipophilic dyes secured, but they do not clear as well as CLARITY and therefore have low visualization depth. It would therefore be an advantage to find alternative dyes for tracing, which would be compatible with CLARITY and similar clearing techniques 15 while also having a strong tie to the cellular membranes to limit the diffusion before fixing. This was demonstrated in axon tracks, which were labelled with DiIâ€"crystals applied onto the tissue surface on post-mortem brain tissue. The tissue was fixed prior to the dyeâ€"application and then fixed again for a week In spite of these improvements, fixability of lipophilic dyes, that remain in the tissue after lipidâ€"clearing, continue to be a desirable quality. The simplest approach to obtain an alternative dye is to chemically change an existing dye, e. DiI, into a DiIâ€"analogue, which possesses both the property of adhering to the cellular membranes and protein structures, such that it would remain in the tissue after lipidâ€"clearing. Such dyes have already been developed, in particular the analogues of DiI: The spinal cord has an envelope of dense white matter and is therefore a difficult tissue to clear of lipids, though it has successfully been cleared previously We also imaged and 3Dâ€"reconstructed the spinal cord and dye traces using confocal microscopy.

DOWNLOAD PDF 27 NEUROANATOMICAL TRACING OF NEURONAL PROJECTIONS WITH FLUORO-GOLD

Chapter 2 : CLARITY-compatible lipophilic dyes for electrode marking and neuronal tracing

Fluoro-Gold (Fluorochrome Inc., Denver CO) is one such highly flexible fluorescent retrograde marker commonly used for neuronal labeling and neuroanatomical tracing. Discover the world's research.

The gonads produce reproductive hormones such as progesterone and estrogen as well as gametes which are essential for reproductive cycle and fertility. The development of gonads is solely dependent on the gonadotopins luteinizing hormone and follicle stimulating hormone released from the pituitary gonadotrophs. The gonadotropin-releasing hormone GnRH neurons within the hypothalamus represent the final common pathway through which the brain regulates the release of gonadotropins from the pituitary. Thus the hypothalamus-pituitary-gonadal HPG axis forms the neuroendocrine system which is indispensable for reproduction and fertility Figure 1. Kisspeptin is a peptide encoded by the Kiss-1 gene and the interaction of kisspeptin and its receptor, G-protein coupled receptor 54 GPR54 is essential for puberty onset and fertility. Functional mutations of GPR54 in both humans and mice cause severely impaired reproductive functions, such as failure to undergo puberty and lack of gonadotropin secretion de Roux et al. A group of neurons in the hypothalamus that express the neuropeptide kisspeptin, known as the kisspeptin neurons, potently stimulate the GnRH neurons Han et al. In situ hybridization and immunohistochemistry studies have identified two populations of kisspeptin neurons located in the rostral periventricular RP3V and arcuate nucleus ARN of rodents, sheep and primates Figure 2. Schematic diagram indicating how kisspeptin neurons regulate GnRH neurons, which are the critical component of the hypothalamic-pituitary-gonadal HPG axis for reproduction. Coronal brain sections indicating the immunostaining of RP3V left and ARN right kisspeptin neurons, kisspeptin-immunoreactive cell bodies appeared as black dots. Our research relies heavily upon the use of a variety of transgenic mouse models, including the global- and neuron-specific gene knockout mice as well as transgenic reporter mice. We have previously established the essential role of RP3V kisspeptin neurons in mediating the estrogen positive feedback on the gonadotropin-releasing hormone GnRH neurons which resulted in puberty onset and ovulation Clarkson and Herbison, ; Liu et al. Whereas much of the RP3V kisspeptin neurons characteristics are well-established, the role of arcuate nucleus ARN kisspeptin neurons remains elusive. Hence, our current research focuses on understanding the functional roles of ARN kisspeptin neurons. We aimed to define the neuroanatomical, morphological and physiological characteristics of the ARN kisspeptin neurons. His main research interest is in defining the electrical properties of ARN kisspeptin neurons. Much of her work involves in vivo models and immunohistochemistry. Exploring electrophysiological aspects of kisspeptin neurons Recently, using gene targeting technology, a mouse line has been created that allows kisspeptin neurons to be fluorescent Mayer et al. We have used dual-label immunohistochemistry to check the relationship between fluorescent neurons and those which are currently expressing kisspeptin. This validates the mouse model as being accurate at reporting kisspeptin neurons, and means that there is a high probability that electrical activity from a fluorescent neuron is that of a kisspeptin neuron. Photomicrographs showing endogenous GFP fluorescence green in the ARN, kisspeptin immunostaining red and a merged image where yellow indicates co-expression of GFP and kisspeptin in a kisspeptin-reporter animal. However, neurons exert their influence by firing action potentials, and there is currently no data available on the effects of estrogen on the firing rate of ARN kisspeptin neurons. Therefore, we are examining the firing-rate of the ARN kisspeptin neurons, by using cell-attached electrophysiology, which is minimally invasive to the cell while recording their activity. Using this technique we record the spontaneous firing rate of ARN kisspeptin neurons during different stages of the estrous cycle since this represents accurately the physiological levels of circulating estrogen. Electrophysiological recording of the spontaneous firing of a kisspeptin neuron in the ARN of a female mouse. Recent evidence showed that ARN kisspeptin neurons co-express neurokinin B NKB and dynorphin Figure 5 , two other neuropeptides which are also essential for normal reproductive function Goodman et al. There is evidence to suggest that there are

physical contacts between ARN kisspeptin neurons, giving rise to the suggestion of a neuronal network existing in the ARN which may be important in regulating GnRH release. The roles of dynorphin and NKB in this neuronal network are currently unknown. Therefore, we are measuring the firing rate of ARN kisspeptin neurons in the presence of NKB and dynorphin, and also in the presence of specific antagonists to the receptors of NKB and dynorphin using cell-attached electrophysiology. Adapted from Lehman et al. Exploring neuroanatomical aspects of ARN kisspeptin neurons Neurons connect to each other by extending their axons into other areas of the brain to provide synaptic inputs to their efferent neurons. The distinctive projection patterns of each kisspeptin neuronal population is not known in any species yet and it is difficult to discern their discreet projection patterns using conventional immunohistochemistry. We undertook neuroanatomical tracing to map out the ARN kisspeptin neuronal projections as a means to envisage the possible physiological roles of these neurons. To explore the projections of kisspeptin neurons, we performed stereotactic surgery to inject the anterograde tracer, Phaseolus vulgaris agglutinin PHA-L into the ARN of adult female mice. The anterograde tracer is used to trace axonal projections from neuronal cell body to their point of termination in the nerve terminal. The retrograde tracer is complementary to the anterograde tracer where it is used to trace neural connections from their termination to the cell bodies. We injected the retrograde tracer, fluorogold into the rostral preoptic area of adult female mice to verify the projection of ARN kisspeptin neurons to the vicinity of GnRH neuronal cell bodies as indicated in our anterograde findings. Dual-label immunofluorescence and extensive confocal microscopy was employed to visualize the distribution of PHA-L-labeled ARN kisspeptin-immunoreactive fibers and kisspeptin-immunoreactive cell bodies labeled with fluorogold. Adapted from Yeo and Herbison, Endocrinology, Schematic, flat-map horizontal view of the female mouse brain showing the organization of kisspeptin neuronal projections. ARN kisspeptin neuron projections are represented by red dotted line on the right, whereas RP3V kisspeptin neuron projections are indicated on the left black solid line. Overall, our neuroanatomical tracing revealed that ARN kisspeptin neurons project widely in the forebrain into multiple hypothalamic nuclei and associated limbic structures Figure 7. Projections of ARN kisspeptin neurons to many hypothalamic areas highlight the diverse neuronal circuits that are likely to be regulated by kisspeptin in addition to the GnRH neurons. However, the fundamental evidence for presynaptic inputs awaits further morphological characterization of these neurons. To examine the detailed morphology of ARN kisspeptin neurons, we are currently employing juxtacellular cell-filling of living kisspeptin neurons with small molecular weight dyes in the acute brain slice preparation. Using triple immunofluorescence labeling for kisspeptin, GnRH and cell-filling dye immunoreactivities, we aim to track the fibres projecting out from the filled kisspeptin neuron, which could possibly lead to the identification of synaptic inputs onto GnRH neurons. With relation to the electrical activity of the ARN kisspeptin neurons, these morphological characteristics are essential to clarify the possible role of ARN kisspeptin neurons in regulating GnRH neuronal activities to maintain tonic GnRH secretion. Our investigations so far have shown that ARN kisspeptin neurons exert spontaneous firing activity and possibly provide efferent inputs to GnRH neurons. However, we are still unsure about i how ARN kisspeptin neurons regulate GnRH neuronal activity ii how estrogen affects the firing activity of ARN kisspeptin neurons iii what the roles of the co- transmitters dynorphin and neurokinin B in these neurons are and iv what the physiological importance of these neurons are. Hence, our quest for elucidating the role of ARN kisspeptin neurons is still ongoing. Clarkson J and Herbison AE Dual phenotype kisspeptin-dopamine neurones of the rostral periventricular area of the third ventricle project to gonadotrophin- releasing hormone neurones. Kisspeptin-GPR54 signaling is essential for preovulatory gonadotropin-releasing hormone neuron activation and the luteinizing hormone surge. Clarkson J, Herbison AE Postnatal development of kisspeptin neurons in mouse hypothalamus; sexual dimorphism and projections to gonadotropin-releasing hormone neurons. Evidence that dynorphin plays a major role in mediating progesterone negative feedback on gonadotropin-releasing hormone neurons in sheep. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. Anatomy of the kisspeptin neural network in mammals. Timing and completion of puberty in female mice

depend on estrogen receptor alpha-signaling in kisspeptin neurons. The GPR54 gene as a regulator of puberty. N Engl J Med Regulation of Kiss1 gene expression in the brain of the female mouse. Projections of arcuate nucleus and rostral periventricular kisspeptin neurons in the adult female mouse brain.

DOWNLOAD PDF 27 NEUROANATOMICAL TRACING OF NEURONAL PROJECTIONS WITH FLUORO-GOLD

Chapter 3 : Hydroxystilbamidine (ultra pure) - enz - Enzo Life Sciences

27 Neuroanatomical Tracing of Neuronal Projections with Fluoro-Gold Lisa A. Catapano, Sanjay S. Magavi, and Jeffrey D. Macklis Summary The study of neuronal connectivity requires the ability to.

Wen Hu and Dan Liu contributed equally to this work. Wen Hu contributed to the study design, tracing surgery, data analysis and manuscript writing. Xiaosong Gu and Jianhui Gu participated in the study design and manuscript writing. Dan Liu was responsible for all animal studies and acquisition of data. All authors approved the final version of the manuscript. Received Feb 20; Accepted Apr This article has been cited by other articles in PMC. Abstract Fluorescent neuronal tracers should not be toxic to the nervous system when used in long-term labeling. Previous studies have addressed tracer toxicity, but whether tracers injected into an intact nerve result in functional impairment remains to be elucidated. A set of evaluation methods including walking track analysis, plantar test and laser Doppler perfusion imaging was used to determine the action of the fluorescent neuronal tracers. Additionally, nerve pathology and ratio of muscle wet weight were also observed. Results showed that injection of Fluoro-Gold significantly resulted in loss of motor nerve function, lower plantar sensibility, increasing blood flow volume and higher neurogenic vasodilatation. Myelinated nerve fiber degeneration, unclear boundaries in nerve fibers and high retrograde labeling efficacy were observed in the Fluoro-Gold group. The True Blue group also showed obvious neurogenic vasodilatation, but less severe loss of motor function and degeneration, and fewer labeled motor neurons were found compared with the Fluoro-Gold group. No anomalies of motor and sensory nerve function and no myelinated nerve fiber degeneration were observed in the Fluoro-Ruby group. Experimental findings indicate that Fluoro-Gold tracing could lead to significant functional impairment of motor, sensory and autonomic nerves, while functional impairment was less severe following True Blue tracing. Fluoro-Ruby injection appears to have no effect on neurological function. True Blue is potentially suitable for long-term neuronal labeling, while Fluoro-Ruby and Fluoro-Gold only allow short-term labeling. The tracer used needs to be non-toxic or of low neural toxicity for long-term labeling. Although the toxicity of neural tracers has been reported to be pathological, it remains unclear whether and how neural function is affected when an intact nerve is exposed to these tracers. Fluoro-Ruby injection did not affect neurological function. INTRODUCTION Neuronal tracing is a common technique used not only in neuroanatomical studies to identify neurocircuitry or axonal projections[1 , 2 , 3 , 4 , 5], but also in neural regeneration research for the evaluation of re-innervation or reconstruction of fiber tracts after spinal cord injury[6 , 7 , 8 , 9 , 10]. Among a variety of neuronal tracers used in tract tracing, fluorescent tracers are particularly useful because they allow direct visualization of labeled neurons[6 , 11 , 12]. Unfortunately, few commercially available neuronal tracers meet all these requirements. For example, Fluoro-Gold is recognized as a potent retrograde tracer with high labeling efficacy and good resistance during tissue preparation, but it has been found to potentially result in neural cell death[13 , 14]. For most neuroanatomical studies, animals are allowed to survive for a relatively short period, i. However, for studies in which animals should be allowed to survive for a longer period of time, e. A typical example is the sequential retrograde tracing for assessing regeneration accuracy of peripheral nerves. In this case, the first tracer should be capable of allowing long-term labeling, and should neither lead to functional loss nor impair neural regeneration capacity[14]. Previous studies have shown that True Blue, Fast Blue, Diamidino Yellow and DiI are all potentially suitable for long-term neuronal labeling while Fluoro-Ruby and Fluoro-Gold are suitably used as the second rather than the first tracer because of rapid fading in vivo[11 , 18 , 19]. However, it remains unclear whether and how neural function is affected when an intact nerve is exposed to these tracers. This is a particularly important issue regarding the selection of the first tracer in sequential retrograde tracing for the assessment of regeneration accuracy. In the present study, we examined the neuronal toxicity of True Blue, Fluoro-Ruby and Fluoro-Gold by evaluating the impairment of motor, sensory and autonomic nerve functions, and observed nerve fiber degeneration and labeling efficacy, using a

rat model of tibial nerve injection. All 60 rats were involved in the final analysis with no loss. Neurogenic vasodilatation following tracer injection Laser Doppler perfusion imaging showed that immediately after injection of Fluoro-Gold solution or True Blue suspension into the tibial nerve, a dramatic increase in skin perfusion was observed in the ipsilateral hind paw, similar to a pattern exhibited after tibial nerve transection. No obvious dermal perfusion increase was found after injection with Fluoro-Ruby solution or saline Figure 1.

Chapter 4 : Understanding the roles of arcuate kisspeptin neurons | The Physiological Society of New Zeal

Neuroanatomical tracing has become particularly important in nervous system regeneration and repair, allowing investigators to follow the axon projections of newly born, transplanted, or axotomized neurons in lesioned or neurodegenerative environments.

Chapter 5 : Jeffrey Macklis | Harvard Catalyst Profiles | Harvard Catalyst

Neuroanatomical Tracing with Fluoro-Gold 27 Neuroanatomical Tracing of Neuronal Projections with Fluoro-Gold Lisa A. Catapano, Sanjay S. P. Magavi, and Jeffrey D.

Chapter 6 : Table of contents for Library of Congress control number

Retrograde tracing with Fluoro-Gold: different methods of tracer detection at the ultrastructural level and neurodegenerative changes of back-filled neurons in long-term studies.

Chapter 7 : Neuronal Tract-Tracers Publications | PubFacts

The anterograde neuronal tracing properties of Fluoro-Gold (FG) were characterized in this study by its ability to label the retinohypothalamic tract (RHT) upon pressure injection of the substance into the vitrous body of the eye in the Djungarian hamster, Phodopus sungorus.