Extracellular unit recording study in characterizing activity of optogenetically identified parvalbumin expressing neurons in dentate gyrus of freely moving mice

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2017

Lit, K. K. (2017). Extracellular unit recording study in characterizing activity of optogenetically identified parvalbumin expressing neurons in dentate gyrus of freely moving mice. Master's thesis, Nanyang Technological University, Singapore.

<http://hdl.handle.net/10356/72720>

<https://doi.org/10.32657/10356/72720>

NANYANG
TECHNOLOGICAL
UNIVERSITY

**EXTRACELLULAR UNIT RECORDING
STUDY IN CHARACTERIZING ACTIVITY
OF OPTOGENETICALLY IDENTIFIED
PARVALBUMIN EXPRESSING NEURONS
IN DENTATE GYRUS OF FREELY
MOVING MICE**

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SCHOOL OF BIOLOGICAL SCIENCES

A thesis submitted to the Nanyang Technological University in partial fulfillment of
the requirement for the degree of
Master of Science

ACKNOWLEDEMENT

I would like to sincerely dedicate my gratitude to my friends and colleagues, without whom none of this work would have ever been accomplished. I would like to thank Asst Prof Ayumu Tashiro, for his patience, guidance and teaching, and most importantly giving me the opportunity to pursue my Masters research in the lab. It had been an eye opening experience in the field of neuroscience research. I have learnt great lot of various specialized skills, techniques and knowledge needed in the research.

Big thanks to everyone in the Tashiro lab, for the enjoyable and unforgettable times during the past 2 years. A very special gratitude to Luis F. Cobar, for the assistance in the course of the project

Last but not least, I am truly grateful to my family for their support, both emotionally and financially. I would have not been able to come this far if not for your support.

TABLE OF CONTENTS

ACKNOWLEDGEMENT.....	I
TABLE OF CONTENTS.....	II
LIST OF FIGURES.....	IV
ABBREVIATIONS.....	VIII
ABSTRACT.....	IX
 INTRODUCTION.....	 1
Organization of the hippocampus.....	1
Cellular Organization of DG.....	4
Role of each neuronal subtype in DG function.....	8
PV expression as a subtype of GABAergic neurons in DG.....	9
Properties of PV expressing neurons.....	10
Role of PV expressing neurons in DG.....	11
Aim of the thesis.....	11
 APPROACH.....	 12
Extracellular unit recording of neuronal activity.....	12
Optogenetics approach to identify neurons.....	14
Cell type specific expression of ChR2.....	17
 METHOD.....	 19
Animals.....	19
Tetrodes implantation*.....	19
In vivo electrophysiology	20

Euthanasia and Perfusion.....	22
Drive removal and Brain extraction.....	22
Histology	22
Estimation of recording sites.....	23
Data Analysis	23
RESULTS.....	27
Histological confirmation of tetrode final resting position.....	27
Identification of light-responsive unit activity.....	29
Characterizing activities of light responsive units during exploration...35	
Identification of recording sites of Group A and B light responsive units	55
Analysis of units not responding to photostimulation.....	57
Identification of recording sites of units not responding to laser.....	74
DISCUSSION.....	76
Distinguishing direct response from ChR2 positive neurons.....	76
Characterizing activities of ChR2 positive neurons.....	80
Determining the possible identity of ChR2 positive neurons.....	81
Characterizing activities of ChR2 negative neurons.....	83
FUTURE WORK.....	84
CONCLUSION.....	86
REFERENCE.....	87

LIST OF FIGURES

Figure 1: Schematic diagram of the strata of the Ca regions and DG.....	2
Figure 2: Nissl stained coronal section of hippocampus of mouse, macaque monkey and human.....	2
Figure 3: Schematic diagram of the organization of the hippocampus and the trisynaptic network.....	3
Table 1: Major morphologically different neurons in the dentate gyrus.....	6
Figure 4: Morphology of different neuronal types in DG.....	7
Figure 5: PV labeled neurons in the dentate gyrus of mouse	10
Figure 6: Spike detection by amplitude threshold.....	13
Figure 7: Schematic overview of unit recording.....	14
Figure 8: Schematic example of optogenetics assisted neuronal identification.....	16
Figure 9: Cell type specific expression of ChR2-YFP using transgenic mouse line.....	18
Figure 10: Recording sites of all units and the light responsive units.....	28
Figure 11: Identification of light response.....	30
Figure 12: Identification of activity of light responsive units during exploration.....	31
Figure 13: Recording sites of all units in the ChR2-YFP negative mouse.....	32
Figure 14: Responses of all units from the ChR2-YFP negative mouse.....	33
Figure 15: Firing location map and rate map of light responsive units.....	36

Figure 16: Dendrogram of cluster analysis of light responsive units using time bin of first peak.....	37
Figure 17: Activities of Group A light responsive units.....	39
Figure 18: Activities of Group B light responsive units.....	40
Table 2 Average firing properties indexes of Group A and Group B units.....	41
Figure 19: Burst like firing responses of Group B light responsive units	42
Figure 20: Dendrogram of cluster analysis of light responsive units using sparseness....	45
Table 3: Average firing properties indexes of Group 1 and Group 2 light responsive units.....	46
Figure 21: Dendrogram of cluster analysis of light responsive units using information....	47
Table 4 Average firing properties indexes of Group α and Group β light responsive units.....	48
Figure 22: Dendrogram of cluster analysis of light responsive units using selectivity....	49
Table 5 Average firing properties indexes of Group i and Group ii light responsive units.....	50
Table 6 Average firing properties indexes of Group i (A) and Group i (B) light responsive units.....	50
Figure 23: Dendrogram of cluster analysis of light responsive units using response latency.....	53

Table 7 Average firing properties indexes of Group AA and Group AB light responsive units.....	54
Table 8 Average firing properties indexes of Group AA1 and Group AA2 light responsive units.....	54
Figure 24: Recording sites of the 2 groups of units.....	56
Figure 25: Recording location of all units without light responses.	57
Table 9 Average firing properties indexes of light responsive and non-light responsive units.....	58
Figure 26: Dendrogram of cluster analysis of non-light responsive units using sparseness.....	60
Table 10. Average firing properties indexes of Group 1, 2 and 3 non-light responsive units by sparseness classification.....	61
Figure 27: Dendrogram of cluster analysis of non-light responsive units using information.....	64
Table 11. Average firing properties indexes of Group 1 and 2 non-light responsive units by information classification	65
Table 12. Average firing properties indexes of Group 1, and the 3 subgroups of Group 2 non-light responsive units by information classification.....	65

Table 13 The significance of comparison between Group 1, and the 3 subgroups of Group 2 non-light responsive units by information classification.....	66
Figure 28: Dendrogram of cluster analysis of non-light responsive units using selectivity.....	68
Table 14. Average firing properties indexes of Group 1 and 2 non-light responsive units by selectivity classification	69
Table 15. Average firing properties indexes of Group 1, and the 3 subgroups of Group 2 non-light responsive units by selectivity classification.....	69
Table 16. The significance of comparison between Group 1, and the 3 subgroups of Group 2 non-light responsive units by selectivity classification.....	70
Figure 29: Firing location map and rate map of units with place cell activities	72
Table 17. Average firing properties indexes of light responsive units and units with place fields.....	73
Figure 30: Distribution of distances between optic fibre and tetrode of non-light responsive units and light responsive units	75

ABBREVIATION

Ca	Cornu Ammonis
ChR2	Channelrhopsin-2
ChR2-YFP	Channelrhopsin-2-Yellow Fluorescent Protein Fusion Protein
DG	Dentate Gyrus
EC	Entorhinal Cortex
GABA	Gamma (γ)-aminobutyric acid
GCL	Granule Cell Layer
LED	Light Emitting Diode
LFP	Local Field Potential
ML	Molecular Layer
PFA	Paraformaldehyde
PSTH	Peristimulus Time Histogram
PV	Parvalbumin
YFP	Yellow Fluorescent Protein

ABSTRACT

Information processing in the DG relies on the interactions between activity of excitatory and inhibitory neurons. Inhibitory neurons are not a homogeneous population. It is interesting to know how each subtype of inhibitory neurons control the activity of excitatory neurons. Traditional extracellular unit recording lacks the ability to identify neuronal types, despite its advantage in simultaneously detecting activity of multiple neurons. Here we used a transgenic mouse line that express Channelrhopsin-2 (ChR2), a light-activated ion channel, in a subtype of inhibitory neurons that also express parvalbumin (PV). By identifying their firing response to optical stimulation and firing during exploration, we were able to characterize the firing pattern of these neurons during spatial exploration of the animal. The result contributes to understanding how processing of spatial information is achieved in the dentate gyrus.

INTRODUCTION

The hippocampus is long associated with a variety of cognitive functions. Normal hippocampal function relies on information processing through a series of interconnected neurons. **(Hebb, 1949)** The trisynaptic circuit of the hippocampus, described by Nobel laureate Santiago Ramón y Cajal back in 1911 **(Ramón y Cajal and Azoulay, 1911)**, had provided a substratum for research to uncover how hippocampal function is attained.

Organization of the hippocampus

The hippocampus is a multi-laminar structure consisting of the Cornu Ammonis (Ca) subregion 1, 2, 3 and 4, and the dentate gyrus (DG). These regions are formed by a series of strata. (Fig. 1) Although the location and orientation of the hippocampus is different from rodents to primates, the multi-laminar organization is preserved. (Fig. 2) **(Hawrylycz et al., 2012)**

Fig. 1 Schematic diagram of the strata of the CA regions and DG

Abbreviation: str. oriens, stratum oriens; str. rad., stratum radiatum; str. l-m, stratum lacunosum-moleculare; str. luc, stratum lucidum; sulc, sulcus; ml, molecular layer; gcl, granule cell layer; pl, polymorphic layer; pcl, pyramidal cell layer

CA4, hilus and polymorphic layer are synonyms often used interchangeably. Sulcus is otherwise known as hippocampal fissure

Fig. 2 Nissl stained coronal section of hippocampus of mouse, macaque monkey and human.

Multi-laminar organization is preserved among the species. Scale bars = 1mm (Reprinted with permission from Hawrylycz et al., 2012)

The trisynaptic circuit had outlined the synaptic connections of the hippocampus. One interesting note is that the DG receives direct input, via the perforant pathway, from the entorhinal cortex (EC) layer II. (Fig. 3) With EC being the relay centre for sensory information, this input suggests that DG serve to pre-process this information. Pattern separation was proposed as one of the function of the DG (**Marr, 1971; O'Reilly and McClelland, 1994; Treves and Rolls, 1994**), where subtle differences in inputs are discriminated before the information is passed down the circuit.

Fig. 3 Schematic diagram of the organization of the hippocampus and the trisynaptic network.

Cellular organization of DG

A vast population of different neuronal subtypes co-exists in the DG. Each segregation criteria can include, but not limited to, neurotransmitter released, morphology, and molecular markers. **(Freund and Buzsáki, 1998; Klausberger and Somogyi, 2008)**

One of the most straightforward classifications is based on the neurotransmitter released. Glutamate and gamma (γ)-aminobutyric acid (GABA) are the two main neurotransmitters released by neurons in the hippocampus. Although the action induced in the postsynaptic neurons is dependent on the receptor being activated, the principal role of glutamate and GABA is to induce and inhibit firing in the respective postsynaptic neurons. Glutamatergic neurons can be otherwise known as excitatory neurons while GABAergic neurons are inhibitory neurons. It had been suggested that information processing in the brain relies on the homeostasis between activity of excitatory and inhibitory neurons. **(Freund and Buzsáki, 1998; Isaacson and Scanziani, 2011; Klausberger and Somogyi, 2008; Markram et al., 2004)**

Another classification can be based on the morphology of the neurons. Several morphologically distinct cell types can be identified in the DG. With the cell bodies densely packed in the granule cell layer, granule cells make up the majority of the cell population in the DG. Granule cells have a distinctive pseudounipolar neurite appearance, with cone-shaped dendritic tree, where the spiny apical dendrites extend into the molecular layer. (Fig. 4) Mossy cells are seen in the polymorphic layer, with often multipolar neurite branches. Each neurite branches further bifurcate into multiple branches, spanning throughout the molecular layer. Thorny excrescences (complex spines)

can be seen in all the proximal dendrites. (Fig. 4) Both granule cells and mossy cells are excitatory neurons. (Table 1) Pyramidal basket cells can be recognized by the cell bodies in the base of the granule cell layer which have a single dendritic tree that extends throughout the molecular layer, and multiple short dendrites that extend into the polymorphic layer. (Fig. 4) They receive input from granule cells and other interneurons and project to other granule cells and basket cells. Axo-axonic cells, or chandelier cells, have an extensive network of axon arbors and can be found in the molecular layer and within the granule cell layer. A few morphologically indistinct cell types are defined by their residing location. Molecular layer perforant path-associated cells are present in the molecular layer, receiving input from the perforant pathway. They synapse with the granule cells and other interneurons, providing a feed forward inhibition to these cells. Hilar perforant pathway associated cells are identified in the hilus, receiving inputs from granule cells and mossy cells and ascend to the outer two-third of the molecular layer to synapse with other granule cells and basket cells to provide a feedback inhibition. Hilar commissural-associational pathway related cells are also cells that are identified in the hilus, receiving inputs from the granule cells and mossy cells. They extend through the granule cells layer to the inner third of molecular layer to make synaptic connection with the other granule cells and basket cells to provide a feedback inhibition. These 5 cell types make up the majority of inhibitory neurons in the DG. (Table 1)

Excitatory (Glutamatergic)	Inhibitory (GABAergic)
Granule Cells	Pyramidal basket cells
Mossy Cells	Axo-axonic cells / Chandelier cells
	Molecular layer perforant pathway-associated cells
	Hilar perforant pathway associated cells
	Hilar commissural-associational pathway related cells

Table 1. Major morphologically different neurons in the dentate gyrus

A sub class of morphological classification can be done to define the GABAergic neurons in DG, divided into those making synaptic connections around the soma (perisomatic) or dendritic region of granule cells. Pyramidal basket cells and axo-axonic cells fall under this category of perisomatic neurons. A minor difference exists between the two, in a way that pyramidal basket cells synapse at the cell body of the granule cells while axo-axonic cells synapse at the initial segments of granule cells axon. The other molecular layer and hilus cells synapse with dendrites of granule cells.

Fig. 4 Morphology of different neuronal types in DG

A) Montage image of cell stained with Lucifer yellow B) schematic drawing of

(*Top*) Granule cell

(*Middle*) Pyramidal basket cell

(*Bottom*) Mossy cell

(Reprinted with permission from: Amaral, Scharfman and Lavenex, 2007)

Neurons can be further classified by the molecular markers expressed. One of the most common molecular markers used to distinguish neurons are the calcium binding proteins. Three calcium binding proteins are often identified to be expressed in neurons within the DG. Calbindin expression was found to be localized in the granule cells (Szabadics et al., 2010) while parvalbumin (PV) expression was confined in the basket cells and axo-axonic cells. (Ribak, Nitsch, and Seress, 1990) Calretinin expression in mossy cells is species specific, observed only in mouse. (Seress, 2007) Cholecystokinin (Freund and Buzsáki, 1996; Houser, 2007; Sik, Penttonen and Buzsáki, 1997), doublecortin (Rao and Shetty, 2004), neurogranin (Singec et al., 2004), neuropeptide Y (Freund and Buzsáki, 1996; Houser, 2007; Sik, Penttonen and Buzsáki, 1997), somatostatin (Amaral, Scharfman and Lavenex, 2007; Freund and Buzsáki, 1996; Houser, 2007; Jinno and Kosaka, 2004; Sik, Penttonen and Buzsáki, 1997) and vasoactive intestinal peptide (Freund and Buzsáki, 1996; Hájos, Acsády and Freund, 1996; Sik, Penttonen and Buzsáki, 1997) are examples of other markers used in distinguishing the neuronal subtypes.

Role of each neuronal subtype in DG function

In order to understand the function of DG, the types of cells in the circuit being activated must be identified and the activities of these cells be characterized. In particular, the vast diversity of inhibitory neurons available to control firing of the excitatory neurons raises an interesting question. Which types of GABAergic neurons are responsible for regulating activities within the DG during different behavior? Coding of spatial information during exploratory behavior is DG dependent (McHugh et al., 2007; Xavier, Oliveira-Filho and Santos, 1999), but it is unclear which types of GABAergic neurons

are activated and what is the activity of these neurons that enables them to regulate the activity in DG serving such function.

PV expressing neurons as a subtype of GABAergic neurons in DG

PV expression was identified in GABAergic neurons in the DG. (**Kosaka et al., 1987; Milenkovic et al., 2013**) They were identified to reside mostly in the base of the granule cell layer, adjacent to the polymorphic layer, or in the molecular layer. (Fig. 5) Morphologically, these neurons resembled pyramidal baskets cells and axo-axonic cells (**Ribak, Nitsch, and Seress, 1990**). PV was reportedly expressed in about 20-25% of these neurons. (**Jinno and Kosaka, 2002**)

PV expressing neurons in the DG are innervated by granule cells (**Blasco-Ibanez, Martinez-Guijarro, and Freund, 2000**) and forms synaptic connection back to the granule cells, providing feedback inhibition and control the duration of neuronal discharge in granule cells. In addition, dendrites of these PV expressing neurons extend through the molecular layer to receive input from EC directly (**Zipp et al., 1989**), thus providing feedforward inhibition to the granule cells to control the timing of neuronal discharge in the granule cells.

Fig. 5 PV labeled neurons in the dentate gyrus of mouse

(Reprinted with permission from: Amaral, Scharfman and Lavenex, 2007)

Abbreviations: M, molecular layer; G, granule cell layer

Properties of PV expressing neurons

PV expressing neurons are well documented to be fast spiking neurons (**Kawaguchi et al., 1987**), characterized by short action potential duration and high frequency firing. Action potentials were reported to propagate at a speed of about 1.5m/s (**Meeks and Mennerick, 2007**) due to an excessively high density of sodium channels in the axon. (**Hu and Jonas, 2014**) Studies had claimed output synapse of PV expressing neurons depends on P/Q-type calcium channels for neurotransmitter release. (**Hefft and Jonas, 2005; Zaitsev et al., 2007**) This type of calcium channels were claimed to show fastest gating as compared to other types (**Li, Bischofberger and Jonas, 2007**), mediating high rate of neurotransmitter exocytosis. These properties allow for fast acting release of GABA neurotransmitters, and precisely timed control over the post synaptic neuron.

Role of PV expressing neurons in DG

It was briefly described recently that blocking activity of PV expressing neurons in the DG impairs spatial recognition (**Murray et al., 2011**), suggesting that regulation of DG activity by PV expressing neurons is essential in achieving coding of spatial information. However, the activity of these neurons during the process is still unknown. It had been difficult to characterize the activity of these neurons, or any other types of neurons per se, due to the fact that in spite of the benefits of extracellular unit recording, the drawback is that it was not yet possible to identify the location of the neurons responsible for the firings, let alone to differentiate the cell types. While intracellular recording can be used to overcome this obstacle, the yield is extremely low. Intracellular recording involves inserting electrode into the cell membrane of the neuron, to measure the current passing through the membrane. The neuron can be labelled by filling the cell with dye, allowing morphological identification to be done during histological analysis.

Aim of thesis

The aim of this thesis is to characterize the activities of PV expressing neurons in the DG during open field exploratory behavior, with the use of an optogenetics assisted neuronal identification approach together with extracellular unit recording. It will contribute as a part of a collaborative effort to characterize the activity of each neuronal type within the dentate gyrus in achieving spatial coding.

APPROACH

Extracellular unit recording of neuronal activity

Extracellular unit recording is a technique that allows simultaneous detection of neuronal discharge from multiple neurons *in-vivo* during behavior. This is achieved by chronically implanting microelectrodes into the brain of an animal, which acquire and measure the summed extracellular electric potential of the vicinity around the tip of the microelectrode, contributed by discharge of all the neurons in the small volume of tissue and background electrical fluctuations. As a neuron fires, a change in extracellular potential will be detected by the microelectrode. The nearer the neuron to the microelectrode, the greater the magnitude of change in extracellular potential will be detected. By measuring this voltage deflection and band pass filtering it, spike of a neuron can be isolated. Start of measurement of the spike depends on method of sampling, but most of the time by defining an amplitude threshold. Whenever the extracellular current exceeds the threshold, measurement is triggered and spikes are sampled. (Fig. 6) Each neuron generates spikes with a characteristic magnitude of depolarization, repolarization and hyperpolarization, resulting in waveform of characteristic shape. Since both the soma and dendrites actively support generation and propagation of spike (**Calvin and Hartline, 1977; Kamondi, Acsády, and Buzsáki, 1998; Regehr et al., 1993; Stuart et al., 1997**), wave shape of the spike is also dependent on location of the microelectrode along the somadendritic axis. (**Gold et al., 2006; Harris et al., 2000**)

Fig. 6 Spike detection by amplitude threshold.

Whenever the detected electrical activity exceeds an amplitude threshold, spike is sampled.

Firing of individual neurons can be identified by sorting the spikes according to their waveform. One of the ways to achieve this is by feature extraction. Feature extraction is a process of quantifying the different features of waveform from each spike, allowing the values of one feature to be plotted against another feature. Since the wave shape of one neuron is unique and similar, the feature values of the same neuron would be similar, and the feature values of a different neuron would be different. This would give rise to clustering of data points on the plot, with each data point representing one spike, thus allowing spikes with similar waveform to form one cluster and spikes with different waveform to form another. Spike sorting can then be done to bin the spikes into individual clusters, with each cluster of spikes representing unit activity of one neuron. This process can be further optimized through the use of multichannel recording with the use of microelectrodes bundled in pair (stereostrodes) or quadruples (tetrodes). The difference in distance between the neuron and microelectrodes in the bundle differently affects wave

shape of spikes from different neuron, reducing ambiguity in separating activity of nearby neurons. (Fig. 7)

Fig. 7 Schematic overview of unit recording. Each channel (microelectrode) in the tetrode detects the extracellular potential. Spikes can be detected whenever the change in extracellular potential, after band pass filtering, exceeds an amplitude threshold. Different neurons generate spikes with different waveform. Spikes with similar features form clusters, when features of different channels are plotted against each other, allowing sorting and identification to be done.

Signal close to tetrode tip (Green sphere) is strong enough to generate distinguishable spikes. Activity of neurons further away (grey sphere) can still be detected but indistinguishable, often masked by the background noise activity. Background noise activity refers to all kinds of random background electrical signal fluctuations. Spikes from neurons even further apart (outside grey sphere) are hardly detectable, but remain as an attribute to the background noises.

Optogenetics approach to identify neurons

Despite the benefits of unit recording to simultaneously monitor activity of multiple neurons; it lacks the ability to provide information about which neuron is responsible for each unit activity. One of the recent approaches being pursued into identifying neuronal subpopulation in awake freely moving animals involves the use of optogenetics. Optogenetics is a biological approach that makes use of genetic

modification of the cells to express light sensitive ion channels, which when subjected to optical stimulation allows for control of cellular activity. Channelrhodopsin-2 (ChR2) is a 7 transmembrane helix light sensitive cation channel (Nagel et al., 2003; Nagel et al., 2005b), that when incorporated into the neurons and photoevoked optimally at 470nm, leads to influx of cations thus resulting in neural activation. (Boyden et al., 2005; Li et al., 2005; Nagel et al., 2005a) ChR2 was initially cloned from algae (Nagel et al., 2005a), and being adapted to manipulate mammalian neuronal activity with precise temporal control. (Boyden et al., 2005) This approach was first designed to study the role of targeted neuronal populations in behavior (Atasoy et al., 2008; Schroll et al., 2006), and map neural circuits (Arenkiel et al., 2007; Petreanu et al., 2007; Zhang et al., 2006) It was later adopted as a method to label neuronal population *in-vivo*, that when combined with large scale electrophysiological recording, can allow study of the physiological activity of individual labelled neurons within the population. This innovation was first detailed by Lima et al. in 2009, in which they named it Photostimulation assisted identification of neuronal populations (Lima et al., 2009)

ChR2 expressing neurons can be identified during recording by their firing in response to light stimulation. When these ChR2 positive neurons are activated with short pulse of laser, the influx of cations will lead to an action potential, which in turn generate a change in extracellular potential, detectable with the use of unit recording. This voltage deflection is tightly coupled to the onset of illumination, triggered within short latency of the illumination onset. In ChR2 negative neurons, illumination will not trigger any response.

Firing of ChR2 positive neuron when the animal was actively exploring can be deduced from the location of the sorted cluster on the plots of 2 same comparing features, in exploration recording and light stimulation recording. This was based on the principle that wave shapes of the spikes by the same neuron were similar, giving similar feature values and thus similar location of the cluster.

Fig. 8 Schematic example of optogenetics assisted neuronal identification. 3 clusters of firing were identified when animal was actively exploring. One of the three (Red cluster) can also be identified during stimulation session when the mouse had little space for exploration. When stimulated with light, cation influx caused an increase in firing rate. . Through cell type specific expression of ChR2, the increase firing rate in short latency of stimulation allows us to know the specific type of neurons expressing ChR2 is responsible for the cluster of firing during behavior and study its activity.

Lima et al. first evaluated this approach to identify and study activity of PV expressing neurons in the auditory cortex (**Lima et al., 2009**), confirming the viability of this approach in electrophysiologically identifying PV expressing neurons. This approach was later utilized by many other studies to investigate activity of inhibitory neurons in other areas of the brain. (**Cardin, 2010; Cardin et al., 2012; Han et al., 2009; Kravitz, Owen, and Kreitzer, 2013; Moore and Wehr, 2013**). They had all reported successful implementation of the approach to achieve results.

Cell type specific expression of ChR2

In this study, ChR2 was controlled to be expressed in neurons expressing PV. This was achieved by cross breeding two transgenic mouse lines, PV-Cre and Rosa-ChR2-YFP. The Rosa-ChR2-YFP mice carried the transgene to express ChR2-YFP, driven by a synthetic “promoter” element known as CAG promoter, in the Rosa26 locus of the mice chromosome. Genes in this locus allow ubiquitous expression under normal circumstances, but this transgene contains a STOP codon before the ChR2-YFP gene preventing expression of ChR2-YFP. This STOP sequence was flanked by 2 direct repeats of short DNA sequences, known as LoxP sequences. LoxP sequence can be cleaved by Cre recombinase enzyme, while direct repeats of LoxP sequences allowed excision of sequences between the two cleavage sites. On the other hand, PV-Cre mice carried the Cre transgene driven by PV promoter, allowing constitutive expression of Cre in PV positive cells. Cross breeding of the two mouse lines resulted in offspring which inherited both transgenes, where in PV positive cells, Cre was expressed to excise the STOP sequence in the ChR2-YFP transgene, allowing expression to proceed in these cells. In cells not expressing Cre, STOP sequence was not removed and ChR2-YFP was not expressed. (Fig. 9)

Fig. 9 Cell type specific expression of ChR2-YFP using transgenic mouse line. (Left) In cells with both transgenes, Cre recombinase expressed under transcriptional control of PV promoter, cleaved the 2 LoxP sequences in the gene expressing ChR2-YFP, allowing the transcription to proceed in cells expressing PV. (Right) In cells which did not carry the Cre gene, expression did not occur.

METHODS

Animals

Our own breeding stock of inbred transgenic mouse line was used. This transgenic line was initially achieved by crossing PV-Cre (B6;129P2-*Pvalb*^{tm1(cre)Arbr}/J; JAX#008069) and Rosa-ChR2-YFP (B6;129S-*Gt(ROSA)26Sor*^{tm32(CAG-COP4*H134R/EYFP)Hze}/J; JAX#0125569), which were both originally purchased from The Jackson Laboratory. This allows ChR2-YFP to be expressed in PV positive cells. (Refer to Approach)

Animals were kept in animal holding facility of Biological Resource Centre in Biopolis. Handling of the animals was performed according to the guidelines provided by the Institutional Animal Care and Use Committee. Mice were individually housed in transparent plastic cages (32 L X 17 W X 18 H cm) in a light controlled room with an inversed 12:12 hour light/dark cycle (light on at 2130 hours) with *ad-libitum* access to water and rodent chow (Altromin 1324 mod. 18% Prot. 6% Fat), unless otherwise described below.

Tetrodes implantation*

Under isoflurane anesthesia, a miniature microdrive device (Neuralynx VersaDrive 8) housing 8 tetrodes and a 245 µm diameter mono fibre-optic cannulas (Doric lenses MFC_200/245-0.37_15mm_ZF1.25_FLT) were stereotactically implanted above right hippocampus (1.9 mm posterior, 1.4 mm right lateral to bregma). The optic fibre and all 8 tetrodes can be independently and vertically moved. Tetrodes were prepared by coiling and heat bonding 4x 0.0007 inch diameter platinum 10% iridium wire

(California Fine Wire Company), and electroplated with chloroplatinic acid to match an impedance of 200-300 k Ω . Each microdrive was secured to the skull with 4x screws and dental cement. Two additional screws were inserted through the skull, with the tip overlying the cortex, and soldered to the grounding wires of each microdrive. Postsurgical prophylaxis (50 mg/mL Enrofloxacin and 5 mg/mL Meloxicam) were added to the drinking water for at least 3 days after the surgery.

(Performed by Ph.D. student colleague Mr. Luis F. Cobar)

In vivo electrophysiology

Prior to recording, at least 3 days of post-operative monitoring with unlimited access to water and rodent chow was carried out. Closely monitored food restriction was then started to reduce animal's body weight to 80-90% free-feeding weight.

Neural activity was recorded by connecting two headstage amplifiers (HS-18-CNR-MDR50, Neuralynx) to the microdrive, amplifying the signals and sending via tether cable to be recorded by the Neuralynx Digital Lynx SX system. The mono fibre-optic patch cord (Doric lenses MFP_50/125/900-0.22_3m_SMA-ZF1.25(F)) to deliver the laser was also connected to the optic fibre. A simple self-assembled pulley system was installed to counterweigh and compensate the added weight of the preamplifiers and cords to allow the animal to move freely. Wide-band signal, acquired at 32 kHz, was band pass filtered between 0.1 to 9000 Hz for local field potential (LFP) and 600-6000Hz for spike activity. Spikes were detected by defining amplitude threshold. (Refer to Approach)

Over the course of 2 to 3 weeks, tetrodes were lowered to the DG (approximately 62.5 μ m increment). It was considered to have reached the DG from the appearance of

high amplitude theta oscillatory pattern in the LFP. In 1 mouse, all 8 tetrodes in each mouse were used to measure the neural activities. Ground screw was used as the reference point to negate the background noises from the environment. In each of the following 3 mice, the references were changed to one of the tetrodes which were left in the corpus callosum. While these reference tetrodes still took reference from the ground screw to make sure no spiking activity was detected, they served to provide reference to other tetrodes.

Activity was recorded when mice were allowed to explore for 10 mins in a 60 L x 60 W x 30 H cm square box test environment. A 21cm x 30cm white area on one wall was provided as a visual cue for the animal to make reference bear. Chocolate cereal sprinkles were distributed throughout the environment as the animal explore for the food reward. To determine the location of the mouse when each spikes were detected, mouse's location was monitored with an overhead camera mounting on the ceiling, tracking one red and one green light emitting diode (LED) integrated on one set of the headstage amplifiers..

Photostimulation regime of 3.7ms pulses of 470nm laser every 2.5s lasting for 10 mins were applied with an absolute power of 15mW through regulation of a shutter, which was in turn controlled by transistor-transistor logic signal from a custom microcontroller circuit (Arduino). Shutter lag, defined by the delay between the signal triggering the shutter and when the laser was delivered, was previously measured as part of another study, and would be compensated in the data analysis. Recordings were carried out while the mouse was on an elevated pedestal, away from the view of the test environment. The signal controlling the shutter was split and sent to the Neuralynx recording system to register the timestamps of the start and end of the stimulations. These

timestamps were integrated, later during data analysis, with the timings of the recorded neural activities to determine if there were responses. As the shutter used was a mechanical diaphragm shutter, a distinct sound was produced whenever it was triggered. This sound was audible to the animal during recording. As a negative control, unit activities were also recorded with the laser source switched off in light off recording session, but the shutter was still triggered as it would when laser light was delivered.

Euthanasia and Perfusion

Mice were euthanised at the end of the experiment with pentobarbital. Intraperitoneal injection of the drug at a concentration of 0.1ml per 10 g body weight was administered. Intracardiac perfusion with saline was then performed, followed by 4% paraformaldehyde (PFA) in 0.1M phosphate buffer. Mice head was immediately decapitated and left submerged in 4% PFA in 0.1M phosphate buffer for at least 24 hours before tetrodes were raised and the drive was removed.

Drive removal and Brain extraction

Tetrodes were fully raised and the brain was extracted by carefully removing the skull without damaging the brain. Extracted brains were left submerged in fresh 4% PFA in 0.1M phosphate buffer for at least further 24 hours and transferred to 30% sucrose in 0.1M phosphate buffer for additional 48 hours minimum.

Histology

Final tetrode position to confirm the sites of recordings were determined by histology. Brain was sliced in 40 μ m coronal sections with Thermo Fischer Microm HM550 Cryostat, and the brain slices were immediately mounted onto gelatin coated

slides. The sections were stained with cresyl violet dye and covered with glass cover slides, by applying mounting medium (Eukitt, Sigma-Aldrich). Images were taken using a camera attached to Zeiss Axioscope A1 microscope to trace the trajectory of tetrodes.

Estimation of recording sites

Recording sites were estimated by first drawing a scale bar on the histology images, from the final tetrode position along the trajectory of the tetrode track, using ImageJ software. Based on the documented distance of tetrode movement during recording, recording sites were deduced.

Data Analysis

Data collected was analyzed offline with Matlab. Feature extraction (Refer to Approach) was done using MClust 3.5A.20 (AD Redish). Spikes were sorted into individual clusters by manually applying boundaries to delimit spikes with similar wave features.

To check the firing pattern of the cluster within the exploration recording session, a Matlab code was used to plot 1) rate maps showing the firing rate in the environment in the form of color scale (warmer color depicts higher firing rate); 2) the firing location maps showing the trajectory and location of the mouse when each spikes fired; and 3) interspikes interval histogram illustrating the counts of interval between consecutive spikes in each 1ms time bin.

Several firing properties of the units recorded during exploration were also computed. The average firing rate was calculated by dividing the number of spikes in the

unit with the duration of the whole session. The peak firing rate was the highest firing rate registered in any spatial bin of the rate map in one session.

Based on Shannon's information theory, incorporated by Skaggs et al. to quantify the information a cell carries, spatial information, in bits per spike, was calculated using

$$\sum_i p_i \frac{\lambda_i}{\lambda} \log_2 \frac{\lambda_i}{\lambda}$$

where p_i is the probability density of the location bin i , λ_i is the mean firing rate when the animal is in the location bin i , λ is the overall mean firing rate of the unit. **(Skaggs et al., 1993)** The value is indirectly proportional to how uniformly the unit fires in the environment

Sparseness was another measure of how diffuse the unit fire in the environment, as a scale of 0 to 1, calculated as following

$$\frac{(\sum_i \lambda_i p_i)^2}{\sum_i \lambda_i^2 p_i}$$

(Skaggs et al., 1993) The value is indirectly proportional to how confined the firing field was.

Selectivity was a measure of selective the unit was firing in the environment, calculated by dividing the peak firing rate by the mean firing rate. To put things into perspective, a unit that fires evenly in the environment will have a low selectivity value, a value of close to 1. This is due to a high average firing rate when the unit fires evenly in the environment.

Spatial coherence was a measure of the “smoothness” of the firing field of a cell and was obtained by calculating a pixel-by-pixel correlation of the smoothed and unsmoothed rate maps.

Time bin of the first peak in the interspike interval histogram was also counted. It is the time bin of which the highest counts of interspike interval between two consecutive spikes were counted.

Response to light stimulation was analyzed by a second matlab algorithm. First, spikes from the isolated clusters were extracted to determine the latency of each spike from each stimulation onset. The extracted spikes were cumulated into bins of 1ms time interval, tabulated and displayed as a peristimulus time histogram (PSTH). A separate raster plot was also plotted illustrating the latency of each spike.

To determine if firing was significantly elevated, a third matlab algorithm, developed and improvised based on method described by Zhang et al. (**Zhang et al., 2013**), randomly shuffle 10,000 times the spikes timing within -100 to +200 msec around each light pulse. The total number of spikes within the time block of three consecutive 1ms bins with the highest number of spikes was compared between real data and 10,000 shuffled data. When the number of spikes in the real data was more than the 99.95th percentile of the probability density of the shuffled data, firing was considered significantly elevated. Mean latency of all spikes contributing to the time block were also calculated.

Based on the principle that wave shape of the spikes from the same neuron were similar (Refer to Approach), it is essential to check the wave shapes of spikes within each

unit that displays light response, to determine if the elevated firing was contributed by spikes from the same neuron. A fourth matlab algorithm was used to plot the waveform of each spikes within the isolated unit cluster and overlap the traces of these waveforms within the light response period and those firing outside of the light response period.

RESULTS

Histological confirmation of tetrode final resting position

Recording was made on 4 mice. Post mortem histology confirmed the final positions of 10 out of 32 tetrodes implanted were within DG. A total of 55 units were recorded from 6 tetrodes in 3 mice. 4 tetrodes in the last of the 4 mice did not record any units. Recording sites of the 50 units were estimated as described in method section. (Fig. 10) These 50 units were recorded by the 6 tetrodes from different location of DG across different days. Some of the units may be generated by the same cell, but no attempts were made to determine whether the units detected by the same tetrode across different days of recording were from the same cells

Fig. 10 Recording sites of all units and the light responsive units.

Recording sites of all 50 units from 6 tetrodes in 3 mice are indicated by the red arrows. Recording sites of the 11 units with light responses are indicated by the yellow arrows.

Identification of light-responsive unit activity

Light responses were confirmed by the existence of peak in the PSTH with the significance percentile value exceed 99.95% as described in Method section. (Fig. 11 D) 11 units were identified to display light response. To determine if the response was evoked by light, spiking response during light off recording was checked. (Fig. 11 C) Absence of peak in the PSTH indicated no response during light off recording. The units between recording sessions were identified to be generated by the same cell based on the corresponding location of the cluster on both plots with the 2 same comparing features. (Fig. 11 A and B)

Based on the principle that wave shape of spikes generated by the same neuron will be identical, wave shape alignment of spikes within the period of response was checked, confirming that the traces were not misaligned but overlapping one another. Misalignment of the wave shape traces could mean that those spikes might be generated by different neurons, which were mistakenly included into the cluster during the clustering process. If these spikes were responses from another unit, including these spikes into the cluster could result in false positive responses of the unit. Therefore, the wave shapes of spikes that fired outside the response period were also visually compared to those fired within the response period to confirm the similarity. (Fig. 11 F) Since the wave shapes were similar, this inferred that the spikes were generated by the same neuron.

The wave shapes of the cluster in the light off recording session were also visually compared to those recorded in stimulation recording. (Fig. 11 E and F) The similarity between the wave shapes substantiated that both units were generated by the same neuron.

Fig. 11 Identification of light response

(A) Isolated unit cluster of firing (Red cluster) from recording during light off recording. (B) Isolated unit cluster of firing (Red cluster) from recording with 15mW photostimulation. (C) PSTH (*top*) and raster plot (*bottom*) of the cluster of spikes in (A). *Yellow bar* indicates the duration of stimulation. (D) PSTH and raster plot of the red cluster of spikes in (B). Note the increase in the number of spikes after stimulation. Light response period, which is the duration of the peak in PSTH, was between 3 to 8 ms. Statistical analysis indicated a percentile value of 100% significance increase in firing and mean latency was 5.4345ms. (E) Wave shape of spikes from (A), across 4 channels (*top to bottom*), within (*left*) and outside (*right*) of light response period found in stimulation session. Note the absence of wave shape on the left due to absence of response. (F) Wave shape of spikes from (B). Note the alignment of the wave shape, and the similarity between those in (*left*) and (*right*).

Firing of the light responsive units during exploration was identified, again by the corresponding location of the cluster on both plots with the 2 same comparing features. (Fig. 12 A and B) Activity of the unit during exploration was determined. (Fig. 12 D, E and G)

Fig. 12 Identification of activity of light responsive units during exploration

(A) Isolated unit cluster (Red cluster) from recording with 15mW photostimulation. (B) Isolated unit cluster (Red cluster) from recording during exploration. Note the similar location of the cluster in (A) and (B). (C) PSTH (*top*), raster plot (*middle*) and statistics (*bottom*) of cluster in (A). (D) Rate map, (E) firing location map and (G) interspike interval histogram of cluster in (B) (F) Color coded scale bar of firing rate in rate map. White depicts that the mouse did not explore the area.

To further validate and exclude the possibility that light stimulation can evoke activity in neurons not expressing ChR2-YFP, recording was also performed on one Cre negative mouse. As the neuron did not express Cre recombinase, the stop codon was not removed and ChR2-YFP could not be expressed. 3 tetrodes tracks were found to end in the dentate gyrus (Fig. 13), having recorded a total of 8 units in 2 of the tetrodes. (Fig.14) Results showed that as no increase in firing in response to the stimulation could be seen, suggesting light stimulation was unable to elicit response in non ChR2-YFP expressing neurons.

Fig. 13 Recording sites of all units in the ChR2-YFP negative mouse.

Recording sites of all 8 units are indicated by the red arrows

Unit #1

Unit #2

Unit #3

Unit #4

Fig. 14 Responses of all units from the ChR2-YFP negative mouse
(left) during light off recording (right) during light on recording

Characterizing activities of light responsive units during exploration

These light responsive units were observed to display characteristic firing patterns during exploration of the animals. The light responsive units appeared to fire throughout the arena, as illustrated by the firing location map (Fig. 15). Pattern of firing between these light responsive units were examined, to determine if there were any differences between these units. Classification was first done using hierarchical clustering (SPSS) with centroid clustering model by square Euclidean distance between each possible clusters. Clustering was performed based on the bin of first peak in the interspike interval since it could depict the relative refractory period of the neuron. Dendrogram obtained showed how the data points can be clustered into individual cluster. (Fig. 16) How to interpret the dendrogram was that starting on the left were the data point for all units, each assigned to individual clusters. The closest clusters were grouped to form a cluster at the next level to the right. This continued until the data points can be classified into 2 clusters towards the right most on the horizontal axis. The horizontal axis represents the distance between clusters in the data space. The decision of the number of clusters that can best depict different groups can be made based on which level of clustering has the largest distance between each clusters. **(Norušis, 2012)** In this case, 2 clusters could be best isolated with the distance between the clusters being the largest.

Fig. 15 Firing location map and rate map of light responsive units

Fig. 16 Dendrogram of cluster analysis of light responsive units using time bin of first peak

The first group of units (referred to as Group A from here on, Fig. 17), consisting of 5 units from 2 mice, exhibited a rise to reaching a peak in the interspike interval histogram within about 6 ms whereas there were 6 units from the same 2 mice in the second group (referred to as Group B from here on, Fig. 18) that first demonstrated the rise to a peak at about 11ms. This indicates that the Group A units were mostly firing at a shortest interspike duration of about 6ms; while the Group B units were rarely firing as short as 5ms apart, but about 11ms. This showed a difference in the relative refractory period of the units in each group, in which Group A units were more likely to fire again at shorter interval, where Group B units were less likely to be able to fire again in short latency after the previous spike. There was no difference in mean firing rate, peak firing rate, information, sparseness and selectivity indexes. Spatial coherence of Group A units were marginally higher than Group B units. (Table 2)

Light responses of the 2 groups of units were also studied. The mean response latency of group A and group B was 6.19 ± 1.57 ms (Mean $\pm$ standard error of mean) and 11.94 ± 2.69 ms (Mean $\pm$ standard error of mean). Statistical analysis showed that there was no significant differences between the response latencies of the both groups. (Table 2)

Fig. 17 Activities of Group A light responsive units

Rate maps, firing location map, interspike interval histogram (x-axis = 20ms), and PSTH, raster plots of Group A light responsive units, which exhibited a rise to peak at about 5ms in the interspike interval histogram

Fig. 18 Activities of Group B light responsive units

Rate maps, firing location map, interspike interval histogram (x-axis = 20ms), and PSTH, raster plots of Group B light responsive units, which exhibited a rise to peak at about 11ms in the interspike interval histogram

	Group A	Group B	Mann-Whitney U test (M-W U)	P value
Average firing rate (Hz)	23.50 ± 6.85	17.67 ± 2.94	12.00	0.662
Peak firing rate (Hz)	35.08 ± 7.90	11.43 ± 4.41	7.00	0.177
Information (bits per spike)	0.10 ± 0.01	0.09 ± 0.01	10.50	0.429
Sparseness	0.92 ± 0.01	0.935 ± 0.006	11.00	0.537
Selectivity	1.77 ± 0.22	1.49 ± 0.08	69.50	0.537
Spatial coherence	0.97 ± 0.004	0.96 ± 0.004	4.50	0.052*
Time Bin of first peak	5.9 ± 0.33	10.91 ± 0.35	0.00	0.004**
Response Latency (ms)	6.19 ± 1.57	11.94 ± 2.69	5.00	0.08

Table 2 Average firing properties indexes of Group A and Group B units.

Data expressed in mean ± SEM

*Marginally significant difference

**Significant difference between the two groups

Units in the group B appeared to fire in burst in response to stimulation, as illustrated by the raster plot. (Fig. 19 A) Spikes were observed to fire at regular interval, resembling burst firing. To determine if spikes were firing in burst, the number of spikes fired after each stimulation was counted (Fig. 19 B). Results showed that in an average of $27.25\% \pm 11.12\%$ of the trials, only one spike was detected. The average percentage of trials with 2 and 3 response spikes were $5.90\% \pm 3.13\%$ and $1.24\% \pm 0.51\%$ respectively. (n=6 units, Mean $\pm$ Standard error of mean) This revealed that the spikes were firing at various latencies, instead of responding in burst firing.

Fig. 19 Burst like firing responses of Group B light responsive units.

(A) PSTH and raster plots of one example unit with burst like firing response. Note the distribution of spikes in the red box at multiple interval, resembling burst firing. (B) Histogram of the average number of spikes fired within the response period in each stimulation trial. (n = 6 units) Error bar represents standard error of mean. Only 1 spike fired in response to stimulation in about 27% of the trials. The average percentages of trials with 2 spikes detected were about 8%, and about 1% of the trials with 3 spikes. This shows that the number of burst firing was very low.

Spatial specific firings of the units were also examined, based on the sparseness, information and selectivity score. Dendrogram of classification by sparseness, information and selectivity score showed that the units were best isolated into 2 clusters each. (Fig. 20, 21, 22)

Using sparseness as a classification parameter, one cluster include 9 units (referred to as Group 1 from here on) with a mean sparseness score of 0.94 ± 0.005 (Mean $\pm$ standard error of mean); and the other cluster with 2 units (referred to as Group 2 from here on) with a mean sparseness score of 0.89 ± 0.008 (Mean $\pm$ standard error of mean). Statistical analysis showed that there were actually no differences between the 2 groups. (Table 3) This showed that there was no differences in how evenly the units were firing within the environment. There were also no significant differences in other firing properties between the 2 groups. (Table 3)

Using information index as classification parameter, one cluster include 9 units (referred to as Group α from here on) with mean information of 0.10 ± 0.005 (Mean $\pm$ standard error of mean); and the other cluster with 2 units (referred to as Group β from here on) with mean information of 0.05 ± 0.003 (Mean $\pm$ standard error or mean). Statistical analysis showed that there were significant differences between the 2 groups. (Table 4) There were also significant differences in the sparseness and selectivity score between the 2 groups. (Table 4) This showed that units in Group α carries more spatial information than units in Group β , at the same time fire less evenly and more selectively in some area of the environment.

Using selectivity as a classification parameter, one cluster include 10 units (referred to as Group i from here on) with a mean selectivity score of 1.53 ± 0.07 (Mean $\pm$ standard error of mean); and the other cluster with 1 units (referred to as Group ii from here on) with selectivity score of 2.48. Perhaps due to a lack of sample size in Group ii, statistical analysis showed that there were no differences between the 2 groups. (Table 5)

If the unit (Mouse 1 Unit 2) were treated as an outlier, group i can be split into 2 groups of units. One group include 3 units (referred to as group i (A) from here on) with a mean selectivity score of 1.83 ± 0.07 (Mean $\pm$ standard error of mean) and the second group include 7 units (referred to as group i (B) from here on) with a mean selectivity of 1.40 ± 0.03 (Mean $\pm$ standard error of mean). Statistical analysis showed the selectivity of both group were significantly different (Table 6)

Fig. 20 Dendrogram of cluster analysis of light responsive units using sparseness

	Group 1	Group 2	Mann-Whitney U test (M-W U)	P value
Average firing rate (Hz)	22.54 ± 3.50	10.30 ± 6.27	5.00	0.44
Peak firing rate (Hz)	30.69 ± 4.41	22.35 ± 14.99	5.00	0.44
Information (bits per spike)	0.08 ± 0.0006	0.13 ± 0.003	2.50	0.15
Sparseness	0.94 ± 0.005	0.89 ± 0.008	2.00	0.15
Selectivity	1.48 ± 0.06	2.23 ± 0.26	2.50	0.15
Spatial coherence	0.96 ± 0.003	0.97 ± 0.01	8.50	0.91
Bin of first peak	9.28 ± 0.90	5.75 ± 0.25	2.50	0.15
Response latency	8.87 ± 1.87	8.93 ± 3.49	8.00	0.91

Table 3 Average firing properties indexes of Group 1 and Group 2 light responsive units.

Data expressed in mean ± SEM

Fig. 21 Dendrogram of cluster analysis of light responsive units using information

	Group α	Group β	Mann-Whitney U test (M-W U)	P value
Average firing rate (Hz)	19.89 $\pm$ 3.42	22.24 $\pm$ 13.32	9.00	1.00
Peak firing rate (Hz)	29.44 $\pm$ 4.42	28.01 $\pm$ 16.59	9.00	1.00
Information (bits per spike)	0.10 $\pm$ 0.005	0.05 $\pm$ 0.003	0.00	3.64E-02 **
Sparseness	0.92 $\pm$ 0.006	0.96 $\pm$ 0.003	0.00	3.64E-02 **
Selectivity	1.69 $\pm$ 0.12	1.30 $\pm$ 0.008	0.00	3.64E-02 **
Spatial coherence	0.97 $\pm$ 0.002	0.96 $\pm$ 0.01	6.50	0.58
Bin of first peak	8.56 $\pm$ 0.99	9.00 $\pm$ 2.00	7.00	0.73
Response latency	8.03 $\pm$ 1.15	12.74 $\pm$ 8.49	8.00	0.91

Table 4 Average firing properties indexes of Group α and Group β light responsive units.

Data expressed in mean $\pm$ SEM

**Significant difference between the two groups

Fig. 22 Dendrogram of cluster analysis of light responsive units using selectivity

	Group i	Group ii	Mann-Whitney U test (M-W U)	P value
Average firing rate (Hz)	20.69 ± 3.63	16.57	3.00	0.73
Peak firing rate (Hz)	28.36 ± 4.58	37.34	2.00	0.55
Information (bits per spike)	0.09 ± 0.008	0.13	0.00	0.18
Sparseness	0.93 ± 0.006	0.89	0.00	0.18
Selectivity	1.53 ± 0.07	2.48	0.00	0.18
Spatial coherence	0.96 ± 0.003	0.98	0.00	0.18
Bin of first peak	8.90 ± 0.89	6.00	2.50	0.55
Response latency	9.23 ± 1.71	5.43	3.00	0.73

Table 5 Average firing properties indexes of Group i and Group ii light responsive units.

Data expressed in mean ± SEM

	Group i (A)	Group i (B)	Mann-Whitney U test (M-W U)	P value
Average firing rate (Hz)	15.49 ± 5.84	22.92 ± 4.55	8	0.67
Peak firing rate (Hz)	25.16 ± 9.00	29.73 ± 5.68	10	1.00
Information (bits per spike)	0.10 ± 0.01	0.08 ± 0.008	4	0.18
Sparseness	0.92 ± 0.01	0.94 ± 0.006	4	0.18
Selectivity	1.83 ± 0.07	1.40 ± 0.03	0	0.02 **
Spatial coherence	0.97 ± 0.005	0.96 ± 0.003	9	0.83
Bin of first peak	7.33 ± 1.59	9.57 ± 1.04	6	0.38
Response latency	8.27 ± 2.26	9.64 ± 2.33	9	0.83

Table 6 Average firing properties indexes of Group i (A) and Group i (B) light responsive units.

Data expressed in mean ± SEM

**Significant difference between the two groups

Classification by the mean latency showed that 2 clusters can be isolated. (Fig. 23) One group contain 10 units (referred to as group AA from here on) with a mean response latencies of 7.65 ± 1.10 ms (Mean $\pm$ standard error or mean), and the second group with 1 unit (referred to as group AB from here on) with a latency of 21.23 ms. Statistical analysis showed no differences between the 2 groups, perhaps due to a lack of sample size for the group AB units. (Table 7)

If the unit (Mouse 1 Unit 5) were treated as an outlier, group AA can be split into 2 groups of units. One group include 3 units (referred to as group AA1 from here on) with a mean response latency of 12.37 ± 0.04 ms (Mean $\pm$ standard error of mean) and the second group include 7 units (referred to as group AA2 from here on) with a mean response latency of 5.63 ± 0.56 ms (Mean $\pm$ standard error of mean). Statistical analysis showed the response latency of both group were significantly different, along with average firing rate, peak firing rate and spatial coherence. (Table 8)

One problem would be that it is unclear if all the spikes were results of direct response to stimulation. A phenomena, known as postinhibitory rebound activation were reported previously where firing of the neuron increases following release from inhibition (**Boehme et al., 2011; Takeuchi et al., 2016**). One report had claimed that direct response from PV interneurons in the entorhinal cortex was 1.42 ± 0.07 ms, and that mean latency to inhibition in the postsynaptic neuron was 3.29 ± 0.07 ms. (**Buetfering, Allen and Monyer, 2014**) By this interpretation, all responses of our units were results of postinhibitory rebound activation from the postsynaptic neurons. However, their experimental design was different in a way that the laser intensity was not specified, the duration of stimulation was longer (10ms) and the neurons were stimulated at higher

frequency (6.67Hz); where the stimulation condition used in this study was 3ms pulse of 15mW laser every 2.5s interval. It is unclear if the difference in stimulation condition will impact the response latency of the neurons.

Fig. 23 Dendrogram of cluster analysis of light responsive units using mean response latency

	Group AA	Group AB	Mann-Whitney U test (M-W U)	P value
Average firing rate (Hz)	21.46 ± 3.43	8.92	1.00	0.36
Peak firing rate (Hz)	30.95 ± 4.23	11.43	1.00	0.36
Information (bits per spike)	0.09 ± 0.006	0.05	0.00	0.18
Sparseness	0.93 ± 0.007	0.96	1.00	0.36
Selectivity	1.65 ± 0.11	1.31	1.00	0.36
Spatial coherence	0.97 ± 0.002	0.95	0.00	0.18
Bin of first peak	8.40 ± 0.87	11.00	2.00	0.55
Response latency	7.65 ± 1.10	21.23	0.00	0.18

Table 7 Average firing properties indexes of Group AA and Group AB light responsive units.

Data expressed in mean ± SEM

	Group AA1	Group AA2	Mann-Whitney U test (M-W U)	P value
Average firing rate (Hz)	11.49 ± 3.89	25.73 ± 3.60	1.00	3.33E-02 **
Peak firing rate (Hz)	16.16 ± 4.62	37.29 ± 3.61	0.00	1.67E-02**
Information (bits per spike)	0.10 ± 0.01	0.09 ± 0.008	7.00	0.52
Sparseness	0.92 ± 0.009	0.93 ± 0.009	6.00	0.38
Selectivity	1.66 ± 0.16	1.64 ± 0.16	7.00	0.52
Spatial coherence	0.96 ± 0.003	0.97 ± 0.002	1.5.00	3.33E-02**
Bin of first peak	10.00 ± 2.29	7.71 ± 0.84	6.00	0.38
Response latency	12.37 ± 0.04	5.62 ± 0.56	0.00	1.67E-02**

Table 8 Average firing properties indexes of Group AA1 and Group AA2 units.

Data expressed in mean ± SEM

**Significant difference between the two groups

Identification of recording sites of Group A and B light responsive units

The recording sites of the two groups of units were identified. Units in the group A were recorded in the molecular layer and near hilus of the DG whereby units in group B were recorded in the granule cell layer and hilus region. (Fig. 24) This helped to provide a clue to account for the possible identity of the neuron. Axo-axonic cells can be found in the molecular layer and the hilus region, where pyramidal basket cells reside in the granule cell layer. (**Amaral, Scharfman and Lavenex, 2007**) Detection of Group A units in the molecular layer indicates the activities could be generated by axo-axonic cells. However, on the other hand, it is hard to tell if the group B units were generated by pyramidal basket cells since axo-axonic cells can also be found in the hilus region, which is adjacent to the granule cell layer where the pyramidal basket cells are.

Fig. 24 Recording sites of the 2 groups of units

Recording sites of units in Group A are indicated by red arrows; units in Group B by the yellow arrows.

Analysis of units not responding to photostimulation

Activities of the other 44 units without light responses were also examined. All 44 units were found across all stratum of DG. (Fig. 25) The firing properties of light responsive and non-light responsive units were first compared. The average firing rate and peak firing rate of the light responsive units were significantly higher than the non-light responsive ones. (Table 9) The information and selectivity scores were also significantly lower, and sparseness index significantly higher. This shows that these light responsive units had notable different firing properties as compared with the non-light responsive ones

Fig. 25 Recording location of all units without light responses.

	Light responsive units	Non light responsive units	Mann-Whitney U test (M-W U)	P value
Average firing rate (Hz)	20.32 ± 3.31	4.82 ± 1.65	41	2.34E-05**
Peak firing rate (Hz)	29.18 ± 4.22	11.06 ± 2.11	57	9.92E-05**
Information (bits per spike)	0.09 ± 0.01	0.40 ± 0.003	59.5	1.22E-04**
Sparseness	0.93 ± 0.01	0.67 ± 0.003	62.5	1.58E-04**
Selectivity	1.62 ± 0.11	4.53 ± 0.05	69.5	2.84E-04**
Spatial coherence	0.96 ± 0.003	0.96 ± 0.001	207.5	0.465

Table 9. Average firing properties indexes of light responsive and non-light responsive units.

Data expressed in mean ± SEM

**Significant difference between the two groups

Cluster analysis using sparseness as classification criteria suggested that the units could be grouped into 3 groups. (Fig. 26) The first group include 1 unit with sparseness score of 1.53, second group include 17 units with a mean sparseness score of 0.90 ± 0.01 (Mean $\pm$ standard error of mean), and a third group including 26 units with mean sparseness score of 0.54 ± 0.02 (Mean $\pm$ standard error of mean). There was no statistical differences in firing properties between Group 1 and 2, and Group 1 and 3; while there was significant differences in average firing rate, peak firing rate, information, sparseness and selectivity index between Group 2 and 3. This suggests that units in group 2 and 3 have different kind of spatial firing activity. There was no difference between group 1 and 2, and group 1 and 3 could be due to a low number of units in group 1 ($n=1$) reducing the statistical significance. (Table 10)

Comparison between the two groups of light responsive units and this 3 groups of non-light responsive units revealed that there were no differences in sparseness firing between any group of the light responsive units and group 1 and 2 non-light responsive units. The mean sparseness measure of Group 3 non-light responsive units was 0.54 ± 0.02 , (Mean $\pm$ standard error of mean) which were significantly lower than both group 1 light responsive units (0.94 ± 0.005 , Mean $\pm$ standard error of mean) with a p value of 5.57^{-13} and Group 2 light responsive units (0.89 ± 0.008 , Mean $\pm$ standard error of mean) with p value of 7.76^{-7} .

Fig. 26 Dendrogram of cluster analysis of non-light responsive units using sparseness

	Group 1	Group 2	Group 3		P value	
				Group 1 vs Group 2	Group 1 vs Group 3	Group 2 vs Group 3
Average firing rate (Hz)	0.85	9.94 ± 1.80	1.50 ± 0.17	0.11	0.44	1.19E-05 **
Peak firing rate (Hz)	9.71	16.13 ± 2.52	7.45 ± 0.69	0.67	0.74	1.00E-03 **
Information (bits per spike)	1.53	0.12 ± 0.01	0.55 ± 0.03	0.11	0.07	7.18E-05**
Sparseness	0.2	0.90 ± 0.01	0.54 ± 0.02	0.11	0.07	1.75E-03 **
Selectivity	14.36	2.01 ± 0.19	5.89 ± 0.38	0.11	0.07	1.75E-03**
Spatial Coherence	0.93	0.95 ± 0.003	0.97 ± 0.002	0.22	0.07	0.31

Table 10. Average firing properties indexes of Group 1, 2 and 3 non-light responsive units by sparseness classification

Data expressed in mean ± SEM

**Significant difference between the two groups

Classification by information showed that two clusters of units could be present. (Fig. 27) The first group contained 1 unit with information score of 1.53, while the second group contained 43 units with a mean information score of 0.37 ± 0.04 . There were significant differences in information, sparseness and selectivity between the two groups. (Table 11) However, the low number of units in this classification also lowered the level of confidence in such classification. I further divided group 2 into 3 subgroups, with the first new subgroup including 17 units with mean information of 0.12 ± 0.01 (Mean $\pm$ standard error of mean), second subgroup with 8 units with mean information of 0.76 ± 0.03 (Mean $\pm$ standard error of mean) and the last subgroup containing 18 units with mean information of 0.44 ± 0.02 (Mean $\pm$ standard error of mean).

There were no significant differences between the first group of units with the 3 subgroups of the second group. (Table 12, 13) The average firing rate, peak firing rate, information, sparseness and selectivity of the first subgroup was significantly different from the second and third subgroup. The first subgroup also had significantly lower coherence than the third. The second subgroup and third subgroup is different in terms of information, sparseness and selectivity. (Table 12, 13) This suggests that 4 main firing pattern can be classified by the information the units carried.

Comparison between Group α of the light responsive units with the 4 groups of non-light responsive units showed that there was significant difference with the last 2 subgroups of non-light responsive units. Group α has a mean information of 0.10 ± 0.005 (Mean $\pm$ standard error of mean), while the second and third subgroup of group 2 non-light responsive units has a mean information of 0.76 ± 0.03 (Mean $\pm$ standard error of mean) and 0.44 ± 0.02 (Mean $\pm$ standard error of mean) respectively. The p value was

6.60^{-13} and 6.67^{-13} respectively. Group β light responsive units has mean information of 0.05 ± 0.003 , which were also significantly lower than the second and third subgroup of the group 2 non-light responsive units. The p value was 6.60^{-13} and 8.86^{-10} respectively. This showed that both group α and β light responsive units each carried less amount of spatial information than the last 2 subgroups of non-light responsive units.

Fig. 27 Dendrogram of cluster analysis of non-light responsive units using information

	Group 1	Group 2	Man-Whitney U test (M-W U)	P value
Average firing rate (Hz)	0.85	4.91 ± 0.95	5	0.27
Peak firing rate (Hz)	9.71	11.09 ± 1.25	21	1.00
Information (bits per spike)	1.53	0.37 ± 0.04	0	4.55E-02 **
Sparseness	0.2	0.69 ± 0.03	0	4.55E-02 **
Selectivity	14.36	4.30 ± 0.37	0	4.55E-02 **
Spatial coherence	0.93	0.96 ± 0.002	1	0.09

Table 11 Average firing properties indexes of Group 1 and 2 non-light responsive units by information classification

Data expressed in mean ± SEM

**Significant difference between the two groups

	Group 1	Group 2A	Group 2B	Group 2C
Average firing rate (Hz)	0.85	9.94 ± 1.80	1.04 ± 0.22	1.89 ± 0.28
Peak firing rate (Hz)	9.71	16.13 ± 2.52	7.43 ± 1.45	7.95 ± 0.90
Information (bits per spike)	1.53	0.12 ± 0.01	0.76 ± 0.03	0.44 ± 0.02
Sparseness	0.2	0.90 ± 0.01	0.42 ± 0.01	0.60 ± 0.02
Selectivity	14.36	2.01 ± 0.19	8.21 ± 0.44	4.72 ± 0.22
Spatial Coherence	0.93	0.95 ± 0.003	0.96 ± 0.05	0.97 ± 0.002

Table 12 Average firing properties indexes of Group 1, and the 3 subgroups of Group 2 non-light responsive units by information classification

Data expressed in mean ± SEM

	P value					
	Group 1 vs Group 2A	Group 1 vs Group 2B	Group 1 vs Group 2C	Group 2A vs Group 2B	Group 2A vs Group 2C	Group 2B vs Group 2C
Average firing rate (Hz)	0.11	0.89	0.32	2.64E-04 **	3.58E-05 **	0.91
Peak firing rate (Hz)	0.67	0.67	0.84	2.05E-02**	4.98E-03**	0.984
Information (bits per spike)	0.11	0.22	0.11	5.10E-09**	5.102E-09**	5.11E-09**
Sparseness	0.11	0.22	0.11	5.102E-09**	5.102E-09**	1.342E-08**
Selectivity	0.11	0.22	1.10E-01	5.102E-09**	5.71E-09**	5.42E-09**
Spatial Coherence	0.22	0.22	0.11	0.64	1.00E-02**	0.28

Table 13 The significance of comparison between Group 1, and the 3 subgroups of Group 2 non-light responsive units by information classification

**Significant difference between the two groups

Classification by selectivity showed that two clusters of units could be present. (Fig. 28) The first group contained 1 unit with selectivity of 14.36, while the second group contained 43 units with a mean information score of 4.30 ± 0.37 . There was significant differences in information, sparseness and selectivity between the two groups. (Table 14) However, the low number of units in this classification had again lowered the level of confidence in such classification. I again further divided group 2 into 3 subgroups, with the first new subgroup including 22 units with mean selectivity of 2.37 ± 0.20 (Mean $\pm$ standard error of mean), second subgroup with 14 units with mean selectivity of 5.24 ± 0.20 (Mean $\pm$ standard error of mean) and the last subgroup containing 7 units with mean selectivity of 0.39 (Mean $\pm$ standard error of mean).

There were no significant differences between the first group of units with the 3 subgroups of the second group. (Table 15, 16) The average firing rate, peak firing rate, information, sparseness and selectivity of the first subgroup was significantly different from the second and third subgroup. The second subgroup and third subgroup is different in terms of information, sparseness and selectivity. (Table 15, 16) This suggests that there could be 4 kind of firing patterns.

Group i (A) and Group i (B) light responsive units were noticed to have significantly different selectivity measure than the last 2 groups of non-light responsive units. Group i (A) had a mean selectivity index of 1.83 ± 0.07 (Mean $\pm$ standard error of mean), which is lower than the second group of light non-responsive units with a mean selectivity index of 2.37 ± 0.20 (Mean $\pm$ standard error of mean) and the third group of light non-responsive units 5.24 ± 0.20 (Mean $\pm$ standard error of mean); while group i (B) units had a mean selectivity index of 1.40 ± 0.03 (Mean $\pm$ standard error of mean).

Fig. 28 Dendrogram of cluster analysis of non-light responsive units using selectivity

	Group 1	Group 2	Man-Whitney U test (M-W U)	P value
Average firing rate (Hz)	0.85	4.91 ± 0.95	5	0.27
Peak firing rate (Hz)	9.71	11.09 ± 1.25	21	1.00
Information (bits per spike)	1.53	0.37 ± 0.04	0	4.55E-02 **
Sparseness	0.2	0.69 ± 0.03	0	4.55E-02 **
Selectivity	14.36	4.30 ± 0.37	0	4.55E-02 **
Spatial coherence	0.93	0.96 ± 0.002	1	0.09

Table 14 Average firing properties indexes of Group 1 and 2 non-light responsive units by selectivity classification

Data expressed in mean ± SEM

**Significant difference between the two groups

	Group 1	Group 2A	Group 2B	Group 2C
Average firing rate (Hz)	0.85	8.30 ± 1.54	1.55 ± 0.15	1.01 ± 0.25
Peak firing rate (Hz)	9.71	14.52 ± 2.12	7.53 ± 0.66	7.42 ± 1.68
Information (bits per spike)	1.53	0.17 ± 0.02	0.49 ± 0.02	0.78 ± 0.04
Sparseness	0.2	0.85 ± 0.02	0.57 ± 0.02	0.41 ± 0.01
Selectivity	14.36	2.37 ± 0.20	5.24 ± 0.20	8.50 ± 0.39
Spatial Coherence	0.93	0.96 ± 0.003	0.97 ± 0.002	0.96 ± 0.005

Table 15 Average firing properties indexes of Group 1, and the 3 subgroups of Group 2 non-light responsive units by selectivity classification

Data expressed in mean ± SEM

	P value					
	Group 1 vs Group 2A	Group 1 vs Group 2B	Group 1 vs Group 2C	Group 2A vs Group 2B	Group 2A vs Group 2C	Group 2B vs Group 2C
Average firing rate (Hz)	0.17	0.27	1.00	1.55E-03**	7.49E-03**	0.97
Peak firing rate (Hz)	0.78	0.80	0.75	2.63E-02**	0.09	1.00
Information (bits per spike)	0.09	0.13	0.25	5.14E-09**	5.10E-09**	5.58E-07**
Sparseness	0.09	0.13	0.25	5.11E-09**	5.10E-09**	7.61E-04**
Selectivity	0.09	0.13	0.25	5.14E-09**	5.10E-09**	8.93E-09**
Spatial Coherence	0.17	0.13	0.25	0.08	1.00	0.20

Table 16 The significance of comparison between Group 1, and the 3 subgroups of Group 2 non-light responsive units by selectivity classification

**Significant difference between the two groups

The various firing indexes of the light responsive units were also compared with those of identified place cells. Place cells are neurons that increase their firing at specific location of the environment. (O'Keefe, 1976; O'Keefe and Conway, 1978; O'Keefe and Dostrovsky, 1971; O'Keefe and Speakman, 1987; Jung and McNaughton, 1993). Units with place cell activity was identified from the rate map with the presence of localized area with high firing rate (place field). (Fig.28) The result showed that firing of these light responsive units were significantly different as compared to place cells. (Table 17) The high sparseness index, and low information and selectivity scores of these light responsive units, as compared to place cells, indicated that their firing were less spatially modulated. This indicates that these light responsive units are functionally different from place cells.

It was discussed previously that group α of the light responsive units carried more spatial information than the other group. I therefore compared the information of each group with that of place cells. Result showed that both group of units carried less spatial information than place cells (Group α : 0.10 ± 0.005 , Group β : 0.05 ± 0.003 , Place cell: 0.65 ± 0.05) This further illustrated that the firing of the light responsive units were less spatially modulated.

Fig. 29 Firing location map and rate map of units with place cell activities

	Light responsive units	Place cells	Mann-Whitney U test (M-W U)	P value
Average firing rate (Hz)	20.32 ± 3.31	1.36 ± 0.17	0.00	6.00E-06**
Peak firing rate (Hz)	29.18 ± 4.22	8.09 ± 0.99	7.00	2.55E-04**
Information (bits per spike)	0.09 ± 0.01	0.65 ± 0.05	0.00	6.00E-06**
Sparseness	0.93 ± 0.01	0.47 ± 0.02	0.00	6.00E-06**
Selectivity	1.62 ± 0.11	6.41 ± 0.48	0.00	6.00E-06**
Spatial coherence	0.96 ± 0.003	0.97 ± 0.002	31.00	0.099

Table 17 Average firing properties indexes of light responsive units and units with place fields.

Data expressed in mean ± SEM

**Significant difference between the two groups

Identification of recording sites of units not responding to laser

Optic fibres were implanted more superficially; tetrodes were sometimes advanced too deeply into the brain that the region does not receive sufficient light power intensity to elicit response. Due to the high lipid content in the brain, largely from myelination of neurons, light is absorbed and scattered, resulting in a reduction of light power intensity as the distance between the laser source (tip of optic fibre) and region to be stimulated (neuron location) increases. Therefore, recording sites of the units without light response was checked, to determine if sufficient laser reached the recording sites. Result showed that the furthest distance light response was recorded was about 687.5 μm . This infers that ChR2 positive neurons within this distance would most likely receive sufficient laser intensity to elicit response. Result also showed that all recording sites were within this distance. (Fig. 30)

Fig. 30 Distribution of distances between optic fibre and tetrode of non-light responsive units and light responsive units

DISCUSSION

Distinguishing direct response from ChR2 positive neurons

By using optogenetics assisted neuronal identification and extracellular unit recording, I was able to electrophysiologically identify PV expressing neurons, and record their activities during exploration of the animal. Before I attempt to characterize the activities of these light responsive units, I need to know if the light responses were the direct responses from ChR2 positive cells, and not due to secondary responses from the postsynaptic ChR2 negative neurons.

One report had claimed that mean latency of direct response from PV interneurons in the entorhinal cortex was $1.42 \pm 0.07\text{ms}$, and that mean latency to inhibition in the postsynaptic neuron was $3.29 \pm 0.07\text{ms}$. (**Buetfering, Allen and Monyer, 2014**) By this interpretation, all responses of our units were results of indirect response most probably from the postsynaptic neurons. However, their experimental design was different in a way that the laser intensity was not specified, the duration of stimulation was longer (10ms) and the neurons were stimulated at higher frequency (6.67Hz); where the stimulation condition used in this study was 3ms pulse of 15mW laser every 2.5s interval. It is unclear if the difference in stimulation condition will impact the response latency of the neurons.

It had been widely known that GABA neurons are inhibitory. (**Curtis et al., 1970; Krnjević and Phillis, 1963**) Although studies tried to argue that GABA has excitatory effect on immature neurons (**Ben-Ari et al., 2007; Li and Xu, 2008**), more recent studies have rebuffed the finding (**Kirmse et al., 2015; Valeeva et al., 2016**) PV was only reported to be expressed in the basket cells and axo-axonic cells in the dentate gyrus (**Jinno and Kosaka, 2002; Ribak, Nitsch, and Seress, 1990**), where the both cell types are GABA neurons (**Buhl et al., 1994; Ribak, 1992**). In this regard, I argue that activation of PV neurons is unable to excite other neurons directly.

Previous morphological study reported that most of the synaptic connections of hippocampal interneurons were made onto pyramidal cells, where 5-15% of connections were made onto other interneurons. (**Cobb et al., 1997; Sik et al., 1995**) Although no study had reported the identity of these postsynaptic interneurons in the hippocampus, Pfeffer et al. reported that in mouse visual cortex, PV interneurons strongly inhibit each other providing little inhibition to other interneurons. (**Pfeffer et al., 2013**) Activation of PV interneurons could affect the activity of the postsynaptic interneurons, suppressing its activity. Studies had shown that following release from inhibition, neurons can respond with increase in firing. (**Boheme et al., 2011; Takeuchi et al., 2016**) This phenomenon is known as postinhibitory rebound activation. The average firing rate of the units were too high to be considered excitatory neurons. However, it remained to be determined if the response was fired by PV interneurons postsynaptic to the ChR2 expressing one.

It had been reported that response latencies of primary response from ChR2 expressing neurons are shorter than indirect responses from postsynaptic neurons (**Zhang et al., 2013**) Therefore, determining the response latencies of the two type of responses is

essential. Future experiments would be needed to determine this piece of information. Patch clamping the ChR2 expressing neurons and its postsynaptic neurons can give a clue to the response latencies of direct and indirect responses. A limitation would be it is difficult to replicate the stimulation conditions in in-vivo experiment. The light scattering properties of the high-lipid containing brain tissue made it hard to know how much laser intensity reached the cell to evoke the response. (Yona et al., 2016) It would be difficult to replicate the condition in in vitro experiment. In addition, spiking output can be affected by input from the circuit which would be non-existent in hippocampal slices. A more accurate, but at the same time more labour intensive approach, would be recording with in vivo patch clamp. Once the response latencies of direct and indirect response are established, the conventional unit recording can be used.

Alternatively, recording from halorhopsin expressing neurons can allow comparison of response latencies of any postinhibitory rebound spiking with the response latencies of units recorded in ChR2 positive animals. This allow us to know the response latencies of direct postinhibitory rebound activation.

In summary, it is unclear yet if the responses were direct responses from ChR2 expressing neurons.

I also considered if the responses were truly neuronal responses evoked by light. For the 11 units determined as light responsive, no responses were identified when laser was not delivered, while the shutter sound was kept on. This shows that the shutter sound was incapable of inducing responses. Recording made on one Cre negative mouse also confirmed that light stimulation was incapable of evoking response in ChR2-YFP negative neurons. There is no increase in firing in response to the stimulation in all 8 units

recorded in the dentate gyrus. One source of non-neuronal responses was photovoltaic artefacts. Contamination from photovoltaic artefacts was repeatedly mentioned in many studies. (**Cardin, 2012; Cardin et al., 2010; Han et al., 2009; Kravitz, Owen, and Kreitzer, 2013; Moore and Wehr, 2013**) These artefacts arises from when light (photon) strikes a material, the energy can excite the electrons of the material and cause it to be ejected from the atom. The resulting movement of electrons in metal can generate current, which will raise the wide-band signal detected, resulting in false positive spike detection. This relationship is dependent on the material and wavelength of the photon, regardless of intensity and duration. With this in mind, I argue that with platinum as the material used in making the tetrodes, there is insufficient photon energy (3 electron-volts, eV) in the 475nm laser I were using to release electrons from platinum to generate current. Platinum has a about 5eV energy barrier which requires a minimum of 5eV of photon energy to release electrons (**Gossenberger et al., 2014**). For these reason, I believe that the responses were neuronal responses evoked by light.

Characterizing activities of ChR2 positive neurons

The light responsive units were classified using different firing properties. Using the time bin of first peak in interspike interval, two major types of activities were identified to be elicited by the light responsive neurons in the DG during exploration of the animal. One group of unit activities displayed a firing pattern with a rise to peak at about 6 ms in interspike interval histogram. This means that, at most of the times, the shortest duration between spikes was about 6 ms. The other group of unit activities demonstrated interspike interval histograms a rise to peak only at about 11ms. This infers most spikes were firing at about 11 ms apart. This finding possibly reflected the difference in the relative refractory period of the units in the two groups.

This also showed a difference in minimum rate of inhibitory control over the postsynaptic neuron. This suggests that the Group A units had frequent control over the firing of the postsynaptic neuron and the second group of units had less frequent control. It is likely that the difference represents different subtypes of PV expressing neurons.

The light responses of the units helped to shed some light into the excitability of the neurons in firing the different pattern. Responses from the units in Group A showed a sharp peak in PSTH, suggesting that the neurons were easy to excite, thus firing at as short as 6 ms interspike interval.

Spatial specific firings of the units were also examined, based on the sparseness, information and selectivity score. It was found that all units were firing throughout the environment, but some units appeared to carry more spatial information than the others. This showed that some of these units have some level of spatial modulation. In fact, it was

reported that firing of PV interneurons in the entorhinal cortex were spatially tuned. **(Buetfering, Allen and Monyer, 2014)** It is yet to know if this is the case for PV interneurons within the dentate gyrus.

Determining the possible identity of ChR2 positive neurons

PV was reported to be expressed in two morphologically distinct neurons, the pyramidal basket cells and axo-axonic cells. **(Ribak, Nitsch, and Seress, 1990)** Expression of PV in other types of neurons within DG was not reported. The electrophysiological properties of the two types of neurons could help to account for the disparity in firing pattern. Whole-cell intracellular recording from the both neuronal types revealed that the input resistance of the axo-axonic cells is higher than the pyramidal basket cells [Axo-axonic cells = $73.9 \pm 23.8 \text{ M}\Omega$ **(Buhl et al., 1994)**, Pyramidal basket cells = $43.0 \pm 5 \text{ M}\Omega$ **(Lübke, Frotscher and Spruston, 1998)**]. Ohm's law states that at a constant input current, resistance is directly proportional to the voltage. A low resistance would therefore result in a low voltage change, thus causing the neuron to be less excitable as more input current is required to induce the same depolarization. With the input resistance of basket cells being lower, it might take longer to produce spikes as compared to the axo-axonic cells. This could lead to spikes firing at a longer interval, as illustrated by the firing pattern of the Group B units. However, this deduction was based upon an assumption that both types of cells were receiving similar strength of input.

The location of the recording sites where the activities of the two groups of units were detected can also provide a clue to account for the identity of the neuron. Group A units were detected in molecular layer and in the hilus region; where the group B units were detected only in the hilus region. Axo-axonic cells can be found in the molecular

layer and the hilus region, where pyramidal basket cells reside in the granule cell layer. (Amaral, Scharfman and Lavenex, 2007) Detection of Group A units in the molecular layer indicates the activities could be generated by axo-axonic cells, consistent with the easier excitable nature of the cell type. On the other hand, it is hard to tell if the Group Bf units was generated by pyramidal basket cells since axo-axonic cells can also be found in the hilus region, which is adjacent to the granule cell layer where the pyramidal basket cells are. However, there is a drawback in this approach. It is not known what is the radius the tetrodes can receive distinguishable signal from, with studies claiming a radius of about 60 μm (Harris et al., 2000) up to about 130 μm (Mechler et al., 2011). This could mean even with the recording at the molecular layer, activity of neurons within the hilus might also be detected. This approach could only serve as rough estimation of the identity of each unit.

The difference in light response between the two groups can also be explained by the property of the two cell types. Most of the response spikes of units in Group A were at similar latency, as illustrated by a sharp peak in PSTH, resembling to the activity of the more excitable axo-axonic cells as it is easier to generate spikes. Response spikes of units in Group B were fired at different latency, illustrated by multiple peaks in the PSTH, resembling the less excitable basket cells as it would take longer to generate a spike following activation.

Characterizing activities of ChR2 negative neurons

Activities of non-light responsive neurons were also identified. To study how differently the PV interneurons in the dentate gyrus fires from the other neuronal types during exploration, the non-light responsive neurons were also examined. Initial comparison between the light responsive units and all the non-light responsive units revealed that most of the firing properties of the two groups were significantly different. Comparison between the light responsive units and identified place cells showed that their activity were also significantly different.

For units without light response, I considered if the stimulation was sufficient for eliciting responses at the recording sites. I had estimated the maximum distance where light response was recorded, and found that all recording sites were within the maximum distance where light response was recorded.

I also considered that in addition to the possibility of insufficient laser intensity, the low number of light responsive units could be due to PV expressing neurons that did not express ChR2. PV was reportedly expressed in about 20-25% of the morphologically distinct axo-axonic cells and pyramidal basket cells. (**Jinno and Kosaka, 2002**) The firing pattern might be a characteristic of these neurons, regardless of PV expression. Another possibility might be that there are other neuronal types which fire with a similar pattern. According to the mouse strain datasheet from The Jackson Laboratory where the PV-Cre mice were originally purchased, recombination of transgene occurs at about 90% of neurons. This means that there is a 10% chance where the PV neurons do not express Cre for ChR2 expression. Expression level of ChR2 in PV neurons was not checked, it is unclear if all PV neurons express ChR2.

FUTURE WORK

To confirm that the responses were results of direct responses from ChR2 expressing neurons, follow-up experiments are needed. Stimulating patch clamped ChR2 expressing neurons and measuring the delay between onset of stimulation and the spiking response can give a clue of the primary response latencies of the neurons. Experiments to confirm the latencies of postinhibitory rebound activation can also be done, by patch clamping the postsynaptic neuron. It had suggested that the response latencies of primary response from ChR2 expressing neurons are shorter than indirect responses from postsynaptic neurons, thus allowing the distinguishing of the two types of responses. **(Zhang et al., 2013)** In this way, responses that are less than the minimal response latency of postsynaptic neurons can be therefore treated as the direct response. A limitation to this approach would be there might be a difference in stimulation conditions (i.e. reduction in laser intensity due to light scattering properties of the high lipid content of whole brain tissue) and contribution of inputs from the circuit in in-vivo brain that affect the spiking output. In this regard, in-vivo patch clamp of the ChR2 neuron might give more accurate result as the aforementioned conditions are being kept constant. While in vivo patch clamp often require the animal to be head fixed, making it difficult to freely explore, once the response latencies of direct and indirect response are established, the conventional unit recording can be re-utilized.

In vivo recording of halorhodopsin expressing PV interneurons can also give clue on the latencies of postinhibitory rebound activation. Stimulation of halorhodopsin leads to suppression of neuronal activity. **(Gradinaru, Thompson and Deisseroth, 2008)** If an

increase in light evoked response is observed, it would be possible to compare the response latencies of the halorhopsin expressing unit with that of channelrhopsin expressing unit to see if there is differences in response latencies.

Further experiments are needed to characterize the activity of other neuronal subtypes to fully understand how processing of spatial information is achieved in the DG.

Alternatively, it would be interesting to explore other methods in identifying neuronal subtypes in awake animals. The yield of electrophysiological unit recording is relatively low, and there is a lack of knowledge about the location of the neuron. It is unclear what is the recording radius unit recording can detect. It is possible that multiple tetrodes are recording from the same neuron. The unit activity can be different in each tetrode based on the distance of the neuron from the tetrodes. An approach such as calcium imaging (**Russell, 2011**) would allow a higher number of neurons to be recorded and also allow identification of the neurons. Following calcium influx during neuronal activation, the calcium ions bind with calcium indicators in the neurons to exhibit a change in fluorescence intensity. Through connecting miniature head-mounted microscope (**Helmchen et al., 2001**) to endoscope implanted into the subject's head, fluorescence can be observed, representing neuronal firing. However, unit recording remains the preferred approach to measure neuronal activity due to a high temporal resolution of spikes timing. The thinner tetrodes, as compared to the endoscope used in calcium imaging, also cause less damage to the brain, especially when recording is done in deep brain structure like the hippocampus.

CONCLUSION

In summary, we had characterized the activities of PV expressing neurons in the DG during exploration of the animal. Two major classes of activities were identified. Result had shown that there is one class of units with firing at short interspike interval of about 5ms, and another class of units with firing at a longer interspike interval of about 10ms. Units in the two classes has different response to light, with the aforementioned first group displaying response at more constant latency and the second group responding at more varied latencies between trials. This gives us some insight about the firing pattern of PV expressing neurons in the DG during spatial exploration.

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